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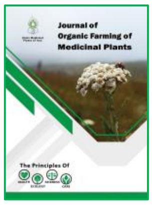


Original Article

# Application of mycorrhiza and phosphorus improve the phosphorus uptake, physiological characteristics and growth of coneflower (*Echinacea purpurea* (L.) Monch) under drought stress

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ABSTRACT

Coneflower is one of the well-known medicinal plants that has been imported to Iran for several years and has attracted the attention of the pharmaceutical industry and researchers. Considering the issue of drought stress and the need to use stress modifiers, this research was conducted to investigate the effect of mycorrhizal fungi and the application of phosphorus under drought stress on nutrient uptake, some physiological characteristics and the growth of coneflower in pot culture. The experiment was carried out in the research greenhouse of Yasouj University as factorial based on a completely random design with three replications. A combination of irrigation levels (irrigation after 10 (control), 30 and 60% soil water depletion (SWD)), inoculation (mycorrhizal fungi and no inoculation) and phosphorus application (phosphorus amount in the soil and twice the amount of phosphorus in the soil) were used. At 10% SWD, phosphorus consumption alone and in combination with mycorrhiza caused an increase, respectively, 105 and 82.83% of phosphorus in root and shoot. The application of mycorrhizal fungus alone caused an increase of 25.59% in leaf soluble proteins and 17.09% in carotenoids. At 30% SWD, the highest amount of proline and catalase was related to the application of mycorrhizal fungus and twice the consumption of soil phosphorus, which were 1.42 and 2.02 times compared to the treatment of not using mycorrhiza and twice the consumption of phosphorus in the soil. Also, at 30% SWD, mycorrhizal inoculation caused a significant increase (44.64%) in root dry weight. At 60% SWD, inoculation with mycorrhiza significantly increased (38.68%) the amount of phenol in aerial parts. In general, stress decreased the uptake of nutrients and increased proline content and catalase activity, but mycorrhiza and phosphorus nutrition could mitigate the effect of stress in potted conditions.



## 1. Introduction

Coneflower (*Echinacea purppurea* (L.) Monch) is a herbaceous and perennial plant of the Asteraceae family, which is used for the treatment of respiratory tract infections due to its antioxidant and antimicrobial activities (Luo et al., 2011), and due to the polysaccharide and polyethylene compounds of the extract, it is effective in treating lung infections and bronchitis (Karsch-Völk et al., 2014). Therefore, due to its many medicinal properties and its high adaptability to different weather conditions, coneflower cultivation has expanded in Iran. Phenolic compounds are the main active ingredients of coneflower. The percentage of phenolic compounds in the extract of coneflower plants is affected by factors such as environmental conditions and different nutritional systems (Jakopic et al., 2007).

Water is important for the production and growth of plants because it plays a significant role in the absorption of nutrients and the solubility and movement of substances in plants (Khalafallah and Abo-Ghalia, 2008). Water limitation is an important factor in the metabolic activities of plants, which generally has a negative effect on the growth and development of plant species (Bettaieb Rebe et al., 2012). The most common factor limiting the production of agricultural plants, especially in arid and semi-arid regions, is drought stress, which affects the physiological, morphological, and biochemical characteristics of plants and causes stomata to close, reduces photosynthesis and growth rate, plant height, and the fresh and dry weight of the crops (Xu et al., 2019).

The occurrence of severe drought stress, together with oxidative damage caused by reactive oxygen species (ROS), through damage to macromolecules and cell membranes can lead to cell death (Ozkur et al., 2009).

Phosphorus is an essential and widely used element for the optimal growth of all plants. Phosphorus is present in the structure of nucleic acids, phospholipids and coenzymes that activate the production of amino acids, so it plays an important role in metabolic processes (Antonios et al., 2016). However, in most agricultural systems, due to the low level of phosphorus mobility in the soil, the absorption of this element by the plant is reduced (Smith, 2002). The limitation of absorbable soil phosphorus restricts the growth of plants, so this element is proposed as the main key to agriculture (Sarikhani et al., 2014). The importance of this issue in Iranian agricultural lands is twofold because due to the calcareous nature of the soils in arid and semi-arid areas, the phosphorus solubility of the soil is very low (Tasdighi et al., 2015). In this regard, the addition of phosphorus fertilizers to the soil environment partially neutralizes the direct and indirect effects of drought on phosphorus absorption and increases the efficiency of water consumption and, as a result, tolerance to drought in the plant (Jones et al., 2003). At the same time, the use of chemical fertilizers has always been associated with environmental risks, and the investigation of renewable inputs with maximum agricultural productivity has become widespread to deal with the consequences of drought stress (Perry et al., 2011).

Soil fertility and plant health depend on the symbiotic relationship in the rhizosphere. An alternative solution to improve tolerance to drought stress and reduce dependence on chemical fertilizers is the symbiosis of mycorrhizal fungi, which has been identified as an integral part of organic agriculture for many crops today (Georgy and Rey, 2023). Mycorrhiza increases the hydraulic conductivity of water in the root and increases the absorption of nutrients from the soil by increasing the effective length of the root (Berude et al., 2015). The colonization of the host plant root by mycorrhizal fungi leads to an increase in seed yield, essential oil yield and the content of phenolic compounds under water stress conditions (Bączek et al., 2019; Weisany et al., 2017). The improvement of the physiological characteristics of plants inoculated with mycorrhiza under stress conditions and the improvement of the plant's water needs have been mentioned by many researchers (e.g. Kapoor et al., 2013; Latef et al., 2016; Rafat et al., 2018). Also, an increase in the height of the plant in lemongrass (De Assis et al., 2020) and an increase in the dry weight of roots and shoots in two species of *Thyme Daenensi* and *Thymus vulgaris* under drought stress (Arpanahi et al., 2020) have been reported with the use of mycorrhiza. Another role of mycorrhizal fungi is to create a synergistic relationship with micro-organisms that dissolve unabsorbable phosphates for various plants, including the chicory family, in such a way that increases phosphorus absorption and, as a result, leads to proper growth (Bauma et al., 2015).

Considering that there is little research on the effect of phosphorus and mycorrhiza under drought stress conditions on the physiological characteristics of coneflower, this research was conducted to investigate the interaction of drought stress, mycorrhiza fungus and phosphorus fertilizer on nutrient uptake, some physiological characteristics and growth of coneflower.

## 2. Materials and Methods

This experiment was carried out based on a completely randomized design with three replications in the research greenhouse of Yasouj University. The experimental factors include irrigation (irrigation after 10 (control), 30 and 60% soil water depletion (SWD)) and mycorrhizal fungi (inoculated and without inoculation (as a control)) and phosphorus application (phosphorus amount in the soil and twice the amount of phosphorus in the soil).

Before the experiment began, sterilized seeds (for one minute in 70% ethanol and also for two minutes in 2% sodium hypochlorite solution) were placed one centimeter deep in special trays filled with peat moss and cared began for two months. Three seedlings were transferred to each pot at the time of 4-5 leaves. In this study, each test pot was prepared with a height of 25 cm and a diameter of 20 cm. The soil used in each pot had a ratio of 2:1 of field soil and sand and had a clay loam texture. Before planting the seedlings, the prepared soil was sterilized by an autoclave device and before transferring of the seedlings to the pots, the mycorrhizal (obtained from the Hamedan herbal medicine clinic) was contacted with the roots and then planting was done. The soil test showed the amount of phosphorus at 8 ppm. The amount of phosphorus in the soil was taken as a control and twice the amount of phosphorus in the soil was considered as the second level of phosphorus. Phosphorous fertilizer, from the source of triple superphosphate, was added to the soil at the time before transferring the seedlings to the pot.

Irrigation levels were applied by weight method. For this purpose, one of the pots was watered to the point of saturation and left for 24 hours until gravity water was removed from it. The amount of available water was considered as field capacity. Then, a 100-gram sample of potting soil was prepared and placed in a 100°C oven until its moisture content reached zero. The difference between moisture at field capacity and zero moisture, indicates the water available for the plant, and according to that, the irrigation treatments were applied after the plant was fully established (Nikbakht et al., 2023).

At the beginning of flowering, three plants in each pot were selected and completely removed from the soil along with the roots. To prevent the chemical composition of leaves from changing, the leaf samples were kept at -40°C until use.

The roots and shoots phosphorus content is determined by a calorimetric method (molybdate-vanadate yellow color) at 420 nm wavelength with a spectrophotometer (SHIMADZO 54A model) (Emami, 1996).

To measure leaf proline content, 1 ml of alcoholic leaf extract (95% ethanol), 10 ml of double distilled water, 5 ml of ninhydrin and 5 ml of glacial acetic acid were added and then the samples were placed in a boiling water bath for 45 minutes. In the next step, 10 milliliters of benzene were added to each sample and the absorbance of the supernatant was read at a wavelength of 515 nm using a spectrophotometer (SHIMADZO 54A model), and the concentration of proline was calculated based on the standard made with L-Proline (Paquin and Lechasseur, 1979).

Measurement of total soluble sugars (TSS) was done as 0.1 ml of the alcoholic extract was selected, then 3 milliliters of freshly prepared anthrone was added to it and placed in a boiling water bath for 10 minutes. The absorbance of the samples was read at a wavelength of 625 nm using a spectrophotometer (SHIMADZO 54A model) (Irigoen et al., 1992).

To measure catalase activity, 100 microliters of enzyme extract were added to 3 ml of 50 mM phosphate buffer with pH=7 containing 30 mM hydrogen peroxide, and the decrease in absorbance was read in 60 seconds at a wavelength of 240 nm by a spectrophotometer (SHIMADZO 54A model) (Aebi, 1984).

Measurement of leaf soluble proteins was done as 0.2 g of leaf tissue was homogenized in a mortar with 2 ml of 0.1 M phosphate buffer with an acidity of 6.8 and centrifuged for 15 minutes at a speed of 13,000 rpm at 4 degrees Celsius. Then, 2.5 ml of Bradford solution was added to 50 microliters of the supernatant and the absorbance of the sample was read at a wavelength of 595 nm with a spectrophotometer (SHIMADZO 54A model) (Kar and Mishra, 1976).

To measure leaf glycine betaine content, aqueous extract was prepared from 0.5 g of leaf tissue and diluted 1:1 with 2N sulfuric acid. After cooling, 0.2 ml of potassium iodide reagent was added to each of the samples, and the samples were centrifuged at 10,000 rpm and 0°C for 15 minutes. Then, the supernatant solution was read at a wavelength of 365 nm with a spectrophotometer (SHIMADZO 54A model) (Grattan and Grieve, 1992).

Measurement of carotenoid content was done as 0.2 grams of freshly chopped leaves were ground in a mortar with 10 ml of cold 80% acetone and 0.5 grams of calcium carbonate powder. The resulting mixture was centrifuged at 3000 rpm for 15 minutes. Then, using a UV-S spectrometer (Lambda EZ210 model), the light absorption of a carotenoid was read at a wavelength of 470 nm (Lichtenthaler, 1987).

Phenolic compounds of aerial parts are measured by extraction of phenol from dry plant samples. Extraction was done with 80% methanol and then staining with Folin–Ciocâlteu reagent. The absorbance of the samples was read at a wavelength of 720 nm. Gallic acid was used as a standard (Haji Mehdipour et al., 2009).

The plant height (average height of the main stem of 3 plants), and the dry weight of the shoots and roots of the plants were measured at the end of the growing season. Also, the components of chlorophyll fluorescence, including Fm (maximum fluorescence), Fo (minimum fluorescence) and Fm/Fv (maximum quantum efficiency) from each test plot were measured and recorded using a fluorometer (OS-1-) (FL) USA. Statistical analysis was done using SAS software (ver. 9.4) and graphs were drawn using Excel software. The LSD test was used to compare the averages of the main effects, and in the case of interactions being significant, the averages were compared using the L. S. Means procedure.

## 3. Results and Discussion

### *3.1 Root and shoot phosphorus content*

The interaction between mycorrhiza and phosphorus and irrigation and phosphorus was significant at the 1% probability level on root phosphorus content (Table 1). The highest phosphorus content in the root was obtained from the treatment of no mycorrhizal inoculation (control) and twice the consumption of soil phosphorus (11.59 mg. $g^{-1}$ dry weight) (Figure 1). At 10% and 60% SWD, phosphorus consumption caused an increase of 105% and 35.05% of phosphorus absorbed by the roots, respectively, although there was no significant difference between controlling phosphorus at 60% SWD (Figure 2). Also, the three-way interaction of irrigation, mycorrhiza, and phosphorus was significant at the five percent probability level on the shoot phosphorus content (Table 1). The highest amount of shoot phosphorus at 10% SWD was obtained from the combination of inoculation with mycorrhiza and the double use of phosphorus in the soil (6.71 mg. $g^{-1}$ dry weight), which is accompanied by an increase of 82.83% compared to the control treatment. The highest amount of this trait was obtained for the second irrigation level (30% SWD) from the combination of no mycorrhizal inoculation and twice the consumption of phosphorus (7.14 mg. $g^{-1}$ dry weight) (Figure 3).

The results indicate that with the consumption of phosphorus, the amount of absorbable phosphorus and phosphorus absorbed by the plant increased. It seems that the low mobility of phosphorus is one of the most important reasons for the reduction of phosphorus absorption from the soil environment, especially in the

Table 1. Analysis of variance (mean square) of the effect of Irrigation, Mycorrhiza and Phosphorus on phosphorus content, some physiological and agronomic traits of *Echinacea purpurea*

| S.O.V | df | Root phosphorus | Shoot phosphorus | Proline | Soluble sugars | Catalase | Soluble proteins | Glycine betaine | Carotenoid | Fm | Fv/Fm | Shoot phenol | Height | Root DW | Shoot DW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Irrigation (I) | 2 | 5.96$^{ns}$ | 1.30$^{ns}$ | 0.06$^{ns}$ | 9.98$^{ns}$ | 1759$^{**}$ | 126$^{ns}$ | 5.66$^{*}$ | 0.51$^{ns}$ | 1597$^{*}$ | 0.001$^{ns}$ | 12.70$^{ns}$ | 75.11$^{ns}$ | 17.64$^{*}$ | 2.29$^{ns}$ |
| Mycorrhiza (M) | 1 | 83.17$^{**}$ | 14.24$^{**}$ | 2.51$^{**}$ | 3.91$^{ns}$ | 17.05$^{ns}$ | 2267$^{**}$ | 6.45$^{*}$ | 1.44$^{*}$ | 2131$^{*}$ | 0.01$^{*}$ | 72.33$^{**}$ | 136.11$^{*}$ | 73.42$^{**}$ | 0.05$^{ns}$ |
| Phosphorus (P) | 1 | 44.66$^{**}$ | 23.52$^{**}$ | 3.19$^{**}$ | 2.29$^{ns}$ | 156$^{ns}$ | 1916$^{**}$ | 0.02$^{ns}$ | 0.01$^{ns}$ | 110$^{ns}$ | 0.006$^{ns}$ | 3.56$^{*}$ | 81.00$^{ns}$ | 115.47$^{**}$ | 13.01$^{*}$ |
| I × M | 2 | 3.68$^{ns}$ | 0.41$^{ns}$ | 0.88$^{**}$ | 1.34$^{ns}$ | 216$^{*}$ | 10.75$^{ns}$ | 1.51$^{ns}$ | 0.58$^{ns}$ | 680$^{ns}$ | 0.006$^{ns}$ | 15.42$^{**}$ | 1.44$^{ns}$ | 26.93$^{*}$ | 14.1$^{*}$ |
| I × P | 2 | 31.37$^{**}$ | 0.23$^{ns}$ | 0.81$^{*}$ | 14.34$^{ns}$ | 248$^{*}$ | 34.67$^{ns}$ | 1.50$^{ns}$ | 0.40$^{ns}$ | 3336$^{**}$ | 0.005$^{ns}$ | 7.62$^{ns}$ | 44.3$^{ns}$ | 50.38$^{**}$ | 9.43$^{*}$ |
| P × M | 1 | 107$^{**}$ | 1.97$^{ns}$ | 0.19$^{ns}$ | 215.16$^{**}$ | 2979$^{**}$ | 193$^{ns}$ | 0.0002$^{ns}$ | 0.50$^{ns}$ | 584$^{ns}$ | 0.0007$^{ns}$ | 6.58$^{ns}$ | 513$^{**}$ | 157$^{**}$ | 44.63$^{**}$ |
| I × M × P | 2 | 0.09$^{ns}$ | 5.85$^{*}$ | 1.25$^{**}$ | 37.05$^{ns}$ | 1454$^{**}$ | 87.19$^{ns}$ | 5.83$^{*}$ | 0.53$^{ns}$ | 1718$^{*}$ | 0.01$^{*}$ | 7.81$^{ns}$ | 3.11$^{ns}$ | 3.96$^{ns}$ | 8.75$^{*}$ |
| Errore | 24 | 3.02 | 1.26 | 0.14 | 12.38 | 833.34 | 129.07 | 1.08 | 0.20 | 494 | 0.002 | 5.77 | 28.63 | 5.48 | 1.81 |
| CV (%) | | 24.06 | 21.07 | 18.60 | 13.17 | 19.79 | 16.24 | 11.48 | 17.83 | 10.06 | 6.69 | 15.03 | 18.31 | 15.17 | 20.97 |

**, * and ns indicate significant at 1, 5 % probability levels and non-significant, respectively

face of water scarcity, but the use of mycorrhizal fungi has increased the absorption of nutrients, especially phosphorus (Kour et al., 2020). Mycelial branching of mycorrhizal fungi can penetrate the soil tissue and openings that are not accessible to the structure of the root hairs, and in this way, by forming a symbiotic relationship with the host plant and increasing the absorption level, it increases the absorption of water and nutrients, especially phosphorus (Mohammadi et al., 2014). In addition to this, the positive effectiveness of mycorrhizal fungi in mobilizing phosphorus and absorbing it, becomes possible with root surface development mechanisms, increasing hydraulic conductivity of water and facilitating the mass transfer of phosphorus, increasing transpiration in high water potential in the soil and helping to release phosphorus, releasing organic compounds that adjust pH and increasing the activity of the polyphosphate kinase enzyme (David et al., 2007).

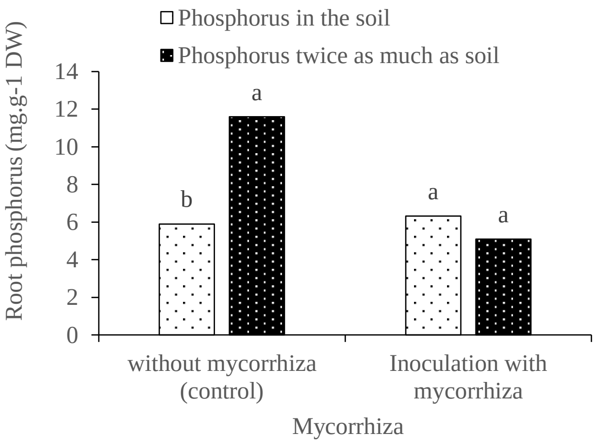


**Fig. 1.** Mean comparison of interaction of mycorrhizal and phosphorus for root phosphorus

### *3.2 Leaf proline content*

The interaction of irrigation, mycorrhiza and phosphorus was significant at the probability level of 1% on leaf proline content (Table 1). At the irrigation levels of 30 and 60%, the highest amount of proline was related to the use of mycorrhizal fungi and twice the use of phosphorus in the soil, with an increase of 1.42 and 1.41 times of proline, respectively, compared to the treatments without the use of mycorrhiza + phosphorus in the soil and control treatment (Figure 4).

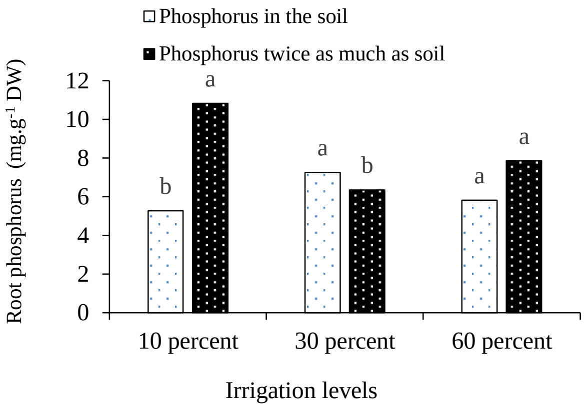


**Fig.2.** Means comparison of interaction of irrigation and phosphorus for root phosphorus

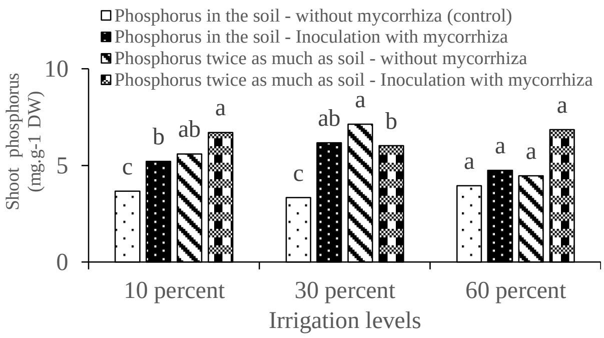


**Fig. 3.** Means comparison of interaction of irrigation, mycorrhiza and phosphorus for shoot phosphorus

Drought stress did not have much effect on proline accumulation in coneflower, but the application of phosphorus and mycorrhiza was able to have a significant effect on proline accumulation. During stress conditions, proline can act as osmotic adjustment, protection of cell membrane and protein structure, and also plays the role of scavenging free radicals. Proline is effective in increasing the water absorption capacity of the cytoplasm of cells and defending macromolecules such as enzymes (Deka et al., 2018). Proline acts as a source of nitrogen in stressful conditions in the plant, Mycorrhiza by absorbing more of these nutrients causes an increase in the production of proline and more resistance of the plant to drought stress. Also, mycorrhiza has caused an increase in proline in sorghum plants under water stress conditions (Attarzadeh et al., 2019).

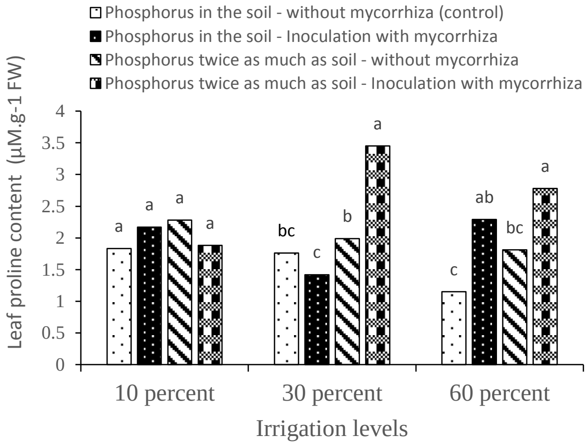


**Fig. 4.** Means comparison of interaction of irrigation, mycorrhiza and phosphorus for leaf proline content

### *3.3 Total leaf soluble sugars content*

The interaction of mycorrhiza and phosphorus was significant at 1% probability level in the leaf's total soluble sugar content (Table 1). In the treatment without inoculation with mycorrhiza, with the increase of phosphorus, the amount of soluble sugars increased by 14.18%, but in the treatment of inoculation with mycorrhiza, the use of phosphorus fertilizer led to a significant decrease in soluble sugars (Figure 5).

In the condition of water stress, the water potential of the soil decreases. As a result, the plant turns to osmotic adjustment to continue the absorption process due to the accumulation of osmotic compounds such as soluble carbohydrates. The symbiosis of mycorrhiza with plants is based on the exchange of carbohydrates and nutrients between the plant and the fungus, and the phosphorus element will also be effective in breaking down carbohydrates and synthesizing polysaccharides such as starch (Ebrahimi et al., 2022). Also, plant hormones such as cytokinin and gibberellin increase in inoculated plants. The increase of these hormone levels due to the effect on the transport of ions affecting the opening of the stomata is the factor in increasing the rate of photosynthesis and the carbohydrate content of plants (Arve et al., 2011). It has been reported that the amount of fructose, α-glucose, β-glucose, and sucrose as well as the total sugar content in pepper plants symbiotic with *Glomus intraradices* was significantly higher than in non-mycorrhizal plants (Demir, 2004).

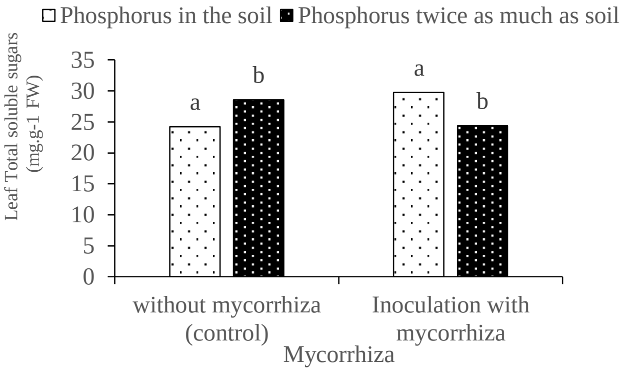


**Fig. 5.** Means comparison of interaction of mycorrhiza and phosphorus for leaf toral soluble sugars

### *3.4 Catalase enzyme activity*

The interaction of irrigation, mycorrhiza and phosphorus at the level of 1% had a significant effect on catalase activity (Table 1). The highest activity of catalase at the irrigation level of 10 and 30% SWD, respectively, corresponds to no mycorrhiza inoculation + no phosphorus consumption (55.58 mmol/g/min) and mycorrhiza inoculation treatment + twice the consumption of phosphorus in the soil (57.08 mmol/g grams per minute). No statistical difference was observed between the treatments at 60% SWD (Figure 6).

In stress conditions, photorespiration increases and leads to the production of ROSs in the plant due to the limitation of $CO_2$ absorption and stabilization and the increase of Rubisco activity. The activity of antioxidant enzymes such as catalase increases to remove these molecules. The increase in catalase activity to scavenge and clean the active oxygen species produced due to water stress is known as a resistance mechanism in plants, and the lower this increase is in plants under stress, it indicates the higher resistance of the plant to stress (Habibi, 2010). In line with this research, Mirzaei et al., (2021) reported that symbiosis with *Piriformospora indica* fungus activated the antioxidant system of garden thyme. In such a way the highest activity of catalase enzyme was obtained in the leaves of plants inoculated with fungus at 30% field capacity, which was a significant difference between non-inoculated plants. Therefore, it can be concluded that arbuscular mycorrhizal fungi modify the risks caused by stress on plant growth in terms of improving the antioxidant defense system.

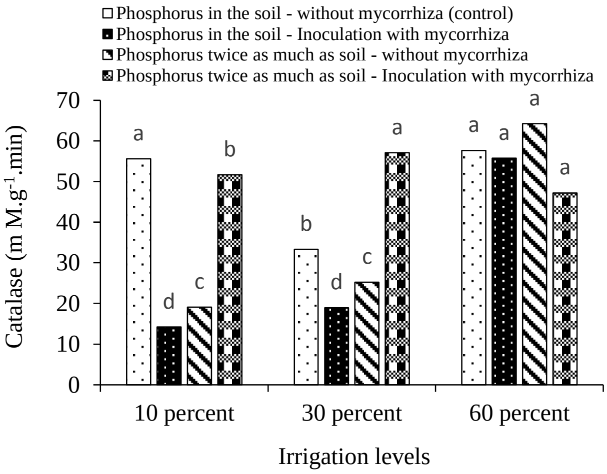


**Fig. 6.** Means comparison of interaction of irrigation, mycorrhiza and phosphorus for catalase enzyme activity

### *3.5 The leaf soluble proteins content*

The main effects of mycorrhiza and phosphorus were significant at the 1% probability level for leaf soluble proteins (Table 1). Application of mycorrhizal fungus and phosphorus fertilizer has increased the content of leaf soluble proteins by 25.59 (Figure 7) and 19.51 (Figure 8) compared to the control treatments.

Dehydration stress destroys the structure of proteins and amino acids by producing oxygen-free radicals. Also, oxygen free radicals have a high affinity with protein and cause their oxidation, and this, in turn, reduces the amount of leaf soluble protein (Parida and Das, 2005), but in this research, the effect of stress was not significant. Mycorrhizal fungi in their host plants increase the absorption of nutrients, especially phosphorus, and increase the amount of proteins against environmental stress. Selvaraj and Chellappan, (2006) showed that Prosopis juliflora plants symbiotic with *Glomus fasciculatum* had more protein in their leaves than the control. The results of the study of Maleki Narg et al., (2013) on sweet corn plants also show a positive and significant effect of phosphorus treatment on the amount of leaf soluble proteins, which is in line with the results of this experiment.

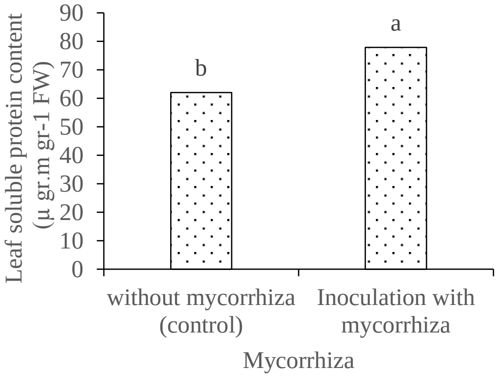


**Fig. 7.** Means comparison of mycorrhizal levels for leaf soluble proteins

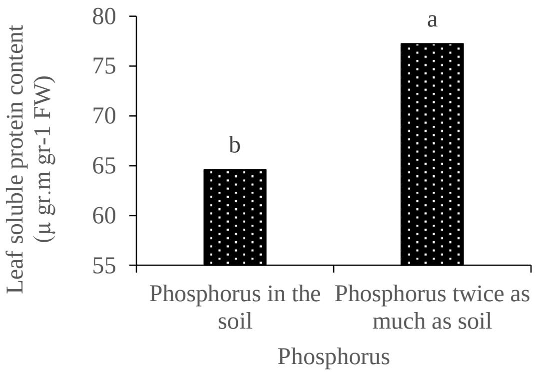


**Fig. 8.** Means comparison of phosphorus levels for leaf soluble proteins

### *3.6 Leaf glycine betaine content:*

Variance analysis shows a significant interaction of irrigation, mycorrhiza and phosphorus at the 5% probability level for glycine betaine content (Table 1). No significant difference was observed between the treatments at 10 and 30% SWD levels. At the level of 60% SWD, the highest amount of glycine betaine was related to the combination of inoculation with mycorrhiza and twice the consumption of phosphorus (with an average of 11.02 μg/mg leaf dry weight). The lowest amount was also observed in the treatment with double phosphorus consumption and without mycorrhizal inoculation (Figure 9).

Plants have different defense mechanisms to survive under environmental stress conditions. The accumulation of compounds with low molecular weight and highly soluble, which are non-toxic in high concentrations, is one of these mechanisms. Glycine betaine appears in plants with stress crises and is considered an effective osmotic regulation solution in plants and has a high correlation with the growth of plants in dry environments (Hanson et al., 2007). Soleymani (2015) showed that the interaction between irrigation and mycorrhizal species on glycine betaine was significant. The highest amounts of glycine betaine were observed in irrigation treatments at 50 and 60% of crop capacity.

### *3.7 Leaf carotenoid content:*

The main effect of mycorrhiza was significant at the 5% probability level for carotenoid content (Table 1). Inoculation with mycorrhiza increased the carotenoid content by 17.09% (Figure 10).

The function of carotenoids is energy collection and photoprotection. So, these pigments are responsible for extinguishing singlet oxygen and preventing lipid peroxidation and finally, oxidative stress (Koyro, 2006).

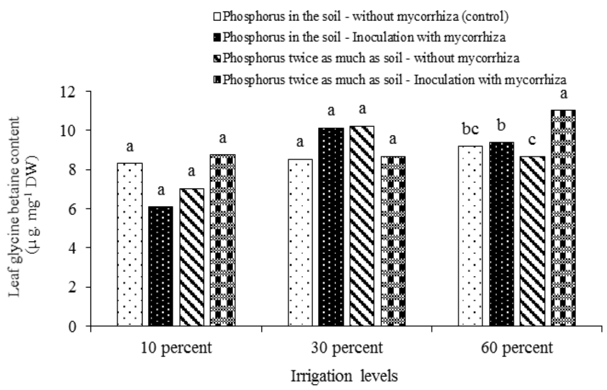


**Fig. 9.** Means comparison of interaction of irrigation levels, mycorrhiza and phosphorus for leaf glycine betaine

Kheiri et al., (2013) stated that the effect of different species of mycorrhizal fungi on carotenoid on evergreen plants was significant, and the highest amount was observed in plants inoculated with *Glomus mosseae* and G. *geosporum* species, which increased by 77.5% and 77.5%, respectively, compared to the control. It caused 84.4% and *G. etanicatum* showed a 62% increase compared to the control. However, research by Rafat et al., (2018) showed in coneflower that the highest content of carotenoids (6.602 mg. $g^{-1}$) was obtained with the treatment of 25% of the recommended dose of phosphorus fertilizer combined with *G. intraradices* in irrigation after 110 mm of pan evaporation. It seems that mycorrhiza has a significant effect on the biosynthesis pathways of secondary metabolites in the plant by creating bioavailability of nutrients and improving plant mineral nutrition, and plays a role in improving plant growth and increasing photosynthetic pigments.

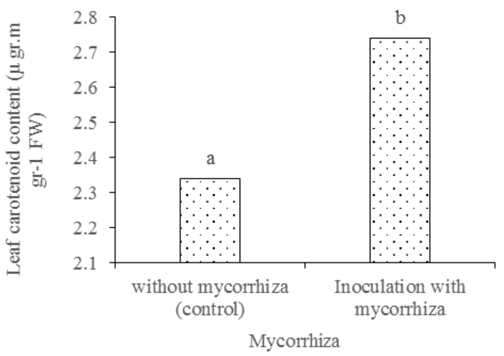


**Fig. 10.** Means comparison of mycorrhizal levels for leaf carotenoid content

### *3.8 Chlorophyll fluorescence parameters:*

The interaction of irrigation, mycorrhiza and phosphorus on Fm and Fv/Fm was significant (Table 1). In general, stress had no noticeable effect on Fm and Fv/Fm. At the irrigation level of 10% SWD, there was no statistical difference between the treatments, but at the stress of 30% SWD, the application of mycorrhiza and/or phosphorus reduced this index. At stress 605 SWD, only mycorrhiza had a significant decrease in Fm (Figure 11). Application of phosphorus and mycorrhiza at the level of 10% SWD caused a sharp decrease in Fv/Fm. However, no difference between the treatments was observed in the other two stress levels. (Figure 12). In line with the current research, Mearajipour et al., (2012) found that drought stress does not cause significant changes in the minimal fluorescence of safflower. The findings of Roshanzamir (2011) on basil plants showed that drought stress had a significant effect on quantum yield efficiency (Fv/Fm) so that at control levels, 80 and 60 percent of the field capacity were equal to 0.85, 0.74 and 0.64, respectively. Fv/Fm is widely used to represent the effects of disruption of photochemical centers, as a decrease in this trait can be the result of photoreduction processes and photodamage to photosystem II reaction centers, both of which reduce the maximum quantum efficiency of photosystem II. (Alizadeh, 2013). Drought stress has caused a decrease in photosynthetic pigments, one of the causes of which is an increase in the fluorescence of pigments, which results in a decrease in the amount of photosynthesis. Karbaschi, (2013) showed that the maximum fluorescence and quantum efficiency of photosystem II belonged to the treatment of mycorrhiza with phosphorus, which reduced the effect of stress on pigments and improved their efficiency.

### *3.9 Total phenolic content of aerial parts:*

The results (Table 1) showed that the interaction of irrigation and mycorrhiza was significant at 1% for the phenolic content of the aerial parts. Although drought had no noticeable effect, there was no difference between inoculation and non-inoculation of mycorrhiza at 10% and 30% SWD, but at 60% SWD, inoculation with mycorrhiza significantly increased the amount of total phenols in aerial parts by 38.68% (Figure 13).

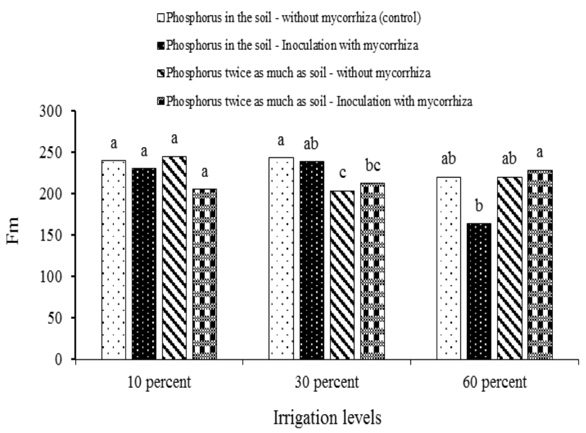


**Fig. 11.** Means comparison of interaction of irrigation, mycorrhiza and phosphorus for Fm

In the conditions of drought stress, due to the weakening of the plant's immune system, phenolic compounds along with other defensive enzymes increase in resistance against microorganisms. Among the antioxidant mechanisms of plants under drought stress is increasing the levels of phenolic compounds, because such compounds act as scavengers of reactive oxygen species and as a result stabilize cell membranes and prevent lipid peroxidation (Lopez-Raez et al., 2010).

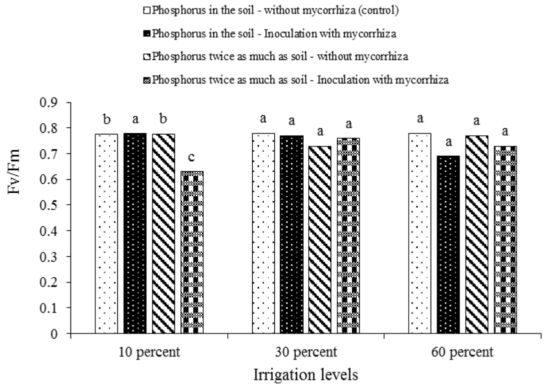


**Fig. 12.** Means comparison of interaction of irrigation, mycorrhiza and phosphorus for Fv/Fm

In a study on *Agropyron elongatum*, Shirali et al., (2020) stated that the phenol content of plants inoculated with the mycorrhizal species *Funneliformis mosseae* and *Rhizophagus intraradices* showed an increase of 134 and 153%, respectively, compared to the control treatment.

### *3.10 Plant height*

The interaction of mycorrhiza and phosphorus for plant height was significant at the probability level of 1% (Table 1). If without mycorrhizal inoculation, twice the phosphorus consumption of the soil caused a significant increase in height. However, mycorrhiza inoculation reduced the effectiveness of phosphorus fertilizer. However, the maximum height of the plant was obtained from the inoculation with mycorrhiza + phosphorus in the soil by 33.44 cm (Figure 14).

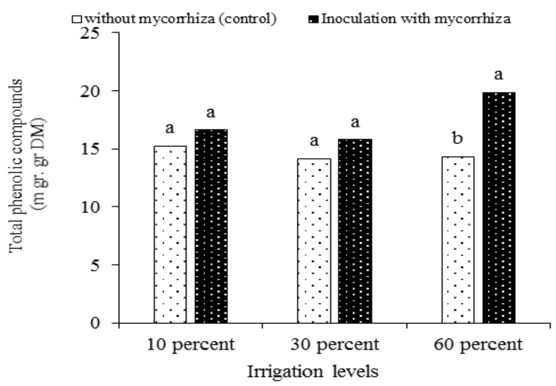


**Fig. 13.** Means comparison of interaction of irrigation and mycorrhiza for total phenolic compounds

Water limitation reduces the division and expansion of cells and thus reduces the growth of organs and the height of the plant. Therefore, one of the negative effects of drought stress is the decrease in plant height due to the decrease in available moisture and the ability to absorb elements and reduce growth. In the study by Gholam et al., (2015), the inoculation of mycorrhizal seeds in fennel increased the height of the plant by three percent compared to the control treatment.

They stated that mycorrhizal symbiosis with plant roots under water stress conditions increased photosynthesis and this caused more growth and improved plant height. In the study of Rezaei Chianeh et al., (2015) also, the use of mycorrhiza, the use of *Azotobacter* + Phosphate-Barvar 2 alone and in combination with mycorrhiza significantly increased the height of plants. It seems that the reason for this is the beneficial effect of this mycorrhiza in absorbing water and nutrients needed by the plant through root production and root development and increasing the amount of nitrogen fixed in the soil for plant use, which has caused an increase in height.

Motahari et al., (2012) reported that phosphorus, as one of the three nutrients required by the plant, caused a significant increase in the height of the marigold medicinal plant, in such a way that by regulating plant hormones, it played an important role in cell division and the production of photosynthetic substances and led to the improvement of the conditions. It has provided plant growth and more height.

### *3.11 Roots and shoots dry weight*

The interactions of phosphorus and mycorrhiza and phosphorus and irrigation at the level of 1% and the interaction of mycorrhiza and irrigation at the level of 5% were significant in root dry weight.

Also, the interaction of irrigation, mycorrhiza and phosphorus was significant at the 5% probability level of shooting dry weight (Table 1).

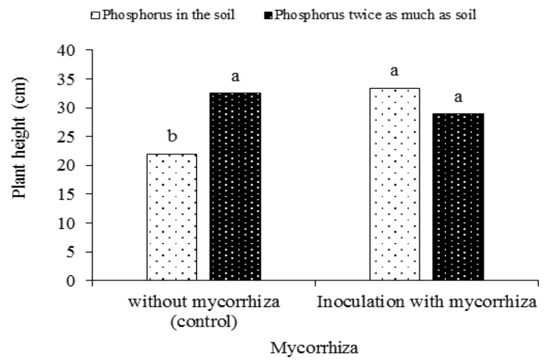


**Fig. 14.** Means comparison of interaction of mycorrhiza and phosphorus for plant height

In general, drought significantly increased the dry weight of the roots. Only at the level of 30% SWD, mycorrhizal inoculation caused a significant increase of 44.64% in root dry weight (Figure 14). Also, at the levels of 10 and 60% SWD, the application of twice the amount of phosphorus in the soil was associated with a decrease of 21.24 and 38.30% of root dry weight, respectively (Figure 15).

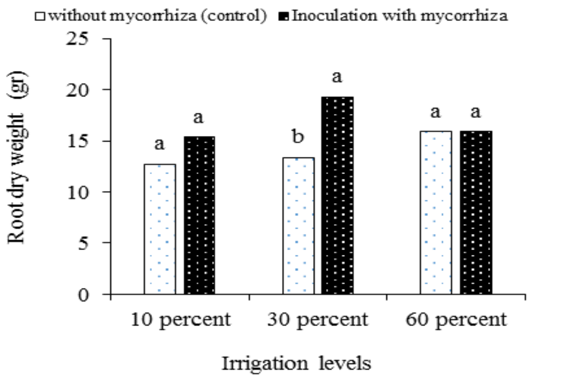


**Fig. 15.** Means comparison of interaction of mycorrhiza and irrigation for root dry weight

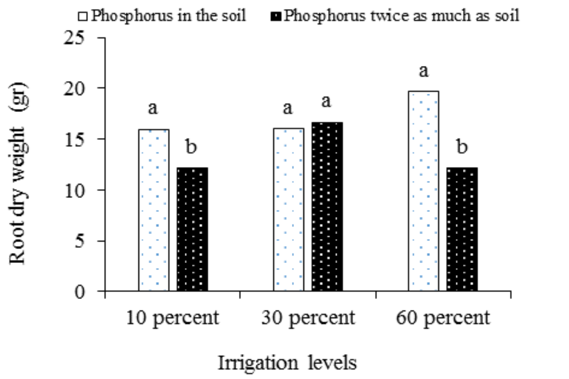


**Fig. 16.** Means comparison of interaction of irrigation and phosphorus for root dry weight

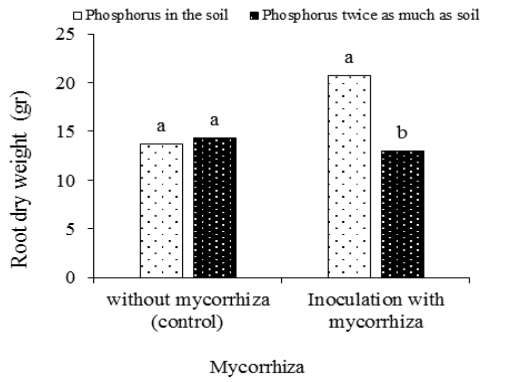


**Fig. 17.** Means comparison of interaction of mycorrhiza and phosphorus for root dry weight

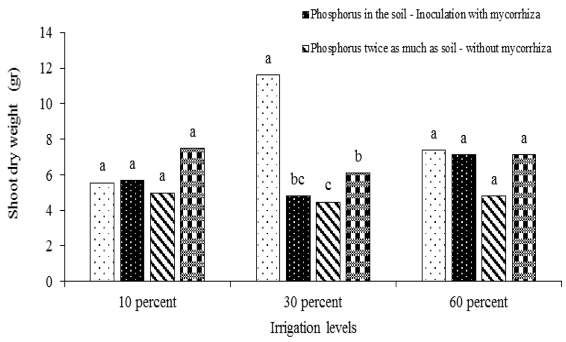


**Fig. 18.** Means comparison of interaction of irrigation and mycorrhiza for shoot dry weight

The mean comparison of the interaction of phosphorus and mycorrhiza showed that in the treatment of inoculation with mycorrhiza, the application of twice the amount of phosphorus in the soil caused a significant decrease of 37.43% of the root dry weight (Figure 16). In addition, the mean comparison for the shoot dry weight indicated that the highest amount of this attributed in the second irrigation level (30% SWD) was related to the treatment combination of no inoculation with mycorrhiza and phosphorus in the soil with the amount of 11.59 grams. There was no statistical difference between the treatments at 10% and 60% SWD (Figure 18).

Rasam et al., (2012) investigated *Hyssopus officinalis* and reported that the application of mild stress compared to the control was associated with a significant increase in the dry weight of the shoot and root, as it is consistent with the results of this research at the treatment level of 30% SWD. However, researchers attributed the decrease in root and shoot growth under conditions of extreme moisture stress to the decrease in root symbiotic relationship and the decrease in absorption of mineral elements (Tadayyon and Soltanian, 2016). In this experiment, under severe stress (60% SWD), mycorrhizal inoculation treatment had no significant effect on the dry weight of roots and shoots. One of the most important effects of mycorrhizal fungi is improving the agricultural characteristics of plants, especially in soils with low fertility. In this regard, Nooshkam, (2015) related the improvement in the growth and development of plants under the application of mycorrhizal fungi with the availability of absorption of nutrients by improving root development and increasing the contact surface with the soil, dissolving phosphate and nutrients from soil organic compounds through acid production of phosphatase and ultimately reducing root disease (elimination of specific pathogens with a special mechanism and increasing plant resistance). Sharifi et al., (2011) found that the average dry weight of aerial parts in mycorrhizal plants increased from 0.57 g to 0.85 g in green basil and from 0.48 g to 0.91 g in purple basil.

Nooshkam (2015) showed that in the treatment containing a mixture of fungi and bacteria, the addition of chemical fertilizer decreased the dry weight of *Satureja* sp. In this study, the double interaction of mycorrhiza and phosphorus showed a significant decrease in root dry weight. Contrary to the current results, Karbaschi (2013) found that the highest dry weight in the fertilizer treatment was due to the level of mycorrhiza with phosphorus in increasing the dry weight of the plant.

## 5. Conclusion

Although drought stress can affect the uptake of nutrients and biochemical characteristics in coneflower plants, the extent of this effectiveness varies in different traits. In this study, the positive role of mycorrhizae and phosphorus nutrition in water-stressed conditions was proved by increasing the phosphorus content of roots and shoots. The use of mycorrhizal fungi and phosphorus improves the negative consequences of dehydration by increasing osmolytes such as proline and soluble sugars. It also increased the activity of the catalase enzyme and increased the content of soluble proteins and glycine betaine. The set of these traits, together with the improvement of carotenoid content, led to the improvement of the phenolic compounds of aerial parts and the dry weight of roots and aerial parts under stressful conditions.

## References


Aebi, H. (1984). Catalase in vitro. Method in Enzymology. 105, 121-126.

Alizadeh, M. (2013). The effect of foliar spraying of zinc sulfate and salicylic acid on some morphological, physiological and performance characteristics of safflower under drought stress in pots. Master's thesis, Faculty of Agriculture, Yasouj University. 86 pages.

Antonios, C., Christakis Panayiotou, C. & Nikos Tzortzakis, N. (2016). Nitrogen and phosphorus levels affected plant growth, essential oil composition and antioxidant status of lavender plant (*Lavandula angustifolia* Mill.). Industrial Crops and Products. 83, 577-586.

Arpanahi, A. A., Feizian, M., Mehdipourian, G. & Khojasteh, D.N. (2020). Arbuscular mycorrhizal fungi inoculation improves essential oil and physiological parameters and nutritional values of *Thymus daenensis* Celak and *Thymus vulgaris* L. under normal and drought stress conditions. European Journal of Soil Biology 100, 103217.

Arve, L.E., Torre, S., Olsen, J.E. & Tanino, K. (2011). Stomatal responses to drought stress and air humidity in abiotic stress in plants mechanisms and adaptations. Journal of Plant Research. 119, 267- 280.

Attarzadeh, M., Balouchi, H., Rajaie, M., Movahhedi Dehnavi, M. & Salehi, A. (2019). Growth and nutrient content of Echinacea purpurea as affected by the combination of phosphorus with arbuscular mycorrhiza fungus and *Pseudomonas florescent* bacterium under different irrigation regimes. Journal of Environmental Management. 231, 182-188.

Bączek, K.B., Wiśniewska, M., Przybył, J.L., Kosakowska, O. & Węglarz, Z. (2019). Arbuscular mycorrhizal fungi in chamomile (*Matricaria recutita* L.) organic cultivation. Industrial Crops and Products. 140, 111562.

Bauma, C., W. El-Tohamy, & N. Gruda. (2015). Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. Scientia Horticulturae. 187, 131-141.

Berude, M., Almeida, D., Riva, M., Cabanêz, P. & Amaral, A. (2015). Mycorrhizae and their agroecological importance. Biosphere Encyclopedia. 11, 132-146.

Bettaieb Rebey, I., Jabri-Karoui, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F. & Marzouk, B. (2012). Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Industrial Crops and Products. 36, 238-245.

de Assis, R.M.A., Carneiro, J.J., Medeiros, A.P.R., de Carvalho, A.A., da Cunha Honorato, A., Carneiro, M.A.C., Bertolucci, S.K.V. & Pinto, J.E.B.P. (2020). Arbuscular mycorrhizal fungi and organic manure enhance growth and accumulation of citral, total phenols, and flavonoids in Melissa officinalis L. Industrial Crops and Products 158.

Deka, D., Singh, A. K. & Singh, A. K. (2018). Effect of drought stress on crop plants with special reference to drought avoidance and tolerance mechanisms: A review. International Journal of Current Microbiology and Applied Sciences. 7, 2703 -2721.

Demir, S. (2004). Influence of arbuscular mycorrhiza on some physiological, growth parameters of pepper. Turkish Journal of Biology. 28, 85-90.

Ebrahimi, F., Salehi, A., Movahhedi dehnavi, M., & Mirshekari, A. (2022). Morpho-physiological responses of two cultivars of German chamomile (*Matricaria chamomilla*) to mycorrhiza application under water deficit stress. Iranian Journal of Horticultural Science. 53(3), 737-749.

Emami, R. (1996). Methods of plant analysis. Publication No. 982 of Iran's Soil and Water Research Institute. first volume. 126 pages.

George, N.P. & Ray, J.G. (2023). The inevitability of arbuscular mycorrhiza for sustainability in organic agriculture A critical review. Frontiers in Sustainable Food Systems 7, 1124688.

Gholami, A., Akbari, I. & Abbas Dokht, H. (2015). Study the effects of bio and organic fertilizers on growth characteristics and yield of Fennel (*Foeniculum vulgare*). Journal of Agroecology. 2, 215-224.

Grattan, S. R. & Grieve, C. M. (1992). Mineral element acquisition and growth response of plants grown in saline environments. Agriculture. Ecology and Environment. 38, 275-300.

Habibi, G. (2012). Exogenous salicylic acid alleviates oxidative damage of barley plants under drought stress. Acta Biologica Szegediensis. 56(1), 57-63.

Haji Mehdipour, M., Khanovi, M., Shekarchi, M., Abedi, Z. & Pir Ali Hamdani, M. (2009). Investigating the best method of extracting phenolic compounds found in Coneflower plant. Journal of Medicinal Plants. 4(32), 152-145.

Irigoen, J. J., Emerich, D. W. & Sanchez-Dias, M. (1992). Water stress induced changes in concentrations of prolins and total soluble sugars in modulated alfalfa (*Medicago sativa*) plants. Physiologia plantarum. 84, 55-60.

Jakopic. J., Colaric, M., Veberic, R., Hudina, M., Solar, A. & Stampar, F. (2007). How much do cultivar and preparation time influence on phenolics content in walnut liqueur?. Food Chemistry. 104 (1), 100 - 5.

Kapoor, R., Evelin, H., Mathur, P. & Giri, B. (2013). Arbuscular Mycorrhiza: Approaches for Abiotic Stress Tolerance in Crop Plants for Sustainable Agriculture, In: Tuteja, N., Singh Gill, S. (Eds.), Plant Acclimation to Environmental Stress. Springer New York, New York, NY, pp. 359-401.

Kar, M. & Mishra. D. (1976). Catalase, Peroxides and poly phenol oxides activities during rice leaf senescence. Plant Physiology. 57, 315-319.

Karbaschi, F. (2013). The effect of mycorrhizal fungus and phosphorus fertilizer on the response of clover to salinity stress. Master's thesis of the Faculty of Agriculture and Natural Resources of Isfahan University of Technology, 78 pages.

Karsch-Völk, M., Barrett, B., Kiefer, D., Bauer, R., Ardjomand-Woelkart, K. & Linde, K. (2014). Echinacea for preventing and treating the common cold. The Cochrane database of systematic reviews, 2: CD000530-CD000530.

Khalafallah, A. A. & Abo-Ghalia, H. H. (2008). Effect of arbuscular mycorrhizal fungi on the metabolic products and activity of antioxidant system in wheat plants subjected to short-term water stress, followed by recovery at different growth stages. Journal of Applied Sciences Research. 4(5), 559-569.

Kheiri, Z., Moghadam, M. & Moradi, M. (2020). Investigation of the effect of different species of mycorrhizal fungi on some growth indices, photosynthetic pigments, flavonoid and carotenoid content of pot marigold flower. Scientific Journal of Garden Nutrition. 3(1), 37-50.

Kormanik, P. P. & McGraw, A. C. (1982). Quantification of vesicular-arbuscular mycorrhizae in plant roots. In Methods and Principles of Mycorrhizal Research (N. C. Schenck, Ed.). pp. 37-45.

Kour, D., Rana, K.L., Sheikh, I., Kumar, V., Yadav, A.N., Dhaliwal, H.S. & Saxena, A.K. (2020). Alleviation of drought stress and plant growth promotion by *Pseudomonas libanensis* EU-LWNA-33, a drought-adaptive phosphorus-solubilizing bacterium. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 90, 785-795.

Koyro, H. W. (2006). Effect of salinity on growth, photosynthesis, water relations and solute composition of potential cash crop halophyte *Plantago coronopus* L. Environmental and Experimental Botany. 56, 136-149.

Latef, A.A.H.A., Hashem, A., Rasool, S., Abd_Allah, E.F., Alqarawi, A.A., Egamberdieva, D., Jan, S., Anjum, N.A. & Ahmad, P. (2016). Arbuscular mycorrhizal symbiosis and abiotic stress in plants: A review. Journal of Plant Biology. 59, 407-426.

Lichenthaler, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology. 148, 350-382.

Lopez-Raez, J.A., Flors, V., Garcia, J.M. & Pozo, M.J. (2010). AM symbiosis alters phenolic acid content in tomato roots. Plant Signaling and Behavior. 5, 1138–1140.

Luo, H.J., Wang, J.Z., Chen, J.F. & Zou, K. (2011). Docking study on chlorogenic acid as a potential H5N1 influenza A virus neuraminidase inhibitor. Medicinal Chemistry Research. 20, 554-557.

Maleki Narg, M., Balouchi, H., Farajee, H., & Yadavi, A. (2013). The Effect of Nitrogen and Phosphorus Chemical and Biological Fertilizers on Grain Yield and Qualitative Traits of Sweet Corn. Journal of Agricultural Science and Sustainable Production. 23(3), 89-104.

Mearajipour, M. (2012). The effect of drought stress on yield and some physiological characteristics of spring safflower (*Carthamus tinctorius* L) in Yasouj region. Master's thesis, Yasouj University. 89 pages.

Mirzaei, M. S., Siadat, S. A., pakdaman sardrod, B., & Moradi Telavat, M. (2021). Effect of Pre formormospora indica and Foliar Application of Salicylic Acid on morphological characteristics and antioxidant enzymes of thyme irrigation cut-off stress. Journal of Crops Improvement. 23(4), 853-839.

Mohammadi, E., Asghari, H. & Gholami, A. (2014). Evaluation the possibility of utilization of bio fertilizer mycorrhiza in phosphorus supply in Chickpea cultivation (*Cicer arietinum* L.). Iranian Journal of Field Crops Research. 11(4), 658-665.

Motahari, M., Hani, A., Moradi, P. & Motahari, H. (2012). The effect of phosphorus fertilizer and mycorrhizal fungi on the yield, yield components and effective substances of *Calendula officinalis* medicinal plant, the first national conference on modern topics in agriculture, Saveh, pp. 1-7.

Nikbakht, J., bakmohammadi, F. & Barzegar, T. (2023). Effect of seed priming and irrigation level on the yield and water productivity of French lettuce. *Iranian Journal of Irrigation & Drainage. 16*(6), 1211-1220.

Nooshkam, A. (2015). Study the effects of biological and chemical fertilizer on quantitative and qualitative characteristics of savory specie (*Satureja khuzestanica* jamzad). *Journal of Crop Production*. *8*(4), 87-103.

Ozkur, O., Ozdemir, F., Bor, M. & Turkan, I. (2009). Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. Environmental and Experimental Botany. 66, 487-492.

Paquin. R. & Le chasseur, P. (1979). Observation surune method dosage Labrie dans lesde plants. Canadian Journal of Botany. 57, 1851-1854.

Parida, A. K., & Das, A. B. (2005). Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety. 60, 324-349.

Perry, T. W., Rhykerd, C. L., Holt, D.A. & Mayo, H. H. (2011). Effect of potassium fertilization on chemical characteristics, yield and nutritive value of corn silage. Journal of Animal Science. 34, 642-646.

Rafat, N., Yarnia, M., Mirshekari, B., Rashidi, V. & Moghadam, A. M. D. (2018). Reduction possibility of phosphorus using with the application of mycorrhiza in purple coneflower (*Echinacea purpurea* L.) through physiological traits and essence yield under different irrigation levels. Applied Ecology and Environmental Research. 16(5), 6453-6468.

Rasam, Gh., Dadkhah, A. & Khoshnoud Yazdi, A. (2012). Evaluation of the effect of water deficit on the morphological and physiological characteristics of the medicinal plant hyssop. Science of Agriculture. 5 (10), 1-12.

Rezaei Chianeh, I., Jalilia, J., Ebrahimian, E. & Mohammad Sidi, S. (2015). The effect of biofertilizers on quantitative and qualitative yield of *Bacillus lentus* at different levels of irrigation. Journal of Crops Improvement. 17, 775-788.

Roshanzamir, F. (2011). Investigating the effect of drought stress and phosphorus fertilizer on the quantitative and qualitative characteristics of basil. Faculty of Agriculture and Natural Resources, Zabul University. 62 pages.

Sarikhani, M. R., Malboubi, M. A. & Ebrahimi, M. (2014). Phosphate solubilizing bacteria: Isolation of Bacteria and Phosphate Solubilizing Genes, Mechanism and Genetics of Phosphate Solubilization. Agriculture Biotechnology Journal. 6, 1. 77-110.

Selvaraj, T. & P. Chellappan. (2006) Arbuscular mycorrhizae: A diverse personality. Journal of Central European Agriculture. 7, 349-358.

Sharifi, M., Mohtashamian, M., Riyahi, H., Aghaee, A. & Alavi, S. (2011). The Effects of Vesicular-Arbuscular Mycorrhizal (VAM) Fungus *Glomus etunicatum* on Growth and some Physiological Parameters of Basil. Journal of Medicinal Plants. 10 (38), 85-94.

Shirali, F., Almasi, R. & Fattahi, B. (2020). Effects of symbiosis with two species of arbuscular mycorrhiza on some morphological and physiological characteristics of rangeland grass. *Agropyron elongatum* (Host). Beauv. Journal of rangeland. 14 (4), 731-741.

Smith F.W. (2002). The phosphate uptake mechanism. Plant and Soil. 245, 105–114.

Soleymani, F. & Pirzad, A. (2015). The effect of mycorrhizal fungi on malondialdehyde concentration and some metabolic processes in hyssop (*Hyssopus officinalis*) under water deficit stress. Iranian Journal of Plant Biology. *7*(24), 15-26.

Tadayyon, A. & Soltanian, M. (2016). Effect of arbuscular mycorrhizal fungi on root colonization and phosphorus uptake of linseed (*Linum ussitatissimum* L.) under drought stress conditions. Plant Process and Function. 5 (15) ,147-156.

Tasdighi, H., Salehi, A., Movahhedi Dehnavi, M. & Behzadi, Y. (2015). Survey of Yield, Yield Components and Essential Oil of *Matricaria chamomilla* L. With Application of Vermicompost and Different Irrigation Levels. Journal of Agricultural Science and Sustainable Production. 25, 3. 62-78.

Weisany, W., Sohrabi, Y., Siosemardeh, A. & Ghassemi-Golezani, K. (2017). *Funneliformis mosseae* fungi changed essential oil composition in *Trigonella foenum graecum* L., *Coriandrum sativum* L. and *Nigella sativa* L. Journal of Essential Oil Research. 29, 276- 287.

Xu, J., Jin, J., Zhao, H. & Li, K. (2019). Drought stress tolerance analysis of *Populus ussuriensis* clones with different ploidies. Journal of Forestry Research. 30, 1267-1275.