Role of Bio-Fertilizer Treatments in Alleviating the Adverse Effect of Water Stress in *Mangifera indica*

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WATER stress is one of the most important abiotic stresses that may limit agriculture production worldwide. This work was carried out on mango trees *(Mangifera indica* L.) to study the effect of exposure to different levels of drought stress (65, 75, 85 and 100 % of full irrigation requirements), in addition to evaluate the role of using some plant growth promoting rhizobacteria (PGRP); such as *Azospirillum* and *Azotobacter*, in alleviating drought-induced changes. Physiological and biochemical changes were determined in mango leaves after two seasons of different treatments. Results indicated that membrane stability, photosynthetic pigments and insoluble sugar contents were significantly decreased with increasing drought levels, while electrolyte leakage, soluble sugars, total carbohydrates and proline content were sharply increased compared to control. Lipid peroxidation level and the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzymes showed progressive increases with drought levels. Application of bio-fertilizers may be effective in alleviating the adverse effect of water stress. Bio-fertilizers caused marked increase in photosynthetic pigments and carbohydrate contents, and decrease in proline content compared to control.

Key words: Drought, Water stress, PGPR, *Mangifera indica,* Chlorophyll, Antioxidant enzymes, Proline.

Abbreviations: PGRP, plant growth promoting rhizobacteria; ROS, reactive oxygen species; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; EL, Electrolyte leakage; MSI, membrane stability index; DW, dry weight; FM, fresh mass; MDA, Malondialdehyde; S, *Azospirillum***;** B, *Azotobacter***.**

Countries in arid and semi-arid regions suffer from water shortage for agriculture usage. Water stress affects plant growth and productivity as it causes various physiological and biochemical changes including hormonal and nutritional imbalance, ion toxicity, desiccation, abscission, senescence and susceptibility to diseases (Nadeem *et al.*, 2014). Also, drought can lead to pigment degradation (Hendry *et al.*, 1987) causing irreversible damage to the photosynthetic apparatus (Clarke *et al.*, 1996).

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On the other hand, water stress can cause rapid damage to plant cells membrane due to an uncontrolled enhancement of reactive oxygen species (ROS) (Moussa and Abdel-Aziz, 2008). Excess accumulation of ROS may initiate destructive oxidative processes such as lipid peroxidation and chlorophyll bleaching as well as oxidation of proteins, deoxyribonucleic acid and carbohydrates (Ashraf, 2009). The degree of damage caused by ROS depends on the balance between the production of ROS and its removal by efficient antioxidant scavenging system which includes nonenzymic and enzymic antioxidants (Azooz *et al.*, 2009). The enzymic antioxidants include superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase and enzymes of ascorbate-glutahione (AsA-GSH) cycle such as ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase (Sharma *et al.*, 2012). Moreover, production and accumulation of proline is one of the most common adaptive strategies to cope with environmental stresses. Proline plays an important role in osmotic adjustment, detoxification of ROS and membrane integrity under stress conditions (Lisar *et al.*, 2012).

Plant growth promoting rhizobacteria (PGPR) plays a significant role in enhancing plant growth and development under both stress and non-stress conditions (Nadeem *et al.*, 2010; Kundu *et al.*, 2011). They can improve plant tolerance towards abiotic stresses like drought, chilling injury, salinity, metal toxicity and high temperature (Grover *et al.*, 2011). PGPR elicit physical and chemical changes that improve plant defense and enzymes activity; such as catalase and superoxide dismutase, which alleviate the oxidative damage induced by drought (Kohler *et al.*, 2008 and Wang *et al.*, 2012). *Azospirillum* and *Azotobacter* strains are non-symbiotic rhizobacteria. *Azospirillum* spp. can exert a positive effects on plant growth including synthesis of phyto-hormones, N_2-fixation, nitrate reductase activity and enhancing minerals uptake (El-Komy *et al.*, 2004). Moreover, *Azospirillum* spp. are associated with biochemical changes in roots, which in turn; promote plant growth and increase the tolerance to water stress (Ilyas *et al.*, 2008). However, *Azotobacter* strains play a key role in harnessing the atmospheric nitrogen through its fixation in the roots and improve fertility condition of the soil (Mahato *et al.*, 2009).

New reclaimed areas in Egypt, where the water sources are limited, are cultivated mainly with horticulture crops. Mango (*Mangifera indica* L.) is the second largest cultivated fruit in the tropical and subtropical regions in terms of cultivated area and quantity of production. In 2012, the mango cultivated areas in Egypt increased to more than 700,000 hectare (FAO, 2012). Irrigation of mango orchards; especially during the period of plant growth and fruit development, is vital to improve fruit size and quality, prevent fruit drop and sustainability of the orchard. Drought stress can cause a great reduction in the vegetative growth, flush length and weight, leaf water content and root growth of mango trees (Tahir *et al.*, 2003).

Therefore, this investigation aimed to 1) assess the biochemical changes in mango leaves; Owais cv., after two years of exposure to four levels of irrigation

(65, 75, 85 and 100 % of full irrigation requirements) and 2) evaluate the role of application of some non-symbiotic rhizobacteria such as *Azospirillum* and *Azotobacter* in alleviating drought-induced changes.

Materials and Methods

Our experiment was carried out in a private mango orchard placed at Cairo-Alexandria Desert Road. Eight to ten years old mango trees (cv. Owais); grafted onto seedling rootstock and planted at 5 meters apart in sandy soil under drip irrigation system, were selected for this study. Agricultural practices and chemical fertilizer were conducted as recommended by Egyptian Ministry of Agriculture and Land Reclamation (2004).

Experimental design

The experiment was laid out with sixteen treatments (three replicates for each) in randomized complete block design to study the interaction between four irrigation levels and four bio-fertilizer treatments. Four levels of irrigation (100, 85, 75 and 65% of full water requirements) were applied in combinations with four bio-fertilizer treatments; T_1: Control (without using bio-fertilizer), T_2: *Azospirillum*, T_3: *Azotobacter* and T_4: *Azospirillum* + *Azotobacter*. Three rows of trees formed the border to the adjacent irrigation treatments.

Irrigation treatments

Water requirements for irrigation was calculated as potential crop evapotranspiration (ETc), based on climatic data obtained from the meteorological station of El-Tahrir, using CROPWAT computer program, which models crop-specific water requirement based on the Penman–Monteith equation (Allen *et al.*, 1998).

$$ET_C = k_C * ET_0$$

where, Kc: the crop coefficient which varies for different crops and their growth stages; ET_0: the reference crop evapotranspiration (FAO, 1993).

The following irrigation treatments (Table 1) were applied starting February 2012 through the two successive growth seasons. Leaf samples were taken in April 2014.

TABLE 1. The amount of applied irrigation water based on crop water requirements.

Irrigation treatments	Water quantity (m^3/fed*/year)
100% of calculated water requirement (full irrigation)	6545
85% of full irrigation requirement	5564
75% of full irrigation requirement	4909
65% of full irrigation requirement	4255

*Fed= 4200 m^2

Bio-fertilizer treatments

Two non-symbiotic rhizobacteria (*Azospirilum lipoferum* and *Azotobacter chroccocum*) were obtained from the microbiology department, Agriculture Research Center, Giza, Egypt. The two strains were grown on semisolid malate media (Dobereiner *et al.*, 1976) and modified Ashby's (Abd El-Malak and Ishac, 1968), respectively. Strains were grown on liquid medium in a rotary shaker at 30°C, then cultures were diluted and added to the trees at a rate of 500 ml/tree (10^3- 10^4 cell. ml^{-1}). Biofertilizers were mixed with the soil and covered at a depth of 30 cm. in a ring one meter away from the trunk. Biofertilizer applications were repeated three times (February, March and April) in two seasons; 2012 and 2013.

Laboratory analysis

In April 2014, a bulk sample of 15-20 fully expanded leaves of mid-shoot was selected for each treatment. Leave samples were free from diseases, insects or mechanical damage. Fresh collected leaves were transferred directly in ice-box to the lab to estimate the following parameters.

Electrolyte leakage

The total inorganic ion leak from the leaves was measured by the method described by Sullivan and Ross (1979) in the form of electrolyte leakage. Twenty leaf discs of 2 ml diameter were placed in a boiling tube containing 10 ml double distilled water. The tubes were heated at 45°C (EC_a) and 55°C (EC_b) for 30 min in a water bath and the electrical conductivity (EC) was measured with a conductivity meter (ME977-C, Max Electronics, India). Subsequently, the tubes were boiled at 100°C for 10 min and the EC was again recorded (EC_c). Electrolyte leakage was calculated with the following formula:

$$\text{Electrolyte leakage (\%)} = (ECb - ECa) / ECc \times 100$$

Membrane stability index

The membrane stability index (MSI) was estimated in two sets, by placing 200 mg of leaves in 10 ml double distilled water in each set. First set was heated at 40°C for 30 min in a water bath and the second set was boiled at 100°C in a boiling water bath for 10 min. Electrical conductivities (C_1 and C_2) were measured, respectively using a conductivity meter (ME977-C, Max Electronics, India)..The MSI was calculated using the formula described by Premchandra *et al.* (1990) and modified by Sairam (1994): $MSI = [1 - (C_1/C_2)] \times 100$

Photosynthetic pigments

The contents of the photosynthetic pigments chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids in fresh leaves were estimated using the spectrophotometric method described by Hassanein *et al.* (2009). The concentration of each pigment was calculated and expressed as $\mu g.g^{-1}$ dry weight (DW) of leaves.

Determination of carbohydrate content

Soluble sugar was extracted from air –dried leaf tissue with 80% ethanol.

One gram of the dried tissues was homogenized with 80% ethanol then put in a boiling water bath for 15 min. After cooling, the extract was filtered and the filtrate was oven dried at 60°C then dissolved in a known volume of water to be ready for soluble sugars determination (Homme, *et al.*, 1992). The soluble sugars were determined by the anthrone sulfuric acid method described by Scott and Melvin (1956). Polysaccharide content was determined in the dry residue left after extraction of soluble sugars. A known weight of dried material was added to 10 ml 1.5N sulphuric acid in sugar tube with air reflux and heated at 100°C in a water bath for 6 hr (Hodge and Hofreiter, 1962). The hydrolysate was made up to a known volume to be ready for polysaccharide determination by the method of anthrone sulphuric acid reagent. Total carbohydrates content was calculated as the sum of the amounts of soluble sugars and polysaccharides in the same sample. All data were calculated as mg 100 g^{-1} DW of leaves.

Estimation of proline content

Free proline was extracted and determined in fresh leaves according to the procedure of Bates *et al.* (1973). Proline contents were determined and calculated as mg.100g^{-1} DW of leaves.

Protein content

The total protein content in the leaves was estimated by adopting the methodology of Lowry *et al.* (1951). The protein was extracted with (0.1 M) NaOH and the Folin phenol reagent was added to develop the blue colour which was read at 600 nm. A calibration curve was plotted by using bovine serum albumin to calculate the percentage protein content in the samples.

Determination of lipid peroxidation

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) contents using the method of Hodges *et al.* (1999).

Antioxidative enzyme assay

For the assay of antioxidant enzymes, the leaf tissue (0.5 g) was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1 % polyvinylpyrrolidone. The homogenate was centrifuged at 27,600×g for 10 min at 4°C, and the supernatant was used as source of the enzymes CAT (EC 1.11.1.6), POD (EC1.11.1.7) and superoxide dismutase (SOD; EC 1.15.1.1). For the estimation of peroxidase activity (Chance and Maehly, 1956), the enzyme extract (0.1 ml) was added to the reaction mixture consisting of pyrogallol, phosphate buffer (pH 6.8) and 1% H_2O_2. The change in the absorbance was read every 20 s for 2 min at 420 nm on a spectrophotometer. A control set was prepared by adding DDW instead of enzyme extract. The reaction mixture for catalase consisted of phosphate buffer (pH 6.8), 0.1 M H_2O_2 and enzyme extract (1.0 ml). H_2SO_4 was added to the reaction mixture, after incubating it for 1 min at 25° C, and was titrated against potassium permanganate solution (Chance and Maehly, 1956). The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium using the method of Beauchamp and Fridovich (1971). The reaction mixture containing 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM

nitroblue tetrazolium, 2 mM riboflavin, 0.1 mM EDTA and 0–50 µl enzyme extract was placed under a 15-W fluorescent lamp. The reaction was started by switching on the light and was allowed to run for 10 min. Fifty percent inhibition by light was considered as one enzyme unit.

Statistical Analysis

Mean values were analysed for differences using one-way ANOVA test. Duncan Multiple Range Test was used to determine significant difference between individual means, at the 0.05 level of significance. All the data were analyzed using SAS and MSTATC software.

Results and Discussion

Electrolyte leakage and membrane stability index

Membrane damage was evaluated indirectly by measuring solute leakage (electrolyte leakage) from cells and also by MSI. The effect of different irrigation levels (100, 85, 75 and 65% of full irrigation) and Bio-fertilizer treatments on electrolyte leakage and membrane stability of mango leaves are demonstrated (Fig 1). Different levels of water stress caused a significant increase ($P<0.05$) in electrolyte leakage and decreased MSI of mango leaves compared to full irrigation treatment (100%). Maximum membrane damage was recorded in the plants exposed to 65% of full irrigation. Water stress caused rapid damage to the plasma membrane due to an uncontrolled enhancement of free radicals that cause lipid peroxidation (Moussa and Abdel-Aziz, 2008). In addition, water stress caused disturbance of the association between membrane lipids and proteins as well as disturbance in enzymes activity and transportation capacity of membranes (Lisar *et al.*, 2012).

Bio-fertilizer applications alleviate the adverse effects of water stress on the plasma membrane denoted by the significant decrease in the electrolyte leakage. These results are in line with Naghashzadeh (2014) who found that mycorrhizal biofertilizer application improved membrane stability in maize plant as a consequence of enhancing nutrient uptake, extension of the root system and water status of the plants.

Changes in photosynthetic pigments content

Photosynthetic pigments content (chl a, chl b, carotenoids and total pigments) estimated in mango leaves are shown in Fig 2. The contents of all photosynthetic pigments were gradually decreased with the increase of water stress compared with full irrigation treatment. The lowest values of chl a, chl b, carotenoids and total pigments were recorded at 65% of full irrigation. Decrease in chlorophyll content under water stress is expected since water stress inhibits chlorophyll (a & b) synthesis along with their inclusion into developing pigment–protein complexes of the photosynthetic apparatus (Lisar *et al.*, 2012).

As regarding to the bio-fertilizer applications; the maximum values in all photosynthetic pigments were recorded with *Azotobacter* (T_3) under full

irrigation (100%), *while Azospirillum* (T_2) increased photosynthetic pigments under water stress treatments. The role of bio-fertilizers in increasing photosynthetic pigments may be due to higher N incorporation which contributed in the formation of chlorophyll (Baset Mia *et al.*, 2010**).**

Changes in carbohydrate contents

Data illustrated in Fig 3. revealed that water stress induced significant increase in soluble sugars and total carbohydrates in mango leaves, as compared with those of full irrigation. Bio-fertilizer applications (T_3 and T_4) stimulated the accumulation of soluble sugar and total carbohydrates in the stress-affected plants. On the other hand, the application of *Azospirillum* (T_2) led to significant decrease in soluble sugar and total carbohydrates, while the insoluble sugars increased, as compared with the control.

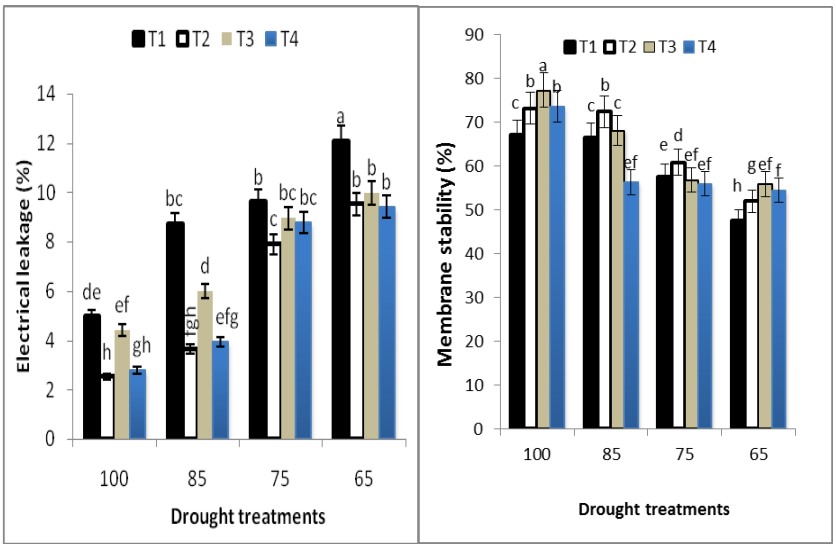

Fig 1. Effect of different irrigation levels (100, 85, 75 and 65% of full irrigation) and Bio-fertilizer treatments on electrolyte leakage (%) and membrane stability (%) of mango leaves.

where T1: control; T2: Azospirillum; T3: Azotobacter; T4: Azospirillum + Azotobacter; Error bars represent the standard deviation.

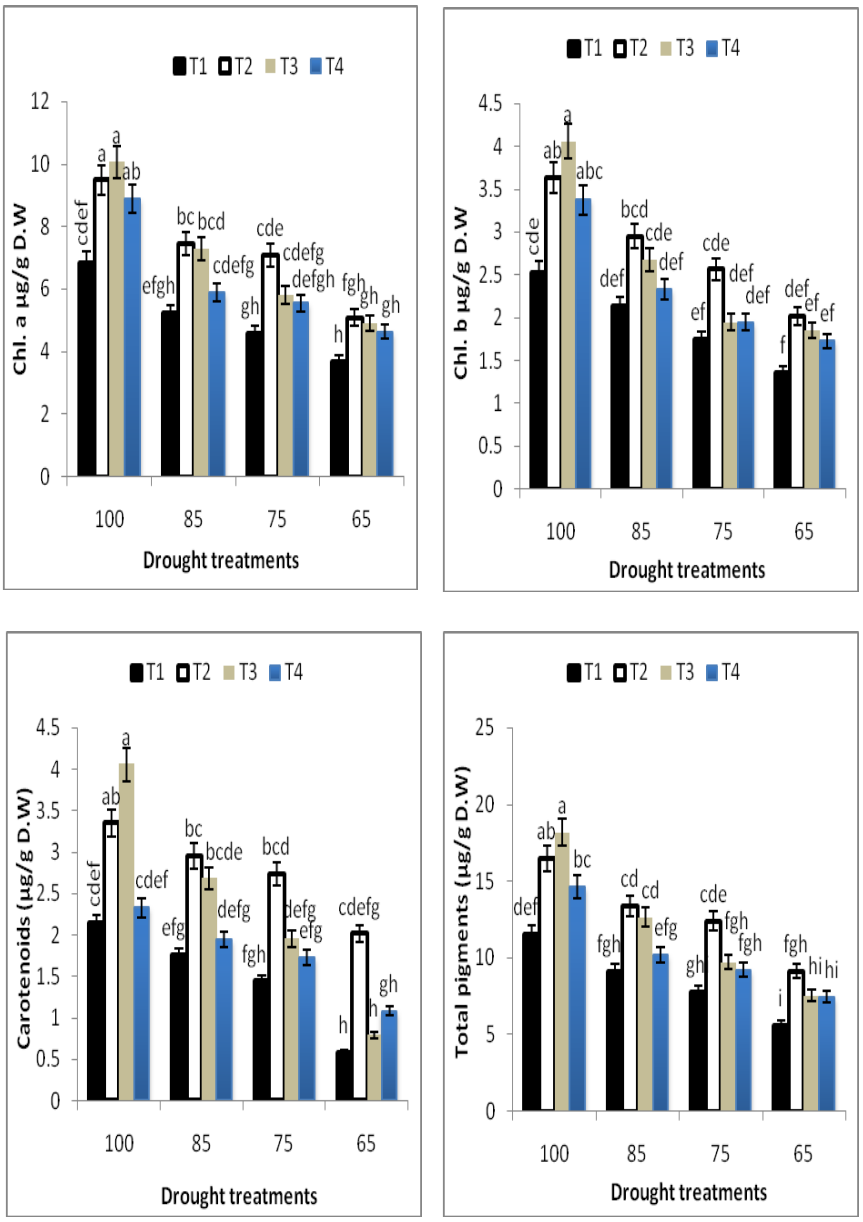

Fig 2. Effect of different irrigation levels (100, 85, 75 and 65% of full irrigation) and Bio-fertilizer treatments on Chl.a, Chl. b, carotenoids and total pigments in mango leaves.

where T1: control; T2: Azospirillum; T3: Azotobacter; T4: Azospirillum + Azotobacter; Error bars represent the standard deviation.

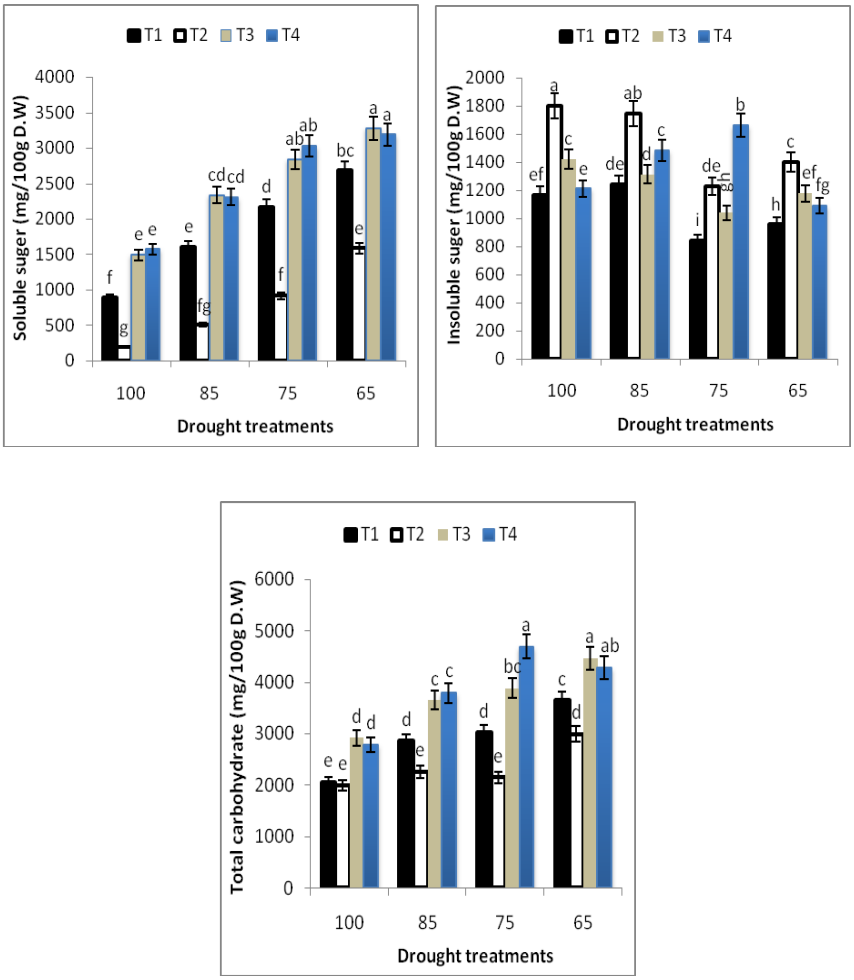

Fig 3. Effect of different irrigation levels (100, 85, 75 and 65% of full irrigation) and Bio-fertilizer treatments on soluble, insoluble sugar and total carbohydrates of mango leaves.

where T1: control; T2: Azospirillum; T3: Azotobacter; T4: Azospirillum + Azotobacter; Error bars represent the standard deviation.

Changes in total protein and proline content

The effects of different levels of irrigation and bio-fertilizer treatments on total protein and proline of mango leaves are presented (Fig 4). The decrease in total protein contents with increasing water stress may be due to proteolysis or decline in some essential minerals for protein synthesis, which are absorbed with water, as nitrogen compounds (Bayramov *et al.* 2010 and Costa *et al.* 2011). Furthermore, Lisar *et al.* (2012) reported that water stress alters gene expression and consequently, the synthesis of new proteins and mRNAs.

On contrast, water stress increased the proline content in mango leaves. This increase was directly proportional to water stress (Kim *et al.*, 2004 and Lee *et al.*, 2009). Proline accumulation in cells leads to increase in the osmotic potential and finally results in higher water uptake capacity by roots and water saving in the cells (Lisar *et al.*, 2012). On the other hand, proline acts as a source of carbon and nitrogen for rapid recovery from the stress and acts as a free radical scavenger and plasma membrane stabilizer (Hartzendorf and Rolletschek, 2001 and Lisar *et al.*, 2012).

Bio-fertilizer treatments resulted in significant increase in the total protein contents in mango leaves, whereas it caused a significant reduction in proline content compared with those of the controls. The minimum proline content was recorded in *Azospirillum* treated plants (T_2) compared to other bio-fertilizer treatments. Our results were in agree with Abdelmoneim *et al.* (2014) who reported that protein content enhancement is related to a relative increases in nitrogen fixation due to PGPR application.

It was noticed that the increase in proline content in drought-stressed plants is associated with the decrease in chlorophyll content. This is consistent with the suggestion that nitrogen might be redirected to the synthesis of proline instead of chlorophyll (Da La Rosa-Ibarra and Maiti, 1995). These results demonstrated the efficiency of using bio-fertilizers; especially *Azospirillum,* in alleviating the adverse effect of water stress on mango.

Lipid Peroxidation

Data presented in Fig 5. showed the effect of different levels of irrigation and bio-fertilizer treatments on the lipid peroxidation as malondialdehyde in mango leaves. Water stress increased the MDA content significantly. This result is in constant with earlier studies which reported lipid peroxidation as a well-known effect of drought and many other environmental stresses via oxidative damage (Abdul Jaleel *et al.*, 2008 and Lisar *et al.*, 2012).

The application of bio-fertilizers led to significant decrease in MDA under low levels of irrigation (75 and 65%) while, no significant differences were observed at (100 and 85%) of irrigation levels, except with T_4 treatment under full irrigation. Bio-fertilizer treatments may improve the membrane stability through the decrease in lipid peroxidation. In this sense, low concentration of MDA has been associated with drought tolerance in tomato (Sanchez-Rodriguez *et al.*, 2010) and maize (Moussa and Abdel-Aziz, 2008). Also, lower lipid peroxidation and higher membrane stability have been reported in salt-tolerant genotypes of rice (Tijen and Ismail, 2005) and sugarcane (Gomathi and Rakkiyapan, 2011).

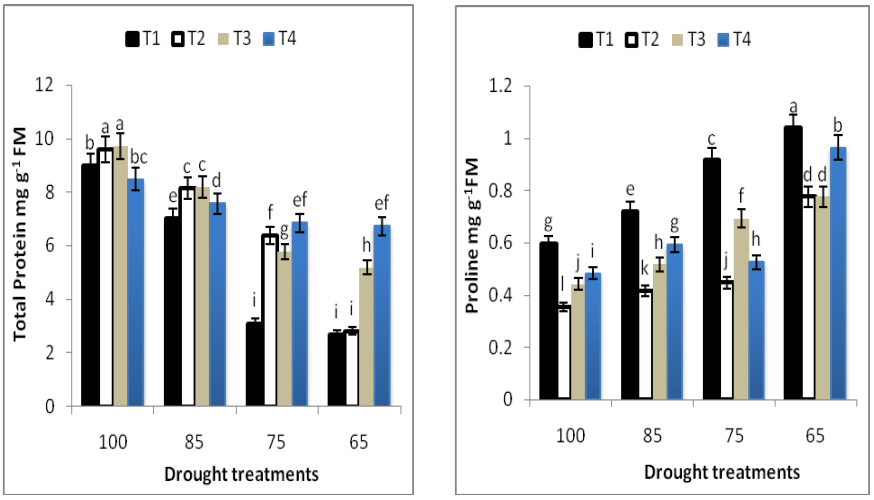

Fig 4. Effect of different irrigation levels (100, 85, 75 and 65% of full irrigation) and Bio-fertilizer treatments on total protein and proline in mango leaves.

where T1: control; T2: Azospirillum; T3: Azotobacter; T4: Azospirillum + Azotobacter; Error bars represent the standard deviation.

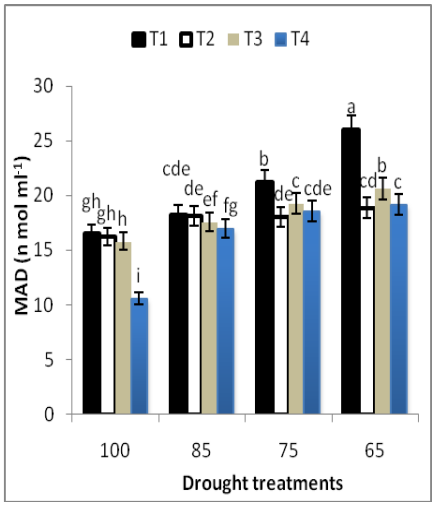

Fig 5. Effect of different irrigation levels (100, 85, 75 and 65% of full irrigation) and Bio-fertilizer treatments on lipid peroxidation as malondialdehyde (MDA) in mango leaves.

where T1: control; T2: Azospirillum; T3: Azotobacter; T4: Azospirillum + Azotobacter; Error bars represent the standard deviation.

Antioxidant enzyme activities

Antioxidant enzymes are very important in scavenging dangerous free oxygen radicals, produced as the usual secondary consequence of environmental stresses, from plant cells. SOD converts the toxic O_2^- radicals to H_2O_2 which scavenged to O_2 and water by the antioxidant enzymes such as CAT and POD (Ozkur *et al.*, 2009). The results illustrated in Fig 6. showed the effect of different levels of irrigation and bio-fertilizer treatments on the activity of the antioxidant enzymes (SOD, POD and CAT) in mango leaves. The activity of all antioxidant enzymes increased with increasing water stress. These results are in harmony with Moussa and Abdel-Aziz (2008) and Lisar *et al.* (2012) who reported that the increase in antioxidant enzymes (SOD, CAT and POD) activity under various stress conditions had been linked with cell protection from oxidative damage.

Application of bio-fertilizer treatments led to significant increase of SOD, POD and CAT activity which could alleviate the adverse effect of water stress on mango trees. The maximum activity of CAT recorded in plants treated with *Azospirillum* (T_2) at different levels of irrigation, compared with the other bio-fertilizer treatments. Similar results concerning antioxidant protective effects of PGPR associated with other environmental stresses where previously reported (Saravanakumar *et al.*, 2011 and Heidari and Golpayegani, 2012). Moreover, earlier reports revealed that SOD activity increased in drought-tolerant cultivars of bean (Turkan *et al.*, 2005), and sesame (Fazeli *et al.*, 2007). Also, high activity of CAT is noticed with drought tolerance in tomato cultivars (Sanchez-Rodriguez *et al.*, 2010), and peanut (Akcay *et al.*, 2010).

Conclusion

Under drought conditions plants adopt certain strategies to overcome the damage caused by water stress through enhanced production of antioxidant enzymes such as; SOD, POD, and CAT, organic solutes (soluble sugar and total carbohydrates) and also increased proline accumulation. Bio-fertilizer applications (*Azospirillum* and *Azotobacter*) could alleviate the adverse effects of water stress by increasing antioxidants activity, reduction of electrolytes leakage, accumulation of carbohydrate content, enhanced photosynthetic pigments, increased total protein contents, reduced proline content and MDA.

Recommendation: Bio-fertilizer applications, especially *Azospirillum* could be effective in mango orchards in arid and semi-arid areas to reduce the adverse effects of water shortage.

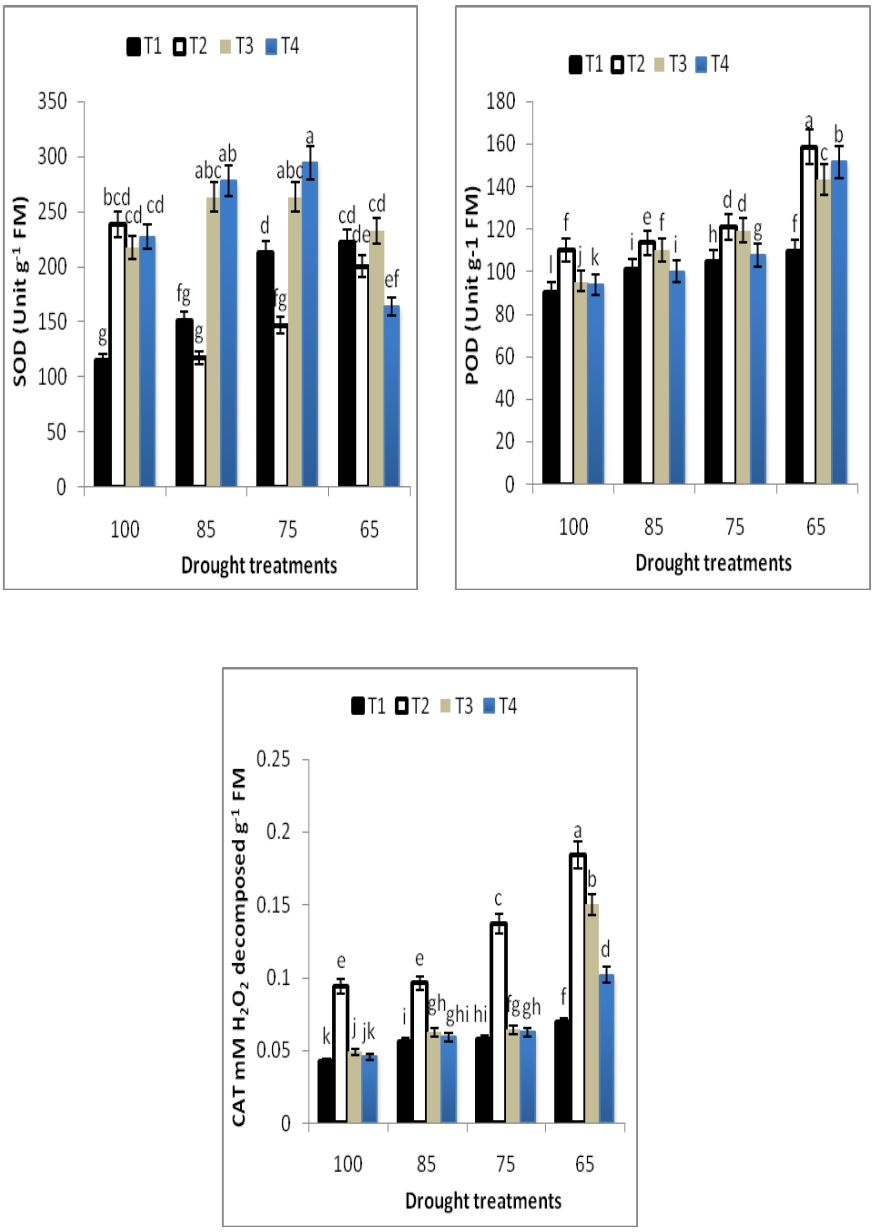

Fig 6. Effect of different irrigation levels (100, 85, 75 and 65% of full irrigation) and Bio-fertilizer treatments on SOD, POD and CAT in mango leaves.

where T1: control; T2: Azospirillum; T3: Azotobacter; T4: Azospirillum + Azotobacter; Error bars represent the standard deviation

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دورالمعامله بالسماد الحيوى في الحد من الآثارالسلبية للإجهاد المائي في اشجار المانجو

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يعد الجفاف واحد من اهم الاجهادات غير الحيوية التى تحد من الإنتاج الزراعي في العالم. أجري هذا العمل بهدف تقييم التغيرات البيوكيميائية في اوراق المانجو (*Mangifera indica L*) بعد تعرضها لمستويات مختلفة من الجفاف (65، 75، 85 و 100٪ من متطلبات الري الكامل), بالاضافة الى تقييم تأثير المعاملة ببعض انواع الريزوبكتريا غير التكافلية المحفزة لنمو النبات - مثل الازوسبريليم والازوتوباكتر- في الحد من التغييرات الناتجة عن الجفاف.تم قياس التغيرات الفسيولوجية والبيوكيميائية في اوراق المانجو بعد مرور موسمين من اجراء المعاملات المختلفة. وقد اظهرت النتائج انخفاض معنوى فى درجة ثبات الاغشية، وصبغات البناء الضوئي ومحتويات السكريات غير الذائبة مع زيادة مستويات الجفاف، بينما حدثت زيادة حادة فى تسريب الاغشية للايونات، ومحتوى كل من السكريات الذائبة، والكربوهيدرات الكلية والبرولين مقارنةً بالكنترول . كما ظهرت زيادة مضطردة فى مستوى فوق اكسدة الدهون ونشاط انزيمات السوبرأوكسيد ديزميوتيز (SOD)، البيروكسيديز(POD)، والكاتاليز(CAT) مع زيادة مستويات الجفاف. استخدام الأسمدة الحيوية يمكن أن يخفف من الآثار السلبية الناتجة عن الجفاف حيث سببت زيادة ملحوظة فى صبغات البناء الضوئي ومحتوى الكربوهيدرات كما حسنت نشاط إنزيمات السوبر أوكسيد ديزميوتيز، البيروكسيديز, والكاتاليز، وكذلك خفضت محتوى البرولين فى النباتات المعاملة مقارنةً بالكنترول.