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RESEARCH PAPER

Changes in morphological and biochemical properties of *Celtis caucasica* L. mycorrhizal fungi-inoculated under drought stress condition

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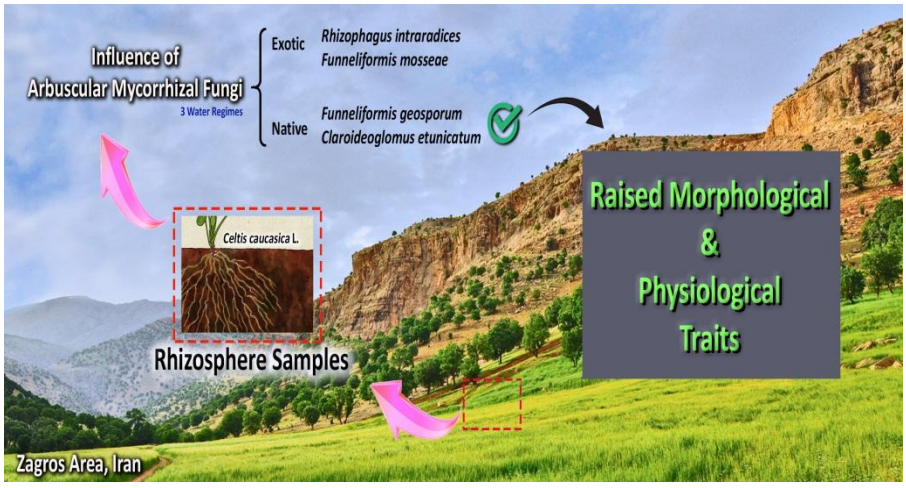
Highlights

- The percentage of colonization on native AMF, isolated from rhizosphere of *Celtis caucasica* L., has been more than exotic.
- The use of mycorrhizal fungi of *Glomus intraradices*, *Glomus mosseae*, *Funneliformis geosporum* and *Claroideoglomus etunicatum* increases the activity of CAT, SOD, and POD enzymes.
- AMF-symbiosis can improve the important morphological traits of *Celtis caucasica* and this can be contributed to the better initial establishment of seedlings.
- AMF decreases the content of H2O2 and MDA. As well as, the activity of antioxidant enzymes in the mycorrhizal plant was more than a non-mycorrhizal plant.

Graphical Abstract



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Abstract

Zagros forest in the west of Iran has been destroying recently due to climate changes, dust, pests, local people using, grazing livestock and used more than ecosystem capacity. Accordingly, these regions need to be recovering by reforestation resistance seedlings. For this purpose, the influence of different arbuscular mycorrhizal fungi (AMF) included; Rhizophagus intraradices and Funneliformis mosseae as exotic fungi and two native, Funneliformis geosporum and Claroideoglomus etunicatum, as well as non-mycorrhizal (control), investigate to produced resistance seedling of Celtis caucasica L. under three water regimes (optimal irrigation, 75% of field capacity and water deficit, 50% and 25% of field capacity) as factorial in a completely randomized design with ten replications. Mycorrhizal seedlings especial natives one reveals that AMF significantly raised morphological and physiological traits such as the fresh and dry weight of above and underground biomass, the length of the root, seedling height, colonization percentage, H_2O_2 and MDA content, CAT, SOD, POD activity, the content of chlorophyll a, b, total chlorophyll, and carotenoid. The concentration of H_2O_2 and MDA in inoculated plants was less than non-mycorrhizal plants under all irrigation regimes. The activity of antioxidant enzymes simultaneously increased with increasing drought stress, application of AMF caused a further increase in their activity. The content of chlorophyll a, b, total chl, and carotenoid decreased with increasing water deficit. In AMF treatments, the content of these pigments was more in respect to control (non-mycorrhizal plants). Generally, we suggest that the effect of native mycorrhizal fungi was more and better than the exotic fungi and control. Thus, identifying the symbiosis between native mycorrhizal fungi and trees could be considered as an eminent step towards the restoration of degraded areas of plantations and forests.

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1. Introduction

Mycorrhizal symbiosis is known as one of the most important symbiotic relationships in nature that has been created during the course of evolution, where roots and fungi as a living unit are active and benefit from each other. The diverse and positive impact of this sort of co-symbiosis on survival and enhancing plant growth in arid and semi-arid areas has been focused on since the 1970s. In these areas lack of sufficient water resources is known as the most important factor that limiting plant growth and crop production (Al-Karaki, 2000). Zagros forests in the west of Iran as a semi-arid region, where covers an area of 5 million ha, has a significant role in the forests ecosystem health and the life of local people. Here, soil degradation, shortage of nutrients and adverse microclimate conditions lead to limitation of establishment and reproduction of plants. Zagros is very invaluable in terms of plant species diversity more than 190 trees and shrub species such as *Querqus* sp. and Celtis sp. are grown in this area (Apel and Hirt, 2004). *Celtis Caucasica* L. (caucasian Hackberry) is a broad-leaved tree species belonging to the *Celtis* genus of Celtidaceae family (Sattarian, 2006) that compared with many species used in afforestation of Zagros forest, has considerable growth and resistance to water dearth and different conditions of soil (Khanhasani et al., 2013; Ardalan et al., 2013). Improving afforestation and accelerating the early growth of seedlings in areas such as Zagros, is very important and collect comprehensive information of natural interaction such as the relationship between soil and plant has an undeniable role in obtaining sustainable management of natural resources (Altieri, 2002). To obviation these problems, different strategies are needed to enhance the ability of trees and plants against environmental stress by improving the absorption of water and nutrients (He et al., 2013; Ortiz et al., 2015). In this regard, the mycorrhizal relationship is a strategy, which has been widely used in recent years in forest areas (Bremner, 1965). Stress is one of the major challenges for plants and agriculture. The positive effects of mycorrhizal fungi in tolerance of plants to abiotic stress such as drought and salinity as well as biotic stress such as pathogens have been known (Berruti et al., 2014). In natural ecosystems, plants have different degrees of dependence on mycorrhizae to uptake nutrients such as phosphorus and nitrogen (Lee et al., 2014; Nouri et al., 2014; Xie et al., 2014). The mobility of nutrients is limited in inappropriate conditions, however, arbuscular fungi had a significant effect on the plant growth and development in this condition because to improve nutrient uptake (Pagano et al., 2013; Xie et al., 2014; Bundrett et al., 1996). Mycorrhizal symbiosis increases relative water content (RWC) and water use efficiency (WUE) under drought stress conditions in *Robinia pseudoacacia* L. (Yang et al., 2014). As well as, the removal of the drought and increased soil water. Mycorrhizal plants absorbed water more quickly than non-mycorrhizal plants and reach balance in the water potential of leaves (Subramanian and Charest, 1997; Subramanian et al., 2006). Drought stress triggers the production of reactive oxygen in plants, and thereby cause the chlorophylls degradation, damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (Cruz de Carvalho, 2008; Anjum et al., 2011; Hasanuzzaman et al., 2013). Plants cope with drought stress by reducing growth parameters, stomatal closure, reducing photosynthesis, changes in ionic performance and increase the activity of antioxidant systems include enzymatic and non-enzymatic scavenging systems (Cruz de Carvalho, 2008; Gill and Tuteja, 2010). Enzymatic scavenging systems include catalase (CAT), superoxide dismutase (SOD), peroxidase (PX), ascorbate peroxidase (APX), and glutathione reductase (GR) (Cruz de Carvalho, 2008; Gill and Tuteja, 2010). The AMF enhances plant water relations, which improve the drought resistance of host plants, therefore, had an important role in sustainable agriculture (Carina et al., 2016; Shah et al., 2008; Leifheit et al., 2014). Research has shown that in the drought stress conditions AMF enhances plant biomass, chlorophyll contents and rate of transpiration compared with plants without AMF infection (Augé et al., 1987; Augé, 2001; Chapman et al., 1999; Shah et al., 2009; Asensio et al., 2012; Yang et al., 2014).Some studies have demonstrated that AMF symbiosis can enhance the activities of antioxidant enzymes under drought and saline conditions (Alguacil et al., 2003; He et al., 2007; Benhiba et al., 2015). The main objectives of this study were to evaluate the effect of AMF symbiosis on growth, biomass production of *Celtis Caucasica* cultivated at either optimal or restricted irrigation, and evaluated the content of H_2O_2 and MDA, activities of antioxidant enzymes include CAT, SOD, POD, and content of chlorophyll *a,b*, total chlorophyll, and carotenoid (Chaghakaboodi et al., 2021).

2. Materials and Methods

2.1. Site description and soil characteristics

Zagros area is located in 50° 51′ 33 ″ E and 29° 49′ 31″ N and 2400 m a.s.l. Soil and root samples (rhizosphere) were collected in September of 2014. For this purpose, 5 trees were randomized selected and soil samples were collected in 0-20 cm depth. Soil samples after passing through a 2 mm sieve, were classified into two classes, a class for identifying Arbuscular mycorrhizal fungi (keep in 4 C) and another for assaying physical and chemical features of soil (keep in room temperature). As seen in Table 1, pH using distilled water (water: soil, 2.5:1), soil texture using hydrometrical method (Kahrizi et al., 2021; Walkley and Black, 1934). Also, the soil features were prepared following its habitat characteristics.

Table 1. The physical and chemical characteristics of the soil.

texture	Sand (%)	Silt (%)	Clay (%)	pH
Clay- loam	40.88	22	37.12	8.22

2.2. Isolation and identification of native spores

The wet sieving method was applied to isolate native spores of soil samples (Gerdemann and Nicolson, 1963). To identify spores, slides were prepared with polyvinyl alcohol (PVLG) and polyvinyl alcohol-Meltzer (PVLG-M). In this method, fungi were determined by morphological features such as existence and absence of sporocarp, size, color, shape, and the number of wall layers of spores (Fan et al., 2008; Farokhian et al., 2021).

2.3. AMF inoculum and inoculation

Arbuscular mycorrhizal fungi are co-symbiosis that its reproduction is impossible in common fungi medium, thus trap plants due to high growth rate and infection to generate fungi were used for this purpose. In this research, *Zea mays* L. were selected as a trap plant (Fan et al., 2008). First, as the above mentioned, the spores of two native mycorrhizal species were isolated and identified from soil samples (rhizosphere). The second, 100 spores inoculated on *Zea mays* for each pot with sterilized soil. Fungi species used in this study included *Funneliformis geosporum*, *Claroideoglomus etunicatum* that is identified for the first time coexistence to *Celtis Caucasica* and combination of them. The seeds were sterilized with bleach water. Four months after planting corn, the Caucasian hackberry (*Celtis Caucasica*) seeds were inoculated by 100 gr rhizosphere materials of corn including spore, hyphae, and root fragments. On the other hand, *G. intraradices* and *G. mosseae* were used as exotic fungi to compare with native ones. After that, the samples were irrigated for three months in a normal condition, and then they were treated under drought stress for three months (Amjadian et al., 2021).

2.4. Experimental design

The effects of mycorrhizal fungi (*Glomus intraradices*, *Glomus mosseae*, *Funneliformis geosporum*, *Claroideoglomus etunicatum*, combination of F&C, and non- mycorrhiza as control) and drought stress (optimal irrigation: 75% FC as a control, 50% FC, and 25% FC) as factorial in a completely randomized design with three replications were evaluated on morphological traits including the fresh and dry weight of shoot and root, length of root, seedling height, colonization percentage, and biochemical traits including the content of protein, catalase activity, superoxide dismutase activity, peroxidase activity and content of chlorophyll a&b, total chlorophyll and carotenoid (Haghshenas and Ghanbari Malidarreh, 2021; Ghasemi et al., 2021)

2.5. AMF colonization

Root samples of Caucasian hackberry were cleared and stained as described by Phillips and Hayman (1970) and the percentage of mycorrhizal root colonization was determined by visual observation of fungal

colonization (Phillips and Hayman, 1970). Percentage of AMF colonization was calculated with the following formula:

$$Colonization\ (\%) = \frac{Number\ of\ mycorrhizal\ root\ pieces}{Total\ number\ of\ observed\ root\ pieces} \times 100$$

2.6. Chlorophyll and carotenoid determination

Before extracting the chlorophylls content, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Extraction was carried out using 1 gr leaf sample ground in 80% acetone in pestle and mortar. The absorbance was measured using a UV/Visible spectrophotometer (Shimadzu UV-160) and the concentration of chlorophylls was calculated based on Arnon (Arnon, 1949).

2.7. Measurement of H2O2 and MDA

H_2O_2 extraction was measure according to Loreto and Velikova (Loreto and Velikova, 2001). So that 0.35 g of leaves was homogenized with 5 ml 0.1% (w/v) trichloroacetic acid (TCA) in an ice bath. The homogenate was centrifuged at 12,000×g for 15 min and then 0.5 ml of supernatant was transferred to a new tube and mixed to 0.5 ml 10 mM phosphate buffer (pH 7.0), and 1 ml 1 M KI. The assay mixture was kept in dark for 1 h and the absorbance was read at 390 nm. The concentration of H_2O_2 was determined from a standard curve and expressed as μmol H_2O_2 g^{-1} fresh weight. By measuring the concentration of malondialdehyde (MDA), Lipid peroxidation in leave was detected (Heath and Packer, 1968). According to this method, 0.2 g of fresh leaves powdered with 5 ml 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 8,000 ×g for 5 min, 1 ml of supernatant transfer to the new tube then added 4.5 ml 20% TCA. The homogenate was centrifuged at 4,000×g for 10 min, the absorbance was read at 532 nm. The unspecific turbidity was corrected by A_{600} subtracting from A_{530}. The concentration of MDA was calculated using an extinction coefficient of 155mM/cm.

2.8. Protein content and antioxidant enzyme activity

Protein extraction was carried out according to Bradford (1976) using Bovine Serum Albumin V as a standard (Bradford, 1976). For enzyme assays, frozen leaf samples were ground to a fine powder with liquid nitrogen and extracted with 50 nM phosphate buffer (pH 7.0). The extracts were centrifuged at 4 C for 30 min at 20000×g and after that supernatant was collected and used for protein content assay and enzyme activities. Catalase activity was estimated. The reaction mixture contained 100 μL crude extracts, 50 μL 30% H_2O_2, and 3000 μL 50 mM potassium phosphate buffer. The decrease in the absorbance was recorded at 240 nm for 1 min by a spectrophotometer. Catalase activity of the extract was expressed as ΔOD mg^{-1} protein min^{-1}. Superoxide dismutase activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium according to the method of (Dhindsa et al., 1982). The reaction buffer containing 100 mM phosphate buffer (pH 8 .7), 12 mM Methionine, 75 mM nitroblue tetrazolium, 100 μM EDTA, and 0-100 ml of enzyme extract was added 2 mM riboflavin. The reaction was allowed to run for 15 min after which the light was switched off and the absorbance read at 560 nm. One unit of superoxide dismutase activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of nitroblue tetrazolium. Superoxide dismutase activity of the extract was expressed as ΔOD mg^{-1} protein min^{-1}. The activity of POD (EC 1.11.1.7) was determined using the guaiacol oxidation method (Chance and Maehly, 1955). That adding an aliquot of the tissue extract (100 μL) to 3ml of assay solution, consisting of 3 ml of reaction mixture containing 13 mM guaiacol, 5mM H_2O_2 and 50 mM k-phosphate (pH 6.5). An increase of the optical density at 470 nm for 1 min at 25 °C was recorded using a spectrophotometer. POD activity was expressed as a change in absorbance min/mg protein. The increase in A470 was measured for 3 min and activity expressed as a 470 mg protein/min (Modanlo et al., 2021; Rabbani and Safdary, 2021).

2.9. Statistical analysis

Data were analyzed using two-way ANOVA (SAS 9.2). AMF and irrigation were used as the first and second factors, respectively. In order to determine the significance of means, Duncan's multiple range test (DMRT) was performed using SAS 9.2 software.

3. Results and Discussion

3.1. Morphological parameters AMF colonization

Effect of drought stress, fungus and interaction of drought stress × fungus on shoot fresh weight, root fresh weight, shoot dry weight, root length and seedling height, and percentages of AMF colonization in roots of inoculated plants were significant at P≤ 0.01, and effect of fungus on root dry weight was significant at P≤ 0.01 (Figs. 1 and 2). As it stands, the use of mycorrhizal fungi in drought stress conditions increased shoot and root fresh weight and shoot dry weight in comparison to the control. Drought stress decreased shoot and root fresh weight and dry weight so that the lowest value of this parameter was observed in the non-mycorrhizal plant (control). In contrast, using exotic mycorrhizal fungi, *Glomus intraradices*, and *Glomus mosseae*, under drought stress increased these morphological properties at least twice and the native fungi and combination of them resulted in a 2.5-fold increase in comparison to the control. Besides, a combination of native fungi (F&C) had the greatest impact on root dry weight (Fig. 1). The highest percentage of colonization was observed in F&C, while the lowest percentage was obtained in exotic fungi and *Funneliformis geosporum* under drought stress of 25% FC (Fig. 2). The results showed drought stress reduced colonization of roots. Like other morphological traits, mycorrhizal fungi had a positive effect on seedlings height. In addition, the highest height of seedling was observed in *Funneliformis geosporum*, *Claroideoglomus etunicatum* and F&C under normal irrigation (Fig. 2). Generally, the drought stress increased the root length so that the highest value was obtained at the F&C in 50% FC and *Funneliformis geosporum* in normal irrigation treatments.

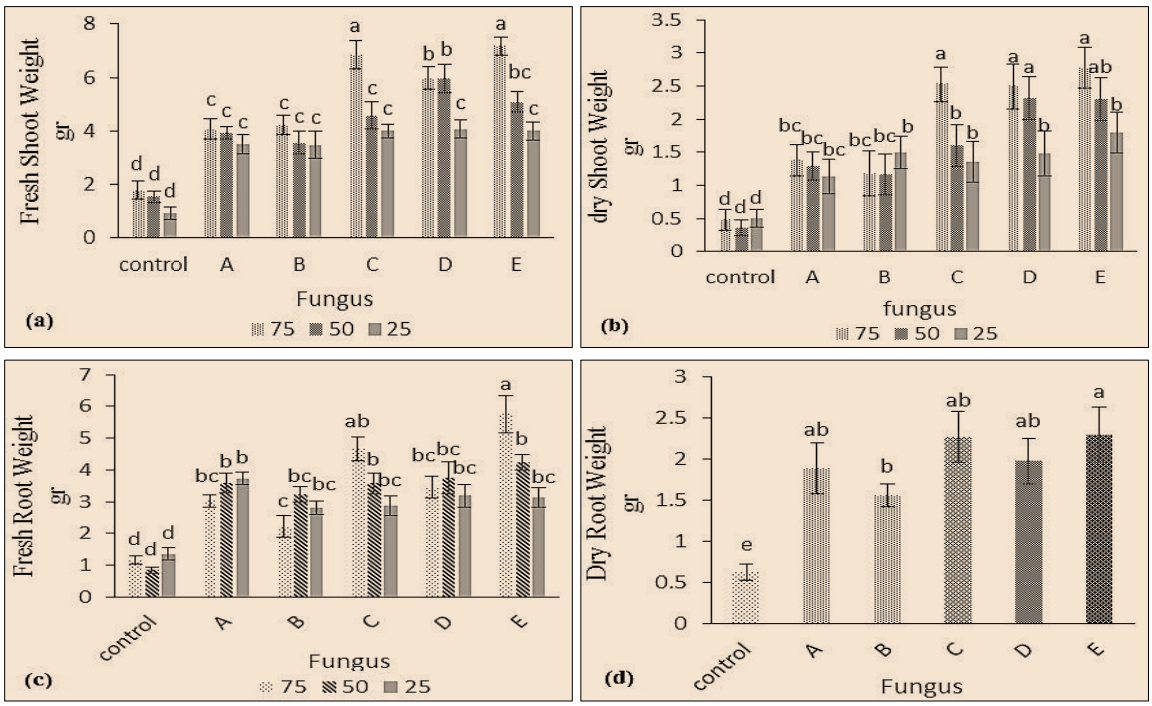

Fig. 1. Effect of drought stress and mycorrhizal fungi on morphological traits; (a) fresh shoot weight, (b) dry shoot weight, (c) fresh root weight, (d) dry root weight, (e) Height and (f) Length of the root. Fungal treatment includes (A) *Glomus intraradices*, (B) *Glomus mosseae*, (C) *Funneliformis geosporum*, (D) *Claroideoglomus etunicatum*, (E) combination of native (F&C) and non-mycorrhiza as a control.

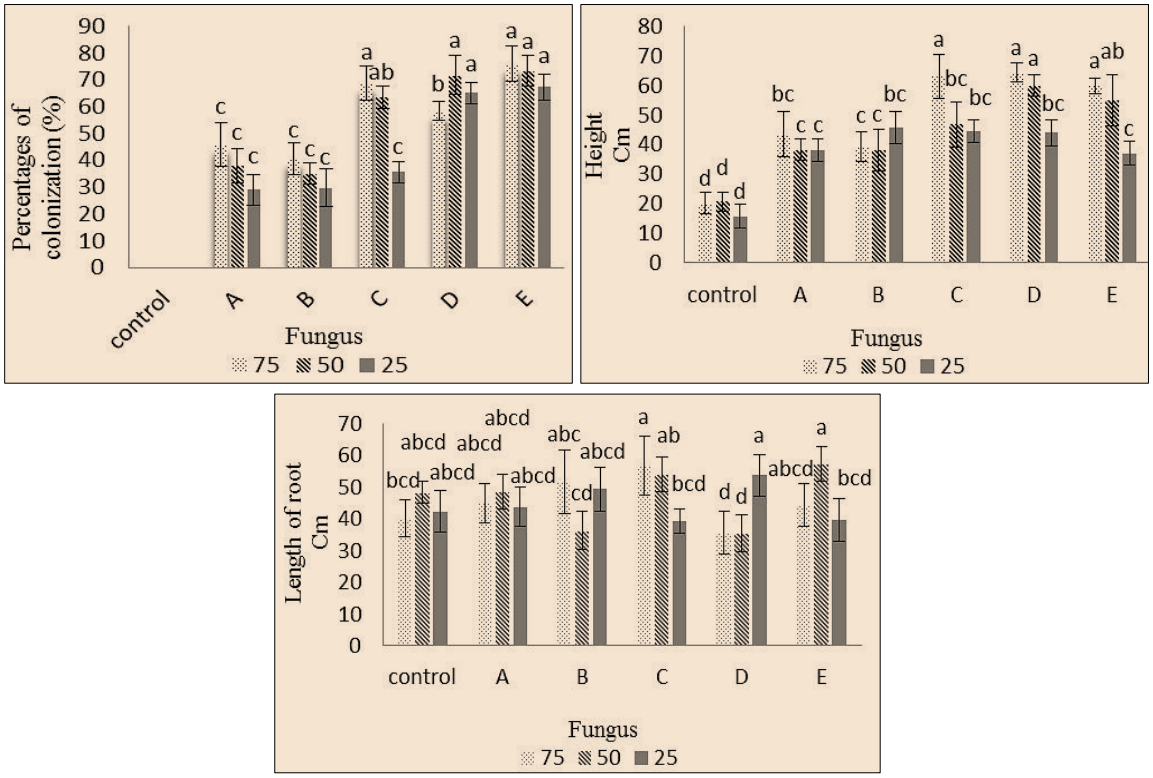

Fig. 2. Effect of drought stress and mycorrhizal fungi on Percentages of AMF colonization in roots of inoculated plants, length of seedling and length of the root. Fungal treatment includes (A) *Glomus intraradices*, (B) *Glomus mosseae*, (C) *Funneliformis geosporum*, (D) *Claroideoglomus etunicatum*, (E) combination of native (F&C) and non-mycorrhiza as a control.

3.2. H2O2 and MDA content

Drought stress increased H_2O_2 in all treatments (Fig. 3), the highest content of H_2O_2 was observed in a non-mycorrhizal treat (control) at 25% FC. Application of *Glomus intraradices*, *Glomus mosseae*, *Funneliformis geosporum*, *Claroideoglomus etunicatum*, and a combination of F&C reduces the H_2O_2 content. As well as, the concentration of MDA increased under drought stress conditions (Fig. 3) so the highest content of MDA was observed at 25% FC of a non-mycorrhizal treat. Using AMF induces the MDA content. Like H_2O_2, the lowest content of MDA, it was found in the combination of F&C at 75% FC.

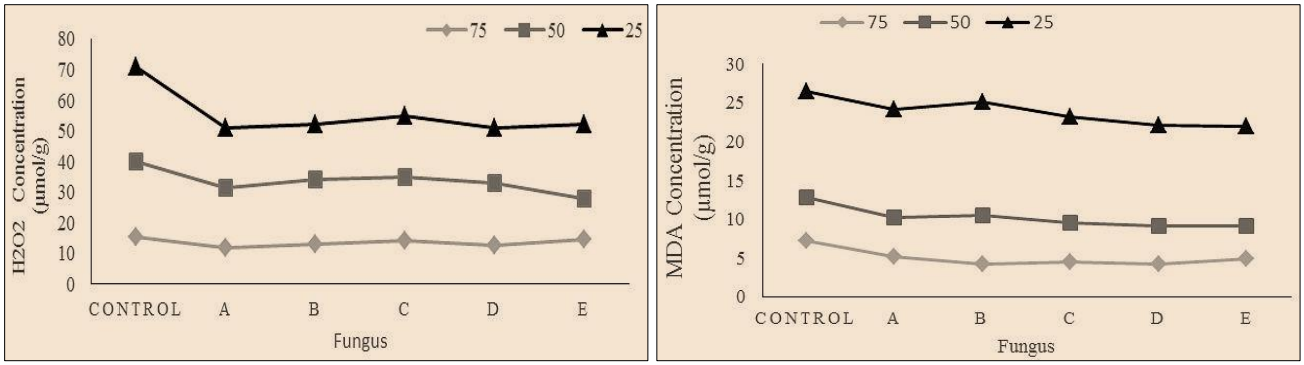

Fig. 3. Effect of drought stress and mycorrhizal fungi on H_2O_2 and MDA content. Fungal treatment includes (A) *Glomus intraradices*, (B) *Glomus mosseae*, (C) *Funneliformis geosporum*, (D) *Claroideoglomus etunicatum*, (E) combination of native (F&C) and non-mycorrhiza as a control.

3.2. Antioxidant enzyme activity

Drought stress increased antioxidant enzyme activity; this trend can be seen in the activity of CAT, SOD and POD (Fig. 4). CAT activity increased under drought stress in both conditions (with and non-mycorrhiza

application). In the control condition, with increasing the stress up to 25% FC, the activity of this enzyme was increased, as well as use of exotic and native fungi increased enzyme activity. The highest catalase activity was observed at 50% of FC and application of F&C, *Claroideoglomus etunicatum* and *Funneliformis geosporum*, respectively. Interestingly, in native fungi, catalase activity was higher than in exotic fungi. Drought stress increased the activity of SOD, with increasing stress up to 50% of FC enzyme activity also reached its greatest extent. The SOD activity was reduced in 25% of FC. Like CAT, the highest SOD activity was observed at 50% of FC and application of F&C, *Claroideoglomus etunicatum*, and application of *Glomus intraradices* in 50 and 25% FC. The levels of POD activity, as well as two other antioxidant enzymes, increased under drought stress. The highest activity of this enzyme was observed in order in 50% FC and F&C and *Glomus mosseae.*

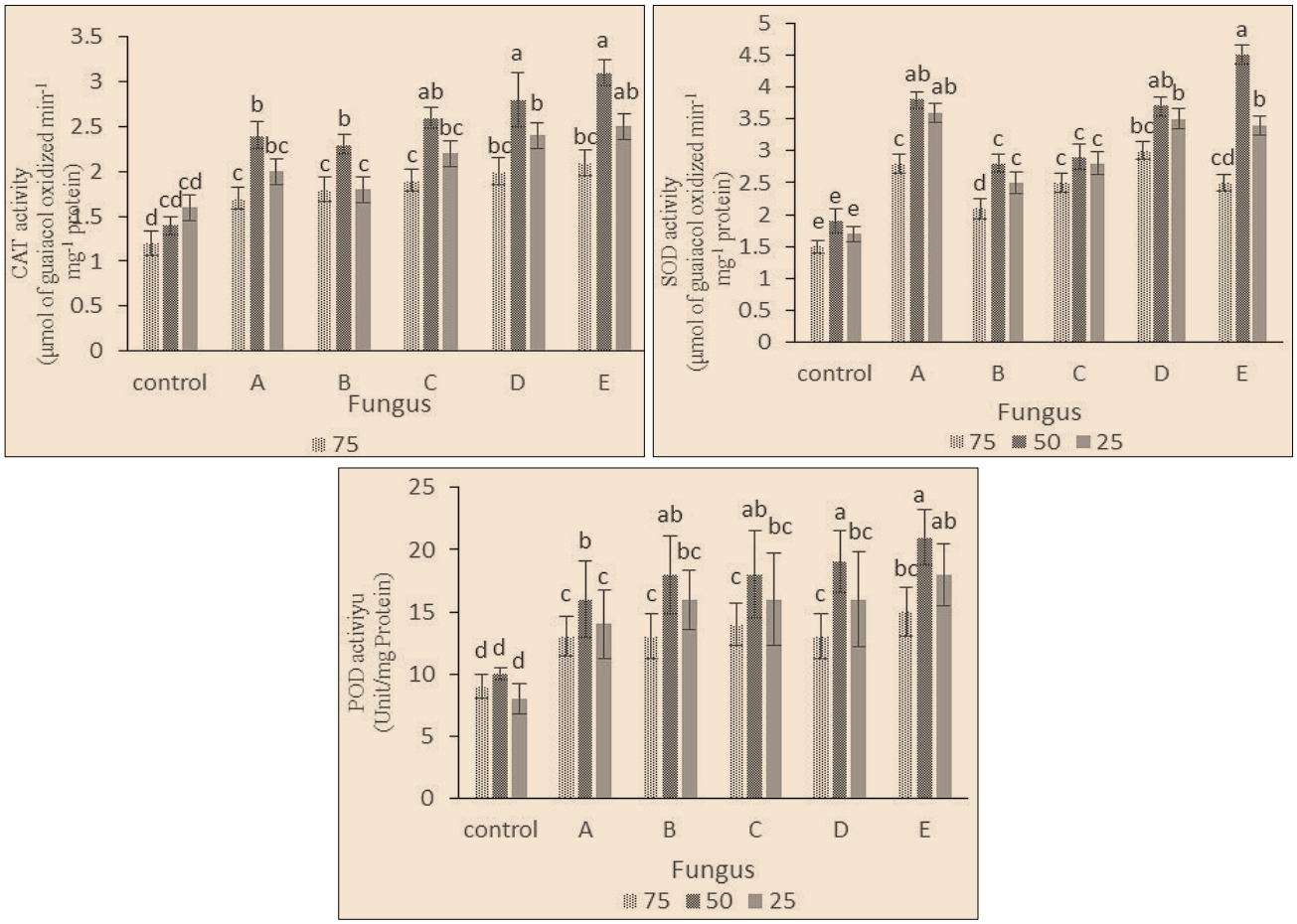

Fig. 4. Effect of drought stress and mycorrhizal fungi on antioxidant activity enzymes; (a) CAT, (b) SOD, (c) POD. Fungal treatments include (A) *Glomus intraradices*, (B) *Glomus mosseae*, (C) *Funneliformis geosporum*, (D) *Claroideoglomus etunicatum*, (E) mix of native (F&C) and non- mycorrhiza as a control.

3.3. Chlorophyll and carotenoid content

In AMF treatments, the content of chlorophyll a,b increased compared to the control (Fig. 5). Drought stress reduced chlorophyll a,b and in all treatments, the highest amount of chlorophyll a,b and total chl was recorded in 75% FC. In addition, the highest content of chlorophyll a and b were in F&C and *Claroideoglomus etunicatum* in 75% FC. As is seen in Fig. 4, there was found no significant difference between fungal treatments at 25% FC and 75% FC of control for chlorophyll a. As an undeniable advantage of fungal treatments, they increased 2 to 3 folds the amount of chlorophyll. There was not recorded a significant difference for the interaction of fungi × drought stress on the carotenoid content. Besides, the highest value of this trait was observed in F&C, and mycorrhizal fungi had no significant difference together.

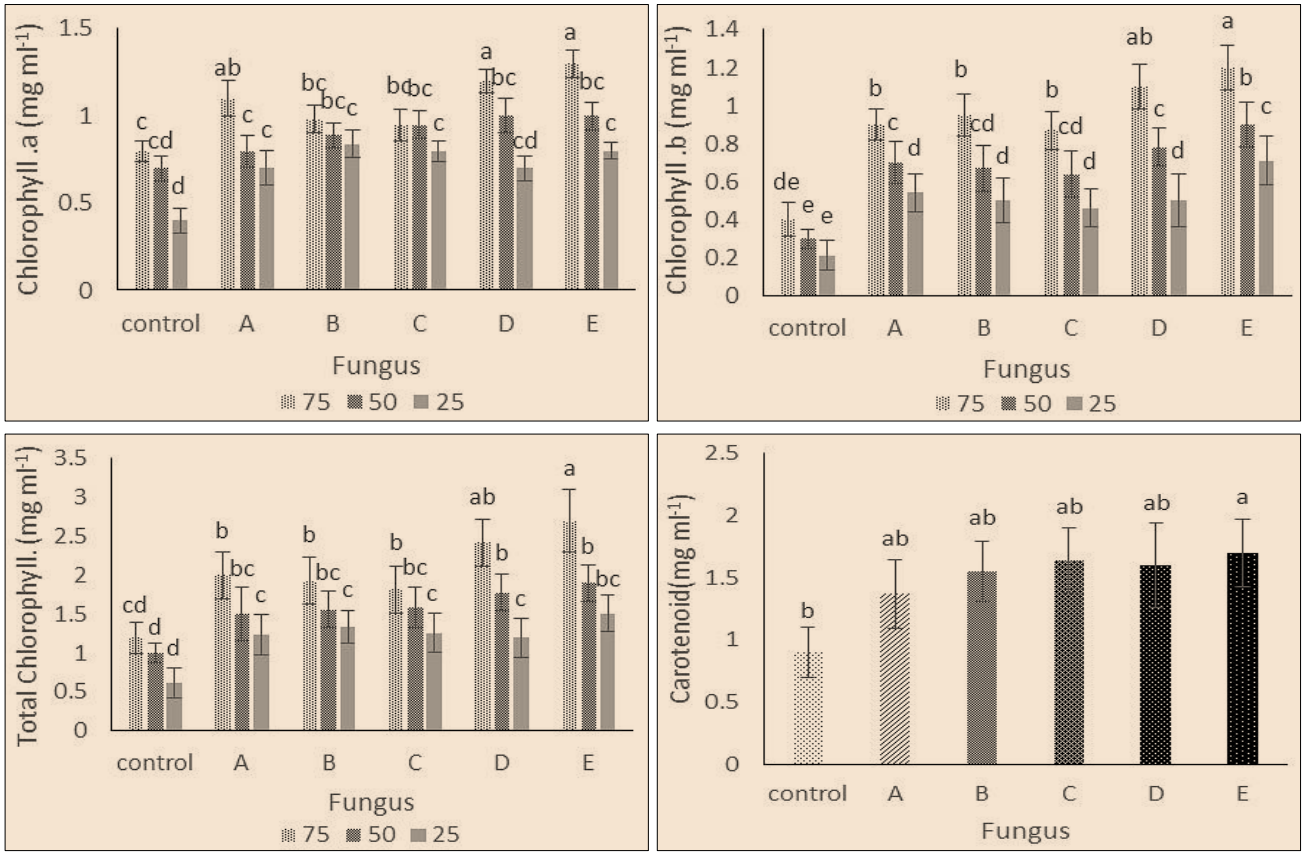

Fig. 5. Effect of drought stress and mycorrhizal fungi on chlorophyll a,b, total chl and carotenoid content; (a) chl a, (b) chl b, (c) total chl and (d) carotenoid . Fungal treatments include; (A) *Glomus intraradices*, (B) *Glomus mosseae*, (C) *Funneliformis geosporum*, (D) *Claroideoglomus etunicatum*, (E) mix of native (F&C) and non-mycorrhiza.

Based on the results of this study, mycorrhizal symbiosis had a significant effect on fresh and dry weight, of above and underground biomass and percentage of colonization. As is clear from Figs. 1 and 2 in all treatments, by increasing water stress, a fallen trend of biomass plants was observed. Using the *Glomus intraradices* and *Glomus mosseae* as exotic fungi have led to a two-fold increase in biomass. It's very impressive; use of mycorrhizal fungi has been increased dry matter so that under drought stress at 50 and 25% FC the reduction in biomass is much less than that found in the control treatment. These two fungi have not a significant difference in normal irrigation treatment. On the other hand, the use of native fungi has a far greater impact on biomass. As previously noted, the highest shoot fresh and dry weight were obtained in F&C and *Funneliformis geosporum* under normal irrigation. Treatment of *Claroideoglomus etunicatum* have less impact on shoot fresh weight compared to *Funneliformis geosporum* and mix of them but have been more stable in 50% FC. In fact, leaves are the main cause of dry matter production and photosynthesis (Kapulnik at al., 1985). Mycorrhizal fungi increase the number and leaf area, leading to increased growth and photosynthesis and more production of carbohydrates for themselves (Cartmill et al., 2008, Wu et al., 2005). They also increase the uptake of nutrients from the soil and enzymatic and non-enzymatic activities, which increase root growth and shoots dry matter yield (Cartmill et al., 2008). Several reports have shown that in 80% of mycorrhizal plants, shoot dry weight was more than that in non-mycorrhizal plants (Giovannetti et al., 1994). Such a process due to a gradual increase in the absorption of solar radiation synchronous increase the percentage of the canopy and thus will increase the rate of dry matter accumulation in plants (Asea et al., 1988). As is observed from Figs. 3 and 4, similar to biomass, the use of mycorrhizal fungi increases the roots fresh, dry weight, root length, and seedling height compared with control. What is interesting here is the significant increase of root weight in *Glomus intraradices*, *Glomus mosseae* and control by enhancing drought stress. This can be interpreted so that with increasing drought

stress, plants increase the length of roots, thus root weight is also increased. The highest root fresh weight was observed in F&C in normal irrigation. (Manjunath et al., 1983) stated that inoculation with mycorrhizal fungi increases the shoot dry weight, roots and most morphological traits of the plant. Mycorrhizal fungi have a significant effect on uptake of nitrogen and phosphorus for plant growth and seedlings height. On the other hand, Mycorrhizal symbiosis delayed the leaf water potential drop under drought stress, also Mycorrhizal plants are much faster than non-mycorrhizal plants in absorbing the water and leaf water potential to reach a balance (Wu et al., 2005). Mycorrhizal plants due to their much-developed leaf area, lead to a decrease in transpiration. In addition, mycorrhizal fungi cause better osmotic adjustment and improve the relationship between water and plant by extending the hyphae around the root system and subsequently increasing the water absorption (Fagbola et al., 2001; Johnson and Hummel, 1985). Mycorrhizal fungi may change the shape and size of the roots in two aspects including: 1) Nutritional status change, 2) Change in the synthesis of plant hormones (Yao et al., 2005). As well as changes in the branches and volume of roots is affected by nutrients, especially phosphorus (López-Bucio et al., 2003). In fact, growth in mycorrhizal plants is due to increasing the enzyme activity such as nitrate reductase and glutamine synthetas and increase the absorption of nutrients such as phosphorus and potassium (Azcón and Tobar, 1998; Poss et al., 1985; Bever et al., 2009).

The concentration of H_2O_2 and MDA increased under drought stress but the application of AMF cause prevents them from rising further. The exotic and native AMF had no significant difference, however, interestingly, native AMF were more effective. Previous works suggest that AMF reduces the H_2O_2 and MDA (Wu and Zou, 2009; Zhu et al., 2010; Benhiba et al., 2015) and this is probably due to the increased activity of antioxidant enzymes in AMF treatment.

Results indicated in non-mycorrhiza treatment, the activity of CAT increased with the increasing of drought stress. As *Celtis caucasica* is tolerant to water deficit, this is expected that this trend can be observed in the mentioned condition. However, the use of mycorrhizal fungi of *Glomus intraradices*, *Glomus mosseae*, *Funneliformis geosporum* and *Claroideoglomus etunicatum* increases the activity of this enzyme. As previously noted, the highest catalase activity was observed in order at 50% of FC and application F&C, *Claroideoglomus etunicatum* and *Funneliformis geosporum*. In fungal treatments, the highest enzyme activity was observed in 50% FC and enzyme activity also decreased with reducing the moisture level to 25% FC, however, it is still higher than that in non-mycorrhizal treatments. Physiological and genetic evidence have clearly defined the antioxidant systems of plants is an important component of protective mechanisms against stress (Sairam et al., 2000). This enzyme plays an important role in the removal of H_2O_2 produced by processes such as β-oxidation of fatty acids, oxidation during photorespiration and electron transport in the mitochondrial respiratory chain (Fumanal et al., 2006). The results of the research presented by (Becana et al., 2000) indicated that the activity of antioxidant enzymes such as CAT, APX, and GPX in legume plants inoculated with mycorrhizal fungi was higher than that in control plants.

The activity of SOD increased by 50% FC in non-mycorrhizal treatments. The highest SOD activity was observed at 50% of FC, application F&C, application of *Glomus intraradices* in 50% FC, *Claroideoglomus etunicatum* and application of *Glomus intraradices* in 25% FC. We can say that the impact of *Glomus intraradices* fungus on the activity of this enzyme has been more than the native fungi. SOD in the cell is the first line of a defense system against ROS (Foyer et al., 1994). This enzyme catalyzes the revival of radical superoxide to hydrogen peroxide and molecular oxygen. Subsequently, hydrogen peroxide generated by SOD will be catalyzed by Ascorbate peroxidase (Zeid and Shedeed, 2006). Similar to CAT, mycorrhizal fungi increase the activity of SOD. The activity of POD increased under drought stress. Similar to other enzymes, the POD activity increased in mycorrhizal treatment. Peroxidase decomposes hydrogen peroxide in the cells and thus decreases ROS production. Many studies have shown that mycorrhizal fungi have a positive effect on host plants and make resistant plants to environmental stresses. As regards, *Celtis caucasica* is resistant to drought stress, the activity of CAT, SOD and POD increased under drought stress in the control condition (non- mycorrhiza), nevertheless, that's a symbiosis with mycorrhizal fungi caused about 1.5 fold increase in the activity of these enzymes under

drought stress. The results suggest that mycorrhizal fungi by increasing the production of biomass enable plants to withstand stress conditions. The fungi lead to increased uptake of phosphorus and potassium by more production of hyphae and better penetration in soil pores, increasing the effective surface area of roots through hyphae, and changing chemical properties in the rhizosphere area (Abbott and Robson, 1985; Bolan, 1991; Tarafdar and Marschner, 1994). Oxidative enzymes in roots shoot and leave are important and the high level from them protection plants against free radicals. mycorrhizal fungi provide material for making proteins so that the plants When necessary at higher levels of the enzyme are expressed in plants. Also when plants are not under drought stress it's not necessary for the expression of these enzymes (Maheshwari and Dubey, 2009).

As shown in Fig. 5, chlorophyll a,b content reduced with increasing drought stress, while using of mycorrhizal fungi increases chlorophyll a,b. Mycorrhizal fungi have increased chlorophyll a,b and total chlorophyll. The lowest value of chlorophyll a,b and total chlorophyll was observed in 25% of FC in non-mycorrhizal treatments. F&C in 75% FC has increased 1.5 fold compared to control. Previous studies have also shown that the concentration of chlorophyll in plants treated with arbuscular mycorrhizal fungi is higher than that of non-mycorrhizal (Kapoor et al., 2008). Increasing the amount of chlorophyll a and b in *Prosopis juliflora* colonized with *G.fasciculatum* has been reported (Selvaraj and Chellappan, 2006). The high concentration of chlorophyll in mycorrhizal samples is due to high phosphorus uptake during photosynthesis as an energy carrier (Selvaraj and Chellappan, 2006). On the other hand, mycorrhizal fungi due to increasing the absorption of essential elements in chlorophyll biosynthesis (including magnesium and iron) can increase the production of these pigments and ultimately increase the rate of photosynthesis (Krishna et al., 2005). The rate of photosynthesis in mycorrhizal plants is more than in non-mycorrhizal plants because of the impact of mycorrhizal symbiosis in keeping open the stomatal (Augé et al., 1987). Different species of mycorrhizae have various performances in accelerating photosynthesis in the host plants under drought stress (Dixon et al., 1997). The effect of fungi × drought stress has no significant effect on the carotenoid content. As cited, mycorrhizal fungi have increased the content of carotenoid (non- mycorrhiza). It is necessary to mention that the highest value of this trait has been observed in F&C and there has not been recorded a significant difference among mycorrhizal fungi. Carotenoids are tetraterpenoids pigments that are produced in chloroplast and Chromoplast (Corona et al., 1996). Generally, we can say that the use of mycorrhizal fungi, especially indigenous ones make a symbiotic relationship with the tree roots so that increase the chlorophyll content, which results in an increase of production rates. Thus, the greenery and the establishment of seedlings in natural conditions are more appropriate in respect to non-natural conditions. Under stress conditions, germination of spores and proliferation of fungal hyphae are limited, so the ability of mycorrhizal symbiosis decreases. The colonization percentage of seedlings under drought stress at 25% FC was less than that at 50 and 75% FC. These results have shown the higher moisture content in soil has increased mycorrhizal activity. Colonization percentage has ranged 45-85% and 15-40% in native and exotic fungi, respectively. Spore germination is correlated with soil moisture content. Increasing soil moisture grows up mycorrhizal activity with the spreading hyphae network in root and soil. So this mechanism increases the level of root to uptake more available nutrients for plants.

4. Conclusion

AMF-symbiosis can improve the important morphological traits of *Celtis caucasica* and this can be contributed to the better initial establishment of seedlings. On the other hand, AMF decreases the content of H_2O_2 and MDA. As well as, the activity of antioxidant enzymes in the mycorrhizal plant was more than a non-mycorrhizal plant. Significantly, the percentage of colonization on native AMF has been more than exotic, therefore it can be concluded the native symbiosis has an efficient effect on *Celtis caucasica* seedlings in respect to exotic fungi.

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