



RESEARCH ARTICLE

The effect of mycorrhizal species on the growth, essential oils, yield and morpho-physiological parameters of Lemon Balm (*Melissa officinalis* L.) under water-deficit conditions in Tabriz region

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Abstract

Two years experiment were conducted in 2016-2017 as split-plot based on randomized completely block design with three replications to morpho-physiological responses of Lemon Balm (*Melissa officinalis* L.) plant to mycorrhizal fungi species treatments (control, *Rhizophagus intraradices*, *Funneliformis mosseae*, *Glomus hoi* and combined application of all three species) under different irrigation regimes (irrigation after 70, 100, 130 and 160 mm evaporation). In results, the highest oil yield was achieved in irrigation at 100 mm+ application of all three species of mycorrhizal fungi. Irrigation after 100 mm evaporation increased this trait by 33% compared to irrigation after 70 mm evaporation due to increase in essential oil percentage under dehydration conditions, however, increasing drought stress led to a significant decrease in essential oil yield. In terms of physiological parameters, dehydration led to an increase in proline content and antioxidant activities. In general and according to the results, modifying the destructive effects of water deficit stress and the use of mycorrhiza can increase the essential oil of lemon Balm. But aggravating water deficiency conditions can drastically reduce the essential oil yield. The findings could be helpful for herbal medicine researchers to achieve high-quality drugs.

Keywords

Essential oil, lemon balm, Medicinal plants, Mycorrhiza

Introduction

Lemon balm (*Melissa officinalis*) that belongs to the Lamiaceae family is an important aromatic medicinal plant for its therapeutic characteristics for example in digestive problems, including upset stomach, bloating, intestinal gas (flatulence), vomiting and colic; relieve of pain, including menstrual cramps, headache and toothache; and for mental disorders, including hysteria and melancholia (5, 11, 27). The therapeutic properties of this plant are derived from the antioxidant, antimicrobial, anti-inflammatory, anxiolytic and anti-depressant (10, 23, 33, 38).

Fresh herbs contain phenolic compounds, l-ascorbic acid, carotenoids, flavonoids and terpenoids. Lemon balm leaves are rich in flavonoids (0.5 % dry weight) consist of quercitrin (a derivative of quercetin), rambocitrin, luteolin and its derivatives (luteolin 7-o-β-d-glucuronopyranoside, luteolin 3'-o-β-d-glucuronopyranoside, apigenin 7-o-β-d-glucopyranoside and luteolin 7-o-β-d-glucopyranoside-3'-o-β-d-glucuronopyranoside). The

major components among terpenoids are neral. Geranyl acetate, ursolic acid and tannins (31). 0.087 g/100 g of caffeic acid and 21.15 g/100 g of rosmarinic acid were detected in the hydroethanolic extract of lemon balm leaves and phenolic compounds constituted 33.97% (30). Also the most important components of the essential oil of this plant are citral, citronellal, geraniol and linalool (2).

Reaching to high volume of essential oils of medicinal plants are always interested for researchers, however, biotic and abiotic stresses could influence the quality and quantity of essential oil. Controlled drought stress could induce more essential oil synthesis, however, suitable application of drought stress at proper time and stage of plant growth has great importance. Drought, like other stress, has detrimental effects on plant performance (25). Drought has various physiological effects on plants. Drought stress reduced leaf potential, stomatal conductance, nitrate recovery and cell elongation. Decreasing in chlorophyll content of plants exposed to water deficit stress is caused by degradation of pigments and chlorophyll (22). Drought is a multi-dimensional stress that affects plants at different levels in space and time. So the physiological response to Drought is very complex and unpredictable. In fact, the signs of drought are the discoloration of the leaves from green to gray. At the same time the stomata are closed and photosynthesis is drastically reduced (25).

Organic farming is dependent on natural soil microflora that enhances plant growth and yield by employing a variety of beneficial bacteria and fungi, including arbuscular fungi and plant growth promoting bacteria (25). According to available reports, 60 to 90 % of the total fertilizer used is lost and only 10 to 40 % is absorbed by the plant. Studies have shown that microorganisms can play an important role in the integrated management of fertilizers to maintain soil productivity and fertility. Plant growth promoting bacteria and mycorrhizal fungi can increase fertilizer use efficiency (3). Bio fertilizers maintain the soil environment through nitrogen fixation, phosphorus and potassium solubilization or mineralization, release of growth stimulants, production of antibiotics and decomposition of organic matter in the soil, rich in macro and micro elements (31). Previous studies have shown that mycorrhizal

portant for ecosystem stability, plant establishment and biodiversity conservation. The role of mycorrhizal fungi in biodiversity and ecosystem function has been well illustrated, in particular by their role in plant diversity and production power. The positive relationship between plant diversity and colonization of mycorrhizal fungi was confirmed (8). Symbiotic fungi increase the nutrient uptake of host plants and can increase plant growth, quality and resistance to environmental stress (6). Mycorrhizal fungi increase the uptake of nutrients, especially phosphorus, thereby enhancing the growth and yield of plants (37). Mycorrhizal fungi, like other fungi, do not spread their spores through the wind, but are transmitted from plant to plant by soil transfer (19). One of the important effects of mycorrhizal fungi is to increase crop yields, especially in low yield soils. Mycorrhizal fungi increase plant resistance to dehydration by increasing water uptake and a number of nutrients such as zinc and copper, improving leaf cell biogenesis, regulating stomata activity, root growth and development. Mycorrhizal fungi also increase plant resistance to dehydration by regulating plant hormonal activities (18). So, this study was conducted for determination of the effect of different levels of irrigation and different mycorrhizal species on growth and yield essential oil yield of lemon balm.

Materials and Methods

According to Domarton climate conditions, the region has a semi-arid climate. The average annual temperature is 10 °C, the average annual maximum temperature is 16 °C, and the average annual minimum temperature is 2.2 °C. The average annual rainfall in this area is 272.3 mm. The pH of the soils in this region is alkaline to medium.

Soil characteristics

Before any agricultural practices to determine the physical and chemical properties of the soil, soil samples were taken from depth of 0-30 cm then transferred to laboratory for analysis. The results of soil analysis showed that the field soil had sandy loam texture, salinity of 1.56 ds/m and acidity of 7.53 (Table 1). Accordingly the proper volume of fertilizers was used but phosphorus and potassium fertilizers were not used in this study.

Table 1. Results of field soil decomposition during two crop years

Clay %	Silt %	Sand %	P (mg kg ⁻¹)	K (mgkg ⁻¹)	N %	Organic carbon	The acidity of saturated mud	EC (ds/m)	Depth (cm)
44	42	14	8.60	420	0.077	70%	7.5	1.02	0-30

fungi affect plants in a variety of ways, including accelerated growth, nutritional status, water intake, disease resistance and resistance to stress. The response of plants to root colonization by mycorrhizal fungi is largely dependent on the plant type and strain of the fungus and environmental conditions such as soil nutrient levels, light intensity and temperature. Also root colonization of plants by several strains of fungi has more positive effect than single strain application (24).

The presence of arbuscular mycorrhizal fungi is im-

Field experiment

The experiment was conducted as split plot in a randomized complete block design with three replications. The experiment was conducted during two cropping seasons of 2016- 2017 at the Agricultural Station of Islamic Azad University, Tabriz Branch (46°17' E, 38°5' N, 1360 m above sea level). Experimental treatments including drought stress as the main factor in three levels (A₁: Irrigation after 70 mm evaporation from Class A pan (control), A₂: Irriga-

tion after 100 mm evaporation from Class A pan, A3: Irrigation after 130 mm evaporation from Class A pan, and A4: Irrigation after 160 mm evaporation from Class A pan) and different species of mycorrhiza at five levels as a byproduct including control (having no treatment), *R. intraradices*, *F. mosseae*, *G. hoi* and mixture of all three mycorrhiza species.

On 11 June, after leveling the ground, the plot was dimensioned 3 × 1.5 m. Each plot had 3 rows of plants. Sub plot was separated by a plot and 1.5 m far from main plot. On June 16, 2016, irrigation was done before planting.

Prior to planting, mycorrhizal species prepared from the Institute of Soil and Water of Iran then, they were added to the soil according to the experimental map as 9 g per plant and in the mixed form 3 g per plant. In this study, 2-4 leaf transplants of lemon balm were used. The transplants were obtained from the Orumieh Golden Plant Institute and transferred to the main field on June 17, 2016, at the first year of the experiment. Row spacing of 60 cm and seedling spacing of 30 cm and plant density of 5.5 plants m⁻² were also considered. Irrigation was performed immediately after planting. In the first week after transplanting, if some of the transplanted crops were destroyed, replanting was attempted. After transplanting, irrigation was done every 2–3 days, and irrigation was performed every seven days until complete plant establishment. The first stage of weed removal took place on July 9, 2016. Weed control was done manually. In order to provide the plant nitrogen requirements, urea was applied at 60 kg/ha.

In the first year of the study, the second stage of weeding was June 22, 2016. After weeding the field on July 26, 2016, the field was re-fertilized. On this date 60 g were given per plot and used as a spread. After full establishment, irrigation levels were applied according to 70, 100, 130 and 160 mm evaporation from Class A pan. The amount of irrigation for each treatment was based on the calculated total water requirement of the plant which was calculated by random sampling from three different parts of each plot. To determine the vegetation coefficient, two equations: $ET_c = K_c \cdot ET_0$; and $ET_0 = K_{pan} \times E_p$ were used, in which ET_c and ET_0 are reference evapotranspiration and reference evapotranspiration. During each year, in the treatments of 70, 100, 130 and 160 mm, almost 27, 18, 12 and 10 irrigation rounds was done respectively.

After flowering morphological parameters were evaluated. The measured traits include leaf chlorophyll content (chlorophyll a and b), and antioxidant enzymes activity. Also, plant height, leaf area index, yield of essential oil content, flower dry weight, dry matter, proline content and root colonization with mycorrhiza were evaluated in two years.

Determinations of Root Mycorrhizal Colonization

Root mycorrhizal colonization was determined using the standard protocol (38) after clearing with 10% KOH solution, bleaching with 30% H₂O₂ solution, acidifying with 0.1 mM HCl solution, and staining with 0.05% trypan blue solution in lactoglycerol. The root mycorrhiza was observed under a binocular biomicroscope. Root mycorrhizal colonization degree was expressed as the percentage of mycor-

rhiza-colonized root lengths against total observed root lengths.

To extract the essential oil from the Lemon balm plant, the middle rows were harvested manually after removing the marginal rows. After harvesting 10 plants, the samples were transferred to the laboratory and air dried in the shade. To measure the humidity, two grams of the powdered sample were placed in a hygrometer and the amount of moisture was recorded. After determining the moisture content of the samples, they were poured into separate one-liter balloons and about 50% of the balloons were filled with distilled water. After ensuring the proper placement of the balloons and the outlet water from the tubes connected to the Clevenger, the essential oil extraction operation was started by heating the balloons at 100°C. From the boiling, the essential oil extraction operation continued for 2.5 hrs. Then, the device turned off and after 30 min the essential oil collected and wrapped in aluminum sheets to avoid any oxidation and color changes. Because some of the water with the essential oil remains in the tube when the essential oil is collected and it will lose the therapeutic properties of essential oil, therefore, dry sodium sulfate was used for dehydration and pure essential oil was collected using syringe. Essential oil percentage was calculated using below equation:

$$\text{Essential oil percentage} = \frac{\text{Essential oil weight (g)}}{\text{Sample weight (g)}} \times 100$$

Also, essential oil yield was resulted from the following formula:

$$\text{Essential oil yield} = \frac{\text{Yield of the essential oil extraction unit (for example, grain, flower and)}}{\text{Sample weight (g)}} \times \text{Essential oil percentage}$$

Statistical analysis

Statistical analysis of data was performed with SAS 9.1 software and means comparison were by Duncan's multiple range test at 5% level. The graphs were drawn with of Excel software.

Results and Discussion

In this study, the main effects of irrigation and application of mycorrhizal fungi on plant height, leaf area, flower dry weight and total dry yield were significant as well the interaction between the 2 treatments was significant in essential oil percentage and essential oil yield (Table 2).

Leaf area, plant height, flower dry weight and total dry matter yield

Drought stress caused a significant decrease in leaf area per plant, plant height, flower dry weight and total dry matter yield in all of these traits, with the greatest decrease being in irrigation after 160 mm evaporation. So that in these traits, irrigation after 160 mm evaporation from the pan reduced 36.5, 32.6, 39.3 and 30.3% respectively (Table 4). Other researchers have also reported a decrease in

Table 2. Composite analysis of variance of traits studied in lemon balm

Sources of Variations	df	Plant height	Leaf area index	Flower dry weight	Dry matter	Essential oil %	Essential oil yield	Rate of colonization
Year(Y)	1	24.752	0.03	0.005	49.408	0.001	0.331	114.27
Repeat year	4	17.615	0.119	18.267	70.094	0.004	4.208	15,497
Irrigation levels(A)	3	1389.125 **	9,765 **	1363.298 **	17829.149 *	0.058 **	212.780 **	1020.528 **
YA	3	9.447	0.167	109.055	30.283	0.004	1.58	6.948
Original error	12	18.506	0.052	33.793	41.611	0.003	3.007	32.643
Mycorrhizae(B)	4	149.268 **	0.755 **	145.344 *	2042.610 **	0.007	30.553 **	670.381 **
YB	4	39.987	0.226	17.308	430.493 *	0.002	10.778 *	58.659 *
AB	12	41.448	0.15	31.123	158.926	0.010 **	15.929 **	102.892 **
YAB	12	22.048	0.159	39.71	88.432	0.002	3.644	17.249
Minor error	64	22.081	0.152	40.52	133.715	0.003	4.139	20.895
CV(%)		12.99	11.49	20.43	12.98	24.46	37.63	7.57

Table 2—Continued

Sources of Variations	Chlorophyll Index	Chlorophyll a	Chlorophyll b	Catalase	Glutathione Peroxidase	Ascorbate	Proline
Year(Y)	7.203	0.574	0.574	0.00013	0.0001	0.061	0.037
Repeat year	4.834	0.202	0.137	0.001 *	0.00025	0.016	6.554
Irrigation levels(A)	292.582 **	16.175 **	5.163 **	0.005 **	0.004 **	0.122 **	112.872 **
YA	0.845	0.478	0.061	0.001	0.0002	0.022	6.769
Original error	5.579	0.272	0.107	0.00025	0.00033	0.019	4.324
Mycorrhizae(B)	31.284 **	1.510 **	0.409 **	0.002 *	0.002 **	0.138 **	21.045 *
YB	6.987	0.204	0.006	0.00025	0.00025	0.009	10.691
AB	3.975	0.286	0.126	0.0005	0.00025	0.017	7.305
YAB	8.482	0.343	0.138	0.00016	0.00041	0.015	5.011
Minor error	6.869	0.366	0.079	0.001	0.00039	0.019	8.047
CV(%)	17.7	18.95	17.08	41.16	36.82	26.16	37.93

** and* represent significant at the 1 and 5 % probability level, respectively

Table 4. Comparison of mean traits affected by irrigation levels

Irrigation levels	Proline	Ascorbate peroxidase	Glutathione Peroxidase	Catalase	Dry matter	Flower dry weight	Chl.b	Chl.a	Chl. Index	Leaf surface	plant height	The amount of Citral
70	5.590 c	0.4533 c	0.04657 bc	0.04707 c	113.9 a	37.92 a	2,099 a	3.823 a	17.72 a	4,033 a	44.78 a	36.06 a
100	6.293 c	0.4967 bc	0.05617 b	0.05317 bc	103.4 b	35.25 a	1.862 b	3.750 a	17.08 a	3.637 b	37.24 b	34.22 a
130	8.127 b	0.5367 ab	0.04343 c	0.05800 b	79.32 c	28.48 b	1.461 c	2.917 b	13.21 b	3.170 c	34.31 c	29.09 b
160	9.907 a	0.6033 a	0.06960 a	0.07710 a	59.63 d	22.99 c	1.166 d	2.280 c	11.20 c	2.717 d	28.42 d	24.20 c

growth due to dehydration. In *Dracocephalum* it was showed that dehydration reduced plant height (13). A significant decrease in the height of *Agropyron elongatum* was under dehydration found (1). It was reported that drought impedes cell development and consequently plant growth and height (35). In *Coriandrum sativum* showed that dehydration caused a significant decrease in leaf area index (16). The researchers stated that dehydration reduced the leaf area by reducing the each leaf area and number of leaves. It

was also found that dehydration significantly reduced the dry weight of *Dracocephalum moldavica* (4).

Mycorrhizal application significantly increased vegetative traits of lemon balm. In leaf area and total dry weight, all mycorrhizal species had similar additive effects, but in plant height and total dry yield, the highest increase was due to combined application of mycorrhizal species. The combined application of mycorrhizal species increased these two traits by 33.4% and 33.5%, respectively (Table 3). The greater the diversity of mycorrhizal fungi found in soil,

Table 3. Comparison of mean traits affected by irrigation levels

mycorrhizal fungi	Proline	Ascorbate peroxidase	Glutathione Peroxidase	Catalase	Dry matter	Flower dry weight	Chl.b	Chl.a	Chl. Index	Leaf area	plant height
without	6.392 b	0.4375 b	0.04117 c	0.04829 b	75.49 c	27.33 b	1.427 b	2.779 b	12.96 b	3.117 b	32.82 d
<i>R. intraradices</i>	7,583 ab	0.5167 b	0.05758 ab	0.05592 ab	88.73 b	31.40 a	1.638 a	3.275 a	14.42 ab	3,525 a	36.14 bc
<i>F. mosseae</i>	6.721 b	0.5083 b	0.05012 bc	0.05717 ab	93.07 b	31.90 a	1.717 a	3.250 a	15.56 a	3.388 a	37.84 ab
<i>G. hoi</i>	8,017 ab	0.5042 b	0.05754 ab	0.06200 ab	87.24 b	31.04 a	1.696 a	3,200 a	15.47 a	3.350 a	34.92 cd
all	8.683 a	0.6458 a	0.06329 a	0.07079 a	100.8 a	34.14 a	1.757 a	3.458 a	15.61 a	3,567 a	39.22 a

the better the absorption of water and nutrients through the mycorrhizas, the better (17). In this study, it was observed that only the combined application of fertilizers caused a significant increase in the dry yield of lemon balm plants. Khalil and El-Noemani (18) investigated the effect of different levels of phosphorus fertilizer and mycorrhizal fungi application on growth characteristics of the medicinal plant *Lepidium sativum*. The researchers observed that mycorrhizal fungi combined with phosphorus fertilizer caused a greater increase in plant height of *Lepidium sativum*. Increasing levels of phosphorus fertilizer resulted in a higher increase in plant height. It was showed that application of mycorrhizal fertilizer significantly increased leaf area of pepper mint, but different strains of mycorrhizal fertilizer had different effect on total fresh weight (9). *G. intraradices* was the most effective strain in increasing this trait. In the present study, application of phosphorus fertilizer also caused a significant increase in the lemon balm leaf area, but the effect was dependent on mycorrhizal strain. The highest leaf area was obtained using phosphorus fertilizer and *G. intraradices*. A plant needs numerous growth factors such as fertilizer, water and nutrients for optimal growth. Studies have shown that mycorrhizal fertilizers increase the uptake of water and various nutrients such as nitrogen, phosphorus, iron, zinc and manganese in plants, as these microorganisms increase the root uptake of plants (13). In a study done on lemon balm, it was found that the combination of mycorrhizal strains had a greater effect on lemon dry weight than either alone (21).

Essential oil percentages

According to the results of the present study, the highest and lowest essential oil percentages were obtained with 0.36% and 0.16% in two irrigation treatments of irrigation after 100 mm evaporation from pan with application of all three species of mycorrhizal fungi and irrigation after 160 mm evaporation without application of mycorrhizal fungi

respectively. These results indicate that the treatments under study caused significant changes in the percentage of essential oil of lemon balm. By application of *F. mosseae*, *G. hoi* and all three mycorrhizal fungi, reducing irrigation water had not significant effect on essential oil percentage, but with no application of mycorrhizal fertilizer and application of *R. intraradices* it was different. In the absence of mycorrhizal fertilizer application and *R. intraradices*, irrigation water reduction from irrigation after 70 and 160 mm evaporation decreased essential oil by 38.4% and 40.9% respectively. Therefore, the results of this study show that application of mycorrhizal fertilizers can reduce the negative effect of dehydration on essential oil percentage (Table 5). While In lemon balm showed that dehydration reduced the essential oil percentage (12). It was showed that in all plant organs, the percentage of essential oil decreased with increasing amount of available water and the lowest essential oil percentage was observed in treatments that were not drought tolerant (7). With the onset of dehydration, growth stops, cells differentiate and secondary metabolite reservoirs and the carbon plant is devoted to the production of effective pharmaceuticals (7). In this study, application of mycorrhizal fertilizer only in irrigation treatment after 100 mm evaporation had significant effect on essential oil percentage. In this irrigation treatment, application of all three species of mycorrhizal fungi resulted in an increase of 43.26% in lemon balm essential oil percentage. An increase in the percentage of essential oils of medicinal plants by using mycorrhizal fertilizer, it was also reported in *Ocimum basilicum* (40). It was also showed that the effect of different strains of mycorrhizal fungi was different in terms of the percentage of essential oil of *Ocimum basilicum* (40).

Essential oil yield

The highest essential oil yield was obtained after 100 mm evaporation from pan + application of all three species of mycorrhizal fungi. In this treatment, essential oil yield was

Table 5. Comparison of mean traits affected by irrigation levels and mycorrhizal species

	Mycorrhizal species	Essential oil yield	The rate of colonization	Essential oil percentage
70	without	7.050 cde	34.60 fg	0.2600 bcd
	<i>R. intraradices</i>	9.983 ab	69.42 b	0.3050 ab
	<i>F. mosseae</i>	5.133 efgh	62.93 cd	0.1933 defg
	<i>G. hoi</i>	6.733	71.87 b	0.2467 bcdef
	all	8.733 bcd	78.63 a	0.2233 cdefg
100	without	4.717 efghi	31.37 g	0.2533 bcde
	<i>R. intraradices</i>	6.233 defg	63.93 c	0.2517 bcdef
	<i>F. mosseae</i>	9.467 abc	62.93 cd	0.3117 ab
	<i>G. hoi</i>	6.483 def	59.95 cde	0.2583 bcd
	all	11.65 a	73.12 b	0.3633 a
130	without	3.733 ghij	32.37 g	0.2250 cdefg
	<i>R. intraradices</i>	2.983 hij	57.08 def	0.1767 fg
	<i>F. mosseae</i>	4.033 fghij	59.77 cde	0.1983 defg
	<i>G. hoi</i>	3.533 hij	56.27 ef	0.2150 defg
	all	6.267 defg	62.67 cd	0.2900 bc
160	without	1.717 j	51.45 p	0.1600 g
	<i>R. intraradices</i>	2.317 ij	32.85 g	0.1800 efg
	<i>F. mosseae</i>	2.683 hij	54.83 ef	0.2133 defg
	<i>G. hoi</i>	2.267 ij	56.15 ef	0.1817 efg
	all	2.400 ij	54.83 ef	0.1783 efg

11.65 g/m², while the lowest essential oil yield was 1.7 g/m² in irrigation treatment after 160 mm evaporation from pan + no application of mycorrhizal fertilizer. According to the results, moderate dehydration not only had not a negative effect on essential oil yield, but also significantly increased this trait. So that in application of *F. mosseae* and application of all three species of mycorrhizal fungi by reducing irrigation water from irrigation after 70 and 100 mm evaporation from pan, essential oil yield increased by 84.4 and 33.4% respectively, while in application of *R. intraradices*, essential oil yield decreased after 100 mm evaporation from pan (Table 5). Moderate drought stress can stimulate essential oil yield by stimulating the production of secondary compounds. The results of the present study also showed that severe drought stress significantly reduced the essential oil content of medicinal plants. In non-application of mycorrhizal fertilizer, application of *R. intraradices*, application of *F. mosseae*, application of *Glomus hoi* and application of all three species of mycorrhizal fungi, with reduction of irrigation water from irrigation after 70 and 160 mm evaporation from pan, the essential oil yield decreased by 75.6%, 76.9%, 47.7%, 66.3% and 72.5%, respectively. In the present study, in irrigation after 70 mm evaporation from pan, only application of *R. intraradices* significantly increased essential oil yield and increased this trait by 41.6%. In irrigation after 100 mm evaporation from pan, two treatments of *F. mosseae* and application of all three species of mycorrhizal fungi increased essential oil yield by 100 and 146% respectively. In irrigation treatments of irrigation after 130 mm evaporation from pan and irrigation after 160 mm evaporation from pan, application of mycorrhizal fertilizers had no significant effect on essential oil yield. The positive effect of mycorrhizal fungi on essential oil yield has been shown in various studies.

Root colonization percentage

Also, the percentage of colonization of lemon balm roots significantly affected the interaction of irrigation levels and mycorrhizal fungi application (Table 2). The highest percentage of colonization of lemon root with 78.6% was in irrigation after 70 mm evaporation from pan with application of all three species of mycorrhizal fungi. In this study, drought stress alone did not have a significant effect on root colonization percentage in the absence of mycorrhizal fertilizer treatment, while drought with mycorrhizal fungi caused a significant decrease in the colonization rate of lemon balm root. In treatments of application of *R. intraradices*, application of *F. mosseae*, application of *Glomus hoi* and application of all three species of mycorrhizal fungi, with reduction of irrigation water from irrigation after 70 mm evaporation from pan to irrigation after 160 mm evaporation from pan, root colonization percentage decreased by 23.8, 12.8%, 21.8% and 30.2% (Table 5). It was reported that colonization of maize roots and *Citrus reticulata* roots by mycorrhizal fungi was reduced by dehydration (29, 32).

These results indicate that drought stress had negative impact on the percentage of root colonization. Considering the fact that in irrigation after 160 mm evaporation from the pan, there was no significant difference between the studied strains and control in root colonization percent-

age, it can be concluded that the strains under study had the potential to colonize the lemon balm rootstocks at low levels. In this study, in the irrigation after 160 mm evaporation from the pan, mycorrhizal application had the least incremental effect. In irrigation after 70 mm evaporation from pan, irrigation after 100 mm evaporation and irrigation after 130 mm evaporation with application of three types of mycorrhizal fertilizer, The percentage of root colonization was increased by 129, 135.3 and 93.6% respectively and these results showed that the effect of mycorrhizal fungi decreased with exacerbation of dehydration. It was obtained a significant increase in the percentage of pepper mint colonization by using mycorrhizal fertilizer (36). In this study, the application of *Escotelospora calospora* caused the highest increase in this trait at low levels of fertilizer.

Chlorophyll index traits

Combined analysis of variance showed that the main effects of irrigation and mycorrhizal application on chlorophyll index traits, chlorophyll a and b were significant, but interaction of studied factors had no effect on chlorophyll content index of lemon balm (Table 1). Severe drought stress caused the highest decrease in chlorophyll index and chlorophyll content. Irrigation after 160 mm evaporation decreased the chlorophyll index, chlorophyll a and b by 40.3%, 40.8% and 44.4%, respectively (Table 4). Similar results have been reported by other researchers. Investigation is on the effect of irrigation levels on chlorophyll content index of *Rosmarinus officinalis* leaves and reported that dehydration at 60% of field capacity reduced chlorophyll content of *Rosmarinus officinalis* by 23.5% decreases (14). Studies have shown that among the stressors of water scarcity, the greatest decrease in chlorophyll content of crop leaves is due to production of active forms of oxygen in thylakoids (20, 21). According to the results, mycorrhizal fertilizer treatments often caused a similar and significant increase in chlorophyll index and chlorophyll content. Mycorrhizal fertilizer application treatments increased chlorophyll index, chlorophyll a and b by 20.4%, 24.4% and 23.1% respectively (Table 3). The effect of different bacterial strains on *Ocimum basilicum* and observed a significant difference between bacterial strains in terms of chlorophyll index (15). This researchers found the highest increase in chlorophyll index using *Pseudomonades* sp. also observed the positive effect of *R. intraradices* on the chlorophyll content of *Ocimum gratissimum*. It was also showed that the use of mycorrhizal biofertilizer significantly increased the chlorophyll b content of their medicinal plant, *Coleus aromaticus* (41).

Enzymes Activity

Proline content, catalase activity, glutathione peroxidase and ascorbate peroxidase were affected by the main effects of irrigation levels and fertilizer application in the present study (Table 2). The results of this study showed that irrigation treatment after 160 mm evaporation from pan caused the highest increase in these traits and increased proline content, catalase activity, glutathione peroxidase and ascorbate peroxidase by 31.2, 42.6, 49% and 28.3% respectively (Table 4). Proline is an amino acid compound that plays a role in the osmotic regulation of cells. Cellular regu-

lation helps maintain the cell's motility and makes cells more open for longer. Drought stress increases the amount of this compound (26). An increase in the proline content of medicinal plants has also been reported in studies by other researchers. In a study on the medicinal plant, *Trigonella foenum-graceum* it was found that dehydration caused a 98% increase in leaf proline content (34). In current study, the activity of other antioxidants also increased under the influence of dehydration. In this study, in most traits of proline content, catalase activity, glutathione peroxidase and ascorbate peroxidase, and all mycorrhizal species application increased significantly and increased these traits by 26.3, 42.7, 53.9 and 43.4% (Table 3). Reports are on the effect of mycorrhizal application on *Silybum marianum* and observed that application of *F. mosseae* and *R. intraradices* significantly increased the content of catalase and glutathione peroxidase in this plant (28). The researchers attributed the decline to improved plant water uptake.

Conclusion

Medicinal plants are always the basis of modern and traditional medicine and are frequently use in herbal and chemical drugs. Lemon Balm (*Melissa officinalis* L.) medicinal plant by having many active compounds showed a wide range of remedy effects on multiple ailments and disorders and is popular in medicinal plants science and in various system of medicine like Ayurveda, Chines and Traditional Iranian Medicine (TIM). Biotic and abiotic stresses could influence on quality and quantity of essential oil. Controlled drought stress could induce more essential oil synthesis, however, suitable application of drought stress at proper time and stage of plant growth has great of importance. One of the most environmental stresses affected plants growth and yield is drought stress. Water deficit condition could decrease the medicinal plant essential oil yield or alter the presence of therapeutic chemical components of essential oils. Moreover, water deficit could induce plant secondary metabolites and increase the essential oil content. Thereby, determination of suitable application of drought stress has great of importance. According to the results, although irrigation treatment had a positive effect on essential oil yield after 100 mm evaporation from pan, but in total drought stress levels caused a decrease in general oil yield and lemon balm oil yield by increasing drought stress intensity. So that drought stress reduced up to 84% of the oil yield of lemon balm. However, the results showed that the application of mycorrhizal fungi, and particularly the combined application of the fungal species, could reduce the negative effect of drought stress on the studied traits. So that use of the all three species of mycorrhizal fungi under irrigation at 100 mm increased the oil yield by 33% compared to irrigation after 70 mm evaporation. The result of this paper has high importance for herbal medicine researchers and device makers to achieve high quality plants and drugs.

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Authors contributions

NSH designed and conducted the experiments; MY developed the study idea and design; NM participated in writing of the article, AF wrote the manuscript; JA analyzed the data.

Compliance with ethical standards

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