AcademicPres

Available online at www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol 2 (3) 2010, 57-66

Notulae Scientia Biologicae

Effect of Microbial Inoculants on Uptake of Nutrient Elements in Two Cultivars of Sunflower (*Helianthus annuus* L.) in Saline Soils

Mostafa SHIRMARDI[1)], Gholam Reza SAVAGHEBI[1)], Kazem KHAVAZI[2)], Ali AKBARZADEH[1)], Mohsen FARAHBAKHSH[1)], Farhad REJALI[2)], Abdolvahab SADAT[1)]

[1)] *University of Tehran, University College of Agriculture and Natural Resources, Faculty of Agricultural Engineering and Technology, Department of Soil Science, Karaj, 31587-77871, Iran; mos_shirmardi@yahoo.com; savagheb@ut.ac.ir; aliakbarzadeh1236@yahoo.com; mfbakhsh@ut.ac.ir; av.sadat@gmail.com*

[2)] *Soil and Water Research Institute, Tehran, 14155-6185, Iran; kkhavazi@yahoo.com; frejali@yahoo.com*

Abstract

A greenhouse experiment was conducted in order to evaluate the interactive effects of microbial inoculants on uptake of nutrient elements (N, P, K, Ca, Mg, Na, Cl, Fe, Zn, Cu, Mn) in two cultivars of sunflower. The trials were carried out on saline (EC = 7.6 dS m^{-1}) calcareous soils taken from Eshtehard (Karaj) region of Iran. In a factorial trial and completely randomized design (CRD), three levels of arbuscular mycorrhizal inoculants (non inoculation, inoculation with *Glomus etunicatum* and *Glomus intradices*) and four levels of *Pseudomonas fluorescens* inoculants (non inoculation and inoculation with *Pseudomonas fluorescens* strains 4, 9, 12) in two cultivars of sunflower with four replications per treatments were applied. Results revealed that all of the treatments increased the N uptake in Euroflor cultivar. Moreover, in Euroflor cultivar, inoculation with *Pseudomonas fluorescens* strains 9 and co-inoculation of *Pseudomonas fluorescens* strains 4 and *Glomus intradices* made a significant different in phosphorous uptake, while did not make any significant change in the Master cultivar. However, bacterial and fungal treatments significantly ($P < 0.05$) increased uptake of micro nutrients such as Fe, Zn and Mn.

Keywords: *Arbuscular mycorrhiza*, plant growth promoting rhizobacteria, salinity, sunflower

Introduction

Today accurate management of soils having salt problems, for achievement to maximum yield has received widespread attention throughout the world. Over than 1000 million hectare of the world's land are affected by the salt problems and these areas including about 7% of the total world's land. Also from 1.5 billion of agricultural lands throughout the world, about 77 million hectare (5% from these areas) is extremely under influence of salt problems (Giri *et al*., 2007). Plants growing in these regions are seriously under salt stress and hence, due to harms received from this stress they never reach to their maximum growth and production. Chemical characteristics of soils affected by soluble salts which show their impact on plant growth are low activity of nutrient elements, high ratio of Na^+/Ca^{+2}, Na^+/K^+, Mg^{2+}/Ca^{+2} and Cl^-/NO^{-3}, nutrition malformation and reduction of overall growth and yield quality (Mirmahammadi-Meybodi and Ghareyazi, 2002). The presence of excessive Na^+ into the soil causes disruption in uptake and transportation of nutrient elements such as Mg^{2+} and Ca^{+2} by plants. Salt stress causes a variety of disruptions in plant nutrition and therefore, will provide adverse condition for plant development. These disruptions are usually because of negative effect of salts on uptake ability of nutrient elements, competitive behavior of elements for uptake, transportation and distribution of these elements to different parts of the plant. Also, salt stress some times leads to physiological non activity of one the essential nutrient element in the plant and therefore, the symptom of deficit will appear in the plant's tissues (Kafi and Damghani, 2000).

There are several methods for reducing of adverse effects of soil salinity. 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 are representing 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).

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 are able to produce ACC-deaminase in plants rhizosphere and they can consume pre-produced ethylene (ACC) and convert it to α-ketobutyrate and ammonium, so they are

Received 17 May 2010; accepted 23 August 2010

able to reduce ethylenes level in plants and hence, increase their growth (Glick *et al*., 1998; Penrose and Glick, 2003). Mayak *et al*. (2004) evaluated the role of a PGPR (*Achromobacter piechaudii*) bacterium in resistance of tomato plant to salt stress in dry salty environments of Israel. This bacterium significantly increased the fresh and dry weights of tomato seedlings grown in the presence of up to 172 mM NaCl salt. The bacterium reduced the production of ethylene by tomato seedlings which were otherwise stimulated when seedlings were challenged with increasing salt concentrations, but did not reduce the content of sodium. However, it slightly increased the uptake of phosphorous and potassium which may contribute in part to activation of processes involved in the alleviation of the effect of salt. 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. Nadeem *et al*. (2006a,b) have observed almost similar results in the case of maize growth under salt stress in response to inoculation with ACC deaminase PGPR. Similarly, Hamdia *et al*. (2004) studied the effect of a PGPR (*Azospirillum brasilense)* on uptake of elements in corn plants as grown in salty conditions. They reported that the Na^+ concentration in the root and above ground parts of corn plants decreased while the Ca^{2+} and K^+ concentration in these organs of plants inoculated by PGPR increased.

Decreasing of ion activity in soil solution and appearance of nutrition problems in plants is the other unfavorable effect of salinity. If AM fungi are used as a symbiosis participant in plants rhizosphere, plants achieve the beneficial effects of this relationship. AM fungi produce hypha networks on the surface of plants roots thus increase the volume of soil which is available for them, so existence of this network helps plants to take more water and nutrient elements. Also, AM fungi are known to increase plant tolerance to abiotic stress, in particular soil salinity. Yano-Melo *et al*. (2003) showed that inoculation of banana plants (*Musa* sp. cv. Pacovan) with specific AM fungi (*Glomus* isolates) reduced plant stress caused by soil salinization. Similarly, Copeman *et al*. (1996) reported that AM fungi can promote tomato plant growth through improvement of plant nutrition and production of osmoregulators in salty environments. Sannazzaro *et al*. (2007) investigated the involvement of *Glomus intraradices* in the regulation of plant growth, polyamines and proline levels of two *Lotus glaber* genotypes differing in salt tolerance, after long-term exposure to saline stress. They suggested that modulation of polyamine pools can be one of the mechanisms used by AM fungi to improve *Lotus glaber* adaptation to saline soils. 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 shoot contents of P, K, Zn, Cu, and Fe were higher in AM compared with nonAM plants grown under nonsaline and saline water conditions. In addition, shoot Na concentrations were lower in AM than nonAM plants grown under saline water conditions. Feng *et al*. (2002) showed that AM fungi are able to increase tolerance of maize plants to salt stress. They reported that mycorrhizal plants had higher electrolyte concentrations in roots and lower electrolyte leakage from roots than non-mycorrhizal plants under given NaCl and P levels. Sannazzaro *et al*. (2006) found that AM fungi (*Glomus intraradices*) improved growth of *Lotus glaber* plants under saline conditions. They showed that mycorrhizal plants had higher values of net growth, shoot/root and K^+/Na^+ ratios and protein concentrations than controls.

In many areas of Iran due to low precipitation and overexploitation of available water resources (e.g., ground water) often soils are saline. In addition, this salinity is mostly due to irrigation practices that bring salts to the soil surface where they can be toxic to crop plants. This salinity probably reduce plants uptake of nutrient elements from soil and hence, leads to reduction in crop growth and yield. Proper cropping techniques to run sustainable agriculture in the presence of irrigation water of poor quality (e.g., saline) becoming essential especially with limiting availability of fresh water for irrigation use (Al-Karaki, 2006). One of these cropping techniques is improvement of plant tolerance to saline condition by microbial inoculants. Therefore, this study carried out with 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 nutrition condition of sunflower plants, (b) to study the role of co-inoculation of sunflower plant by PGPRs and AMF in increasing uptake of nutrient elements and (c) to evaluate and compare the nutrition response of two cultivars of sunflower (Euroflor and Master) to microbial inoculation.

Materials and methods

Plant selection

One of the best characteristic of sunflower is its high aptitude to establishing of symbiosis with mycorrhizal fungi. This characteristic often can not observe easily in other plants. Therefore, sunflower may benefit from this symbiosis. This nature of sunflower along with its economic importance that introduce this plant as an industrial oilseed crop having main source of high quality of edible oil, were adequate reasons for selecting of this plant in present research. 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 javelle water (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

Microbial inoculants including micorrhizal fungi (AMF) and bacteria (PGPR) 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* (Tab. 1).

Tab. 1. Some characteristics of bacteria used in this study

Bacterium	Activity of ACC deaminase (μmoles mg^{-1} h^{-1})	Auxin (μg ml^{-1})	Bacterial population in inoculants (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

Cultural practices

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 treatment design, in each pot 4 small holes were shaped and then, 2 g fungi inoculants and 1 g bacterial inoculants were added to the inside of 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°C 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.

Soil type

It was needed a salty soil for the experiments. Therefore, a pre-sampling performed from different locations in Eshtehard (Karaj) region of Iran. After determining the salinity of these pre-samples, the best place was selected. The sampling spot is located 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, 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.

Plant analysis

For measuring of different elements in plant tissues, the dry ashing method was used. In this method, the grinded plants were dried in the oven with the temperature of 70 °C for 48 hours. In the next stage 2 g of these dry matter that were tranfered into the ceramic vessels were subjected slowly to 450°C heat in the oven. The final product was a white ash. After cooling the white ashes in room temperature, 20 ml 1N HCl was added to each sample and followed by the sand bath for 30 minutes. Then the samples were elutriated in a 100 ml volumetric balloon (Cottenie, 1980).

After providing the plant extracts, the concentration of K and Na was measures by flamephotometer apparatuses (ELE) directly in the volumetric balloons. Also, the concentration of Ca and Mg were measured by complexometery method and Fe, Zn, Cu and Mn were measured by atomic apparatus (Shimadzu AA6600) directly in the balloons. For measuring of plant phosphorus percentage, the yellow method was used. In this technique nitrovanado molybdate as an indicator was applied and the P concentration measured in the wave length of 430 nm using spectrophotometer apparatus (Shimadzu UV3100). The total plant N in the plant was measured using digestion

method by the Kejeldahl apparatus (Cottenie, 1980). The percentage of Cl in plant tissues was measured by the Hipp and langdal (1971) method using a chlorometer apparatus (METEROHM-781). This apparatus has an ion selective electrode (ISE) and therefore, is able to measure the concentration of Cl in extracts directly. After measuring of element concentration (% for macroelements and mg kg^{-1} for microelements) in aerial parts (above ground organs) of the plant tissues, the rate of uptake (mg pot^{-1}) for different elements was also calculated on the basis of plant dry matter.

Experimental design and statistical analysis

The analysis of variance (ANOVA) was carried out with randomized complete block design with sub split plot. Main plot were three levels of arbuscular mycorrhizal inoculants including non inoculation (F_0), inoculation with *Glomus etunicatum* (F_1) and *Glomus intradices* (F_2). Sub plot were 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). Sub sub plot were two cultivars of sunflower including Euroflor (C_1) and Master (C_2) (Tab. 2). The ANOVA 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

Major effect of fungus, bacterium and cultivar on uptake of elements

The result of analysis of variance (ANOVA) for the major effect of fungus, bacterium and cultivar of sunflowers on uptake of macro elements showed that the fungus and cultivar inoculants had no significant effect on uptake of N, while the effect of bacterium was significant ($p < 0.01$). Bacterium and cultivar had significant effect ($p < 0.05$) on uptake of P to above ground parts of the plants, while the fungus had not any influence. Fungus, cultivar and bacterium had significant effect on uptake of K by the plants.

Tab. 2. Different treatments

Treatments		
Fungi	Without fungus	(F_0)
	Glomus etunicatum	(F_1)
	Glomus intradices	(F_2)
Bacteria	Without bacterium	(B_0)
	Pseudomonas fluorescence strain 4	(B_1)
	Pseudomonas fluorescence strain 9	(B_2)
	Pseudomonas fluorescence strain 12	(B_3)
Cultivar of sunflower	Euroflor	(C_1)
	Master	(C_2)

Fungus and bacterium had significant effect on uptake of Ca, whereas the cultivar had not any influence. In the case of Mg, the bacterium and cultivar had significant effect on its uptake by the plants ($p < 0.01$), while the fungi had no influence (Tab. 3). The comparison of mean indicated that none of the fungus inoculants as well as sunflower cultivars had no significant effect on uptake of N to the above ground parts of the plants, whereas the bacteria inoculants of B_1 and B_3 significantly ($p < 0.05$) increased and decreased the N uptake by plants, respectively. Fungi treatments had not any effect on uptake of P. Also between the bacteria inoculants, the B_1 treatment had significant effect on enhancing the P uptake compared with the control treatment. The lowest uptakes were related to control (B_0) and B_1 treatments. In addition the cultivar of Euroflor (C_1) had higher P uptake compared with the Master (C_2). Addition of treatment of F_1 had a significant decreasing effect ($p < 0.05$) on uptake of K between other fungi treatment. Between bacteria inoculants, the B_3 treatment had a significant effect ($p < 0.05$) on decreasing the K uptake compared with its control treatment. In addition, between the cultivars of sunflower, the Master (C_2) had higher uptake of K than to the Euroflor (C_1). The comparison of mean for fungi treatments showed that the most level of Ca uptake was related to control treatment. Therefore, the fungi inoculants did not increased the Ca uptake by the plants and even inversely reduced the Ca uptake. Between the bacteria treatments, the B_3 had the highest level of Ca uptake and had significant difference ($p < 0.05$) with the B_1 and B_2 treatments. Moreover, application of B_1 treatment reduced significantly uptake of Mg (Tab. 4).

The result of ANOVA for the major effect of fungus, bacterium and cultivar of sunflowers on uptake of micro elements (Fe, Zn, Cu, and Mn) and elements of Na and Cl showed the cultivar had significant effect ($p < 0.01$) on uptake of all of these elements (micro and Na and Cl). The effect of bacterium and fungus was significant on uptake of the entire micro element except Fe and Cu, respectively. Also, the fungus had no any significant effect of uptake of Cl (Tab. 3). The result of comparison of mean for the major effect of fungus, bacterium and cultivar of sunflowers on uptake of micro elements and elements of Na and Cl showed that between the fungi treatment, the most uptake of Na was observed in the F_1 treatment. Application of F_2 treatment had significant decreasing effect ($p < 0.05$) of uptake of Fe. The most and least levels of Zn uptake was related to F_2 and F_1 treatments, respectively and there was no significant difference between F_0 and F_1 treatments. Also, using of F_1 treatment significantly ($p < 0.05$) reduced the uptake of Mn compared with the control (F_0) treatment and the most level of Mn uptake was observed in the F_0 treatment (Tab. 4). In addition, all of the three bacterial inoculants (B_1, B_2 and B_3) had significant effect on increasing Na uptake by plants compared with the control treatment (B_0). The treatment of B_3 had the lowest level of Cl uptake between other treatments and had significant dif-

Tab. 3. ANOVA for uptake of macro and micro elements and elements of Na and Cl

S.O.V.	df.	Mean square				
		N	P	K	Ca	Mg
Fungus (F)	2	395.821ns	11.076ns	44220.618**	2195.906**	39.65ns
Bacterium (B)	3	3096.918**	38.262*	29221.314*	787.683*	393.328**
Cultivar (C)	1	24.837ns	71.826*	189126.616**	406.722ns	6649.678**
F × B	6	403.486ns	10.299ns	7958.785ns	2073.474**	871.125**
F × C	2	269.628ns	28.944ns	4907.554ns	177.344ns	46.879ns
B × C	3	2701.637**	18.696ns	62835.263**	471.306ns	826.532**
Error	72	499.862ns	12.244ns	7654.433ns	278.88ns	73.582ns

S.O.V.	df.	Mean square					
		Na	Cl	Fe	Zn	Cu	Mn
Fungus (F)	2	97.141**	2791.874ns	13.342**	0.96*	0.087ns	3.219**
Bacterium (B)	3	107.528**	20553.466**	6.65ns	0.96*	1.1**	2.328**
Cultivar (C)	1	778.148**	195877.466**	32.83**	5.175**	2.908**	27.8**
F × B	6	42.309*	7507.172*	8.718**	0.499ns	0.243ns	0.604ns
F × C	2	18.233ns	9995.483*	0.222ns	0.21ns	0.002ns	0.454ns
B × C	3	63.479**	33481.074**	6.628ns	0.533ns	1.602**	2.65**
Error	72	14.36ns	2476.343ns	2.531ns	0.279ns	0.269ns	0.521ns

** and * significant at 1 and 5%, respectively; ns non significant

ference ($p < 0.05$) with control treatment (B_0). The B_2 and B_3 treatments showed the maximum and minimum level of Fe uptake by plants but none of them had no significant difference with the control treatment (B_0). All of the three bacterial inoculants (B_1, B_2 and B_3) were increased Zn and Cu uptake whereas, application of bacterial inoculants significantly decreased uptake of Mn by the plants. So that, the most levels of Mn uptake was related to B_0 and B_1 treatments and the least was related to B_3 treatment. Also, Master cultivar (C_2) of sunflower had higher uptake of Na

Tab. 4. The comparison of mean for the major effect of different levels of fungus, bacterium and cultivar on uptake of macro and micro elements and elements of Na and Cl (mg pot^{-1})

Treatments	N	P	K	Ca	Mg
(F_0)	302.234A	30.099A	675.03A	216.169A	63.137A
(F_1)	295.240A	29.031A	601.88B	202.256B	63.423A
(F_2)	298.09A	29.993A	649.95A	201.422B	61.368A
(B_0)	294.64BC	28.649B	659.14A	208.441AB	64.143A
(B_1)	312.966A	31.259A	669.6A	202.130B	64.451A
(B_2)	300.575AB	30.213AB	648.89A	201.968B	65.356A
(B_3)	285.905C	28.708B	591.51B	213.923A	56.619B
(C_1)	299.03A	30.572A	597.9B	208.674A	54.32B
(C_2)	298.013A	28.842B	686.67A	204.557A	70.965A

Treatments	Na	Cl	Fe	Zn	Cu	Mn
(F_0)	12.540B	399.74A	11.848A	4.73AB	5.093A	6.647A
(F_1)	15.232A	384.11A	11.308AB	4.489B	5.069A	6.014B
(F_2)	11.97B	400.79A	10.562B	4.826A	5.169A	6.372AB
(B_0)	10.524C	403.12A	11.35AB	4.401B	4.807B	6.52A
(B_1)	15.386A	422.49A	11.44AB	4.735A	5.231A	6.631A
(B_2)	14.341AB	400.37A	11.688A	4.875A	5.286A	6.02AB
(B_3)	12.740B	353.54B	10.479B	4.715A	5.117A	5.925B
(C_1)	10.400B	349.71B	11.824A	4.449B	4.936B	5.806B
(C_2)	16.095A	440.05A	10.654B	4.914A	5.284A	6.883A

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

Tab. 5. The comparison of mean for the interaction effect between fungus and bacterium on uptake of macro and micro elements and elements of Na and Cl (mg pot^{-1})

Treatments	N	P	K	Ca	Mg
(F_0B_0)	294.1ABCD	27.72AB	680.1ABC	210.7BC	71.87AB
(F_0B_1)	316.4A	31.17AB	724.4A	212.9ABC	67.25BC
(F_0B_2)	309/4ABC	31.64A	662.1ABC	219.9AB	64.07BC
(F_0B_3)	289.0BCD	29.87AB	633.5ABC	221.1AB	49.36E
(F_1B_0)	289.8BCD	28.02AB	595.9CD	190.8D	51.30DE
(F_1B_1)	307.5ABC	31.63A	625.9ABCD	197.6CD	59.86CD
(F_1B_2)	304.1ABCD	29.13AB	651.7ABC	190.3D	77.03A
(F_1B_3)	279.6D	27.34B	533.9D	230.3A	65.50BC
(F_2B_0)	300.0ABCD	30.21AB	701.4AB	223.8AB	69.27ABC
(F_2B_1)	315.0AB	30.98AB	658.5ABC	195.9CD	66.24BC
(F_2B_2)	288.2CD	29.87AB	632.8ABC	195.7CD	54.96 DE
(F_2B_3)	289.1BCD	28.91AB	607.1BCD	190.3D	55.00DE

Treatments	Na	Cl	Fe	Zn	Cu	Mn
(F_0B_0)	8.139F	379.2BCD	11.09AB	4.434BCD	4.680C	6.766AB
(F_0B_1)	17.11AB	429.9AB	12.34A	5.033AB	5.405A	6.910A
(F_0B_2)	14.00ABCD	418.1ABC	12.25A	4.880ABC	5.336 A	6.768AB
(F_0B_3)	10.91DEF	371.8CD	11.71AB	4.573BCD	4.950ABC	6.144ABCD
(F_1B_0)	12.37CDE	381.3BCD	10.67AB	4.146D	4.720BC	5.983BCD
(F_1B_1)	15.00ABCD	396.6ABCD	11.20AB	4.516BCD	5.191ABC	6.361ABC
(F_1B_2)	15.86ABC	415.4ABC	12.49A	4.911ABC	5.300AB	6.233ABCD
(F_1B_3)	17.71A	343.1D	10.88AB	4.384CD	5.065ABC	5.481D
(F_2B_0)	11.07DEF	448.9A	12.29A	4.624BCD	5.021ABC	6.813AB
(F_2B_1)	14.05ABCD	441.0A	10.78AB	4.658ABCD	5.098ABC	6.621ABC
(F_2B_2)	13.16BCDE	367.6CD	10.32BC	4.834ABC	5.223ABC	5.908CD
(F_2B_3)	9.602 EF	345.7D	8.855C	5.188A	5.335A	6.149ABCD

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

and Cl than to the Euroflor cultivar (C_1). This result also was observed for all of the microelements except Fe hence, the C_1 treatment just had higher Fe uptake compared with the C_2 treatment (Tab. 4).

Interaction effect between fungus and bacterium (F × B)

The result of ANOVA (Tab. 3) showed that the interaction effect between fungus and bacterium (F × B) had no any significant effect on N, P and K uptake by the plants while this interaction (F × B) had significant effect ($p < 0.01$) on uptake of Ca and Mg. The comparison of mean for the interaction effect between fungus and bacterium (F × B) on uptake of macro elements showed the F_0B_1 and F_1B_3 treatments contained the maximum and minimum levels of N uptake but, none of these treatments had no significant differences with the control treatment (F_0B_0). At the level of F_0, application of B_1 and B_2 inoculants led to partial enhancement in N uptake. At the levels of F_1 and F_2, none of the bacterial inoculants (B_1, B_2 and B_3) had any significant differences. In all of the F × B treatments except F_1B_3, the level of P uptake by the plants increased. In addition, the maximum and minimum levels of K uptake were related to treatments of F_0B_1 and F_1B_3, respectively. The F_1B_3 treatment had the most level of Ca uptake between other treatments. At the levels of F_0, application all of the bacterial inoculants (B_1, B_2 and B_3) insignificantly increased Ca uptake, while at F_2 all of the bacterial inoculants significantly reduced Ca uptake. In associated with Mg uptake, the comparison of mean showed that the F_0B_3, F_1B_0, F_2B_2 and F_2B_3 treatments had significant decrease compared with the control treatment (F_0B_0) (Tab. 5).

The result of ANOVA for interaction effect between fungus and bacterium (F × B) in uptake of micro elements and elements of Na and Cl showed that this interaction had significant effect on Na, Cl and Fe uptake, whereas this interaction had no significant effect on Zn, Cu and Mn uptake (Tab. 3). The comparison of mean for the interaction effect between fungus and bacterium (F × B) on uptake of micro elements and elements of Na and Cl showed that all the treatments except F_0B_3, F_2B_0 and F_2B_3 increased significantly ($p < 0.05$) Na uptake by the plants compared with the control treatment (F_0B_0). The F_2B_0 and F_2B_1 treatments also significantly increased Cl uptake. The most and the least level of Cl uptake were related to F_1B_3 and F_2B_3, respectively. The results of comparison of

mean demonstrated that the F_2B_3 treatment reduced significantly Fe uptake than to the control (F_0B_0) treatment. For the Zn, the treatments of F_1B_2 and F_2B_3 significantly ($p < 0.05$) increased its uptake. In addition, the treatments of F_0B_1, F_0B_2, F_1B_2 and F_2B_3 significantly increased Cu uptake compared with the control (F_0B_0) treatment. For Mn, just the F_1B_3 and F_2B_2 treatments decreased significantly its uptake compared with the control treatment (Tab. 5).

Interaction effect between fungus and cultivar (F × C); bacterium and cultivar (B × C)

The result of ANOVA indicated that the interaction effect between fungus and cultivar (F × C) had no significant effect on uptake of none of the macro elements (N, P, K, Ca and Mg), while the interaction effect between bacterium and cultivar (B × C) had significant effect ($p < 0.01$) on all of the macro elements except P and Ca (Tab. 3). The comparison of mean showed that none of the FC treatments had any significant differences in N, P uptake. The treatment of F_0C_2 had more K and Mg uptake than to the F_0C_1 treatment and application of F_1C_2 treatment significantly decreased K uptake by the plants. Similarly, the treatments of F_1C_1 and F_2C_1 significantly decreased Ca uptake (Tab. 6). Furthermore, the B_0C_2 treatment had more N, P, K and Mg uptake than to the B_0C_1 treatment. However, this increasing uptake level in B_0C_2 treatment for P was not significant. Also, B_0C_2 treatment had lower Ca uptake compared with the B_0C_1 treatment. The B_1C_1 and B_2C_1 treatments significantly increased N, P and Mg uptake by the plants but, B_3C_2 treatment significantly decreased the N uptake. Application of B_2C_2 and B_3C_2 treatments significantly decreased K uptake. The B_1C_1 and B_2C_1 treatments significantly reduced Ca uptake by the plants (Tab. 7).

The result of ANOVA for interaction effect between fungus and cultivar (F × C) did not show significant effect on uptake of none of the micro elements (Fe, Zn, Cu and Mn) as well as Na, but this interaction had significant effect ($p < 0.05$) on Cl uptake. In addition, the interaction effect between bacterium and cultivar (B × C) had significant effect ($p < 0.01$) on Na and Cl uptake as well as all of the micro elements except Fe and Zn (Tab. 3). The comparison of mean showed that the treatment of F_0C_2 had higher Na, Cl and Mn uptake compared with the F_0C_1 treatment. However with respecting to the Fe, Zn and Cu uptake, there were no significant differences between two treatments. In addition, the treatment of F_1C_1 significantly increased and decreased the Na and both Zn and Mn uptake respectively compared with its control (F_0C_1) treatment. The treatment of F_2C_1 just significantly decreased the Fe uptake and two treatments of F_1C_2 and F_2C_2 also significantly decreased the Cl and Fe uptake respectively (Tab. 6). Furthermore, the treatment of B_0C_2 had higher Na, Cl, Zn, Cu, Mn uptake compared with the B_0C_1 treatment. Moreover, with respecting to Fe uptake there was no significant differences between these two treatments. The treatment of B_2C_1 significantly enhanced the Na, Cl and Fe uptake compared with its control (B_0C_1) treatment. A similar result was observed for the Cl and Mn uptake by the B_1C_1 treatment. Moreover, two treatments of B_1C_2 and B_2C_2 significantly increased the Na uptake compared with their control (B_0C_2) treatment. The treatments of B_2C_2 and B_3C_2 significantly reduced the Cl and Mn uptake compared with their control (B_0C_2) treatment. At the level of C_1 all of the bacterial inoculants (B_1C_1, B_2C_1 and

Tab. 6. The comparison of mean for the interaction effect between fungus and cultivar on uptake of macro and micro elements and elements of Na and Cl (mg pot^{-1})

Treatments	N	P	K	Ca	Mg
(F_0C_1)	299.4A	30.94AB	617.7BC	220.9A	53.45B
(F_0C_2)	305.1A	29.25ABC	732.4A	211.4AB	72.82A
(F_1C_1)	297.6A	28.96BC	558.7C	203.4B	56.06B
(F_1C_2)	292.9A	29.11BC	645.1B	201.1B	70.79A
(F_2C_1)	300.1A	31.82A	617.3BC	201.7B	53.45B
(F_2C_2)	296.1A	28.17C	682.6AB	201.1B	69.29A

Treatments	Na	Cl	Fe	Zn	Cu	Mn
(F_0C_1)	8.981E	336.3C	12.36A	4.548B	4.916B	6.179B
(F_0C_2)	16.10AB	463.2A	11.34ABC	4.911AB	5.270AB	7.115A
(F_1C_1)	12.31CD	356.0C	11.98AB	4.164C	4.889B	5.339C
(F_1C_2)	18.16A	412.2B	10.63CD	4.815AB	5.249AB	6.690A
(F_2C_1)	9.914DE	356.8C	11.13BCD	4.636AB	5.004AB	5.902B
(F_2C_2)	14.03BC	444.7AB	9.993D	5.015A	5.334A	6.843A

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

Tab. 7. The comparison of mean for the interaction effect between bacterium and cultivar on uptake of macro and micro elements and elements of Na and Cl (mg pot^{-1})

Treatments	N	P	K	Ca	Mg
(B_0C_1)	281.4BC	28.25C	551.7D	214.6AB	47.84D
(B_0C_2)	307.9A	29.05ABC	766.6A	202.3B	80.45A
(B_1C_1)	312.4A	32.20A	609.3CD	201.5B	56.77C
(B_1C_2)	313.5A	30.32ABC	729.9AB	202.8B	72.13B
(B_2C_1)	304.2A	31.76AB	628CD	199.2B	58.24C
(B_2C_2)	297AB	28.66BC	669.7BC	204.7AB	72.47B
(B_3C_1)	298.1AB	30.08ABC	602.5CD	219.4A	54.44CD
(B_3C_2)	273.7C	27.34C	580.5D	208.4AB	58.80C

Treatments	Na	Cl	Fe	Zn	Cu	Mn
(B_0C_1)	8.754E	315.2D	11.37BC	4.002C	4.338C	5.493 E
(B_0C_2)	12.29CD	491A	11.33BC	4.801AB	5.276AB	7.548A
(B_1C_1)	10.12DE	358.8C	12.03AB	4.48B	4.927B	6.261CD
(B_1C_2)	20.66A	486.2A	10.85BC	4.991A	5.536A	7.001AB
(B_2C_1)	12.16CD	377.6C	12.97A	4.638AB	5.270AB	5.846DE
(B_2C_2)	16.52B	423.2B	10.40C	5.112A	5.303AB	6.759BC
(B_3C_1)	10.57DE	347.3CD	10.93BC	4.678AB	5.210AB	5.626DE
(B_3C_2)	14.91BC	359.8C	10.03C	4.752AB	5.023B	6.223CD

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

B_3C_1) increased Zn and Cu uptake by the plants compared with the control (B_0C_1) (Tab. 7).

Discussions and conclusions

Soil salinity can cause lots of nutrition problems for the plants. The main reason for these nutrition problems can be related to the abundant presence of a special ion in soil solution like Na and Cl. The abundance of these soluble ions can decrease the activity of other essential elements in the soil and lead to reduction in accessibility and uptake of elements by the plants. Lots of researchers reported that with increasing in soil salinity, the concentration of micro and macro elements in above ground parts of the plants as well as their roots will reduce (Feng *et al*., 2002; Al-Karaki, 2006; Giri *et al*., 2007).

AM fungi form symbiotic associations with the roots of most plant species, and they aid those plants in uptake of nutrients especially those immobile in soil like P (Al-Karaki and Al-Raddad, 1997; Marschner and Dell, 1994; Cantrell and Linderman, 2001). However, the results of the present study showed that the inoculation of the soil by the *Glomus etunicatum* and *Glomus intradices* did not affected P uptake by the sunflower. One of the reasons for this observation was probably because of the small volume of soil. Usually the AM fungi hyphae show their effectiveness in a large volume of the soil and hence, into the limit environment of pots the hyphae could not work properly and did not their functions correctly. On the other hand the fungi had high population into the soil of the pots and therefore, the fungi could not show their role appropriately. The behavior of Cl uptake by the plants was almost similar to the P uptake in the present study, which is in contrast with the findings by Graham and Syvertsen (1989). They reported that root colonization by AM fungi increased Cl uptake by the citrus plants in the saline condition. The AM fungi also did not affect the K uptake by the plants. It seams that the high concentration of Na in soil solution and fungi inability in reduction of Na uptake by the plants was its reason. Similar results were observed by the Poss *et al*. (1985) and Al-Karaki (2006) in tomato plants. They stated that K uptake was affected little by AM fungi root colonization in plants grown under saline conditions. In addition, in the present study, application of fungi inoculants reduced Fe and Mn uptake by the plants. Similarly, Pacovsky and Fuller (1998) showed that AM fungi reduced Fe concentration by the plants. This might indicate that Fe and Mn were retained in roots without being translocated to the above grounds parts of the plants and suggest that they might be retained in intraradical AM fungal hyphae or were compartmentalized in the root cell vacuoles (Cantrell and Linderman, 2001). Also, the lower Fe and Mn concentrations in AM plant tissues compared to nonAM plants might be explained by dilution effects due to growth enhancement by AM fungi colonization (Al-Karaki, 2000). In addition, in rhizosphere of AM plants, the population of Mn reduction bacteria will reduce and lead to deficit of exchangeable Mn in the soil (Sharma and Johri, 2002). However, different result was also reported by the Clark and Zeto (1996) in related to Fe uptake by corn plants. They pointed out that using of AM fungi significantly increased Fe uptake.

Inoculation with PGPR containing ACC deaminase activity could be helpful in sustaining plant growth and development under stress conditions by reducing stress-induced ethylene production (Saleem *et al*., 2007). In the present study, the *Pseudomonas fluorescens* strain 4 could significantly increase the uptake of K and P by the plants. Similarly, Mayak *et al*. (2004) concluded that the uptake of P and K increased at the present of a PGPR in a salty environment at tomato plants. They also reported that the PGPR reduced the content of Ca, Mg, K and S in the salt environment; the content of Na was increased; and the content of Ba and Fe was unchanged. In the present study all of the bacterial inoculants (strains 4, 9 and 12) significantly increased Zn and Cu compared with none inoculated plants. This improvement is probably because of the addition of root growth by the PGPRs that cause the root has more contact with the soil particles. Moreover, the PGPRs due to creation of special compounds may able to enhance the Zn and Cu uptake by the roots. The plants inoculated by PGPRs also had more Na uptake than to the noneinoculated plants while, the PGPRs did not increased the K, Ca and Mg uptake by plants. On the other hand the *Pseudomonas fluorescens* strain 12 significantly decreased the uptake of Cl by the plants. This bacterium probably inhibits from Cl uptake by the roots or prevents from its translocation to the above ground parts. With lowering the Cl uptake probably uptake of other anions such as soleplate and nitrate by plants will increase and hence, the nutrition condition of the plant will increase.

Acknowledgements

We wish to thank University of Tehran for supporting field studies and samplings. We would like to gratefully thank all the members of the Soil Science Laboratory of Faculty of Agricultural Engineering and Technology of University of Tehran, for providing the facilities to carry out this work and for their suggestions.

References

Al-Karaki, G. N. (2006). Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Scientia Horticulturae 109:1-7.

Al-Karaki, G. N. (2000). Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza 10:51-54.

Al-Karaki, G. N. and A. Al-Raddad (1997). Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance. Mycorrhiza 7:83-88.

Cantrell, I. C. and R. G. Linderman (2001). Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. Plant and Soil 233:269-281.

Cheng, Z., E. Park and B. R. Glick (2007). 1-Aminocyclopropane-1-carboxylate (ACC) deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. Canadian Journal of Microbiology 53(7):912-918.

Clark, R. B. and S. K. Zeto (1996). Mineral acquisition by mycorrhizal maize grown on acid and alkaline soil. Soil Biology and Biochemistry 28:1405-1503.

Copeman, R. H., C. A. Martin and J. C. Stutz (1996). Tomato growth in response to salinity and mycorrhizal fungi from saline or nonsaline soils. HortScience 31:341-344.

Cottenie, A. (1980). Methods of Plant Analysis. In: Soil and Plant Testing. pp. 64-100. FAO Soils Bulletin 38/2.

Feng, G., F. S. Zhang, X. L. Li, C. Y. Tian, C. Tang and Z. Rengel (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 12:185-190.

Gamalero, E., L. Fracchia, M. Cavaletto, J. Garbaye, P. Frey-Klett, G. C. Varese and M. G. Martinotti (2003). Characterization of functional traits of two fluorescent pseudomonads isolated from basidiomes of ectomycorrhizal fungi. Soil Biology & Biochemistry 35:55-65.

Giri, B., R. Kapoor and K. G. Mukerji (2007). Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, Glomus Fasciculatum may be partly related to elevated K/Na ratios in root and shoot tissues. Microbial Ecology.54:753-760.

Glick, B. R., D. M. Penrose and J. Li (1998). A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. Journal of Theoretical Biology 190:63-68.

Graham, J. H. and A. Syvertsen (1989). Vesicular-Arbuscular Mycorrhiza increase chloride concentration in citrus seedling. New Phytologist 113:29-36.

Hamdia, M. A., M. A. K. Shaddad and M. M. Doaa (2004). Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense* inoculation on maize cultivars grown under salt stress conditions. Plant Growth Regulation 44:165-174.

Hipp, B. W. and G. W. langdal (1971). Use of a solid-state chloride electrode for chloride determination in soil extracts. Soil Science and Plant Analysis 2:237-240.

Kafi, M. and A. M. Damghani (2000). The mechanisms of plant resistance to salt stress. Mashhad Ferdosi University Press, Mashhad, Iran (In Persian).

Marschner, H. and B. Dell (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159:89-102.

Mayak, S., T. Tirosh and B. R. Glick (2004). Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiology and Biochemistry 42:565-572.

Mirmahammadi-Meybodi, S. and B. Ghareyazi (2002). Physiological aspects of salt stress in plants. Isfahan University Press, Isfahan, Iran (In Persian).

Nadeem, S. M., I. Hussain, M. Naveed, H. N. Ashgar, Z. A. Zahir and M. Arshad (2006a). Performance of plant growth

promoting rhizobacteria containing ACC-deaminase activity for improving growth of maize under salt-stressed conditions. Pakistan Journal of Agricultural Sciences 43:114-121.

Nadeem, S. M., Z. A. Zahir, M. Naveed, M. Arshad and S. M. Shahzad (2006b). Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress. Plant, Soil and Environment 25:78-84.

Pacovsky, R. S. and G. Fuller (1998). Mineral and lipid composition of Glycine-Glomuse-Bradyrhizobium symbiosis. Physiologica plantarum 72:733-746.

Penrose, M. and B. R. Glick (2003). Methods for isolating and characterizing Acc deaminase-containing Plant Growth-Promoting Rhizobacteria. Plant Physiology 118:10-15.

Poss, J. A., E. Pond, J. A. Menge and W. M. Harrell (1985). Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. Plant and Soil 88:307-319.

Russo, A., C. Felici, A. Toffanin, M. Götz, C. Collados, J. M. Barea, Y. Moënne-Loccoz, K. Smalla, J. Vanderleyden and M. Nuti (2005). Effect of *Azospirillum* inoculants on arbuscular mycorrhiza establishment in wheat and maize plants. Biology and Fertility of Soils 41:301-309.

Saleem, M., M. Arshad, S. Hussain and A. S. Bhatti (2007). Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. Journal of Industrial Microbiology and Biotechnology 34:635-648.

Sannazzaro, A. I., M. Echeverria, E. O. Alberto, O. A. Ruiz and A. B. Menendez (2007). Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza. Plant Physiology and Biochemistry 45:39-46.

Sannazzaro, A. I., O. A. Ruiz, E. O. Alberto and A. B. Menendez (2006). Alleviation of salt stress in *Lotus glaber* by *Glomus intraradices*. Plant and Soil 285:279-287.

Saravanakumar, D. and R. Samiyappan (2007). ACC deaminase from *Pseudomonas Xuorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. Journal of Applied Microbiology 102:1283-1292.

Sharma, A. K. and B. N. Johri (2002). Arbuscular Mycorrhiza, Interactions in Plant, Rhizosphere and Soil. Oxford and IBH Publishing. New Dehli, India.

Yano-Melo, A. M., O. J. Saggin and L. C. Maia (2003). Tolerance of mycorrhized banana (*Musa* sp. cv. Pacovan) plantlets to saline stress. Agriculture, Ecosystems and Environment. 95:343-348.