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Morphophysiological and Biochemical Responses of *Zinnia elegans* to Different Irrigation Regimes in Symbiosis with *Glomus mosseae*

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Abstract
Water deficit conditions, in addition to reduced water uptake, result in a reduction in nutrient uptake, and consequently, a substantial reduction in growth parameters, yield and quality. Substrate inoculation with arbuscular mycorrhizal fungi (AMF) as an environmentally friendly biofertilizer is an important strategy to improve the growth parameters and quality of products in such conditions. Therefore, an experiment was conducted on zinnia, under different irrigation regimes (40, 70 and 100% of field capacity; FC) and different substrate inoculation levels with *Glomus mosseae* (0, 2.5 and 5%), in order to examine their possible symbiosis and root colonization on morphophysiological and biochemical parameters in a factorial experiment based on a completely randomized design with three replications. The results showed the positive and significant effects of AMF on morphophysiological traits under all irrigation regimes compared to the control treatment (without AMF) such as flower longevity and antiradical properties. With increased drought stress, root:shoot ratio, flower diameter, flower longevity, water use efficiency (WUE), the chlorophyll content and nutrient uptake were significantly decreased, while the decreasing trends of these parameters were much lower in pots treated with AMF. The lowest cell membrane stability and highest free radicals were observed in 40% FC without AMF. The maximum antiradical and antioxidant properties occurred at the lowest irrigation level with the highest AMF inoculation level. The results indicated a significant increase in flower diameter and longevity, in 70% FC with 5% AMF inoculation treatment compared to the control. Compared with the control, WUE was significantly increased at a 5% AMF inoculation level.

Keywords: antiradical properties, bedding plant, flower longevity, mycorrhizal symbiosis, water deficit irrigation.

Introduction

Deficit irrigation is one of the most important factors limiting plant growth, particularly in arid and semi-arid areas (Rodriguez, 2006). Zinnia (*Zinnia elegans*) is one the useful ornamental flowers of the Asteraceae family with a wide range of colours (Dole, 2005), which mostly grown as a bedding plant and summer specialty cut flower (Nau, 1991). One of the most limiting factors in the cultivation of bedding and landscaping plants, especially seasonal and annual garden flowers, mainly with shallow roots, is access to water. Water deficit, through restricting cell turgor, leads to a disruption of

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physiological processes (Sofo *et al*., 2004), reduction of photosynthesis (Arora *et al*., 2002), stomatal closure (Mafakheri *et al*., 2010) changes in plant metabolism, and plant death (Kalantari, 1989). Arbuscular mycorrhizal fungi (AMF) are as the most important microorganism for a soil-plant stable system (Smith and Read, 2008). One of the zinnia important diseases that limit its cultivation is powdery mildew. Pythium and Phytophthora fungi can cause root rot in this plant (Ghasemi Ghahsareh and Kafi, 2010). Mycorrhizal increases plant resistance to pathogens (Tangaswami *et al*., 2006). The positive role of coexistence in the agricultural economy is the main cause for extensive research in this field (Abbot and Robson, 1991). Smith and Read (2008) stated that Haifa fields with development of the mycorrhizal plants' root systems have increasing effect on the absorption of nutrients such as phosphorus, nitrogen, zinc and copper and transferring them to the host plant. These fungi can absorb and transfer all 15 elements necessary for plant growth (Jeffries *et al*., 2003; Mortimer *et al*., 2008). More than 95% of the plants can establish symbiosis with mycorrhiza and about 80% for Arbuscular mycorrhiza and exchange carbon, water, and nutrients with them (Smith *et al*., 2009). AMF play a significant role in the absorption of nutrients (Smith and Read, 2008; Asrar and Elhindi, 2011), plant hormone biosynthesis (Helena, 2008), increasing plant resistance against pathogens (Song, 2005), reducing root damage, enhancing biological nitrogen fixation and also improve plants' qualitative and quantitative traits (Mohammad *et al*., 1995). Song (2005) showed positive effects of AMF inoculation in drought conditions on rhizosphere and root development and thereby in improve water and nutrient absorption and oxidative stress reduction.

Despite studies, on the effect of mycorrhizal fungi on some crops and garden plants, there is a paucity of research on bedding plants, particularly zinnia, which appears to have a close relationship between irrigation and its quality parameters such as flower longevity and flower diameter. Therefore, the main objective of this study was to investigate the effect of AMF on the morphophysiological parameters of zinnia flowers, especially the quality of the flower and water use efficiency under water deficit conditions.

Materials and Methods

This study was conducted during the spring and summer of 2012 in Islamic Azad University of Mahabad with an average daily temperature 29ºC and 45% relative humidity, on zinnia (*Zinnia elegans* 'Dreamland Red') in a factorial experiment based on the completely randomized design with three replicates. Possible symbiosis between zinnia and *Glomus mosseae* (*Glomeromycota*) as one of the most prevalent and important arbuscular Mycorrhizal Fungi (AMF) and its effects on growth and flower quality under different inoculation levels (0, 2.5 and 5%, w/w) and different irrigation regimes (40, 70 and 100% of field capacity; (FC)) were the main goals of the current trail. The substrate soil (sandy loam texture with 0.5% organic matter) after sterilization in an autoclave (121°C/20 min), was inoculated by *Glomus mosseae* prepared in the department of soil biology (soil and water research institute, Tehran, Iran), and transferred to the pots (3L/volume). Finally transplanting was performed at the four-leaf phase.

The soil used in this experiment was autoclaved (sterilized) at 121°C for 20 min, pots were also disinfected with alcohol, and they were rinsed with sterile water. Then, depending on the pots treatment with bed inoculated with fungus, control treatment (without fungus) was filled and zinnia seeds were planted in each pot. After the establishment of plants in the 4-leaf stage, different irrigation regimes were applied. For verification the fungi species, spores were mounted on glass slide using

polyvinyl alcohol-lactic acid glycerol (PVLG) and observed under type Hund 600 compound microscope (Hund 600) (100X) equipped with drawing tube for study the morphologic and morphometric characteristics. Spores were identified according to the manual of identification of VAM fungi by (Schenck and Perez, 1990). The International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) worksheet was used for verifying the spores. The soil used was 3 kg for each pot, the amount of fungus used was 2.5% equivalent to 75 g and it was 150 g for 5%. The spore amount was 80 spores per gram.

Irrigation regimes were applied based on the field capacity (FC) method (soil moisture content); briefly, for 48 hr. To prepare crop capacity of pots, three pots were selected before planting and they were irrigated to saturation level; then opening of pots was covered with aluminum foil (to prevent evaporation). According to soil texture and fixed weight loss of pot after saturated irrigation (withdrawal of excess water), after 48 hr, pots were weighted and the difference between initial weight and gained weight, crop capacity 100% and rest of treatments were calculated (Henry, 1990). During the whole period of growth, all pots were weighted daily by accurate scale and weight differences in each pot were compensated based on treatment and irrigation regime. About a month after the treatment with irrigation, traits such as Cell wall stability, Total chlorophyll, and Free radical were calculated.

Growth parameters such as Shoot dry weight and root: shoot ratio, flower diameter, flower longevity (from anthesis to flower wilting) and water use efficiency (WUE) Karkanis *et al*. (2011) were measured during the experiment.

Water use efficiency (WUE) was calculated as total plant dry weight (g) per used water (ml) during the experiment.

$$WUE = \frac{Plant\ performance\ (g)}{The\ amount\ of\ water\ used\ during\ the\ experiment\ (ml)}$$

The total chlorophyll content of young mature leaves at the flowering stage was also determined after extraction in 80% acetone (Gross, 1991). Absorption of each sample after extraction by acetone was measured using a spectrophotometer (Perkin Elmer, UV/VIS, Lambda 25), at 645 and 663 nm, and thus total chlorophyll was determined.

1. Chlorophll a (g L^{-1}) = (0/0127×OD_{663}) - (0.00269×OD_{645})
2. Chlorophll b (g L^{-1}) = (0/0229×OD645) - (0.00468×OD663)
3. Total chlorophll (g L^{-1}) = (0/0202×OD645) + (0.00802×OD663)

Cell membrane stability was measured using an electrical conductivity meter based on the Lutts *et al*. (1996) method. Leaf samples were placed in individual stoppered vials containing 25 ml of deionized water. These samples were incubated at room temperature (25°C) on a shaker (150 rpm per 30 min). Electrical conductivity of solutions (EC_1) was read after shaking. Leaf samples were then placed in a thermostatic water bath at 95°C 15min^{-1}, and the second reading (EC_2) was determined. Finally membrane permeability was calculated using EC_1/EC_2*100, and expressed as a percentage. Phosphate buffer (pH=7) and potassium iodide (KI per 1M) were used for the determination of hydrogen peroxide (Velikova *et al.*, 2000). Absorbance of each sample was recorded at 390 nm, and H_2O_2 was calculated by extinction coefficient (0.28 $\mu M^{-1}cm^{-1}$) and expressed in terms of $\mu M\ g^{-1}$ fresh weight.

The stable 1, 1-Diphenyl-2-picryl-hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity (Koleva *et al*., 2002). After addition of treatment extract to a methanolic solution of DPPH (100 M, 15 min at room temperature), the absorbance was recorded at 517 nm. BHT and

quercetin were used as standard controls. IC 50 values denote the concentration of the sample required to scavenge 50% of DPPH free radicals. The total phenol content was also measured according to folin–ciocalteu reagent (Pourmorad *et al*., 2006).

Sample extracts (1 g DW per 10 ml ethanol) of each treatment or gallic acid (standard phenolic compound) was mixed with Folin–Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). The mixture was allowed to stand for 15 min and total phenols determined by colorimetry at 765 nm. Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference compound. The aluminium chloride colorimetric method was used for leaf and petal flavonoid determination (Chang *et al*., 2002).

The root colonization percentage Trouvelot *et al*. (1986) was measured by coloration. Phosphorus uptake was determined based on vanadate-molybdate reagent (Olsen and Sommers, 1982), and calcium and magnesium uptake were measured based on titration by EDTA (0.01 M) (Anonymus, 1990).

Data were analysed using SAS (Version 9.1, Cry, N.C., USA). Analyses of variance (ANOVA) were performed and mean comparisons were made using Duncan's multiple range test at1% and 5% probability levels.

Table 1. Chemical and physical analysis of substrate soil

Chemical characteristics						Physical characteristics			
Total carbonate (meq L^{-1})	Organic matter (%)	EC (dS m^{-1})	pH	N (%)	P (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
2	0.5	1.5	7.3	0.06	5	59	26	15	Sandy loam

Result and Discussion

Shoot dry weight and root: shoot ratio

According to the results of the analysis of variance the effect of Irrigation regimes and AMF on Shoot dry weight was significant ($P<0.01$), also root to shoot ratio in zinnia were significantly affected by the interaction of Irrigation regimes (FC) and AMF ($P<0.05$).

Accordingly, with increased stress levels (40%) shoot dry weight showed a decreasing trend, but by inoculating the substrate with AMF, the Shoot dry weight significantly increased. The maximum root:shoot ratio occurred in 70% FC and 5% AMF treatments, and the lowest ratio was observed in 40% FC and without AMF (Table 2). A decrease in cell growth and consequently overall plant growth is the first reaction to drought conditions (Rodriguez, 2006).

Accordingly, in drought conditions (40% FC) the aerial parts are influenced more than the roots; in other words, the growth of the aerial part of the plant retarded earlier than roots, leading to an increased root to shoot ratio (Chiatante *et al*., 2006). Moreover, the minimum biomass production in the lowest irrigation regime (40% FC) may occur because of a reduction in water uptake by the plant and consequently a decreasing trend in the absorption of nutrients such as phosphorus (Fig. 6), as well as increased levels of free radicals (Table 3), and as a result reduced dry matter. The results of this study are consistent with those of Al-Qarawi and Alshahrani (2010) regarding the symbiosis of jujube with mycorrhizal fungi and increased root to shoot ratio in marigolds (Asrar and Elhindi, 2011).

Table 2. Effect of interaction irrigation regimes (FC) and AMF (*Glomus mosseae*) levels on shoot dry weight and root to shoot ratio of *Zinnia* 'Dreamland Red'.

FC (%)	AMF (%)	Shoot dry weight (g DW plant^{-1})	Root:shoot ratio
100	0	$1.77^{bc\dagger} \pm 0.18^{\dagger\dagger}$	$0.12^{bc} \pm 0.014$
	2.5	$2.29^{ab} \pm 0.12$	$0.14^{bc} \pm 0.008$
	5	$2.42^{a} \pm 0.11$	$0.17^{b} \pm 0.006$
70	0	$2.27^{ab} \pm 0.03$	$0.13^{bc} \pm 0.008$
	2.5	$2.5^{a} \pm 0.2$	$0.25^{a} \pm 0.006$
	5	$2.59^{a} \pm 0.21$	$0.27^{a} \pm 0.04$
40	0	$1^{d} \pm 0.01$	$0.1^{c} \pm 0.005$
	2.5	$1.48^{cd} \pm 0.28$	$0.12^{bc} \pm 0.006$
	5	$1.67^{c} \pm 0.26$	$0.17^{b} \pm 0.017$

† The means are marked with the same letters in each column, do not have a significant difference according to Duncan's multiple range test (P<0.05).

†† Indicates standard errors (Mean ±SEM; n=4).

Flower diameter and flower longevity

Flower diameter and flower longevity are the important zinnia flower quality components. The results showed the significant effect of Irrigation regimes and AMF on Flower diameter; also flower longevity was significantly affected by the interaction of FC and AMF (P<0.01).

Accordingly, with increased drought stress, flower diameter and longevity demonstrated a decreasing trend, especially in high stress levels (40% FC). Compared with the control (without AMF treatment), substrate inoculation with *Glomus mosseae* increased flower diameter and longevity about 18.5% and 26.5%, respectively Figures 1A, B and Figure 2. Given the role of phosphorus as one of the most essential and effective elements in flowering Taiz and Zeiger (2000) and calcium's effect on cell stability Barker *et al*. (2007) and delaying flower senescence (Ferguson, 1988), the results of the present experiment indicated the improved uptake of phosphorus and calcium at 100 and 70% FC levels containing AMF compared with the control treatment (Fig. 6); thus, improving the absorption of these minerals is amongst the effective factors enhancing the diameter and longevity of zinnia flowers (Fig. 3). The results of this study are in line with those of Garmendia and Mangas (2012) on rose, and of Koltai *et al*. (2010) on *Lisianthus* regarding the positive effect of mycorrhizal fungi on flower diameter and longevity compared to the control treatment (without AMF). Application of AMF in both favourable irrigation and drought conditions also led to an increase in flower diameter in comparison with the control treatment (without mycorrhizal fungi), which was agree with the findings of Asrar and Elhindi (2011).

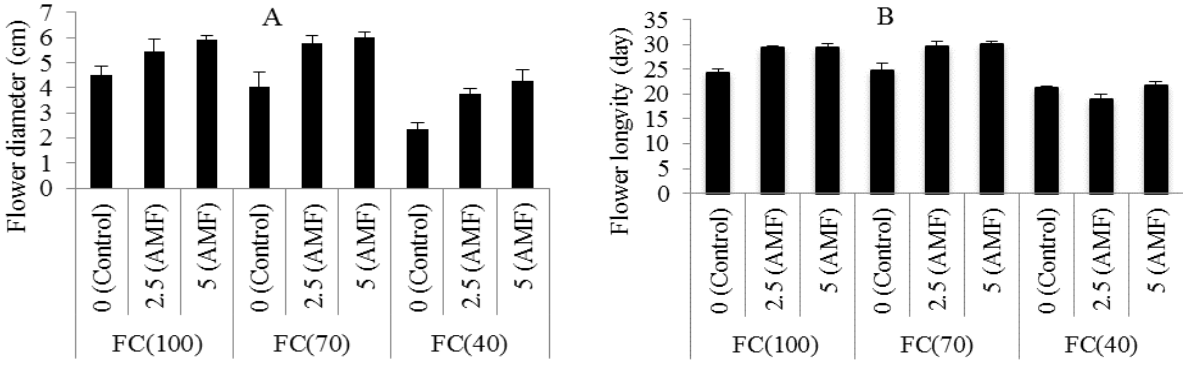

Fig. 1. Flower diameter (A) and flower longevity (B) of *Zinnia* 'Dreamland Red' with *Glomus mosseae* symbiosis (AMF) under different irrigation regimes (FC), Bars indicate standard error (Mean ±SEM; n=4)

Fig. 2. Zinnia flowering in inoculated with *Glomus mosseae* (+M; left) and control plants (-M; right) under different irrigating regimes (FC)

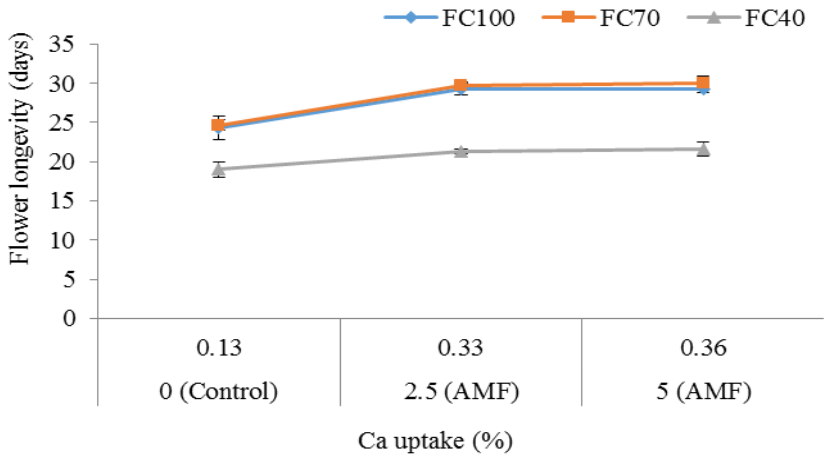

Fig. 3. Effect of AMF (*Glomus mosseae*) symbiosis on Ca uptake and flower longevity of zinnia under different FC levels, Bars indicate standard error (Mean ±SEM; n=4)

Root colonization rate

Based on the results, root colonization rate was significantly affected by FC levels, AMF symbiosis and their interaction effects ($P<0.05$). According to mean comparisons, with increased levels of water stress and AMF root colonization rate has increased, although no significant difference was observed between the different inoculation levels (Fig. 4). As shown in Figure 5, a symbiosis between zinnia root and *Glomus mosseae* has occurred. Colonization rate depends on several factors, including rhizosphere conditions and plant species (Koide, 1991). In this symbiotic relationship the fungi take carbon from the plant and in return help the plant with the absorption of nutrients. Khalvati *et al*. (2005) in their research on barley, concluded that the percentage of root colonization in stress conditions was much higher compared to normal conditions; *Glomus mosseae* increased water uptake and phosphorus content in aerial organs in drought conditions, and root colonization rate decreased with increased application of phosphorus. Shamshiri *et al*. (2011) in their experiments on petunia, and Abdel Fattah *et al*. (2002) in their study on broad beans (*Vicia faba*), reported that along with increased drought stress, the root colonization rate, biomass production and the volume of photosynthetic pigments significantly increased, which is agree with the findings of the present study.

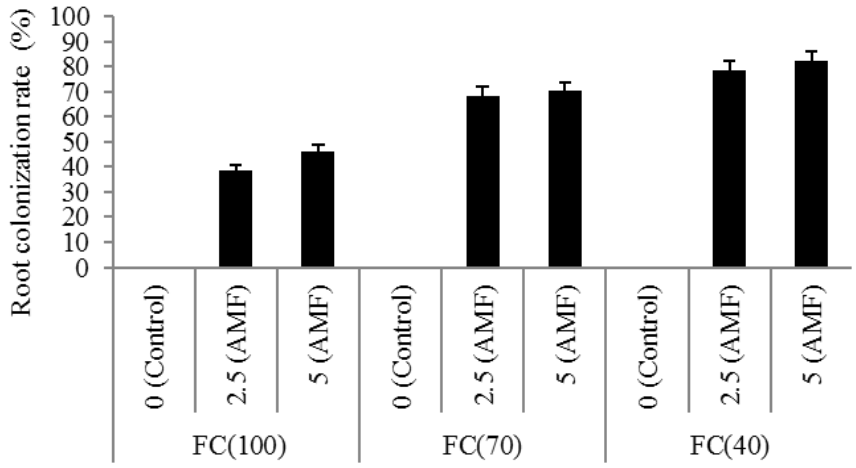

Fig. 4. Effects of different inoculation levels of *Glomus mosseae* on root colonization rate of zinnia under different irrigation regimes (%FC), Bars indicate standard error (Mean ±SEM; n=4)

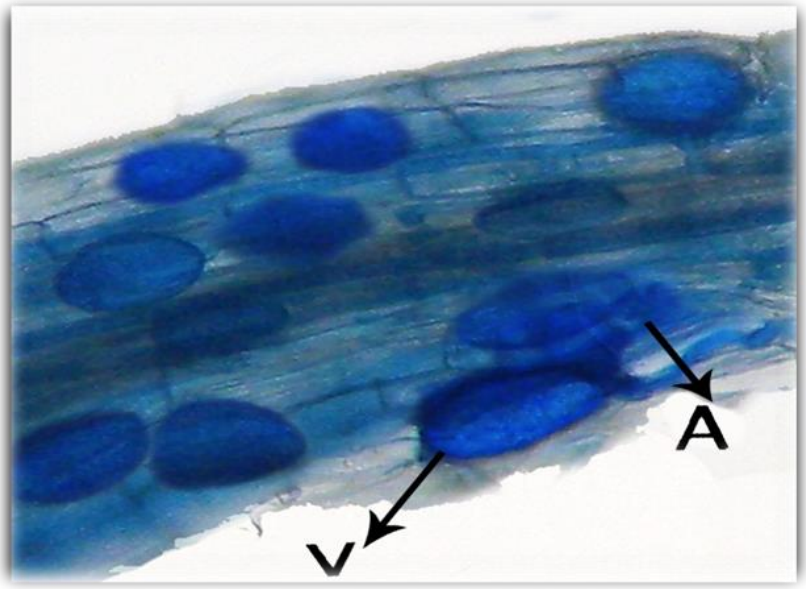

Fig. 5. Vesicles (V) and Arbuscule (A) formation in zinnia roots inoculated by *Glomus mosseae*

Total chlorophyll content

With decreased field capacity, leaf chlorophyll content demonstrated a decreasing trend; however, the application of mycorrhizal fungi led to the moderation of the decreasing trend (Table 3). The reason may be related to water amount and efficiency of nutrient use. Increased drought has led to a decrease in water use efficiency, and chloroplasts and chlorophyll are probably destroyed. In addition, due to the impact of drought stress on magnesium uptake (Fig. 6) in the symbiotic treatments compared to the control (without AMF), and the direct impact of this element in chlorophyll structure, the improved magnesium uptake is one of the reasons for the decreasing declining trend of chlorophyll levels in severe drought stress (40% FC) with mycorrhizal fungi inoculation. The results of this study are consistent with Smith *et al*. (2003) on maize, and (Demir, 2004) on pepper, indicating a close relationship between water stress, nutrient uptake and impact on chlorophyll as a result of symbiosis with mycorrhizal fungi.

Cell membrane stability, hydrogen peroxide, and antiradical properties

The results of the mean comparison indicate a significant effect of irrigation regimes and mycorrhizal fungi symbiosis on cell membrane stability (Table 3). Accordingly, increased FC has been shown to lead to an increase, and AMF treatments to lead to a decrease, in electrolyte leakage compared to the control (without AMF). No significant differences were found between different AMF inoculations, although substrate inoculation with *Glomus mosseae* reduced cell damage due to better water uptake. Furthermore, because of the essential role of calcium for cell wall stability Barker *et al*. (2007), the results demonstrate the positive effects of mycorrhizal fungi symbiosis in increasing leaf calcium content, and thereby moderating the damage caused by drought. Relative water content is an appropriate indicator of leaves' water status, so in case of increased drought stress it is reduced, which leads to changes in the cell membrane, and consequently increases electrolyte leakage from cells (Fu *et al*., 2004). In anthurium flowers ion leakage increased with increasing water stress, but by application of mycorrhizal fungi this index decreased (Asrar *et al*., 2012).

Moreover, the results (Table 3) showed that free radicals (hydrogen peroxide) and antiradical properties were significantly affected by the interaction effects of FC and AMF ($P<0.05$). The highest levels of free radicals were observed in severe drought stress and the lowest at a100% FC level with 5% AMF application. The maximum antiradical properties were obtained with severe drought stress treatment without fungi treatment, and the minimum antiradical properties at a100% FC level without fungi treatment. The increased level of free radicals in response to drought stress Mafakheri *et al*. (2010) coinciding with increased defence ability and the production of antioxidant and antiradical compounds (such as O_2, O_2^-, HO, RO, ROOH, H_2O_2 Free radical (in English: free radical) to atoms, molecules or ions with unpaired electron is called) in response to drought stress Jaleel *et al*. (2007) to reduce the adverse effects of free radicals, are consistent with the results obtained in this study regarding reduced water uptake with a 40% irrigation regime and consequently an increase in free radicals. Obviously, substrate inoculation with *Glomus mosseae* improved the root system and absorption and enhanced the water status, thereby reducing the adverse effects caused by severe stress and oxidative stress in treated plants compared to the control. Wu and Zhu (2009), in their investigation on the effect of drought stress in citrus, demonstrated increased drought stress to lead to an increase in free radicals (hydrogen peroxide) in citrus, while mycorrhizal fungi treatment reduces free radicals compared to the control (without fungi). The results indicate a close relationship between antiradical properties and free radicals under drought stress and symbiosis with mycorrhizal fungi; thus, along with increased water stress, free radical generation and the defence and antioxidant system of the plant (in order to reduce the detrimental effects caused by hydrogen peroxide) demonstrate an increasing trend.

Moreover, mycorrhizal symbiosis contributed to the strengthening of the relationship and ultimate improvement of conditions for the zinnia flower, which is cordinated with the research of Ordookhani and Zare (2011) regarding the increase in the antioxidant capacity of tomato under mycorrhizal fungi treatment.

Table 3. Effects of interaction irrigation regimes (FC) and *Glomus mosseae* inoculation (AMF) levels on some physiological properties of *Zinnia* 'Dreamland Red'

FC (%)	AMF (%)	Total chlorophyll (g L^{-1})	Cell wall stability (%)	Free radical (µM per g FW)	Antiradical properties (%)
100	0	0.00126^{a+} ± 0.0000087††	39.24^{d} ± 0.76	11.92^{cd} ± 0.66	80.4^{d} ± 0.82
	2.5	0.00138^{a} ± 0.000012	27.71^{f} ± 0.52	10.26^{de} ± 0.62	86.73^{c} ± 0.96
	5	0.00135^{a} ± 0.000067	27.59^{f} ± 0.79	10.05^{e} ± 0.54	87^{c} ± 0.92
70	0	0.00097^{ab} ± 0.000034	43.4^{c} ± 0.39	16.51^{b} ± 0.07	89.02^{c} ± 0.7
	2.5	0.00113^{a} ± 0.000012	32.06^{e} ± 0.77	13.58^{c} ± 0.49	93.37^{b} ± 1.21
	5	0.00112^{a} ± 0.000066	31.84^{e} ± 0.46	12.57^{c} ± 0.67	94.29^{ab} ± 0.99
40	0	0.00059^{bc} ± 0.000036	57.65^{a} ± 0.21	24.9^{a} ± 1.01	94.72^{ab} ± 0.76
	2.5	0.00056^{c} ± 0.00001	46.62^{b} ± 0.75	17.56^{b} ± 0.16	96.34^{a} ± 0.64
	5	0.00066^{bc} ± 0.000059	46.43^{b} ± 0.34	17.41^{b} ± 0.05	96.43^{a} ± 0.94

† The means are marked with the same letters do not have a significant difference according to Duncan's multiple range test (P<0.05).
†† Indicates standard errors (Mean ±SEM; n=4).

Total phenol and flavonoids of leaves and petals

As shown in Table 4, increased drought stress and mycorrhizal fungi application has led to an increasing trend in the total phenol and flavonoid levels of leaves and petals compared to control treatment (100% FC, without AMF). Increasing total phenol and flavonoid levels as non-enzymatic antioxidant capacity in 40% FC and 5% AMF treatments can be explained as a response to reduced irrigation or increased drought stress levels, as oxidative stress (Sofo *et al*., 2004) leads to damage of cell membranes (Vannozi and Larner, 2007) and decreasing free radicals (Mafakheri *et al*., 2010), on the one hand, and stimulates the immune system and enzymatic Catalase and Peroxidase (Mittler, 2002) and non-enzymatic antioxidant activity (phenolic compounds; Delitala *et al*., 1986; Szabo *et al*., 2003), on the other hand, to moderate stress levels and improve the plant's defence mechanism. This corresponds with the results obtained in this study regarding the decreased stability of cell membranes, and increased free radicals and phenolic and flavonoid compounds, as a non-enzymatic antioxidant system to improve the adverse effects of drought stress and enhance the antioxidant system under mycorrhizal treatment (Tables 3 and 4). Saneoka *et al*. (2004) reported that drought stress in olive trees led to an increase of phenylalanine ammonia lyase activity and total phenol content of their fruits.

Table 4. Effects of different irrigation regimes (FC) and *Glomus mosseae* inoculation (AMF) on physiochemical properties of *Zinnia* 'Dreamland Red'

FC (%)	AMF (%)	Leaf		Petal
		Total phenol (mg per g DW)	Flavonoid (mg per g DW)	Flavonoid (mg per g DW)
100	0	40.85^{h} ± 1.32††	2.95^{g} ± 0.29	0.78^{f} ± 0.06
	2.5	50.01^{g} ± 1.74	4.19^{f} ± 0.28	1.22^{ef} ± 0.18
	5	56.35^{f} ± 2.02	4.99^{f} ± 0.45	1.57^{e} ± 0.13
70	0	87.85^{e} ± 1.15	9.61^{e} ± 0.49	4.35^{d} ± 0.27
	2.5	102.68^{d} ± 1.58	14.12^{d} ± 0.3	5.63^{c} ± 0.22
	5	103.35^{d} ± 2.02	15.07^{cd} ± 0.36	6.23^{c} ± 0.22
40	0	110.01^{c} ± 1.74	16.05^{c} ± 0.53	7.82^{b} ± 0.35
	2.5	130.35^{b} ± 2.02	20.27^{b} ± 0.21	9.92^{a} ± 0.28
	5	136.01^{a} ± 1.58	21.54^{a} ± 0.45	10.51^{a} ± 0.23

† The means are marked with the same letters; do not have a significant difference according to Duncan's multiple range test (P<0.05).
††: Indicates standard errors (Mean ±SEM; n=4).

Mineral uptake

Figure 6 shows that FC, AMF, and their interaction effects significantly affect Ca, Mg and P uptake ($P<0.01$). Along with decreased FC and access to water, Ca, Mg and P absorptions in this study demonstrated a decreasing trend, while the application of AMF inhibited the decrease in the uptake of these elements (Fig. 6). Increased nutrient uptake in treatments with appropriate field capacity is due to mobility and the possibility of absorbing more nutrients in more favourable water conditions (Demir, 2004). It is also a result of the application of mycorrhizal fungi through an expanding zinnia root system as a result of the expansion of the fungus' hyphal network (Song, 2005), thereby increasing mineral uptake capacity from the soil compared to lower field capacities and lower and non-symbiotic treatments (Fig. 6). This is consistent with the results obtained by (Boomsma and Vyn, 2008; Turk *et al*., 2006; Asrar *et al*., 2012) regarding increased mobility and nutrient uptake, especially phosphorus and calcium as elements that have very low mobility in water stress conditions (Wiley and Inc, 1984).

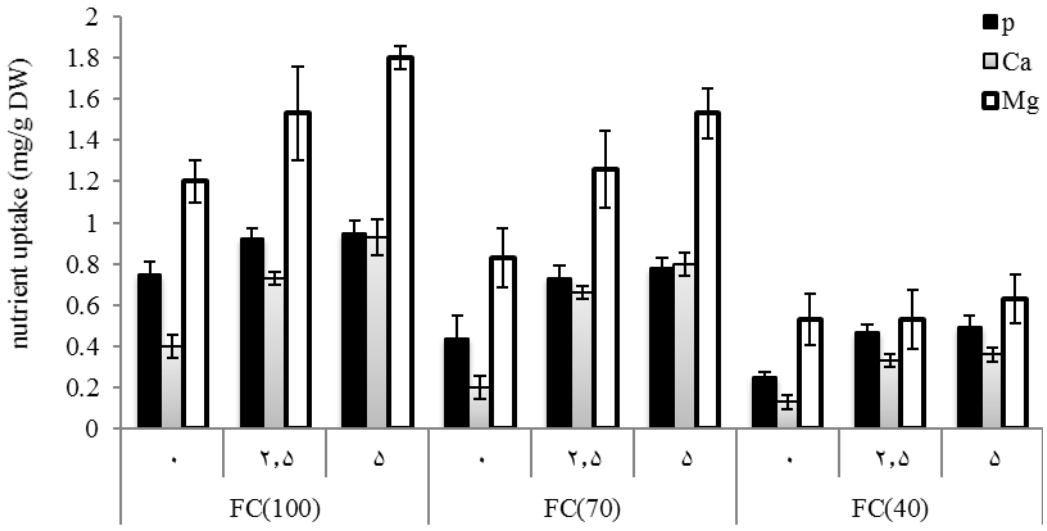

Fig. 6. Effects of *Glomus mosseae* inoculation levels (AMF) on mineral uptake of *Zinnia* 'Dreamland Red', under different irrigation regimes (FC), Bars indicate standard error (Mean ±SEM; n=4)

Water use efficiency (WUE)

Based on the results, FC, AMF and the interaction effects of FC and AMF significantly affected WUE ($P<0.05$). As shown in (Fig. 7) the maximum WUE was observed in 5% mycorrhizal fungi application with 70% FC, with an average of 0.035 g mL^{-1}, and the minimum efficiency in 40% FC without mycorrhizal fungi with an average of 0.011 g mL^{-1}. The maximum WUE in 5% AMF and 70% FC treatments can be attributed to the maximized biomass production in this treatment (Table 2) with respect to more balanced water use compared to other treatments. In fact, taking into account the dry material produced with respect to the water used, or water use efficiency indicators, it can be concluded that substrate inoculation with *Glomus mosseae* leads to increased access to water and thereby increases the development of the plant root system, leading to increased water uptake, helping to maintain cell turgor, increasing nutrient uptake and ultimately improving dry matter production, especially in more balanced irrigation conditions in this experiment. The plants with mycorrhizal symbiosis, through controlling the opening and closing of their leaf stomata, increasing their water uptake by the expansion of their hyphal network, and, thereby, developing

their root system and leaf area and moderating transpiration in comparison to non-mycorrhizal plants, increase their water use efficiency and performance (Sohani, 2000).

Gindaba *et al*. (2005) reported a decrease in WUE due to an increase in drought stress in greenhouse conditions for plants such as *Cordia millettia*, *Africana ferruginea*, and *Croton macrostachyus*. Similar results of increased water use efficiency in mycorrhizal colonization in onions have been reported by Bolandnazar *et al*. (2007).

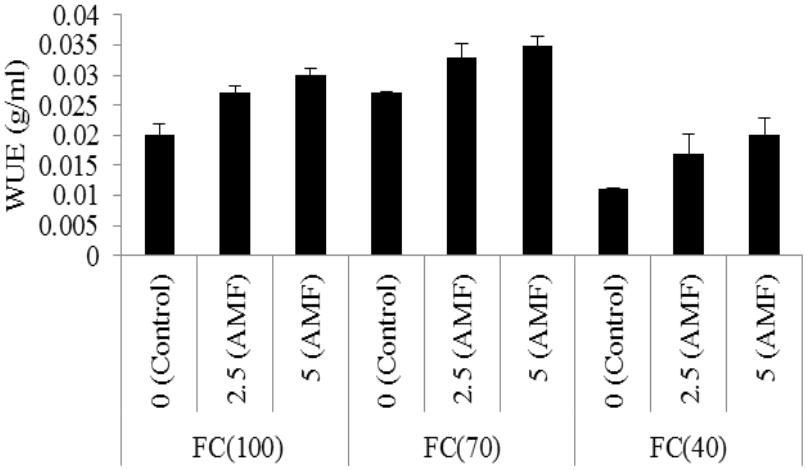

Fig. 7. Zinnia water use efficiency (WUE) in symbiosis with *Glomus mosseae* (AMF) under different irrigation regimes (FC), Bars indicate standard error (Mean ±SEM; n=4)

Conclusions

According to the results of the present experiment, compared to control treatments (without fungi), arbuscular mycorrhizal fungi treatments led to improved growth parameters, nutrient uptake, flower diameter and longevity (as the most important flower quality components), especially in terms of moderating and reducing the adverse effects of oxidative stress in drought conditions (40% FC) in zinnia flowers. Accordingly, the inoculation of the substrates or zinnia 'Dreamland Red' seedling roots with *Glomus mosseae* at a 2.5% level is advised when planting, particularly in low water conditions.

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