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Single and Dual Arbuscular Mycorrhiza Fungi Inoculum Effects on Growth, Nutrient Absorption and Antioxidant Enzyme Activity in *Ziziphus spina-christi* Seedlings under Salinity Stress

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ABSTRACT

***Ziziphus spina-christi* are distributed in arid and semi-arid regions of world. Most of these areas are subjected to soil salinity. So, the aim of this study was to find out the effects of different NaCl concentrations on *Z. spina-christi* seedlings growth, in the presence of a number of single and dual AMF inoculums, to provide some information about possible effects of AMF under salinity condition. For this purpose, the study was conducted in nursery using 4×3 factorial scheme (4 salinity levels; 0, 50, 100 and 150 mM and 3 mycorrhiza treatments; non-mycorrhizal plant, *G. fasciculatum* and *Funneliformis mosseae+G. fasciculatum* inoculated plants). Our results revealed that salinity has negative effects on root length colonization, growth parameters, chlorophyll content, nutrients absorption and results in Na, proline, superoxide dismutase, peroxidase and catalase activity increment but mycorrhizal plants maintained higher growth characteristics, chlorophyll content, nutrients, root colonization and enzymes activity, proline and Na ion reduction and these effects in dual inoculum were significantly different from single inoculum fungi. According to the results dual-inoculated plants are more tolerative to salinity compared to single-inoculated and not-inoculated seedlings. Indicating that *Z. spina-christi* plantation in saline soil would give us the best result if we use dual inoculated plants. It could be said that in saline soils *Z. spina-christi* dual-inoculated with mycorrhiza is tolerative and more efficient.**

Keywords: Enzyme, Mycorrhiza, Proline, Salinity, *Ziziphus spina-christi*.

INTRODUCTION

One third of the world is arid and semiarid which is characterized by receiving less than 400 mm of annual precipitation (Wickens, 1998). In this vast area of the world soil salinity is the major problem affecting forest plantations establishment. One of the common plant species is *Ziziphus spina-christi* which is used for plantation of these regions.

Z. spina-christi is distributed in vast regions of the world including North Africa, South Europe, the Mediterranean, South and East of Asia and the Middle East (Yossef *et al.,* 2011) and most of these areas are facing salinity conditions.

To reduce the negative effects of salinity, Arbuscular Mycorrhizal Fungi (AMF) could serve as a useful strategy. Because, first they are known as microorganisms that can increase plant torrent under biotic and abiotic stresses (Hrynkiewicz and Baum, 2012), second these fungus have association with more than 80% terrestrial plant species (Smith and Read 2008). Researches showed

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that plant tolerance is enhanced by the AM fungi in salt condition (Peng *et al.,* 2010; Abdel Latef and Chaoxing, 2011, 2014) but the mechanism are not clear and also the ability of different AMF varied to alleviate salt stress (Al-Karaki *et al.,* 2001). There are reports about some mechanisms such as increasing dry weight, nutrition acquisition, water uptake (Evelin *et al.,* 2009) and chlorophyll content (Giri and Mukerji 2004) which can cause an increase in salinity tolerance of plants.

Although AMF can alleviate salinity stress but they could be negatively affected by salinity stress in colonization, sporulation and hypha growth (Porcel *et al.,* 2012) which could depend on different AMF and host plant species (Carvalho *et al.,* 2001). They are also effective mechanisms for increasing antioxidant enzyme activity (Zhu *et al.,* 2010; Abdel Latef and Chaoxing, 2011). In the dry and salt condition plants are subjected to low water availability resulting in oxidative stress induction but mycorrhizal fungi can decline reactive oxygen species and increase drought stress tolerance (Fouad *et al.,* 2014) by inducting antioxidant enzymes that can protect plants from oxidative condition (Mittler, 2002).

The major problem of plantations of some parts of the world is soil salinity. So, the objective of this study was to find out the effects of NaCl concentrations on *Z. spina-christi* seedlings in the presence of a number of single and dual AMF inoculums to provide some information about possible effects of AMF on nutrients absorption, growth parameters, chlorophyll content, proline and enzyme activity of *Z. spina-christi* seedlings. We hypothesized that, using dual arbuscular mycorrhizal fungi (*Glomus mosseae+G. fasciculatum*) as inoculum could have better results in alleviative salinity condition compared to single (*G. fasciculatum*) mycorrhizal fungi inoculum and non-mycorrhizal plants.

MATERIALS AND METHODS

Growth Conditions and Methodology

In order to overcome seed dormancy, seed coats were scarified by nail clippers. Seeds were germinated in a mix of clay, sand and perlite (2:1:1 v/v) (Table1). Seedling were planted in nursery using 4×3 factorial scheme experiment design (4 salinity levels and 3 mycorrhizal treatments). Growth condition of seedlings was adjusted as natural photoperiod condition (13 hours of light), between February and August of 2013 (6 months). There were 20 plants selected for each treatment, but for enzyme and nutrient elements analysis only 6 plant per treatment was used. Each pot (25×25×15 cm) contained sterilized soil by autoclave (30 minutes at 121°C) and 100 grams of single (G. *fasciculatum*) and dual (*G. mosseae+G. fasciculatum*) AM inoculum. The inoculum contained at least 50 spore per gram soil, hyphae and root fragments. Plants were established for 5 weeks before being subjected to 4 NaCl levels (0, 50, 100 and 150 mM NaCl) (Zamani *et al.,* 2011) by addition of a salt solution to soil with the irrigation water.

Colonization, Growth Parameters, Nutrient Analysis and Enzymatic Assays

Growth indicators (height and basal diameter) and shoots and roots dry weight

Table 1. Physico-chemical characteristics of soil used in this experiment.

Soil characteristics	Value	Soil characteristics	value
pH	7.32	N (g kg^{-1})	0.12
EC (dS m^{-1})	0.52	P (ppm)	19.6
Organic carbon (%)	1.5	K (ppm)	601
Calcium (%)	5.4	Mg (ppm)	0.6
Na (g kg^{-1})	1.1		

(Meloni *et al.,* 2004) was measured six months after plantation time. Root length colonization was performed according to the method of Phillips and Hayman (1970). Dry Weight (DW) of shoot (leaves and stems) and roots were obtained by oven-drying at 70ºC for 72 hours (Meloni *et al.,* 2004).

Ionic content including total NNitrogen (N) was estimated by semi-micro Kjeldahl method (Nelson and Sommers, 1982) and the other nutrients [total potassium (K), total Phosphorus (P) and sodium (Na)] were determined by atomic absorption spectrophotometry (UV/VIS 9000) according to Xu *et al.* (2006).

Leaf sand roots were grounded in liquid nitrogen for enzyme assays and extraction was performed with ice-cold 50 nM phosphate buffer (pH 7.0). Crude extracts were obtained by the method of Mirzaei and Yousefzadeh (2013). The total superoxide dismutase (SOD) (Giannopolitis and Ries, 1977), Peroxidase (POD) and Catalase activity (CAT) (Cakmak and Horst, 1991) were determined. Proline content was determined by the ninhydrin and sulfosalisilic acid method (Bates *et al.,* 1973).

Chlorophyll content of semi-mature leafs were determined using spectrophotometer (UV/VIS 9000) at 645 and 663 nm for chlorophyll a and b.

Experimental Design and Statistical Analysis

SPSS software (version 19) was used for data analyzing, all parameters were analyzed through two-way ANOVA for main effects (S, Salinity and M, arbuscular mycorrhizal inoculation). Means comparison was done by Duncan's test at 0.05 confidence level.

RESULTS

Colonization Assessment

The mean root length colonization for dual inoculum treatments were 59, 57, 45 and 44 percent at 0, 50, 100 and 150 mM concentration of NaCl, respectively. Our results indicated a 25 percent reduction of root length colonization in dual inoculum by salinity stress. These results were 40, 36, 23 and 18 percent in single inoculum respectively which caused 55 percent decrease in root length colonization. As shown in Figure 1, dual arbuscular mycorrhizal fungi had a better result in root length colonization compared to single mycorrhizal fungi. In both single and dual mycorrhizal inoculums the highest and lowest colonization rate were observed in control and 150 mM respectively. In dual

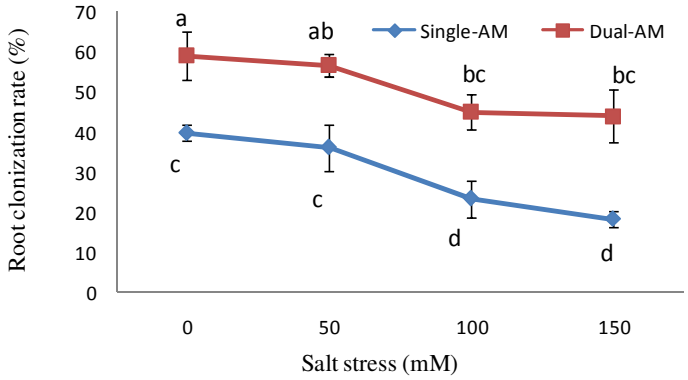


Figure 1. Root colonization rate in different NaCl concentrations. (Means followed by the same letters are not significantly different at P< 0.05 (n= 20)].

inoculum, there was no significant difference (P> 0.05) in root length colonization between control and 50 mM. In addition to that there was a decreasing trend from 50 mM to 150 mM in dual inoculum but this trend was not significant (P> 0.05) between 100 and 150 mM. According to our results root colonization in single inoculated plants at all levels of salinity is generally lesser than dual inoculated seedlings. The lowest colonization rate for both single and dual inoculums was observed in 100 and 150 mM for single inoculum (Figure 1).

Growth Parameters

Growth parameters in AMF treated seedlings were increased. The increase in these parameters in dual inoculated plants was significantly higher than single inoculated seedlings (Table 2). In control treatment (0 mM NaCl) dual inoculation caused increasing in all studied growth parameters significantly except shoot dry weight. There is no significant difference between single inoculation and non-treated seedlings in mentioned parameters (Table 2). As salinity increased, growth parameters deceased, and this loss in control was higher than single inoculated, and in single inoculated it was higher than dual inoculated seedlings. According to the results, growth parameters (shoot and root diameter, shoot and root dry weight) in AMF treated plants at all levels of salinity have been increased. Although the increase in growth at high level of salinity caused by dual inoculation is higher than single and non- inoculated seedlings significantly (Table 2), we found that in low levels of salinity there were no significant differences between single inoculated plants and non- mycorrhizal in growth parameters, but in higher levels of salt these indicators in single inoculated plants were higher than non-mycorrhizal plants.

Chlorophyll Content

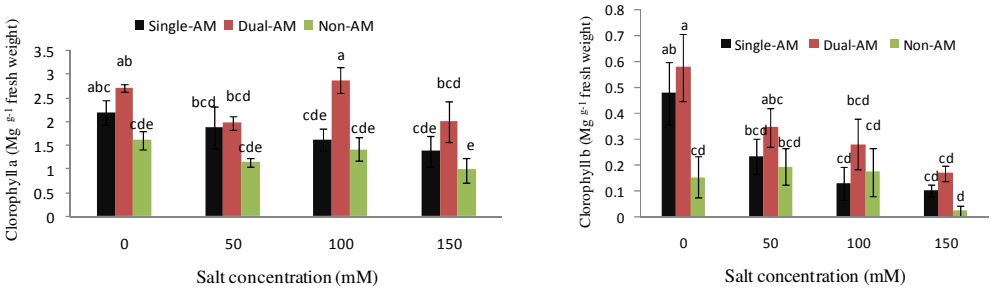
The highest value of chlorophyll content both for a and b were observed in control. Increase in salinity levels from 0 to 150 mM caused reduction of both a and b chlorophyll content significantly. At control, dual inoculated seedlings had the highest level of chlorophyll content in comparison to single and non-inoculated plants (Figures 2 and 3).

In double inoculated plants, chlorophyll a content, at 0 and 50 mM, 1.7 times and in 100 and 150 mM, has been 2 times higher

Table 2. Effect of salt stress and arbuscular mycorrhiza on growth parameters of *Z. spina-christi.*[a]

Salt stress	AM inoculation	Height (cm)	Diameter (mm)	Shoot dry (g)	Root dry (g)
0	Single-AM	23.1±4.5 bc	3.23±0.31 b	7.12±1.5 a	3.42±0.45 b
	Dual-AM	32.8±4.53 a	4.33±0.25 a	6.42±0.25 ab	4.52±0.38 a
	Non-AM	28.8±4.8ab	1.33±0.25 c	5.02±1.5 ab	1.52±0.38 d
50	Single-AM	8.3±1.6de	2.47±0.26 b	3.2±0.38 cd	2.26±0.27 cd
	Dual-AM	13±2.02de	3.17±0.41 b	4.1±0.38 bc	3.38±0.36 b
	Non-AM	15.4±1.9cd	0.63±0.17 cd	1.1±0.38 ef	0.62±0.24e
100	Single-AM	6.3 ±1.14ef	2.55 ±0.4 b	2.34 ±0.48 de	1.6±0.2 d e
	Dual-AM	16.8±1.4cd	2.85±0.37 b	3.24±0.48 cd	2.5±0.2 c
	Non-AM	7.8±2.9de	0.61±0.31 cd	0.38±0.43 f	0.3±0.15 e
150	Single-AM	3.1±0.46e	1.5±0.26 c	2.34±0.45 de	0.68±0.22 e
	Dual-AM	12±0.44 de	2.6±0.22 b	3.44±0.25 cd	1.98±0.23 cd
	Non-AM	7.4±1.98de	0.2±0.17 d	0.72±0.21 f	0.04±0.02 e

[a] Means±*SE*, same letters at each treatment are not significantly different at $P< 0.05$ (n= 20).



(a) (b)

Figure 2. Mean chlorophyll a and b content at different salinity levels. [Bars represent standard error, means followed by the same letters are not significantly different at P< 0.05 (n= 6)].

than that in non-inoculated plants. The same trend was observed for single inoculated plants in comparison to control. Just like chlorophyll a, the highest amount of chlorophyll b was observed in dual inoculated plants.

Nutrients and Na Absorption

By increasing the salinity level, the seedling shoot N content in control was reduced but in mycorrhizal plants adverse effects of salinity were alleviated. Dual inoculated plants had the highest level of N content at all levels of salinity, single inoculated and non-inoculated plants had a lesser level of N content respectively. Although salinity results in N content reduction but dual inoculated plants can enhance N content in comparison to non-mycorrhizal plants. Our results revealed that in 150 mM salinity treatment dual inoculated plants had 93 percent more N content in comparison to non-mycorrhizal plants. This result showed how these fungi can enhance N absorption in higher salinity levels. Like shoot N content, the highest value of N content for root was observed in non-salinity level, in the presence of dual inoculum. Also for root N content at non-salinity level there was no significant difference between single, dual and non mycorrhizal treatments (Table 3) but dual

Table 3. Effect of salinity and arbuscular mycorrhiza on some leaf nutrients content.[a]

Salt stress	AM inoculation	N (g kg^{-1})		P (ppm)		K (ppm)	
		Shoot	Root	Shoot	Root	Shoot	Root
0	Single-AM	2.92±0.3bcd	2.22±0.48abc	4.05±0.65 b	3.71±0.24b	3.09±0.96a	2.58±0.83ab
	Dual-AM	4.62±0.69 a	3.12±0.48 a	5.15±0.54 a	5.21±0.61a	3.99±1.07a	3.48±0.76a
	Non-AM	2.72±0.27 cd	2.02±0.36abc	3.05±0.47bcd	3.31±0.2bc	2.31±1.01a	2.18±0.83ab
50	Single-AM	2.74±0.28 cd	1.68±0.44bc	3.72±0.57bc	3.05±0.5bc	2.36±0.68a	2.79±0.5ab
	Dual-AM	3.84±0.32ab	2.38±0.28abc	3.82±0.3bc	3.55±0.3bc	3.46±0.76a	3.29±0.5a
	Non-AM	2.74±0.28 cd	1.28±0.1 c	2.72±0.3cde	2.2±0.12cd	1.96±0.68a	1.79±0.4bc
100	Single-AM	2.13±0.29 de	1.62±0.24bc	1.72±0.38ef	1.53±0.27d	1.3±0.65a	1.19±0.32cd
	Dual-AM	3.52±0.37bc	2.88±0.27 a	2.22±0.42def	2.2±0.27cd	2.8±1.42a	1.49±0.16bc
	Non-AM	2.32±0.2 de	1.62±0.24bc	1.32±0.38fgh	1.13±0.17d	1.74±1.41a	0.79±0.32d
150	Single-AM	2.2±0.37 de	1.58±0.39bc	0.58±0.17gh	1.51±0.67d	0.79±0.28a	1.04±0.09cd
	Dual-AM	3.1±0.37bcd	2.48±0.39ab	1.28±0.17fgh	2.2±0.67cd	1.69±0.3a	1.74±0.07bc
	Non-AM-	1.6±0.23 e	1.58±0.39bc	0.28±0.12 h	1.27±0.59d	0.45±0.25a	0.64±0.09d

[a] Means±*SE*, same letters at each treatment are not significantly different at *P*< 0.05 (n= 6).

inoculated plants in 100 mM and more salinity levels had significantly higher amount of N in roots.

As shown in Table 3 the increase in salinity caused P content deficit in shoots. The lowest reduction was observed in dual inoculated plants but in non-mycorrhizal plants the highest changes in P content happened. In 150 mM, P content of dual inoculated plants was about 78 percent higher than non-mycorrhizal plants. This increase in 0 salinity level was only 40 percent which indicates the importance of dual inoculation in P absorption at higher levels of salinity. Root P content showed the same results, but there are no significant differences of root P content between 100 and 150 mM of salt.

As shown in Table 3, K was also evaluated. The increase in salinity to 150 mM caused reduction in shoot K content but not significantly. In all treatments under salinity stress, mycorrhiza inoculation in comparison to non-mycorrhizal plants caused a decrease in K absorption but this change was not significant. According to the results shoot K content in dual mycorrhizal plants at 150 mM salinity, is at least 73 percent more than non-mycorrhiza plants. Similar results about N and P indicate the importance of dual AMF inoculation at higher levels of salinity. Unlike shoot, the root K content had a significant decrease by increasing the salinity level. Mycorrhizal treatments had the same results in roots as shoot in K absorption under salinity stress. In addition to that dual inoculum had more effect in root K absorption compared to single mycorrhiza inoculum.

As shown in Figures 4 and 5, Na content increased by increasing the salinity but mycorrhizal inoculation caused significant reduction in Na content at all levels of salinity in comparison to control. There was a significant difference between dual inoculated plants with single and non-mycorrhizal seedlings in Na reduction at higher levels of salinity.

Antioxidant Enzyme

The activity of some antioxidant enzymes known as the most important enzymes expressed in response to salinity stress were evaluated both for root and shoot. Our results revealed that shoot and root CAT activity increased by increasing the salinity levels. In addition to that, there is an increasing trend for CAT in both root and shoot but this is not significant in shoot. There was no significant difference between mycorrhizal and non-mycorrhizal plants in CAT activity both in root and shoot (Table 4).

As our results showed POD activity in shoot increased by increasing the salinity levels. The highest level of POD activity in shoot was observed in 150 and 100 mM of salt but there was no significant difference

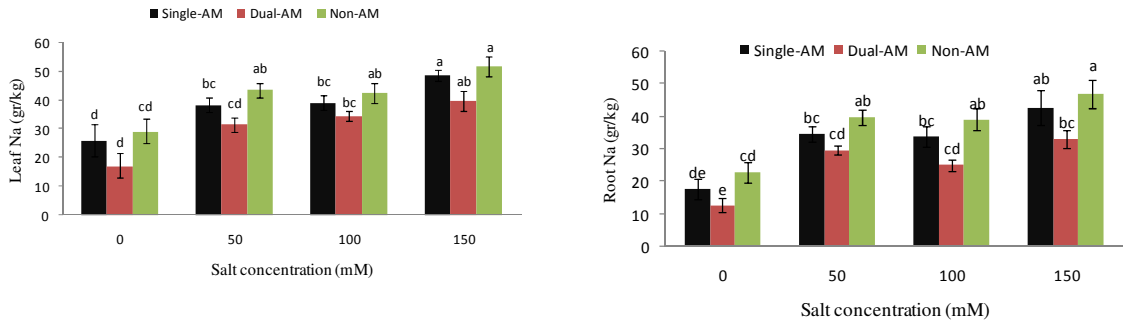


Figure 4. Mean leaf Na (a) and root Na (b) content at different salinity levels. [Bars represent standard error, means followed by the same letters are not significantly different at P< 0.05 (n= 6)].

Table 4. Effect of salinity and arbuscular mycorrhiza on some antioxidant enzyme activity.

Salt stress	AM inoculation	CAT [a] (U mg^{-1} protein)		POD [b] (U mg^{-1} protein)		SOD [c] (U mg^{-1} protein)	
		Shoot	Root	Shoot	Root	Shoot	Root
0	Single-AM	53.18±17.2a	99.56±27.3b	69.98±16.07b	71.5±27.9c	13.82±6cd	19.78±4.29cd
	Dual-AM	48.18±17.24a	94.56±27.3b	56.98±15.11b	46.54±12.3c	5.66±1.8d	11.82±4.83d
	Non-AM	72.18±17.24a	118.5±27.3b	80.98±15.11b	70.54±12.3c	28.82±2.34bcd	34.78±5.4bcd
50	Single-AM	79.64±29.2a	166±27.8b	86.12±26.5b	94.76±27.5bc	27.16±6bcd	23.42±7.34bcd
	Dual-AM	70.6±29.7a	141±37.18b	65.12±19.4b	89.7±27.5bc	16.16±4.13cd	18.4±7.34cd
	Non-AM	114.6±27.5a	189±34.69b	85.12±21.32b	113.76±27.5bc	44.16±5.17ab	42.42±7.34ab
100	Single-AM	173.6±74.18a	130.3±16.2b	170.04±48.4ab	105.3±34bc	32.82±10.8bc	32.56±5.66bcd
	Dual-AM	148.6±79.4a	105±22.05b	135.04±27.4ab	76.3±12.7c	27.82±10.81bcd	25.56±6.61bcd
	Non-AM	190.68±77.5a	149.3±23.7b	171.04±49.6ab	100.3±12.7bc	51.82±10.81ab	49.5±6.61ab
150	Single-AM	235.7±86.7a	350.9±75.5a	219.84±61.6a	214.2±4.4a	46.7±14.4ab	64.3±15a
	Dual-AM	220.74±109a	345.9±75.5a	154.8±39.1ab	189.2±48.8ab	31.7±7.42bcd	45.3±11.9ab
	Non-AM	224.74±89.9a	409.9±71.2a	218.84±52.4a	213±48.8a	61.7±8.56a	67.82±11.2a

[a] Catalase activity; [b] Peroxidase, [c] SuperOxide Dismutase. Means±*SE*, same letters at each treatment are not significantly different at $P< 0.05$ (n= 6).

between 0, 50 and 100 mM salinity level. Similar to shoot, increasing in salinity level results in root POD increment but dual mycorrhiza inoculation caused POD reduction (Table 4).

The highest SOD activity was observed at 150 mM salt in non-mycorrhizal plants and the lowest SOD activity for both shoot and root was observed in dual mycorrhizal seedlings at control (Table 4).

Proline Content

As shown in Table 5 in both shoot and root tissue proline content was increased significantly by increasing the salinity level. The highest proline content was observed both for shoot and root at 150 mM of salt. At all levels of salinity for both root and shoot, the lowest proline content was observed in non-mycorrhizal, single inoculated and dual mycorrhizal plants respectively.

DISCUSSION

Salinity stress had adverse effects on *Z. spina-christi* seedlings growth, nutrients absorption and AMF symbiosis. As we know salinity is a major problem in arid and semiarid parts of the world (Ruiz-Lozano *et al.,* 2012). Data analysis for salinity stress and mycorrhiza inoculation revealed that arbuscular mycorrhizal fungi alleviate negative effects of salinity stress in *Z. spina-christi* and such alleviation effects were more in dual arbuscular mycorrhizal inoculated plants. Therefore these kinds of fungi can be used as bio ameliorator under salinity condition (Chandrasekaran *et al.,* 2014). According to the results this amelioration effect is higher in dual AMF inoculated plants.

Root Colonization

In this study the application salinity suppressed the root length colonization in both single and dual mycorrhiza inoculated plants especially at higher levels of salinity but this suppression in dual inoculated plants is lesser than single ones. As Jahromi *et al.* (2008) mentioned our results showed a significant decrease in root length colonization in non-mycorrhizal plants at 100 and 150 mM salinity levels. Decrease in single inoculated was more than dual inoculated plants which indicates that multiple inoculation under salinity stress could result in better root colonization rate.

Table 5. Effect of salinity and arbuscular mycorrhiza on free proline content.[a]

Salt stress	AM inoculation	Proline	
		Shoot	Root
0	Single-AM	3.33±0.69de	1.73±0.23f
	Dual-AM	2.23±1.65ef	0.92±0.26f
	Non-AM	4.63±0.73cd	3.23±0.32de
50	Single-AM	2.56±0.48ef	4.16±0.63cd
	Dual-AM	1.66±1.37f	2.86±0.52ef
	Non-AM	4.06± 1.06cd	6.06±1.41ab
100	Single-AM	5.23±0.73bc	6.43±0.94ab
	Dual-AM	4.13±0.51cd	5.33±0.99bc
	Non-AM	6.53±0.51ab	7.73±2.21ab
150	Single-AM	6.75±1.36ab	7.75±0.79ab
	Dual-AM	5.45±0.94bc	6.05±1.02ab
	Non-AM	7.85±0.94a	8.45±1.02a

[a] Means±*SE*, same letters at each treatment are not significantly different at $P< 0.05$ (n= 6).

It is possible that different levels of colonization in dual and single mycorrhiza inoculation are because of different arbuscular mycorrhiza behavior (Klironomos *et al.,* 1993).

Growth Parameters

In accordance to Johnson-Green *et al.* (2001) our results showed that salinity had negative effects on plants growth and biomass production. But as it is shown mycorrhizal fungi inoculation results in alleviation of adverse effects on plant growth parameters under salinity stress. In almost all studied parameters in inoculated plants, either single or dual, there was no significant difference between 0 and 50 mM of salt. It probably shows that *Z. spina-christi* could tolerate 50 mM of salt. It could be said that there are no significant differences between 50, 100 and sometimes even 150 mM of salt in dual inoculated plants. It simply shows that using dual mycorrhizal inoculation in saline soil would result in better biomass production and growth rate in *Z. spina-christi*. In higher salinity levels growth parameters significantly decreased and since in our results higher salinity levels lead to an insignificant decrease of nutrients, it might be the reason for growth parameters reduction (Evelin *et al.,* 2009).

Nutrient and Na Absorption

Generally by increasing the salinity, the nutrient content decreased, but in this study Na content in both shoot and root was increased by increasing the salinity levels. As shown in results mycorrhizal treatment especially dual inoculation results in higher nutrient content both in root and shoot which could be due to stability of membrane by increasing mineral absorption (Beltrano *et al.,* 2013) in salinity levels compared to non-mycorrhizal plants. It could be said that decrease in Na content in mycorrhizal plants in comparison to non-mycorrhizal plants is due to positive effects of mycorrhizal in K absorption which can keep high K/Na ratio (Chandrasekaran *et al.,* 2014).

In this study non-mycorrhizal plants had lower NPK and higher Na content which is in agreement with Beltrano *et al.* (2013). The increase in mineral uptake by mycorrhiza is the main mechanism for plants facing limiting conditions (Moucheshi

et al., 2012) and by this mechanism plants tolerate unfavorable conditions like salinity (Estrada *et al.,* 2013).

Chlorophyll Content

The increase in chlorophylls a and b compared to non-mycorrhiza plants might be because of positive effects of mycorrhizal on N absorption at higher levels of salinity compared to control (Evelin *et al.,* 2009; Garg and Chandel, 2011) and in the present study increasing nutrients absorption in mycorrhizal plants were observed compared to non-mycorrhizal plants. This finding is in agreement with other studies (Sheng *et al.,* 2008; Beltrano *et al.,* 2013) which report positive effects of mycorrhizal fungi on chlorophyll content under salinity stress.

Free Proline

Proline is a free amino acid that plays an important role in keeping osmatic pressure (Ruiz-Lozano *et al.,* 2012) and the higher proline content shows the higher osmatic pressure. Arbuscular mycorrhizal fungi have known to be a good mechanism to decrease proline content in stress conditions (Sheng *et al.,* 2008; Abdel Latef and Chaoxing, 2014). Similar to other studies (Beltrano *et al.,* 2013) free proline content is increased with increasing salinity levels which causes better osmotic statues of plants (Martinez *et al.,* 1995; Abdel Latef *et al.,* 2009). We found out that proline content was higher in root compared to shoot which indicate that roots need more proline to overcome osmatic pressure and absorb water from the soil (Ruiz-Lozano *et al.,* 2012) and this is also in agreement with findings of Zhu *et al.* (2010).

Antioxidant Enzymes

Some antioxidant enzymes including CAT, POD and SOD by increasing salinity levels and in the presence of mycorrhizal fungi were evaluated. Our study revealed that salinity increased antioxidant activity in both roots and shoots of *Z. spina-christi* seedlings which is in agreement with findings of Ghorbanli *et al.* (2004). Also similar to some other findings (Wu *et al.,* 2006; Zhu *et al.,* 2010), our study showed that AMF reduces the activity of antioxidant in high salinity levels in comparison to control. As a reason for this we could say that antioxidant enzymes upregulation is one of the plants defense mechanisms against oxidative damage caused by salinity stress, also proline is one of the compatible solutes contributing osmoregulation of cell wall (Ahanger *et al.,* 2014). But as we mentioned in the results, AMF inoculation resulted in higher proline content compared to control which could possibly result in fewer plant needs for antioxidant enzymes production in comparison to control because proline can act as a hydroxyl radical scavenger (Alia *et al.,* 1995).

The increase in proline content under abiotic stresses such as salinity results from protein degradation (Bagdi and Shaw, 2013). In this study proline and antioxidant activity decreased in the presence of mycorrhiza especially dual mycorrhizal fungi which indicates that these fungi have the ability to decrease protein degradation under salinity stress.

CONCLUSIONS

Z. spina-christi is a native species of arid and semi-arid parts of the world. This species can tolerate soil salinity up to 50 mM but dual mycorrhizal fungi inoculation levels would be a good strategy to have successful plantation in higher salinity levels. These results suggest that by dual mycorrhiza inoculation *G. mosseae*+*G. fasciculatum* could have better results in alleviation effects of mycorrhiza in *Z. spina-christi* seedlings under high salinity levels. Mycorrhizal plants (single and dual inoculated) maintained higher growth

parameters, chlorophyll content, nutrients, growth parameters and root colonization. Our results indicate that dual mycorrhiza inoculated plants are less susceptible to salinity conditions compared to single inoculated plants and non-mycorrhizal plants. Finally AMF especially dual mycorrhizal inoculation can increase the tolerance of *Z. spina-christi* in high salinity levels and could be an effective mechanism for having successful plantation in arid and salt conditions. It could be concluded that *G. fasciculatum* as a single mycorrhiza inoculum and *G. mosseae*+*G. fasciculatum* as dual mycorrhiza inoculums had significant effects in alleviation of adverse effects of salinity conditions for *Z. spina-christi* seedlings but these alleviative effects were higher in dual mycorrhiza inoculum.

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تاثیر مایع تلقیح خالص و آمیخته قارچ های میکوریز بر رشد، جذب مواد غذایی و فعالیت آنزیم های آنتی اکسیدان در نونهالهای کنار تحت تنش شوری

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چکیده

کنار گونه‌ای است که در نواحی خشک و نیمه خشک دنیا گسترش دارد. بیشتر این مناطق تحت تنش شوری قرار دارند. بنابراین، هدف این بررسی مطالعه تاثیرات غلظت‌های مختلف شوری بر نونهالهای کنار بود که در حضور برخی گونه‌های قارچ‌های میکوریز به صورت خالص یا مخلوط انجام گرفت. برای این منظور این مطالعه در شرایط نهالستان و با استفاده از طرح فاکتوریل ۳×۴ (چهار سطح شوری: ۰، ۵۰، ۱۰۰ و ۱۵۰ میلی‌مولار؛ و سه تیمار قارچ شامل: گیاهان بدون قارچ، با مایع تلقیح G. fasiculatum و مایع تلقیح مخلوط (G. mossea + G. fasiculatum انجام گرفت. نتایج نشان داد که شوری تاثیرات منفی بر کلنیزاسیون ریشه، پارامترهای رشد، محتوای کلروفیل، جذب عناصر غذایی دارد .همچنین باعث افزایش میزان سدیم، پرولین و فعالیت آنزیم‌های سوپر اکسیدازدیسموتاز، پروکسیداز و کاتالاز شد. اما گیاهان همراه با قارچ‌های میکوریز رشد، محتوای کلروفیل، مواد غذایی، کلنیزاسیون و فعالیت آنزیم‌های بیشتری داشتند. همچنین میزان پرولین و سدیم در نهال‌های میکوریزی

کمتر از نهالهای غیر میکوریزی بود. این تاثیرات در حضور تیمار محتوی قارچ‌های مخلوط به‌طور معنی داری بیشتر از تیمار قارچ خالص داشت. نتایج نشان داد که گیاهان تلقیح شده با مخلوطی از قارچ‌های میکوریز در مقایسه با گیاهان تلقیح شده با گونه قارچ خالص و گیاهان بدون قارچ، حساسیت کمتری نسبت به شوری دارند. این نتایج نشان دهنده این مسئله است که نهاکاری‌های کنار در مناطق شور، در صورتی که همراه با تیمار قارچ‌های میکوریز، به ویژه مخلوط قارچ میکوریز باشد، موفقیت بیشتری خواهند داشت.