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Water relationships and agronomic indices of sunflower infection by microbial inoculants under saline condition

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Abstract. Salinity is one of the most important limiting factors for plant growth and crop production. Saline soils with having low activity in nutrient elements and high osmotic pressure in solution phase make so much nutrition problems and physiological drought in plant. In this study, the effects of arbuscular mycorrhiza fungi and plant growth promoting bacteria with ability to produce ACC deaminase enzyme on water relationships and agronomic indices of sunflower cultivars were investigated. The saline soil (EC = 7.6 dS m^{-1}) used in this investigation was taken from Karaj region (Eshtehard) of Iran. In a factorial experimental design on the basis of randomized complete block samples, three levels of arbuscular mycorrhizal inoculants (non inoculation, inoculation with *Glomus etunicatum* and *Glomus intradices*), four levels of *Pseudomonas fluorescens* inoculants (non inoculation, inoculation with *Pseudomonas fluorescens* strains 4, 9 and 12) on two cultivars of sunflower (Euroflor and Master) with four replications per treatment were applied. Results showed that all of the treatments significantly ($P < 0.05$) increased relative water content (RWC) of two cultivars. Single inoculation of *Pseudomonas fluorescens* strains 12 and *Glomus etunicatum* and also co-inoculation of fungi with each one of the bacteria enhanced fresh and dry weight of tray in Euroflor cultivar, compared with the control treatment. In addition, all of the treatments except *Pseudomonas fluorescens* strains 4 and also *Glomus intradices* significantly ($P < 0.05$) increased dry weight of above ground part in Euroflor cultivar.

Key Words: Salinity, Plant growth promoting rhizobacteria, Arbuscular mycorrhiza fungi, Sunflower.

چکیده (In Persian). شوری یکی از عوامل مهم محدود کننده رشد و تولید محصول می‌باشد. خاک‌های شور با داشتن فعالیت اندک عناصر غذایی و فشار اسمزی بالا در فاز محلول خود مشکلات تغذیه‌ای فراوانی را برای گیاه ایجاد می‌کنند و موجب خشکی فیزیولوژیکی گیاه می‌گردند. در این تحقیق تأثیر قارچ میکوریز آربسکولار و باکتری دارای توانایی تولید آنزیم ACC دآمیناز بر روابط آبی و شاخص‌های زراعی دو رقم آفتابگردان در خاکی با قابلیت هدایت الکتریکی (EC) عصاره اشباع برابر با ۷/٦ دسی‌زیمنس بر متر از منطقه اشتهارد کرج بررسی شد. این تحقیق در قالب طرح کاملاً تصادفی با چهار تکرار به صورت آزمایش فاکتوریل انجام شد. فاکتورها شامل چهار سطح باکتری (سطح بدون باکتری، تلقیح با باکتریهای سودوموناس فلورسنس سویه‌های ٤، ۹ و ۱۲)، سه سطح قارچ (سطح بدون قارچ، تلقیح با قارچهای گلوموس اتونیکاتوم و گلوموس اینترادیسز) و دو رقم آفتابگردان (مستر و یوروفلور) بودند. نتایج نشان داد که تمام تیمارها محتوای نسبی آب برگ را در هر دو رقم به طور معنی‌داری ($p < 0.05$) افزایش دادند. تلقیح مجزای سودوموناس فلورسنس سویه ۱۲ و گلوموس اتونیکاتوم و همچنین تلقیح مشترک قارچ‌ها با هر سه باکتری توانست وزن تر و خشک طبق را در رقم یوروفلور نسبت به شاهد افزایش دهند. در مورد رقم یوروفلور نیز تمام تیمارها توانستند وزن تر گیاه را نسبت به شاهد افزایش دهند. تمام تیمارها به جز تلقیح گیاهان با باکتری سودوموناس فلورسنس سویه ٤ و همچنین قارچ گلوموس اینترادیسز توانستند وزن خشک اندام هوایی را در رقم یوروفلور به طور معنی‌داری ($p < 0.05$) افزایش دهند.

واژه‌های کلیدی: شوری، باکتری‌های ریزوسفری محرک رشدگیاه، میکوریز آربسکولار، آفتابگردان.

Introduction. The environmental stress of salinity reduces growth and agricultural productivity more than any other factors (Karakas et al 1997). The direct effects of salt on plant growth may involve (1) a reduction in the osmotic potential of the soil solution that reduces plant-available water, and (2) toxicity of excessive Na^+ or Cl^- towards the plasma membrane. Osmotic effects are associated with inhibition of cell wall extension and cellular expansion, leading to reduced plant growth (Feng et al 2002). Plants being immobile cannot evade salt stress in the same way as mobile organisms. So, they show many morphological and physiological alterations to acclimatize to unfavorable environment (Sakamoto & Murata 2002). For reducing of adverse effects of soil salinity there are several methods. One of these methods is using of biological fertilizers. Due to many bioenvironmental problems caused by chemical fertilizers, these days many farmers tend to use biological fertilizers for achievement to sustainable agriculture. Plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal (AM) fungi represent two main groups of beneficial microorganisms of the rhizosphere which known as biological fertilizers (Russo et al 2005). The beneficial effect of PGPRs as well as AM fungi on plants is well documented (Gamalero et al 2003).

Sunflower (*Helianthus annuus* L.) is an important oilseed crop grown in different parts of the world. It has C3 photosynthetic pathway and is mostly cultivated in arid and semiarid regions (Iqbal et al 2005). Although many studies on salt tolerance of sunflower has been carried out, basic research on role of microbial inoculants in salt tolerance of sunflower is scarce. Most investigations regarding PGPRs and AM fungi efficiency in increasing plant tolerance to salinity are related to other agricultural crops. However, in all of these investigations it is clear that reducing salinity-induced ethylene by any mechanism can decrease the negative impact of salinity onto plant growth. In another word, plant inoculation with PGPR containing ACC deaminase activity is so helpful in sustaining plant growth and development under stress conditions by reducing stress-induced ethylene production (Saleem et al 2007). Mayak et al (2004) evaluated the role of a PGPR (*Achromobacter piechaudii*) in resistance of tomato plant to salt stress in dry salty environments of Israel. They reported that this bacterium significantly increased the fresh and dry weights of tomato seedlings grown in the presence of up to 172 mM NaCl salt. Also, in the presence of salt the bacterium increased the water use efficiency (WUE). They suggested that the bacterium act to alleviate the salt suppression of photosynthesis. Similarly, Hamdia et al (2004) studied the role of a PGPR (*Azospirillum brasilense)* in enhancing of corn growth at saline conditions. They reported that inoculating of corns varieties that were sensitive to salt stress had significant effect on increasing their dry root and shoot yield as compared with the control treatments. In addition, Saravanakumar and Samiyappan (2007) reported that *Pseudomonas Xuorescens* strain TDK1 containing ACC deaminase activity enhanced the saline resistance in groundnut plants and increased yield as compared with that inoculated with *Pseudomonas* strains lacking ACC deaminase activity. Cheng et al (2007) have also pointed out that ACC deaminase bacteria conferred salt tolerance onto plants by lowering the synthesis of salt-induced stress ethylene and promoted the growth of canola in saline environment. Yano-Melo et al (2003) showed that inoculating of banana plants (*Musa* sp. cv. Pacovan) by an AM fungi (*Glomus clarum*) improved the dry weight of root (80%), shoot (83%), and the total leaf area (60%) compared to non-inoculated plants. The salt tolerance of banana as measured by leaf number and plant height increased considerably in presence of *Glomus* isolates. They suggested that inoculation with specific AM fungi therefore constitutes an alternative method to reduce banana plant stress caused by soil salinization. In addition, Al-Karaki (2006) indicated that pre-inoculation of tomato transplants with AM fungi improved yield and can help alleviate deleterious effects of salt stress on crop yield. He showed that pre-inoculated tomato plants with AM fungi irrigated with both saline and nonsaline water had greater shoot and root dry matter (DM) yield and fruit fresh yield than nonAM plants. The enhancement in fruit fresh yield due to AM fungi inoculation was 29% under nonsaline and 60% under saline water conditions. Also these results indicated that pre-inoculation of tomato transplants with AM fungi improved yield and could help alleviate deleterious effects of salt stress on crop yield. Sannazzaro et al (2006) found that AM fungi (*Glomus intraradices*) improved growth of *L. glaber*

plants under saline conditions. They showed that mycorrhizal plants had higher values of net growth, shoot/root and protein concentrations than controls. Tolerant AM plants also showed higher chlorophyll levels than non-AM ones. Feng et al (2002) observed that the concentrations of chlorophyll were higher than in non-mycorrhizal plants compared with mycorrhizal plants under salty environments. However, mycorrhizal plants maintained higher root and shoot dry weights. The study of the antagonic or synergic effects of the different microbial inoculants when co-inoculated is a crucial step in the development of effective host-microorganism combinations. Kohler et al (2007) studied the interactions between the inoculation of lettuce plants with an arbuscular mycorrhizal fungus (*Glomus intraradices*) and a plant growth-promoting rhizobacterium (*Bacillus subtilis*). They reported that this co-inoculation synergistically increased plant growth compared with singly inoculated (about 77% greater with respect to the control plants). Other similar results confirmed the advantages of co-inoculation with PGPRs and AM fungi in increasing of plant growth (Germida & Walley 1996; Toro et al 1997; Caravaca et al 2005).

Drought and salinity are already widespread in many regions of Iran, and are expected to cause serious salinization of the most arable lands of this region. Also, nowadays Iran is so dependent to other countries in the case of oily seeds. The cost of imports for edible oils or oily seed at Iran is more than 800 million dollar per year. This figure indicates the vital role of sunflower as an industrial crop having main source of high quality edible oil in Iran's economy. Agro climatic condition of the most parts of Iran is such type that this crop can be grown successfully. But according to present information provided from Iranian soil's map about 44.5 million hectare of soils in Iran have large amount of different salts (Banaei 2001). Hence, despite the economic importance of sunflower plant in Iran, there is limited study on the improvement of its tolerance, growth, yield and other its agronomic indices to the infecting by microbial inoculants under saline condition. This study was therefore carried out with three main objectives: (a) to investigate the effect of PGPRs (Strains of *Pseudomonas fluorescens*) and AMF (*Glomus etunicatum* and *Glomus intradices*) on reducing salt stress and improvement of growth, yield and other agronomic indices of sunflower plants, (b) to study the role of co-inoculation of sunflower plant by PGPRs and AMF in increasing its agronomic indices and (c) to evaluate and compare the growth response of two cultivars of sunflower (Euroflor and Master) to microbial inoculation.

Material and Method. This study was carried out at greenhouse condition. Plastic pots having 22 cm height and 20 cm opening mouth diameter were selected for planting of sunflowers. The weight of each vacant pot was about 250 g. Approximately 4 kg of air-dried soil passed through a 4.8 mm sieve was added to each plastic pot. Two cultivars of sunflower seeds (Euroflor and Master) were provided from institute of seed and seedling of Karaj for this research. Before sowing the seeds into the pots, the seeds that their shape was similar were selected and then, were kept in the trays containing javellewater (2.5%) for 7 minute. Then the seeds were washed 6-7 times by distilled water and were held several days on the sterile papers inside the incubator for germination.

Microbial inoculants including micorrhizal fungi and bacteria were prepared from soil & water research institute of Iran as powdery forms and separated boxes. The mycorrhizal inoculants including *Glomus etunicatum* and *Glomus intradices* were isolated from saline soils of Tabriz plane which located in northern Iran. The fungi population in these inoculants was about 1.6×10^4 fungus per 1 g soil. The bacterial inoculants were consisted of strains 4, 9, 12 of *Pseudomonas fluorescens* (Table 1).

Before transferring the seedlings into the plastic pots, the soil was irrigated to the field capacity level. When the soil moisture was suitable for planting, according to the treatment design, in each pot 4 small holes were shaped and then, 2 g fungi inoculants along with 1 g bacterial inoculants were added these holes. After that in these entire holes, one seedling of sunflower was planted and pots were irrigated at the level of field capacity on the basis of the weight of each pot. According to soil analysis, the essential elements as chemical fertilizers were added to the soils. Just the phosphorous applied at the half of its optimum level due to proper studding the effect of micorrhizal fungi. After suitable establishing of plants in the plastic pots, the number of plant was reduced to two

plants in each pot by removing them. The air temperature inside the greenhouse was hold approximately fixed at 25 ºC during the growth stages of sunflower plants. Also, the period of sunshine was about 12 hours throughout the plant growth. Approximately 90 days after transplanting, the sunflower plants were cut form their bottom.

It was needed to a salty soil for doing the experiments. Therefore a pre-sampling performed from different locations in Karaj (Eshtehard) region of Iran. After determining the salinity of these pre-samples, the best place was selected. The sampling location was between 35°, 43′ in eastern latitude and 50°, 18′ in northern longitude. Silage maize is the major feeder crop followed by alfalfa and wheat are the main cereal crop in this region. Due to the lack of animal manure, crop production is based on mineral NPK fertilizers. This area is also situated in a river alluvial plain and the soil of this region belongs to xeric haplocambids. The soil texture is loamy with an average pH of 7.8, electrical conductivity (EC) of 7.6 dS m^{-1}, organic matter (OM) content of 0.5%, $CaCO_3$ content of 13.6%, gypsum content of 3.1%, total N content of 0.045, saturation percentage (SP) of 37.3%, sodium adsorption ratio (SAR) of 9.24, exchangeable sodium percentage (ESP) of 11% and cation exchange capacity (CEC) level of 16.9 meq per 100 g of soil. The levels of soluble Na, Mg, Ca, K, Cl and HCO_3 in the soil were 45.6, 10.0, 39.7, 14.1, 46.5 and 5.3 meq L^{-1}, respectively. Also the level of Fe, Zn, Cu and Mn extracted by DTPA in the soil were 2.8, 1.6, 1.3 and 10.0 mg kg^{-1}, respectively.

Table 1

Some characteristics of bacteria in inoculants

Bacterium	*Activity of ACC deaminase (µmoles mg^{-1} h^{-1})*	*Auxin (µg ml^{-1})*	*Bacterial population (Cfu ml^{-1})*
Pseudomonas fluorescence strain 4	8.17	2.38	7.7×10^9
Pseudomonas fluorescence strain 9	4.45	0.93	2.1×10^9
Pseudomonas fluorescence strain 12	4.61	1.2	2.5×10^9

In this study the leave water potential was measured using a digital plant water potentiometer apparatus (EL540-300). Also, the plant chlorophyll was measured using a chlorophyll meter apparatus (SPAD-502) according to Fox et al (1994) method. For calculating the relative water content (RWC), the latest expanded plant's leaves were cut and their weight measured. After that, the leaves soaked into the distilled water for 24 hours. After this saturation the leave surfaces was dried using a clean towel and their weights measured again. Ultimately the leaves were dried in the oven at 70 ºC temperature and the level of RWC calculated by following equation:

RWC = Fresh leaf weigh – Dry leaf weigh / Saturated leaf weigh – Dry leaf weigh (1)

At the end of plant growth (90 day after transplanting) the leaf area was measured by means of a leaf area meter (GATE HOUSE). Also at this time other growth parameters including dry and fresh weight of above ground parts or organs (consisting of: leaf, stalk and tray), dry and fresh weight of trays, tray diameter, dry weigh of root and percentage of root colonization were also measured. For measuring the root colonization the Grindline Intersect method was used. In this method for segregation of roots from soil particles, the pot's soil was saturated and then soil particles washed gently away by water. After clean-up the roots, 1 gr samples were taken from different parts of roots. These samples were maintained into vessels having a mixture of alcohol and water. For performance of colour-blending, the roots were heated at 90 ºC temperature into the 10% KOH solution for 1 hour. The washed roots were floated in a

basic solution of 10% H_2O_2 for 20 minutes for doing the discolouration and then, the roots were washed for several times and soaked into a 1% HCl solution for 3 minutes for doing the acidification process. At the last stage, the roots transferred into the lactoglycerin-triphane blue for 48 hours for completion the coloration process (Philips & Hayman 1970). Ultimately for measuring the percentage of root colonization, the coloured roots were divided into the 1 cm fragments (Giovannetti & Moss 1980).

The experiments were conducted in the statistical method of factorial design with the basis of randomized complete block samples in four replicates. The treatments were: (a) three levels of arbuscular mycorrhizal inoculants including non inoculation (F_0), inoculation with *Glomus etunicatum* (F_1) and *Glomus intradices* (F_2), (b) four levels of *Pseudomonas fluorescens* inoculants including non inoculation (B_0), inoculation with *Pseudomonas fluorescens* strains 4 (B_0), *Pseudomonas fluorescens* strains 9 (B_2), *Pseudomonas fluorescens* strains 12 (B_3) and (c) two cultivars of sunflower including Euroflor (C_1) and Master (C_2). The analysis of variance was performed using SAS and Minitab softwares. Comparison of mean values was done using Duncan Multiple Range Test by MSTATC software at 5% probability level.

Results and Discussion. The results of analysis of variance (ANOVA) on the basis of the major effect of fungus (F), bacterium (B) and cultivar (C) showed that the fungus (F) had significant effect on the fresh weight of above ground part, dry weight of root, fresh and dry weight of tray, tray diameter, leaf water potential, RWC, root colonization and leaf chlorophyll, while other indices did not affected by the fungus. In addition, the bacterium (B) had significant effect on all of the parameters except dry weight of root, leaf weight, leaf area, leaf water potential and leaf chlorophyll. Moreover, cultivar (C) significantly affected all of the parameters except leaf weight and RWC (Table 2). The comparison of mean for the major effect of fungus (F) showed that the F_1 and F_2 treatments had no significant difference with the control (F_0) for the traits of fresh and dry weight of above ground part, leaf area and leaf weight. For the traits of dry weight of root, leaf water potential and leaf chlorophyll just the F_2 treatment had significant difference with the control (F_0). For the traits of fresh weight of tray, plant length and tray diameter just the F_1 treatment had significant difference ($p < 0.05$) with the control (F_0), while for the traits of dry weight of tray, root colonization and RWC both of the F_1 and F_2 treatments had significant differences ($p < 0.05$) with the control (F_0). In related to comparison of mean for the major effect of bacterium (B), the B_1 treatment significantly ($p < 0.05$) increased by itself the fresh weight of above ground part compared with the control (B_0) treatment. A similar result was observed for the trait of dry weight of above ground part. Also, all of the bacterial treatments (B_1, B_2 and B_3) significantly ($p < 0.05$) increased the fresh and dry weight of tray, tray diameter and RWC compared with the control treatment (B_0). The B_2 and B_3 treatments significantly ($p < 0.05$) decreased the root colonization compared with the control treatment. For the trait of plant length, the comparison of mean showed that the B_3 treatment significantly ($p < 0.05$) reduced this trait compared with the control and also, the maximum and minimum plant length were related to B_1 and B_3 treatments respectively. In addition the comparison of mean for the major effect of bacterium (B) on other characteristics such as dry weigh of roots, leaf chlorophyll, leaf water potential, leaf area and leaf weight did not showed significant differences. The comparison of mean for the major effect of cultivar (C) revealed that the C_2 treatment (Master) had higher levels of fresh and dry weight of above ground part, dry weight of root, fresh and dry weight of tray, tray diameter, plant length, leaf water potential and root colonization than to the C_1 treatment (Euroflor). Also, the C_1 treatment had higher values of leaf area and chlorophyll than to the C_2 treatment. The traits of leaf weight and RWC did not show significant differences between two cultivars (C_1 and C_2) of sunflowers (Table 3).

The ANOVA for the interaction effect between fungus and bacterium (F × B) on the water relationships and agronomic indices of sunflower plants showed that this interaction was significant for all of the studied traits except fresh and dry weight of above ground part, dry weight of root and leaf chlorophyll (Table 2). Also the comparison of mean showed that, just the treatment of F_2B_1 had significant increasing difference ($p <$

0.05) with the control treatment (F_0B_0) for the trait of fresh weight of above ground part. Similarly for the trait of dry weight of above ground part the treatments of F_1B_1 and F_2B_1 and for the trait of leaf water potential the treatments of F_2B_0 and F_2B_2 had significant difference ($p < 0.05$) with the control treatment (F_0B_0). In addition, all of the F × B treatments except F_2B_0 showed significant increasing difference with the control treatment (F_0B_0) for the traits of fresh weight of tray and tray diameter. The treatments of F_0B_3, F_1B_1, F_1B_3, F_2B_1 and F_2B_3 significantly increased the dry weight of tray compared with the control treatment (F_0B_0). Similarly, the treatments of F_1B_0, F_1B_1, F_1B_3, F_2B_0, F_2B_1 and F_2B_2 significantly increased the root colonization compared with the control treatment (F_0B_0). All of the F × B treatments had significant increasing difference ($p < 0.05$) with control treatment (F_0B_0) for the trait of RWC. Moreover, the treatment of F_2B_2 significantly reduced the plant length compared with the control treatment (Table 4).

Table 2

ANOVA for the effect of treatments on the water relationships and agronomic indices of sunflower plants (data are the mean squares)

S.O.V.	*df.*	*Fresh weight of above ground part*	*Dry weight of above ground part*	*Dry weight of root*	*Fresh weight of tray*	*Dry weight of tray*
Fungus (F)	2	227.188**	3.156ns	2.259**	50.619*	4.179*
Bacterium (B)	3	217.818**	12.211**	0.182ns	121.868**	3.094*
Cultivar (C)	1	963.68**	191.507**	12.177**	313.168**	13.635**
F × B	6	56.202ns	1.398ns	0.132ns	71.594**	3.166**
F × C	2	12.689ns	3.708ns	1.638*	28.314ns	6.949**
B × C	3	153.957*	15.538**	0.847ns	60.685**	5.618**
Error	72	42.633ns	1.997ns	0.406ns	14.875ns	0.899ns

S.O.V.	*df.*	*Tray diameter*	*Plant length*	*Leaf weight*	*Leaf area*
Fungus (F)	2	0.55**	52.768ns	23.163ns	61209.885ns
Bacterium (B)	3	0.6**	88.656**	9.462ns	22091.038ns
Cultivar (C)	1	1.283**	7428.081**	1.862ns	129140.01*
F × B	6	0.316**	53.960*	33.526**	84480.288**
F × C	2	0.192ns	6.142ns	4.021ns	10330.385ns
B × C	3	0.087ns	76.078**	33.645*	84354.844*
Error	72	0.081ns	17.755ns	8.478ns	21839.941ns

S.OV.	*df.*	*Leaf water potential*	*RWC*	*Root colonization*	*Leaf chlorophyll*
Fungus (F)	2	0.236**	137.402**	1526.321**	10.345*
Bacterium (B)	3	0.076ns	33.547**	205.930**	1.566ns
Cultivar (C)	1	0.586**	8.766ns	298.144**	715.151**
F × B	6	0.135*	99.477**	145.700**	2.238ns
F × C	2	0.099ns	0.137ns	32.422ns	1.599ns
B × C	3	0.206**	22.132**	5.488ns	2.228ns
Error	72	0.047ns	2.824ns	40.039ns	2.744ns

** and *: Significant at 1 and 5%, respectively and ns: Non significant

The ANOVA for the interaction effect between fungus and cultivar (F × C) on the water relationships and agronomic indices of sunflower plants showed that, none of the studied traits except dry weight of root and tray did not affected by the interaction between fungus and cultivar (F × C) (Table 2). Also, the comparison of mean showed that between control treatments (F_0C_1 and F_0C_2), the F_0C_2 treatment significantly had higher levels of dry weight of above ground part and root, fresh and dry weight of tray, tray diameter, plant length and root colonization than to the F_0C_1 treatment, while F_0C_1

treatment significantly had higher levels of leaf chlorophyll, leaf water potential and leaf area compared with the F_0C_2 treatment. Moreover, the F_1C_1 and F_2C_1 treatments had significant increasing difference ($p < 0.05$) with their control treatment (F_0C_1) for the traits of dry weight of above ground part and root, fresh and dry weight of tray and tray diameter. Similarly, the F_1C_2 and F_2C_2 treatments had significant increasing difference ($p < 0.05$) with their control treatment (F_0C_2) for the trait of leaf water potential. Furthermore, the F_2C_2 treatment had significant increasing difference ($p < 0.05$) with its control treatment (F_0C_2) for the trait of dry weight of root and leaf chlorophyll and both of the two cultivars (F_1C_1, F_2C_1, F_1C_2 and F_2C_2) significantly ($p < 0.05$) increased the root colonization and RWC compared with their control treatments (F_2C_1 and F_0C_2) (Table 5).

Table 3

The comparison of mean for the major effect of different levels of fungus, bacterium and cultivar on the water relationships and agronomic indices of sunflower plants (weight of above ground parts and roots are in g)

Treatments	*Fresh weight of above ground part*	*Dry weight of above ground part*	*Dry weight of root*	*Fresh weight of tray*	*dry weight of tray*
(F_0)	85.948AB	14.58A	2.404B	35.689B	6.034B
(F_1)	83.968B	14.935A	2.514B	38.134A	6.741A
(F_2)	89.243A	15.206A	2.909A	37.422AB	6.521A
(B_0)	84.934B	14.503B	2.64A	33.915B	5.903B
(B_1)	90.573A	15.886A	2.634A	39.241A	6.681A
(B_2)	86.408B	14.966AB	2.681A	37.724A	6.626A
(B_3)	83.631B	14.273B	2.482A	37.448A	6.518A
(C_1)	83.218B	13.495B	2.253B	35.276B	6.055B
(C_2)	89.555A	16.319A	2.965A	38.888A	6.809A

Treatments	*Tray diameter (cm)*	*Plant length (cm)*	*Leaf weight (gr)*	*Leaf area (cm^2)*
(F_0)	4.503B	57.398A	23.199AB	1175.22AB
(F_1)	4.762A	55.128B	22.103B	1117.88B
(F_2)	4.6B	57.303A	23.778A	1203.75A
(B_0)	4.388B	57.569AB	23.864A	1206.13A
(B_1)	4.667A	58.796A	23.148A	1171.33A
(B_2)	4.717A	55.506CB	22.611A	1145.58A
(B_3)	4.717A	54.569C	22.485A	1139.42A
(C_1)	4.506B	47.814B	23.166A	1202.29A
(C_2)	4.738A	65.406A	22.888A	1128.94B

Treatments	*Leaf water potential (– Bar)*	*RWC (%)*	*Root colonization (%)*	*Leaf chlorophyll (SPAD value)*
(F_0)	1.841A	74.220B	58.426B	43.488B
(F_1)	1.784A	77.502A	69.493A	43.747B
(F_2)	1.672B	78.053A	71.118A	44.576A
(B_0)	1.733A	74.885B	70.335A	43.869A
(B_1)	1.742A	77.149A	66.753AB	44.313A
(B_2)	1.738A	77.560A	64.155B	43.754A
(B_3)	1.850A	76.773A	64.140B	43.811A
(C_1)	1.688B	76.894A	64.583B	46.666A
(C_2)	1.844A	76.290A	68.108A	41.207B

Means, in each column, with at least one similar letters are not significantly different at the 5% probability level using Duncan multiple range test

The ANOVA for the interaction effect between bacterium and cultivar (B × C) showed that, all of the traits related to water relationships and agronomic indices except dry weight of root, tray diameter, root colonization and leaf chlorophyll affected by the interaction between bacterium and cultivar (B × C) (Table 2). In addition, the comparison of mean showed that between control treatments (B_0C_1 and B_0C_2), the B_0C_2 treatment significantly had higher levels of fresh and dry weight of above ground part, dry weight of root, fresh and dry weight of tray, tray diameter, plant length and leaf weight than to the B_0C_1 treatment. Where as the treatment of B_0C_1 significantly showed higher levels of leaf chlorophyll, RWC and leaf water potential compared with the other control treatment (B_0C_2). Moreover in the Euroflor cultivar (C_1), all of the bacterial treatments (B_1C_1, B_2C_1 and B_3C_1) had significant increasing difference ($p < 0.05$) with their control treatment (B_0C_1) for the traits of dry weight of above ground part, fresh weight of tray, tray diameter and RWC while, all of bacterial treatments (B_1C_1, B_2C_1 and B_3C_1) significantly reduce the leaf water potential. Similarly, the B_1C_1 and B_2C_1 treatments showed significant increasing difference ($p < 0.05$) with their control treatment (B_0C_1) for the trait of fresh weight of above ground part. Also, the B_2C_1 and B_3C_1 treatments had significant increasing difference ($p < 0.05$) with their control treatment (B_0C_1) for the traits of dry weight of tray and the B_3C_1 treatment significantly decreased root colonization compared with the control treatment (B_0C_1). However, in the Master cultivar (C_2), the B_1C_2 treatment had significant increasing difference ($p < 0.05$) with its control treatment (B_0C_2) for the traits of fresh and dry weight of tray. Also, the B_2C_2 treatment had significant increasing and decreasing difference ($p < 0.05$) with its control treatment (B_0C_2) for the traits of leaf water potential and leaf area respectively. The B_3C_2 treatment had significant decreasing difference ($p < 0.05$) with its control treatment (B_0C_2) for the traits of fresh and dry weight of above ground part. A similar result was observed in the B_2C_2 and B_3C_2 treatments for the traits of root colonization and leaf weight. The B_1C_2 and B_2C_2 treatments had significant decreasing difference ($p < 0.05$) with their control treatment (B_0C_2) for the trait of plant length and also, the B_1C_2 and B_2C_2 treatments significantly increased tray diameter (Table 6).

Besides having difficulties for uptake of elements by plants, salinity can cause problems in water uptake. Increasing of soil salinity and therefore, enhancing of soluble salts concentration into the soil solution will leads to rising of osmotic pressure of soil solution. With increasing of osmotic pressure in soil solution, water uptake by roots will reduce and hence, leaf water potential and relative water content (RWC) will decrease. These disorders that occur in plant water relationships by salinity have a positive link with the plant photosynthesis and dry yield. Consequently, the problems of soil salinity for plant growth are almost similar those that caused by the drought stress (Blumwald 2000; Davies et al 2001). The results of this research confirmed that the inoculating of sunflower plants by AM fungi (*Glomus etunicatum* and *Glomus intradices*) can be so helpful in enhancing of leaf RWC. So that, the plants that were inoculated by *Glomus intradices* significantly showed higher levels of RWC than to the non-inoculated plants. The AM fungi with their complex networks of hyphae, can increase the available soil volume for roots. Hence, the plant available water and water relationships will improve at the presence of AM fungi and hereby plants can easily overcome to stresses caused by salt and drought (Hardie & Leyton 1981; Azcon 1987; Subramanian et al 2005). In the present research, the inoculated plants by *Glomus intradices* had higher levels of leaf chlorophyll compared with non-inoculated plants. It is shown that the rate of photosynthesis in mycorrhizal plants is more than non-mycorrhizal plants, which is probably due to the influence of this symbiosis on opening of leaf stomata because, closure of stomata limits gas exchange resulting in a decrease in photosynthesis (Wright et al 1998; Goss & de Varennes 2002; Sannazzaro et al 2006). The results of this study also showed that both of the AM fungi (*Glomus etunicatum* and *Glomus intradices*) are able to increase the root colonization and therefore, the sunflower plants may benefit from this important advantage. In addition, the *Glomus etunicatum* could slightly increase the dry weight of roots of sunflower plants. Other similar findings for increasing of dry weight of roots in mycorrhizal plants under saline condition were also reported before (Davies et al 2001). So that Feng et al (2002) believe that the higher

accumulation of soluble sugars in mycorrhizal plant tissue, especially in roots, could make mycorrhizal plants more resistant to osmotic stress induced by exposure to salt.

Table 4

The comparison of mean for the interaction effect between fungus and bacterium on the water relationships and agronomic indices of sunflower plants (weight of above ground parts and roots are in g)

Treatments	*Fresh weight of above ground part*	*Dry weight of above ground part*	*Dry weight of root*	*Fresh weight of tray*	*dry weight of tray*
(F_0B_0)	83.48BC	14.02CD	2.401A	30.85C	5.332C
(F_0B_1)	88.56BC	15.42ABC	2.365A	36.54B	6.086ABC
(F_0B_2)	88.60BC	14.67BCD	2.359A	36.99B	6.004BC
(F_0B_3)	83.16BC	14.22CD	2.491A	38.38AB	6.715AB
(F_1B_0)	81.59C	14.85ABCD	2.534A	39.48AB	7.066AB
(F_1B_1)	86.77BC	15.92AB	2.575A	39.73AB	7.008AB
(F_1B_2)	84.43BC	15.31ABC	2.665A	36.48B	6.696AB
(F_1B_3)	83.08BC	13.66D	2.284A	36.84B	6.192ABC
(F_2B_0)	89.74B	14.64BCD	2.985A	31.42C	5.310C
(F_2B_1)	96.39A	16.32A	2.961A	41.45A	6.949AB
(F_2B_2)	86.20BC	14.92ABCD	3.019A	39.70AB	7.178A
(F_2B_3)	84.65BC	14.95ABCD	2.672A	37.12AB	6.646AB

Treatments	*Tray diameter (cm)*	*Plant length (cm)*	*Leaf weight (gr)*	*Leaf area (cm^2)*
(F_0B_0)	4.112B	57.59ABC	23.87ABC	1205ABC
(F_0B_1)	4.538A	59.28AB	23.79ABC	1205ABC
(F_0B_2)	4.612A	57.47ABC	23.88ABC	1211ABC
(F_0B_3)	4.750A	55.25BCD	21.26BC	1080BC
(F_1B_0)	4.850A	54.41BCD	20.90BC	1057BC
(F_1B_1)	4.738A	56.15ABCD	21.60BC	1091BC
(F_1B_2)	4.725A	57.00ABC	23.45BC	1185BC
(F_1B_3)	4.738A	52.95CD	22.47BC	1138BC
(F_2B_0)	4.200B	60.70A	26.83A	1356A
(F_2B_1)	4.725A	60.96A	24.05AB	1218AB
(F_2B_2)	4.812A	52.05D	20.51C	1040C
(F_2B_3)	4.662A	55.51BCD	23.73ABC	1201ABC

Treatments	*Leaf water potential (– Bar)*	*RWC (%)*	*Root colonization (%)*	*Leaf chlorophyll (SPAD value)*
(F_0B_0)	1.962A	66.63D	59.30DE	43.92AB
(F_0B_1)	1.688BCDE	76.06C	60.69DE	43.83AB
(F_0B_2)	1.812ABCD	77.57ABC	55.46E	43.20B
(F_0B_3)	1.90AB	76.63C	58.26DE	42.99B
(F_1B_0)	1.638CDE	78.80AB	74.21AB	43.07B
(F_1B_1)	1.762ABCDE	76.22C	69.05BC	43.87AB
(F_1B_2)	1.875ABC	77.87ABC	63.57CD	44.20AB
(F_1B_3)	1.862ABC	77.12BC	71.13AB	43.84AB
(F_2B_0)	1.600DE	79.23A	77.49A	44.61AB
(F_2B_1)	1.775ABCD	79.17A	70.52AB	45.24A
(F_2B_2)	1.525E	77.24BC	73.43AB	43.86AB
(F_2B_3)	1.788ABCD	76.57C	63.04CD	44.60AB

Means, in each column, with at least one similar letters are not significantly different at the 5% probability level using Duncan multiple range test

With regarding to the role of AM fungi in facilitating of plant water relationships, increasing of plant yield at present of AM fungi is inevitable. Whereas in the greenhouse condition achievement to actual yield of sunflower plant in not possible therefore, the parameters that were related to yield of sunflower were used. One of these parameters is fresh and dry weight of tray. Our results revealed that both of the AM fungi (*Glomus etunicatum* and *Glomus intradices*) used in this study increased significantly the dry weight of tray. Also, the plants that were inoculated by *Glomus etunicatum* had higher fresh weigh of tray than to the none-inoculated plants. Jalaluddin (1993) observed that under salt stress, corn plants inoculated by *Glomus intraradices* showed a 400% increase in dry matter over control plants.

Table 5

The comparison of mean for the interaction effect between fungus and cultivar on the water relationships and agronomic indices of sunflower plants (weight of above ground parts and roots are in g)

Treatments	*Fresh weight of above ground part*	*Dry weight of above ground part*	*Dry weight of root*	*Fresh weight of tray*	*dry weight of tray*
(F_0C_1)	83.45BC	12.78C	1.924C	32.80C	5.137B
(F_0C_2)	88.45AB	16.38A	2.883B	38.58AB	6.932A
(F_1C_1)	80.22C	13.78B	2.419B	36.77AB	6.742A
(F_1C_2)	87.72AB	16.09A	2.609B	39.50A	6.739A
(F_2C_1)	85.98B	13.92B	2.416B	36.25B	6.286A
(F_2C_2)	92.50A	16.49A	3.403A	38.59AB	6.755A

Treatments	*Tray diameter (cm)*	*Plant length (cm)*	*Leaf weight (gr)*	*Leaf area (cm^2)*
(F_0C_1)	4.30C	48.36B	23.74A	1232A
(F_0C_2)	4.706AB	66.44A	22.66A	1118B
(F_1C_1)	4.675AB	46.84B	21.97A	1140AB
(F_1C_2)	4.85A	63.42A	22.24A	1096B
(F_2C_1)	4.544B	48.24B	23.79A	1235A
(F_2C_2)	4.656AB	66.36A	23.76A	1173AB

Treatments	*Leaf water potential (– Bar)*	*RWC (%)*	*Root colonization (%)*	*Leaf chlorophyll (SPAD value)*
(F_0C_1)	1.70BC	74.58B	55.71C	46.46A
(F_0C_2)	1.981A	73.86B	61.15B	40.52C
(F_1C_1)	1.750BC	77.82A	68.78A	46.43A
(F_1C_2)	1.819B	77.18A	70.21A	41.06BC
(F_2C_1)	1.612C	78.28A	69.27A	47.11A
(F_2C_2)	1.731BC	77.82A	72.97A	42.04B

Means, in each column, with at least one similar letters are not significantly different at the 5% probability level using Duncan multiple range test

One of the major effects of salinity on plants is the ethylene accumulation in their roots which decrease root growth and finally reduce the yield of crops. PGPRs those are able to produce ACC-deaminase in plants rhizosphere, can consume pre-produced ethylene (ACC) and convert it to α-ketobutyrate and ammonium, so they are able to reduce ethylenes level in plants and hence, increase their growth (Glick et al 1998; Penrose & Glick 2003). When the PGPR contains the enzyme ACC deaminase, the bacterial cells act as a sink for ACC, the immediate biosynthetic precursor of ethylene thereby lowering plant ethylene levels and decreasing the negative effects of various environmental stresses (Stearns et al 2005). The results of the present study showed that the plant inoculating by *Pseudomonas fluorescence* strain 4 increased the fresh and dry weight of

above ground part. Therefore, it seems that the *Pseudomonas fluorescence* strain 4 is able to increase plant biomass by reducing of ethylene level in sunflowers. All of the bacterial inoculants used in this study (*Pseudomonas fluorescens* strains 4, 9 and 12) significantly enhanced the fresh and dry weight of tray of sunflowers. Therefore, it is expected that with application of these bacteria, the yield of sunflower plants will increase per unit area under saline condition. Similar findings were pointed out in other plants grown under drought and salt stress at the present of PGPRs having the ability of producing ACC deaminase enzyme (Mayak et al 2003; Mayak et al 2004).

Table 6

The comparison of mean for the interaction effect between bacterium and cultivar on the water relationships and agronomic indices of sunflower plants (weight of above ground parts and roots ar in g)

Treatments	*Fresh weight of above ground part*	*Dry weight of above ground part*	*Dry weight of root*	*Fresh weight of tray*	*dry weight of tray*
(B_0C_1)	78.18D	12.16F	2.098C	31.14C	5.152C
(B_0C_2)	91.68AB	16.84AB	3.182A	36.69B	6.653B
(B_1C_1)	87.41BC	14.06DE	2.204C	35.71B	5.843BC
(B_1C_2)	93.74A	17.71A	3.063AB	42.77A	7.518A
(B_2C_1)	84.95C	14.12DE	2.582BC	37.16B	6.630B
(B_2C_2)	87.87BC	15.82BC	2.780AB	38.28B	6.622B
(B_3C_1)	82.33CD	13.63E	2.128C	37.09B	6.594B
(B_3C_2)	84.93C	14.91CD	2.837AB	37.80B	6.442B

Treatments	*Tray diameter (cm)*	*Plant length (cm)*	*Leaf weight (gr)*	*Leaf area (cm^2)*
(B_0C_1)	4.225C	46.37C	22.43B	1164ABC
(B_0C_2)	4.55B	68.77A	25.30A	1248A
(B_1C_1)	4.508B	49.83C	23.07AB	1197AB
(B_1C_2)	4.825A	67.77A	23.23AB	1146ABC
(B_2C_1)	4.683AB	48.44C	23.84AB	1237A
(B_2C_2)	4.750AB	62.57B	21.38B	1054C
(B_3C_1)	4.608AB	46.62C	23.33AB	1211A
(B_3C_2)	4.825A	62.52B	21.46B	1068BC

Treatments	*Leaf water potential (– Bar)*	*RWC (%)*	*Root colonization (%)*	*Leaf chlorophyll (SPAD value)*
(B_0C_1)	1.525D	76.52A	68.54AB	46.73A
(B_0C_2)	1.942A	73.25B	72.13A	41B
(B_1C_1)	1.708C	76.49A	64.78BC	47.13A
(B_1C_2)	1.775ABC	77.81A	68.73AB	41.49B
(B_2C_1)	1.742BC	77.70A	63.07BC	46.71A
(B_2C_2)	1.733BC	77.42A	65.24BC	40.80B
(B_3C_1)	1.775ABC	76.88A	61.95C	46.09A
(B_3C_2)	1.925AB	76.67A	66.33BC	41.53B

Means, in each column, with at least one similar letters are not significantly different at the 5% probability level using Duncan multiple range test

With respecting to positive advantages of AM fungi and PGPRs for plants, it seems that with application of these two microorganisms simultaneously the plant growth will significantly improve under saline condition. So that, in the present study the co-inoculatation of sunflowers by *Glomus intradices* and *Pseudomonas fluorescence* strain 4 significantly increased their fresh and dry weight of above ground part compared with the control treatment. Also using of *Glomus intradices* alone did not increase significantly

fresh and dry weight of tray compared with the control, while addition of *Pseudomonas fluorescence* strain 4, 9 and 12 and AM fungi combination significantly increased these traits of sunflowers. In addition co-inoculation had significant effect on leaf water potential whereas, the traits of RWC, leaf chlorophyll and dry weight of root did not effect. Hence, the PGPRs with producing phytohormones and cohabiting in the rhizosphere with AM fungi could play a helper role in the plant-fungus interaction. However, Vázquez et al (2000) showed that that PGPRs like *Pseudomonas* did not exert an antagonistic effect against AM fungi in corn plants.

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