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Effect of Mycorrhizal fungi inoculation on the water consumptive use, growth, and yield of barley (*Hordeum Vulgare* L.) under drought stress in Gypsifereous soil

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Mycorrhiza, inoculation, drought stress, water consumption, barley, gypsiferous soil

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ABSTRACT

A field experiment was conducted to evaluation effects of mycorrhizal fungi inoculation on the water consumptive use to the growth and yield traits of Barley (Hordeum Vulgare L.) under drought stress in gypsiferous soil. The experimental station of the Soils and Water Resources Department. College of Agriculture-Tikrit university, is located at 34°40′ 49″ and 43° 38′ 40″ and (129 m) above sea level , The experiment was (RCBD) randomized complete block design with two factors : mycorrhizal fungi inoculation, Glomus spp. And The second factor, three irrigation levels, still benefited 25, 50, and 75 % of the available water. The best results were that water consumption ranged between 649.5 and 758.9 mm. The post-depletion irrigation treatment and the inoculation treatment outperformed all growth traits and yielded all other traits. The interaction between mycorrhizal inoculation and post-depletion irrigation gave the best results in all traits which reached plant height, leaf area, number of branches, grain weight, dry mass yield of the vegetative system, and grain yield, were 84.33 cm, 4.84 cm^2, 464.36, 5.69 $ton.ha^{-1}$ and 471.30 kg m^{-2} respectively outperforming all other treatments.

تأثير التلقيح بفطريات المايكورايزا في الاستهلاك المائي و نمو و حاصل الشعير تحت اجهاد الجفاف في تربة جبسية

الخلاصة

أُجريت تجربة حقلية لدراسة تأثير التلقيح بالفطريات الجذرية (المايكورايزا) على استهلاك الماء، ونمو، ومحصول الشعير (Hordeum Vulgare L.) تحت إجهاد الجفاف في تربة جبسية. المحطة التجريبية التابعة لقسم التربة والموارد المائية. تقع كلية الزراعة بجامعة تكريت على خطي طول 34°40 49 و43°38 40، وعلى ارتفاع 129 مترًا فوق مستوى سطح البحر. أُجريت التجربة بتصميم القطاعات الكاملة العشوائية (RCBD) مع توليفتين بعاملين : تلقيح الفطريات الجذرية المايكورايزا (Glomus spp.). أما العامل الثاني، وهو ثلاثة مستويات ري، فقد استفاد منه 25 و50 و75% من المياه المتاحة. وأظهرت أفضل النتائج أن استهلاك المياه تراوح بين 649.5 و758.9 ملم. وتفوقت معاملة الري بعد الاستنزاف ومعاملة التلقيح على جميع صفات النمو، وأعطت جميع الصفات الأخرى. وقد أعطى التفاعل بين التلقيح بالفطريات الجذرية والري بعد الاستنفاد أفضل النتائج في جميع الصفات التي بلغت ارتفاع النبات ومساحة الورقة وعدد الفروع ووزن الحبوب وإنتاج الكتلة الجافة للنظام الخضري وإنتاج الحبوب 84.33 سم^{2} و4.84 سم^{2} و464.36 و5.69 طن.هكتار$^{-1}$ و471.30 كغم. م$^{-2}$ على التوالي متفوقة على جميع المعاملات الأخرى.

الكلمات المفتاحية : الفطريات الجذرية، التلقيح ، الإجهاد الناتج عن الجفاف، استهلاك المياه، الشعير، التربة الجبسية.

INTROUCTION

Barley (Hordeum Vulgare L.) is one of the important of the Poaceae, and it's a complete food suitable and elements for humans and animals, as the plant that can provide basic human food (Alsharqi *et al.,* 2023; Mohammed and Baldwin, 2023). Barley is a traditional crop and has recently gained worldwide attention, thus the ability to grow under various stress and hard conditions like drought, salinity, acidity, wide range temperatures, etc. (Verma *et al.*, 2022). The higher nutritional content of its plants is high in protein and good amino acids (Batîr Rusu *et al.*, 2024). The plant can grow in arid and semi-arid regions and has a high potential for the environment (Qader *et al.*, 2018). Drought Stresses are considered the cause of decreased growth and yield of crops in the fields (Khairo, 2024; Hummadi and Khalaf,2024). Drought limits the production of agriculture due to morphological, biochemical, and physiological changes in the plant, and this stress will probably increase in the future. The water stress on barley reduces plant height, yield, and grain (Elakhdar *et al.*, 2022; Hama *et al.*, 2024).

The gypsum soil can be define that soil contains in the surface layer about 5% gypsum (Farhan *et al.*, 2024; AL-Hamandi *et al.*, 2025). In iraq there is 88,000 km^2 gypsum soils , It have many problems, such as weak structure, hard layer, poor fertility , salinity and sinkholes, so that it needs special management scientific methods to exploit them in agriculture (Algnaby and Aldawri,2023; Musrhed *et al.,* 2023). Also, high level of gypsum in the soil causes reduce water retention, and its content of available water and the appearance of hardening and cracking (Mahmoud and Ismaeal, 2024). Gypsum particles occupy most of the soil particles and reduce the presence of clay and loam particles, the microorganisms adsorb on the clay and loam surfaces of particles, so that we expect weakness of its biological activity As well as being located in the arid and semi-arid regions that Unsuitable for most living organisms (Jerbi *et al.*, 2022; Al-Janabi and Al-Mudalalawi,2023; Mahmoud and Ismaeal, 2024).

Biofertilizers are natural mixtures that include beneficial microorganisms such as mycorrhizae, fungi, and bacteria, which are key in improving crop productivity(Noaema *et al.*, 2024). These microorganisms improve nutrient availability by developing long-term symbiotic relationships with plants, which enhances soil fertility and health and reduces its negative environmental impacts (Iqbal *et al.,* 2023).

In this context, vesicular arbuscular mycorrhizal fungi plant growth-promoting fungi (PGPF) are used as a biological fertilizer to stimulate crops to cope with ecological stresses, such as drought, and it is described as an important tool for sustainable agriculture and food security (Wahab *et al.,* 2023). Mycorrhizal fungi are also one of the mechanisms for resisting drought or rationalizing water use when there is water scarcity(Madouh and Quoreshi,2023; Mousa *et al.,* 2024). Soil application of biofertilizers can average the reverse effects of drought stress on the plant because mycorrhiza fungi cause an increase in the micro and macro elements in the shoot system(Alotaibi *et al.,* 2024). Mycorrhizal fungi have been shown to increase the ability of roots to absorb NPK as well as micronutrients (Chinnathambi *et al.,* 2024).

Due to the lack of water resources and huge increasing population with the need for more food on the other side and droughts stress recent as well as reducing ecological impacts caused by the use of chemical fertilizers and scarcity of enough barley research, the aim of this study to investigate the effect of mycorrhizal fungi and limited-irrigation on water consumption, growth and yield of barley under gypsiferous soil conditions.

MATERIAL AND METHODS

Study location and soil analyses: -

This Study was carried out in the Agriculture College field, Tikrit University/ Salahaddin- Iraq that located at 49° 40′ 34″ N and 40° 38′ 43″ E and an altitude of (129 m) above sea level. Before planting, the soil samples were taken from five field sites at a depth of 0-0.3 m to estimate the soil's physical and chemical properties according to (Page *et al.,* 1982; Khalaf *et al.,* 2021; Aljumaily *et al.,* 2022) , and table (1) shows some physical and chemical characteristics of experiment soil.

Table 1 . Some soil physical and chemical characteristics .

Soil characteristics	**Quantity**	**Unit**
field moisture capacity FMC33 kPa	1.3	mm cm^{-1}
permanent wilting point moisture at1500 kPa	2.3	
available water capacity	3.6	
bulk density	1.34	µg m^{-3}
electrical conductivity , EC	1.5	dS m^{-1}
pH	7.22	
organic carbon SOC	5.4	g kg^{-1}
total N	0.8	
carbonate minerals	118	
available N	44	mg kg^{-1}
available P	4.5	
extractable K	17	
cation-exchange capacity	5	cmolc kg^{-1}
sand	611	gkg^{-1}
Loam	98	
Clay	291	
Texture	Sandy clay Loam	

The experiment in field was tilled and divided into (three main blocks) and plots area (9 m^2) Seeds were planted in a Quantity of seeds 120 kg ha^{-1}, Planting date was on the 4th of December 2022, fertilizers added as urea (46% N), Super Phosphate (21% P), and potassium sulfate (43% K) 90, 120, and 120 kg ha^{-1} respectively for each treatment in the experiment when planting. The second batch of Nitrogen 90 kg ha^{-1} was added after germination. The experiment was performed as a randomized complete block design with three replicates. The first factor included biofertilizer mycorrhizal fungi 2 levels with inoculation and non-inoculation as control. The second factor included irrigation 25, 50, and 75 % depletion of available water respectively.

Not. M0 = without mycorrhizal fungi inoculation , M1 = with mycorrhizal fungi inoculation and I = irrigation levels 25 , 50 , 75, R = replicates.

Mycorrhizal fungi inoculant addition.

The start inoculant was prepared in the laboratory of the Department of Soil Sciences and Water Resources at Tikrit University and it consists of isolated and identified spores, *Glomus spp.*

1- Soil preparation: Mixed soil was taken and sifted with a sieve with a hole diameter of 2 mm, and sterilized with an autoclave at a temperature of 121 °C and a pressure of 15 pounds / ing2 for an hour, Adholeya, *et al.* (2005).

2- Adding the inoculation: Isolated spores were added to the pots for each genus separately while leaving other pots without inoculation (Control) for comparison.

3- The host plant: garlic was planted as a host plant, then it was placed in the greenhouse for three months with continuous service.

4- Diagnosis of fungal infection: The roots of plants were examined to confirm infection with mycorrhizal fungi. by the Ink and Vinegar method described in Vierheilig *et al.*, (1998).

5- Investigation of mycorrhizal spores: The soil surrounding the roots was examined to ensure the presence of mycorrhizal spores and its preparation was done by wet sieving and decanting according to what was mentioned by Gerdemann, and Nicolson (1963). The fungal inoculum should contain 4-5 spores/gm of soil for the inoculum to be effective, in ensuring a typical infection of the roots of the host plant.

6- Mycorrhiza inoculum was added at a rate of 5 g per 5 kg pot in a layer under the lobes when planting.

Irrigation water calculation: -

The drip irrigation method used by one irrigation line with a planting line in the field distance of 5 cm from the plant line, discharge for dripper was 3 L h^{-1} . depth irrigation water were depending on reference evapotranspiration values (ET_0) by the pan class A method was calculated using the following equation (Allen et al., 1998):

$$ET_0 = Epan \times Kp \quad \text{.......(1)}$$

Where, ETo: potential evapotranspiration (mm), Epan: evaporation from pan class A (mm) , Kp: pan coefficient (0.75).

Eta **,** calculated by following equation (Allen et al., 1998):

$$ETa = ET_0 \times Kc \quad \text{.......(2)}$$

Where, ETa: actual evapotranspiration (mm), Kc : coefficients crop

Field water use efficiency (WUE_C) calculated by following equation (Hansen et al., 1980):

$$WUEc = Y/ ETa \quad \text{.......(3)}$$

WUEc : crop water use efficiency kg m^{-3} Y: seed yield kg

Statistical analysis:

The studied traits were statistically analyzed by the Statistical Analysis System Software (SAS Institute, Version 9.2). The differences mean among separated using Duncan's statistical level probability.

RESULTS AND DISSCUSION

The results in figure 1 (a) show the testing of the percentage of mycorrhizal fungi Glomus spp. infection of barley roots showed that it was between 75-85% of the infected roots, while no infection was shown in the uninoculated plants. Figure 1 (b) The number of spores in the soil was between 50-60 spores. g soil-1 in the inoculation treatment, and this confirms the effectiveness of the inoculum to obtain accurate results in improving plant growth and studying the water effects of mycorrhizal fungi on barley plants. The interaction between irrigation treatments and inoculation with mycorrhizal fungi shows non-significant differences in the percentage of mycorrhizal infection at all irrigation levels. This confirms the important role of mycorrhizal fungi in improving plant growth in all environmental conditions. (Rapparini and Peñuelas ,2014).

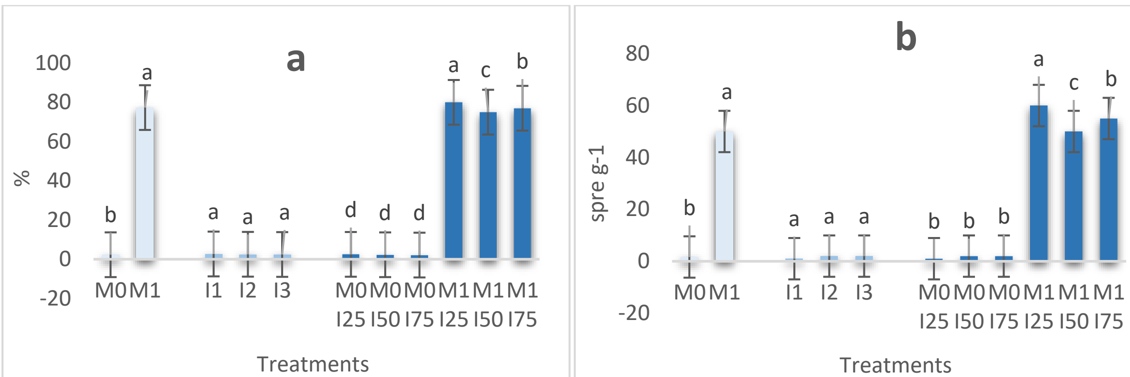

Figure 1. Effect of Mycorrhizal Fungi Inoculation on the (a) percentage of mycorrhizal fungi *Glomus spp*. infection of barley roots % (b) The number of spores g soil^{-1} in the Gypsiferious Soil.

Figure 2 shows the water consumption of the research treatments over the months. The figure shows that the water consumption of barley started low due to the small size of the plant, in addition to the decrease in its water requirements at the beginning of growth. Then, the water consumption increased with the increase in growth, and the plant reached the vegetative growth stage and increased the plant biomass. Then, the plant reached high water consumption in March, because the flowering stage of barley was within this month, which is the highest in the water requirements of the plant. Then, the water consumption decreased after this stage because the plant reached the pubescence stage and completed its growth stage. The treatment gave the highest water consumption for the irrigation treatments because this treatment received the largest amount of irrigation water, while the other two treatments were less compared to this treatment, and the treatment was the least in the irrigation treatments. The role of inoculation with mycorrhizae was also positive in reducing water consumption and reducing the amount of irrigation water used.

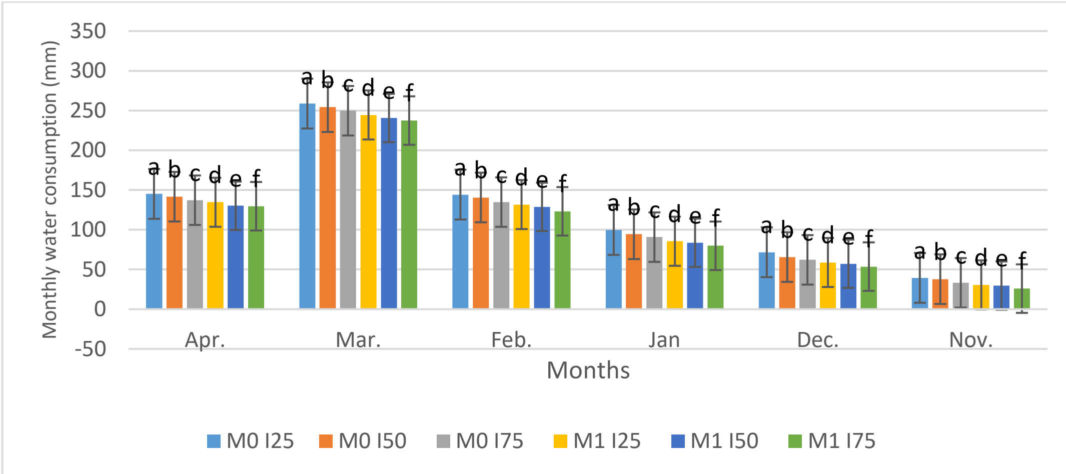

Figure 2. Effect of Mycorrhizal Fungi Inoculation on the Monthly Water Consumption of Barley (*Hordeum Vulgare* L.) under Drought-Stress in Gypsiferious Soil

Figure (3) also shows the total water consumption of barley, the treatment was 758.9, 734.2, 707.8, 685.7, 670.1, and 649.8 mm of M0 I25, M0 I50, M0 I75, M1 I25 M1 I50, and M1 I75 respectively water consumption increased with the increase in added water (Khairo, 2024). Inoculation with mycorrhizae also played a role in reducing water consumption (Gholinezhad *et al.*, 2020). The role of inoculation with mycorrhizae increased the efficiency of water absorption from the soil resulting from increased root branching and increased surface area of the roots increasing the plant's ability to absorb water (Wahab *et al.*, 2023; Owiny and Dusengemungu,2024).

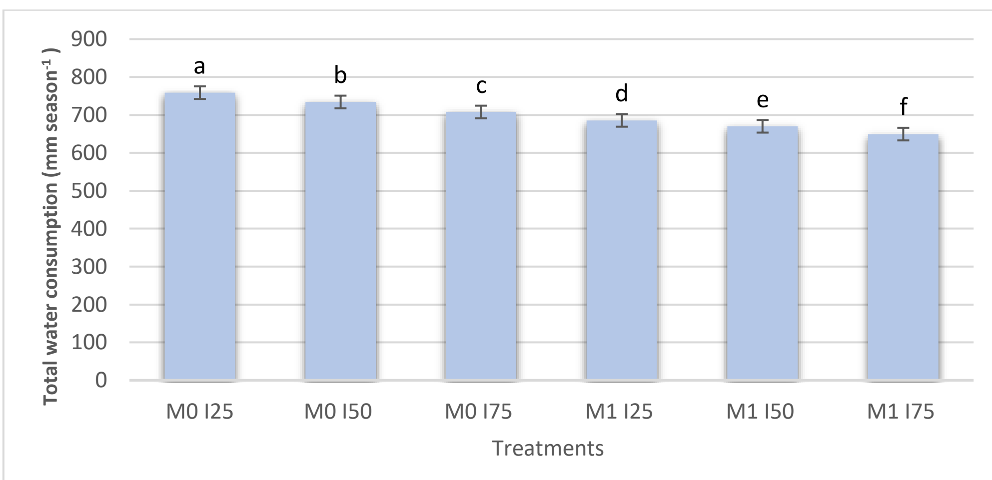

Figure 3. Effect of Mycorrhizal Fungi Inoculation on the Total Water Consumption of Barley (*Hordeum Vulgare* L.) under Drought-Stress in Gypsiferious Soil

Figure (4,5, and 6) show the effect of Irrigation and mycorrhiza on the growth and yield traits of barley. The irrigation treatment I25 increased significantly compared to all other treatments in plant height, number of grains per spike, 100-grain weight, and dry weight of the vegetative mass, while the difference was not significant in leaf area, the difference was not significant between I25 and I50 in the number of branches and grain yield. The percentage of increase in the yield was 12.29% for treatment I25 compared to treatment I75. The treatment of inoculation with mycorrhiza was significantly superior compared to the treatment without inoculation in plant height, leaf area, number of grains per spike, 100-grain weight, and dry weight of the vegetative mass. The percentage of increase in the yield was 99.66 % for treatment M1 compared to treatment M0. As for the interaction treatments, M1 I25 increased significantly compared to all other treatments in growth and yield traits. The percentage of increase in the yield was 32.35% for treatment M1 I25 compared to treatment M1 I75, so irrigation in field is the most important factors that can greatly affect crops growth and productivity. Therefore, choosing the appropriate irrigation system for the cultivating crops is very important to improving and obtaining the highest productivity, irrigation systems includes number of irrigations or their intervals between it (Fan and Schütze,2024). Therefore, the correct decision of irrigation should be regarding made based on a comprehensive study and understanding of the environmental factors that affect plant growth , various characteristics, production, and quality of yield ,Water shortage it is negative effects, reducing photosynthesis, reducing cell division, accelerating leaf aging, leaf area, and transpiration, all of which lead to decrease yields in both grain and straw (Andrianasolo *et al.*, 2016; Yang *et al.*, 2023; Khairo, 2024).

Mycorrhizal fungi and bacteria itis important field practices used for promoting plant growth. Different types of biofertilizers are used to improve productivity and economic , The most important of microorganisms. is mycorrhiza, which stimulates the growth of root hairs, the longitudinal growth of mycorrhizal fungi and their penetration into the deeper layers of the soil. It increases the efficiency of the roots in absorbing water and nutrients, which is becomes clear in the growth and production of the crop (Shi *et al.*, 2023). In addition, several studies have explained the positive effects of biofertilizers and the importance of practices that improve crops productivity and soil fertility (Demir *et al.*, 2023).

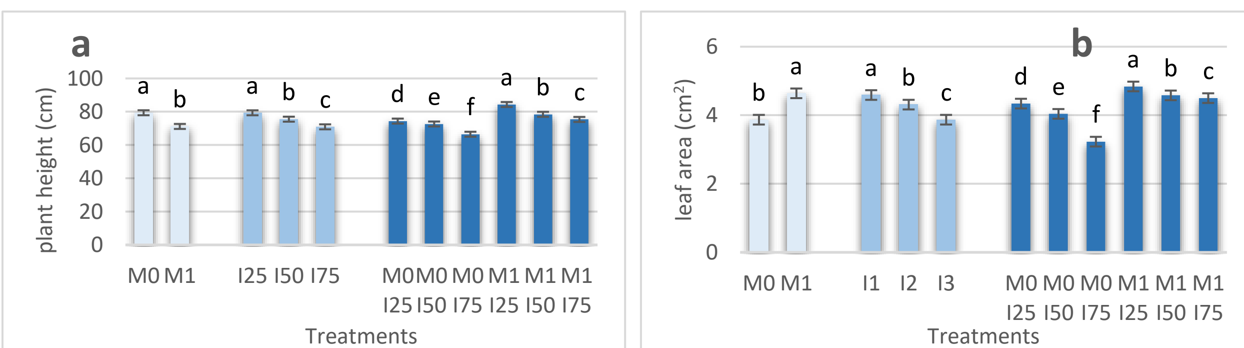

Figure 4. Effect of Mycorrhizal Fungi Inoculation on the (a) plant height (b) leaf area of Barley (*Hordeum Vulgare* L.) under Drought-Stress in Gypsiferious Soil

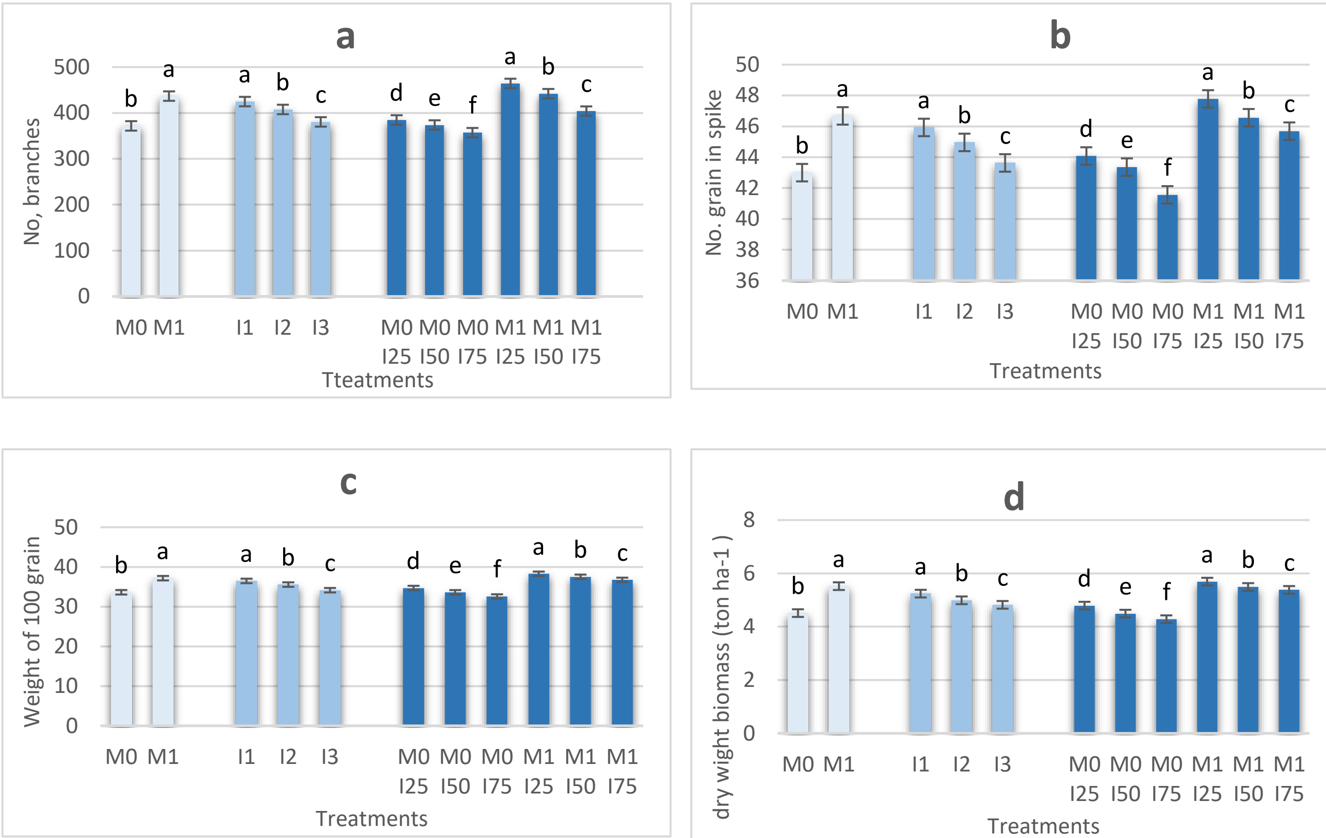

Figure 5. Effect of Mycorrhizal Fungi Inoculation on the (a) No. of branches (b) No. grains per spike (c) 100-grain weight (d) dry weight of the vegetative mass of Barley (Hordeum Vulgare L.) under Drought-Stress in Gypsiferious Soil

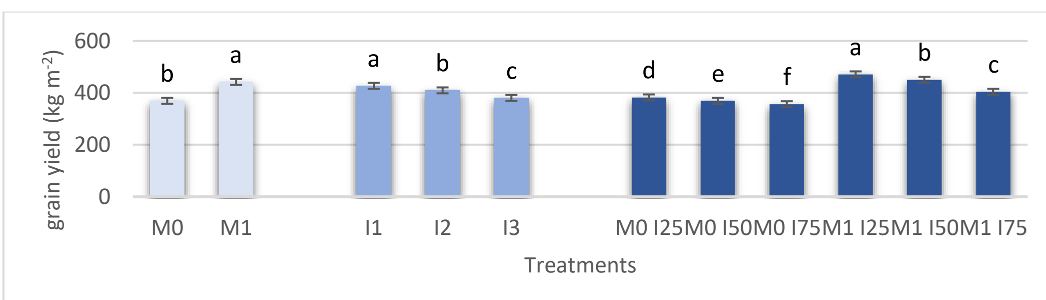

Figure 6. Effect of Mycorrhizal Fungi Inoculation on the grains yield of Barley (Hordeum Vulgare L.) under Drought-Stress in Gypsiferious Soil

Figure (7) shows the Effect of Irrigation and mycorrhiza on growth and yield characteristics of barley. The irrigation treatment I25 increased significantly by 0.89% compared to I75 treatments, but the difference was not significant with I50. The effect of inoculation with mycorrhiza was significantly superior compared to the treatment without inoculation. The percentage of increase in the WUE was 31.07 % for treatment M1 compared to M0. As for the interaction treatments, M1 I25 increased significantly compared to all other treatments in WUE. The percentage of increase in the yield was 37.20% for treatment M1 I25 compared to treatment M1 I75. Water productivity increases when the added irrigation water is reduced, at any rate, from the plant's total requirements(Jiao *et al.*, 2024). When irrigation water is reduced, water use efficiency increases to a certain extent, because efficiency is the result of the relationship between the economic yield of the plant and the amount of water added to the field or crop, when the crop's water requirements are significantly reduced, water use efficiency decreases, mainly due to the significant decrease in water and crop productivity(Bobojonov *et al.*, 2016; Muroyiwa *et al.*, 2023; Işik and Ortaş, 2024).

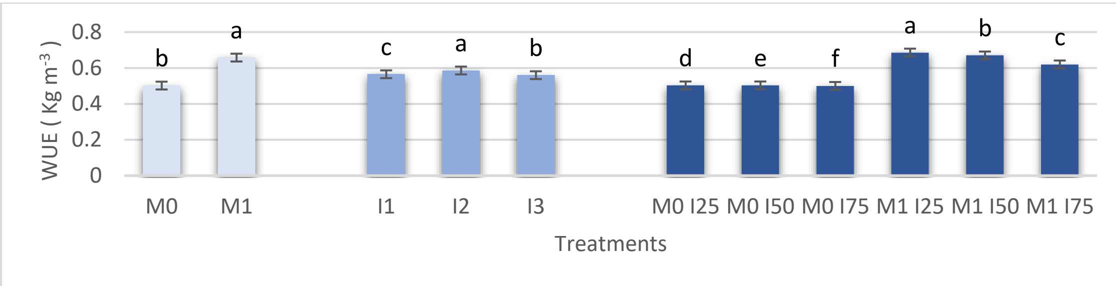

Figure 7. Effect of Mycorrhizal Fungi Inoculation on the Water use efficiency of Barley (Hordeum Vulgare L.) under Drought-Stress in Gypsiferious Soil

Concentration of , N , P , K , elements and percentage of protein % in barley seeds - Figure (8) shows the effect of irrigation and mycorrhiza on N , P , K , elements and percentage of protein % in barley seeds . The irrigation treatment I1 increased significantly compared to all other treatments in N, P, K, and protein % in seeds with an increase rate of 10.83, 17.24, 10.28, and 10.60 % for everyone in N, P, K, and protein % respectively for treatment I1 compared I3. The percentage of increase in 22.02, 33.33, 21.30, and 21.76 % for N, P, K, and protein % respectively for treatment M1 compared to M0. The interaction M1 I1 increased significantly compared to all other treatments in N, P, K, and protein % in seeds with an increased rate of 36.96, 52.00, 35.21, and 36.79 % for everyone in N, P, K, and protein % respectively for treatment M1 I1compared to treatment M1 I3. Irrigation levels were lower than the amount needed by the plant reducing the transport of photosynthesis products and materials absorbed by the roots to the grains and storage areas, which in turn greatly affects the absorption of nitrogen and other nutrients (Ru *et al.*, 2024). The positive effects of the symbiosis microorganisms especially mycorrhizal fungi to promoting growth of barley roots and increasing the ability and absorption of water and nutrition (Farhan *et al.*, 2021; Wahab *et al.*, 2023; Alotaibi *et al.*, 2024).

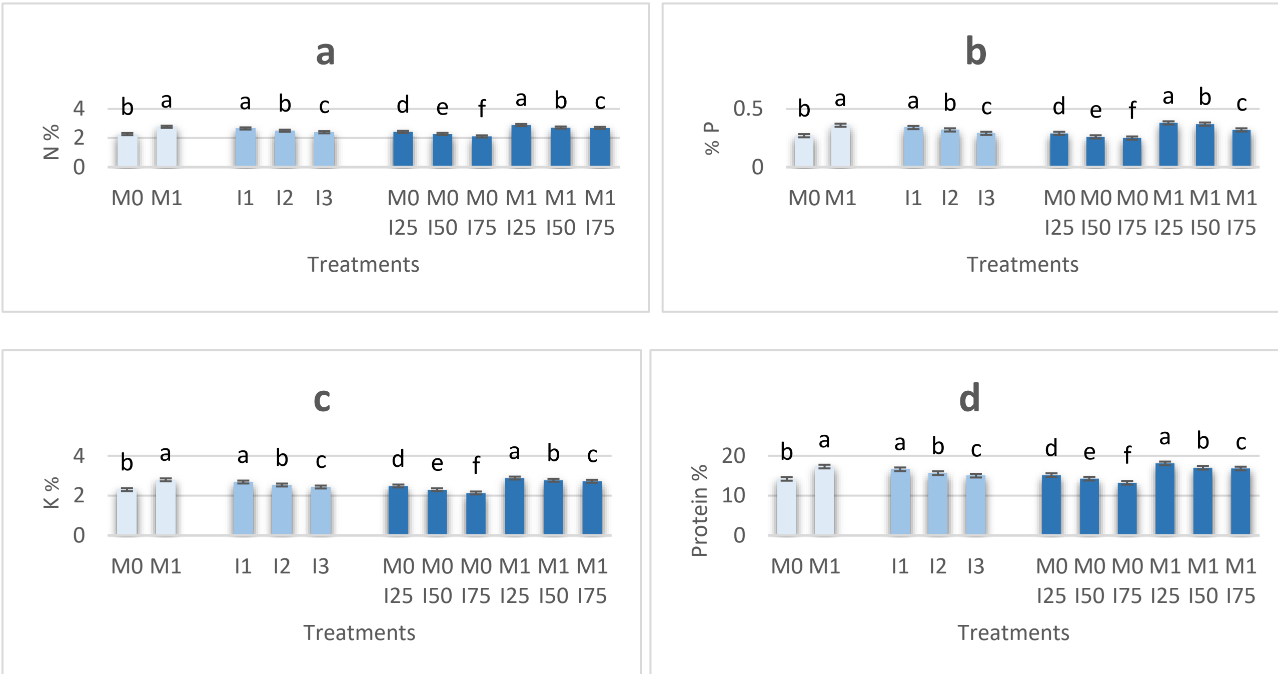

Figure 8. Effect of Mycorrhizal Fungi Inoculation on the (a) N % (b) P % (c) K % (d) Protein % of Barley (Hordeum Vulgare L.) under Drought-Stress in Gypsiferious Soil

CONCLUSIONS

Finally, it could also be concluded that Inoculation with Mycorrhiza has a significant effect in improving growth characteristics and increasing barley yield, in addition to reducing water consumption, increasing water consumption efficiency, and increasing the plant's ability to tolerate low irrigation water and drought stress. Reducing irrigation water certainly leads to reducing the crop's water consumption, in addition to the fact that good management of the irrigation process can give the best water use efficiency, which is what happened in the second irrigation treatment, which achieved the highest efficiency.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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