Pak. J. Agri. Sci., Vol. 53(2), 399-406; 2016
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/16.2425
http://www.pakjas.com.pk

THE INFLUENCE OF MYCORRHIZAL SPECIES ON SOUR ORANGE (*Citrus aurantium L.*) GROWTH UNDER SALINE SOIL CONDITIONS

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A two-staged experiment was conducted to investigate the effects of indigenous and predefined mycorrhizae inoculation on sour orange (*Citrus aurantium L.*) growth under saline soil conditions. In first stage, indigenous mycorrhizae that existed in the rhizosphere of Mediterranean halophytic plants propagated by using a trap culture method. *Trifolium sp.* was used as the host plant. In the second stage, the effects of propagated indigenous mycorrhizae and predefined morphological species (*Glomus clarum, G. caledonium* and *G. mosseae)* on citrus plant growth were evaluated with high levels of salt (2000 µmhos/cm NaCl) under greenhouse conditions. These species are produced in the method of grafting on a regular basis exists Cukurova University. Andesitic tuff: soil: compost (6:3:1) mixture were used as growth media. Shoot and root dry matter, root infections, spore production, and concentrations of N, P, K, Zn, Mn and Cu in plant tissues were analyzed. The results demonstrated that indigenous mycorrhizae, especially spores extracted from the rhizospheres of *Euphorbia paralias* and *Ambrosia maritima,* had a significant effect on citrus growth and nutrient uptake. Citrus plants inoculated with *G. clarum* and *G. caledonium* grew more efficiently than those inoculated with *G. mosseae*.
Keywords: Indigenous mycorrhizae, mycorrhizal species, trap culture, halophytic plant, salinity, citrus growth.

INTRODUCTION

There is a salinity problem in agriculture due to mismanagement from high inputs of fertilizer, inappropriate irrigation and deficient drainage. This is one of the most significant agricultural problems in the world, especially in arid and semi-arid soil ecosystems. Soil salinization can be controlled by a set of factors related to environmental conditions (climate, hydrology), water supply, control systems (irrigation, drainage) and cropping practices (type and density of plant cover and the plant's rooting characteristics) (Metternicht and Zinc, 2008). Salinity stress is a major factor limiting plant growth and productivity in many regions of the world. These stresses include the accumulation of ion toxicity (e.g., Na and Cl), ionic imbalances, osmotic stress and soil permeability problems (Epstein *et al*., 1980; Flowers, 1999). Salinity stress on plant systems is manifested by lowered levels of photosynthesis (Ashraf *et al*., 2002).

Takacs and Voros (2003) reported that arbuscular mycorrhizal fungi (AMF) might play an important role in agriculture by increasing the stress tolerance of the host plants. Among the biological approaches for enhancing plant growth under saline conditions, the role of AMF has been well established. There is considerable evidence that AMF can enhance plant growth and vigor under conditions of saline stress (Dixon *et al.*, 1993; Juniper and Abbott, 1993; Kim and Weber, 1985; Pfeiffer and Bloss, 1988; Pond *et al*., 1984; Tsang and Maun, 1999). Most native plants and crops in arid and semi-arid areas are mycorrhizal, and it has been suggested that AM fungal colonization might enhance the salt tolerance of some plants (Tain *et al.*, 2004). Furthermore, under saline conditions, AMF can increase plant growth and the uptake of nutrients while decreasing yield losses (Al-Karaki, 2000; Al-Khaliel, 2010; Ruiz-Lozano *et al.*, 1996). Wu *et al.* (2010) have shown that mycorrhizal citrus seedlings exhibited more efficient antioxidant defense systems, which provided better protection against salt damage. The significance of mycorrhizal functions, as an extension of the root system of a plant, can be understood as increasing the absorptive area and improving the nutrients uptake such as P, Zn, and Cu (Cho *et al.*, 2009; Ortas *et al*., 2002a,b). The influence of salinity on plant P concentration is especially important in the context of the plant's response to AM (Graham, 1986; Poss *et al*., 1985). In many crops, as salinity increases, plant P concentrations decrease; therefore, these crops might benefit from inoculation with efficient strains of AM that are tolerant to high salinity. In addition, AMF develops an extensive additional hyphal network with the plant root system, which makes a significant contribution to the improvement of soil texture and water exchange (Bethlenfalvay and Shuepp, 1994; Wu *et al.*, 2009). Furthermore, AM fungi can positively influence the establishment and growth of plants by improving their nutrient uptake, increasing their tolerance to drought and salt stress, and increasing their resistance to soil-

borne pathogens (Azcón-Aguilar and Barea, 1997). Syvertsen and Levy (2005) reported that mycorrhizae can affect the salt tolerance of citrus roots and may increase chloride (Cl) uptake. Improvements in physiological processes, such as photosynthetic activity or water use efficiency, have also been demonstrated in mycorrhizal plants growing under salt-stress conditions (Ruiz-Lozano *et al.,* 1996). Many different methods have been used to propagate AM fungi, but these methods involve the growing of fungi in association with a living root system (Douds *et al.,* 2000; Ortas, 1996; Sylvia and Jarstfer, 1994). Currently, different propagation techniques are being developed. One of these techniques is trap culture. In trap culture, after rhizospheric soil (including spores, hyphae and roots) is collected from nature, AM fungi are propagated under sterile sand + soil greenhouse conditions using host plants (INVAM, 2011). Several host plants are used in trap culture. Liu and Wang (2003) reported that the highest spore numbers and the highest number of mycorrhizal species were obtained using *Trifolium repens* grown in coal mine tailings. Trap cultures can be very helpful for revealing fungal community members that were undetected during the initial extraction of spores from soil in the field (Morton *et al.,* 1995). Additionally, when spores are collected directly from soils in the field, they have many problems, such as a loss of spores and changes in the appearance of their structure. According to Maas (1987), samples that were collected from sand dunes indicated that the nourishment of sand dune plants is supported by mycorrhizae. While moderately salt-tolerant crops might continue to grow as much as 8 dS m^{-1}, only halophytes can survive at higher salinities, $\geq$ 30 dS m^{-1} (Flowers and Yeo, 1986; Maas, 1987).

Citrus plants are strongly dependent on mycorrhizae (Ortas *et al*., 2002a,b). In Turkey, 75% of Turkish citrus plantations are located in the Mediterranean region, and soil salinity is one of the major problems in this region. The use of mycorrhizae is important to increase the plants' resistance to the effects of salt. Indigenous mycorrhizae, which are located in the rhizosphere of Mediterranean halophytic plants, can be useful for the growth of citrus seedlings. It would be a great agricultural strategy to collect indigenous mycorrhizae from the rhizosphere of halophytic plants in order to utilize them on citrus plantations.

The aim of this research was to evaluate the effects of indigenous mycorrhizae, which were isolated from the rhizosphere of halophytic plants and termed mycorrhizal inoculums, on the growth and nutrient uptake of sour orange plants under soil conditions where salt was added experimentally. The research was based on the hypothesis that the indigenous mycorrhizal spores, which were cultured in an area with relatively high salinity, could increase the resistance of citrus plants against the effects of added salinity better than the described mycorrhizal species.

MATERIALS AND METHODS

This research was performed in two stages. The aim of the first stage was to propagate the indigenous mycorrhizae by applying the trap culture method. According to this method, rhizospheric soils from six different types of halophytic plants (*Euphorbia paralias (Euphorbiaceae*), *Cakile maritima (Brassicaceae*), *Conyza canadensis (Compositae*), *Echium angustifolium (Boraginaceae), Inula viscosa (Compositae),* and *Ambrosia maritima (Brassicaceae*)) were collected along the southern coast of Turkey. Roots were chopped into small fragments and the pieces were mixed with rhizospheric soil. This mixture was thoroughly homogenized (1:1, V:V), and the sand was sterilized twice in an autoclave (121°C for 1 hour with intervals of 24 h between sterilization steps). Mixtures were transferred to sterilized 1.5 kg plastic pots (15 cm wide and 13 cm high). *Trifolium sp.,* which is highly dependent on mycorrhizae and is grown extensively in the Cukurova region, was chosen as the host plant (Ortas *et al*., 1999). The trap culture experiment was continued for 4 months. Root and soil mixtures were kept inside the pots at a temperature of 4°C for 1-2 weeks after the harvest.

Mycorrhizae were isolated at the end of the harvest. The remaining roots, mycorrhizal hyphae, soil and spores were kept under cold conditions at 4°C until the second stage.

In the second stage of the experiment, seedlings of citrus plants *(Citrus aurantium L.)* were chosen as test plants and andesitic tuff: soil: compost (6:3:1) mixture were used as growth media (Table 1). Citrus seedlings were grown from seeds. When citrus seedlings had three leafs, they were transferred to three kilograms of sterilized growth medium. Mycorrhizae were inoculated at 1000 spores per pot during transferred. Growth continued for 16 weeks. The experiment was done three replicates. The plants were irrigated by 2000 mmhos salty water after eighth month to provide salinity stress.

Table 1. Chemical input of compost and andesitic tuff used in experiment.

	pH	Fire	Total		P_2O_5	K	Ca	Mg	Zn	Fe	Cu	Mn
	(1:1 H_2O)	lost	N	P								
Unit			(%)						(mg/kg)			
Compost	7.91	54.2	1.13	0.18	-	0.98	2.50	0.27	40	1005	12	141
Andesitic tuff	-	-	-	-	0.03	4.3	-	-	0.1	2	0.2	3.6

Table 2. According to Tukey HSD test effects of propagated mycorrhizae with Trap Culture on shoot dry weight, root dry weight, spore count and % mycorrhizal infection of *Trifolium sp*.

	Shoot DM	Root DM	Infection (%)	Spore	Spore numbers before produce
Euphorbia paralis	6.06 ± 0.91a	2.13 ± 0.31ab	57 ± 11.55a	67 ± 14.43bc	16 ± 1.73 bc
Cakila maritima	2.05 ± 0.51c	0.33 ± 0.06c	57 ± 20.82a	42 ± 18.77c	8 ± 2.00 cd
Conyza canadensis	4.92 ± 1.19b	1.56 ± 0.21b	73 ± 05.77a	109 ± 24.43ab	20 ± 4.36ab
Echium angustifolium	6.11 ± 0.72a	2.33 ± 0.38a	63 ± 11.55a	50 ± 13.23c	10 ± 2.00 cd
Inula vicosa	2.64 ± 0.39bc	2.20 ± 0.26ab	86 ± 23.09a	40 ± 05.00c	7 ± 2.64d
Ambrosia maritima	3.71 ± 1.01bc	0.80 ± 0.20c	60 ± 17.35a	110 ± 9.02 a	25 ± 7,28a
P	***	***	NS	***	***

Mean of the three replicates *, **, *** significant at P < 0, 05 - 0, 01 - 0,001 respectively

Dry matter from plant shoots and roots, nutrient content, number of spores and root colonization (%) were determined at the time of harvest. Root samples were first washed with tap water and then with de-ionized water, subsequently, sub-samples were taken to determine mycorrhizal colonization rates.

***Root colonization and spore counting*:** The sub-samples of root material treated with colonizing mycorrhizae were collected and cleared in a 10% KOH solution (w/v) and stained using trypan blue according to the method of Phillips and Hayman (1970). The root clearing and staining procedure as well as the degree of mycorrhizal infection in the root cortex were assessed according to the method of Koske and Gemma (1989). Mycorrhizal colonization was determined using the grid-line intersection method of Giovannetti and Mosse (1980). The percentage of root infection was calculated as follows: Infection % = 100 x total mycorrhizal root intercepts/total root intercepts.

Spores were isolated from the rhizospheric soil samples by using the wet sieving technique (Gerdermann and Nicolson, 1963). Each fraction of soil debris and spores were passed through filter of 53 microns and transferred to a nematode counting dish. Spores were counted using a stereo microscope at a magnification of 25X.

***Plant tissue analysis*:** The shoots (leaves and stems) and roots were dried at 65°C, and the relevant data were recorded. The dried citrus leaves were milled, and the concentration of P was determined by the Murphy and Riley (1962) method using a spectrophotometer. The concentrations of Zn, Cu, Mn and K were determined using an atomic absorption spectrophotometer. Total N concentration was measured by the semi-kjeldahl method, according to Bremner (1965).

***Statistical analysis*:** The data were analyzed with the one-way ANOVA model to test for significant differences between the mycorrhizal applications. All statistical analyses were performed using the SPSS 10.0 program package. Tukey's Multiple Range tests were used to determine the significant differences between treatment means.

RESULTS

In the first stage of the experiment, collected healthy indigenous mycorrhizae were propagated successfully by applying the trap culture method. The best result was observed in *Conyza canadensis.* The spore numbers

Table 3. According to Tukey HSD test effects of propagated mycorrhiza with Trap Culture and different mycorrhiza types at sour orange on shoot dry weight, root dry weight, spore number and % mycorrhizal infection.

	Shoot DM	Root DM	Spore	Infection (%)
Control	0.60 ± 0.08 b	0.20 ± 0.48 bc	0	0
G. clarum	2.08 ± 0.16 ab	1.12 ± 0.23 a-c	43.3 ± 12.58bc	67.5 ± 9.57 ab
G. caledonium,	2.16 ± 0.63 ab	1.20 ± 0.11 ac	54.5 ± 17.06bc	57.5 ± 5 a-c
G. mosseae	0.68 ± 0.23 b	0.33 ± 0.08 c	40.3 ± 6.34bc	35.0 ± 10 c
Euphorbia paralis	2.94 ± 0.53 a	1.51 ± 0.12 a	44.0 ± 7.07 bc	62.5 ±17.08 ab
Cakila maritima	0.99 ± 0.89 b	0.41 ± 0.85 ac	37.5 ± 3.11 c	35 ± 5.77 c
C. canadensis	1.85 ± 0.41 ab	1.02 ± 0.37 ac	45.3 ± 11.59bc	65 ± 5.77 ab
E. angustifolium	1.33 ± 0.64 ab	0.97 ± 0.26 ac	35.6 ± 2.5 cd	42.5 ± 5 bc
Inula viscosa	1.56 ± 1.54 ab	0.85 ± 0.67 ac	42.3 ± 4.03 bc	70 ± 21.60 a
A. maritima	2.33 ± 0.18 ab	1.55 ± 0.14 a	100.6 ± 42.56 a	45 ± 12.91 ac
P	***	***	***	***

Mean of the three replicates *, **, *** significant at P < 0, 05 - 0, 01 - 0,001 respectively

increased from 20 to 109 and infection was determined 73%. As a result of that shoot dry matter was 4.92 g, root dry matter was 1.56 g. Nevertheless, *Euphorbia paralias* plants had 57% infection, *Echium angustifolium* plants had 63% infection and also these plants had the best results of shoot dry matter and root dry matter. *Euphorbia paralias* plants had 6.06 g shoot dry matter, 2.13 g root dry matter. *Echium angustifolium* plants had 6.11 g shoot dry matter, 2.33 g root dry matter. Although, the highest spore numbers were recorded from *Ambrosia maritima* plants, this result did not affect root and shoot growth as much as those from *Euphorbia paralias* and *Echium angustifolium*. On the other hand, the lowest spore count was obtained from rhizospheric soil inoculation of *Inula viscosa, Cakile maritima* plants (Table 2). Nevertheless, *Inula viscosa* had 86% infection, *Cakile maritima* had 57% infection. However, these indigenous mycorrhizae did not affect root and shoot growth.

All of the results demonstrated that spores collected from halophytic plants were propagated successfully and that indigenous mycorrhizae from *Euphorbia paralias* had a significant effect on plant growth.

In the second stage of this experiment, propagated indigenous mycorrhizal spores and predefined mycorrhizal spores were inoculated into citrus seedlings to compare together. The best results were obtained in plants that were inoculated with indigenous mycorrhizae from *Euphorbia paralias* (Table 3). These mycorrhizae adapted to citrus seedlings successfully and determined 44 spore, 62% infection. As a result of that, 2.94 g shoot dry matter, 1.51 g root dry matter were obtained. *Ambrosia maritima* had 100 spore count and 45% infection and *Conyza canadensis* had 45 spore count and 65% infection. Also these infection results affected to shoot and root growth positively.

In general, the lowest root growth was measured in plants that were inoculated with *G. mosseae* and the indigenous mycorrhizae obtained from *Cakile maritima* (Table 3).

When comparing described mycorrhizae with indigenous mycorrhizal inoculations in plants such as *Cakile maritima,* we found that they produce as much dry weight of *G. mosseae* as other inoculated plants. However, when root growth and other values were considered, indigenous mycorrhizae that had been found on *Cakile maritima* were more successful than *G. mosseae*. Although indigenous mycorrhizae that were propagated from *Ambrosia maritima* had the highest number of spores, the results were similar to those of the other indigenous species (Table 3).

The effects of indigenous mycorrhizae collected from halophytic plants such as *Euphorbia paralias, Ambrosia maritima, Inula viscosa,* and *Echium angustifolium* on citrus plant growth were assessed (Fig. 1). Mycorrhizal species from *Cakile maritime* were not successful than the other indigenous species.

Although the spore numbers from *Inula viscosa* were found to be lower than those from other plants, *Inula viscosa* had the highest root colonization and performance scores (Fig. 1). Furthermore, *G. caledonium* and *G. clarum* AM-inoculated plants were observed to have much better shoot and root growth than the other species.

The nitrogen concentrations in the tissues of citrus plants differed between the mycorrhizal inoculums, and it was found that the control plants had a 2.80% N concentration, the indigenous mycorrhizae obtained from the rhizosphere of *Euphorbia paralias* had a 2.90% N concentration, *Cakile maritima* had a 3.34% N concentration and the *G. mosseae*-inoculated plants had a 2.65% N concentration. Plant tissue in the control plant was determined to have a 1.50 K %; in the inoculated *Euphorbia paralias* and *Cakile maritima* plants, K levels were recorded at 2.72 K % and 2.49 K %, respectively, while *G. mosseae*-inoculated plants had 1.60 % K concentrations.

Figure 1. Root and shoot growth of sour orange inoculated with *Euphorbia paralias, Cakile maritima, Conyza canadensis, Echium angustifolium, Inula viscosa, Ambrosia maritime* and *Glomus. clarium, G. mosseae, G. caledonium*.

Table 4. According to Tukey HSD test effects of propagated mycorrhiza with Trap Culture and different mycorrhiza types at sour orange on N, P and K concentration.

	N (%)	P (%)	K (%)
Control	2.80 ± 0.16 bc	0.05 ± 0.01 b	1.50 ± 0.15 c
G. clarium	2.15 ± 0.10 e	0.09 ± 0 ab	1.50 ± 0.14 c
G. caledonium	2.65 ± 1.50 b-d	0.06 ± 0.02 b	1.34 ± 0.14 c
G. mosseae	2.65 ± 0.59 b-d	0.12 ± 0.03 ab	1.60 ± 0.28 c
Euphorbia paralis	2.90 ± 0.19 ab	0.15 ± 0.03 a	2.72 ± 0.21 a
Cakila maritima	3.34 ± 0.26 a	0.07 ± 0.05 ab	2.49 ± 0.42 ab
Conyza canadensis	2.18 ± 0.17 de	0.07 ± 0.03 ab	1.88 ± 0.36 bc
Echium angustifolium	2.46 ± 0.73 c-e	0.13 ± 0.04 ab	1.94 ± 0.26 bc
Inula viscosa	2.75 ± 1.55 b-d	0.11 ± 0.07 ab	1.38 ± 0.42 c
Ambrosia maritima	2.32 ± 0.18 c-e	0.13 ± 0.01 ab	1.88 ± 0.09 bc
P	***	**	***

Mean of the three replicates *, **, *** significant at P < 0, 05 - 0, 01 - 0,001 respectively

Table 5. According to Tukey HSD test effects of propagated mycorrhiza with Trap Culture and different mycorrhiza types at sour orange on Mn, Cu, and Zn concentration.

	Zn (ppm)	Mn (ppm)	Cu (ppm)
Control	13.12 ± 0.67 b	25.00 ± 3.92 b-d	23.05 ± 0.57 ab
G. clarium	18.55 ± 4.61 ab	13.25 ± 2.99 d	23.60 ± 1.76 ab
G. caledonium	15.63 ± 4.12 b	16.75 ± 3.86 cd	20.80 ± 0.52 a-c
G. mosseae	13.68 ± 1.64 b	27.50 ± 1.91 a-c	23.95 ± 0.33 a
Euphorbia paralis	26.18 ± 5.17 a	39.00 ± 5.03 a	17.03 ± 1.28 d
Cakila maritima	19.08 ± 5.03 ab	30.25± 8.1 ab	17.10 ± 3.27 cd
Conyza canadensis	15.50 ± 2.1 b	22.50 ± 4.99 b-d	17.87 ± 1.17 cd
Echium angustifolium	22.62 ± 5.82 ab	33.50 ± 3.51 ab	20.80 ± 1.92 a-c
Inula vicosa	15.43 ± 6.19 b	25.25 ± 5.97 b-d	20.18 ± 1.59 b-d
Ambrosia maritima	18.7 ± 2.91 ab	23.00 ± 6.68 b-d	23.03 ± 0.95 ab
P	***	***	***

Mean of the three replicates *, **, *** significant at P<0, 05 - 0, 01 - 0,001 respectively

According to N% and K% concentration, inoculated and non-inoculated plants had close results. However, when considered P% concentration, inoculated plants had remarkable results.

While the non-inoculated control plant was found to have a 0.05% P concentration, the plants that were inoculated with cultured mycorrhizae, such as *Echium angustifolium*, *Inula viscosa*, *Euphorbia paralias* and *Ambrosia maritime*, were found to have 0.13, 0.11, 0.15 and 0.13% P concentrations, respectively. The plants inoculated with *G. mosseae* were found to have a 0.12% P concentration. (Table 4).

In addition, Zn, Cu and Mn concentrations were examined. While *Euphorbia paralias* (plants inoculated with cultured indigenous mycorrhizae) was found to have a 26.18 mg kg^{-1} zinc concentration, control plants had a 13.12 mg kg^{-1} Zn concentration and the *G. clarum*-inoculated plants were found to contain 18.55 mg kg^{-1} of Zn (Table 5). The non-inoculated control plants had 25.0 mg kg^{-1} Mn, while *the G. mosseae*-inoculated plants had 27.5 mg kg^{-1} Mn, *Euphorbia paralias*-inoculated plants had 39 mg kg^{-1} Mn and *Echium angustifolium*- inoculated plants had 33.5 mg kg^{-1}. The citrus tissue Cu concentration in the non-inoculated control plants was 23.05 mg kg^{-1} and the *G. mosseae*, *G. clarum* and *Ambrosia maritima* -inoculated plants had similar results (Table 5).

DISCUSSION

Salinity is one of the main problems that affect agricultural practices in the Mediterranean. However, there has been some research on methods to reduce salinization, such as establishing drip irrigation systems. This strategy is financially supported by the government; however, reduced salinity will not be enough to protect the citrus farms from salinization risk in the future.

Isolating indigenous mycorrhizal spores from the rhizosphere of naturally salty habitats, and using these mycorrhizal spores to improve citrus seedling growth and nutrient uptake under salt stress conditions could be an important agricultural

strategy, when compared with using described mycorrhizal species.

Mycorrhizal spores were collected from the rhizosphere zones of halophytic plants located in and near natural saline regions, and the spores were propagated using trap culture method in order to use the mycorrhizae to inoculate citrus seedlings. In the greenhouse experiment, significant growth was detected in the mycorrhiza-inoculated citrus treatments compared with that of the control treatments (Fig.1, Table 3). Indigenous mycorrhizae that existed in the rhizosphere of *Euphorbia paralias* inoculated citrus seedlings were found to have the highest root and shoot growth. However, *G. mosseae* and indigenous mycorrhizae that existed in the rhizosphere of *Cakile maritima* did not demonstrate similar performance (Table 3), as expected. Cakan *et al.* (2006) reported that *Euphorbia paralias* plants had 85% root colonization at the same sand dunes.

Inoculations with the indigenous and the described mycorrhizae were found to significantly increase root colonization when compared with the non-inoculated control plants. However, *G. mosseae* inoculated plants and spores cultured in the *Cakile maritima* rhizosphere were found to have less root colonization (35%) than other inoculations. Previously, Ortas *et al.* (2002) found that, for unknown reasons, *G. mosseae*-inoculated sour orange seedlings had less root colonization. Because the indigenous mycorrhizal fungi recovered from the halophytic plant rhizosphere were not taxonomically classified, it is not known which specific types of mycorrhizal species exist in the inoculums. It is important to research the type of mycorrhizal species in the region in different citrus orchards and halophytic plants as well.

The spore counts taken from the *Ambrosia maritima* and *Euphorbia paralias* plant rhizospheres did more than described mycorrhizae in citrus plantations. The ability of mycorrhizal fungi to enhance citrus growth has been described many times, and the differences among fungi have been reported by several authors (Ortas *et al.*, 2002a; Vinayak and Bagyaraj, 1990).

In all of the experimental treatments, the Zn concentration in the citrus plants was found to be higher than in the control treatments. Mycorrhizal inoculation was increased the concentrations of Zn (Tables 5). In addition, the results showed that *Euphorbia paralias*-inoculated citrus plants had the highest P, Zn and K uptake. It appears that the P uptake is accompanied by Zn uptake in *Euphorbia paralias*-inoculated plants. Similarly, Ortas *et al.* (2002b) found that mycorrhizal inoculation increased P and Zn content in citrus plants. It has been reported that mycorrhiza-inoculated citrus seedlings had high plant growth and P, K, Fe, Cu and Zn uptake. The influence of salinity on plant P concentration is especially important for many crops (Poss *et al.*, 1985), and it is suggested that selecting efficient strains of mycorrhizae may increase their tolerance of soil salinity.

The enhanced accumulation of these micronutrients is vital to physiological processes that impact yield of citrus plants. It has been indicated by Poss *et al.* (1985) that greater nutrient acquisition in response to AMF colonization is a plant strategy for improving salt stress tolerance as well.

This study showed that indigenous mycorrhizae that exist in halophytic plants are even more efficient at improving plant growth than selected known mycorrhizal types. The results encouraged us to collect indigenous spores from marginal soil conditions and to re-inoculate them in salt-sensitive plants, such as citrus. Because rhizospheric soil contains other soil organisms, the factors linked to the biological and chemical characteristics of soil, even the physical parameters, must have a large influence on mycorrhizal performance (Schreiner, 2003; Schreiner and Mihara, 2009) and plant growth. Additionally, mycorrhizal inoculation has been reported to directly improve plant nutrition (Gerdermann, 1968) through the fungal hyphae and thereby affect root architecture (Linderman, 1992) by increasing the number of finer lateral roots. On the other hand, (Wu *et al.*, 2010) indicated that the suppressed growth and colonization of arbuscular mycorrhizae under higher salinity might be attributed to the reduced hyphal extension of the AMF.

The results show that indigenous spores collected from the rhizosphere of *Euphorbia paralias* are the most efficient variety. Therefore, further study of this plant's rhizosphere rather than the mycorrhizal spores is necessary. It is also important to examine the effects of other soil beneficial organisms on plant growth.

Citrus inoculation with selected AM fungi and indigenous spores was found to be beneficial for the establishment and growth of the rootstock of sour orange. It is important to isolate and classify the rhizospheric soils of the indigenous halophytic plants to inoculate citrus seedlings with selected inoculations. As a result, applied mycorrhizae should be detected for better citrus seedling production. Indigenous mycorrhizal usage could be a new agricultural strategy for the management of citrus growth in naturally saline soils for sustainable agriculture.

***Conclusions*:** Inoculation with indigenous mycorrhizae increases citrus growth and nutrient concentrations. Plant growth and nutrient concentrations were significantly higher in soils receiving described mycorrhizal species. Indigenous mycorrhizal species were as efficient as described mycorrhizal species. Indigenous mycorrhizal spores extracted from saline areas significantly affected the growth of citrus plants under high salt application. The effect of indigenous mycorrhizal species extracted from different halophytic plant

species should be further studied as remedies for stress factors.

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