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Enhancing drought resistance in *Dracocephalum moldavica* L. through mycorrhizal fungal inoculation and melatonin foliar application

Behvar Asghari[1,2]✉, Marziyeh Hoseinzadeh[1] & Sudabeh Mafakheri[1]

This research focused on improving the drought tolerance of *Dracocephalum moldavica*, a plant vulnerable to water stress, by exploring the combined effects of melatonin spray and mycorrhizal fungus *Glomus intraradices* inoculation. The experiment was designed as a factorial randomized study to evaluate the plant's morphological, physiological, and phytochemical responses under different drought conditions (100%, 75%, and 50% field capacity). The findings revealed that the combination of melatonin and mycorrhizal inoculation significantly improved the morphological traits of Moldavian balm under drought conditions. Under severe drought (50% field capacity), chlorophyll a and b levels increased by 26.3% and 35.5%, respectively, when both treatments were applied. Stress indicators, including electrolyte leakage and malondialdehyde content, were substantially reduced with the simultaneous application of melatonin and mycorrhizal symbiosis, indicating decreased cellular damage. Moreover, the combined treatment resulted in the highest activities of the antioxidant enzymes catalase and peroxidase, suggesting that these treatments bolster the plant's oxidative stress defense mechanisms. Additionally, drought stress alone led to an increase in secondary metabolites like phenolic and flavonoid compounds, which were further amplified by the treatments. The study also observed significant alterations in the essential oil composition of the plant. Drought stress increased the levels of α-pinene, 1,8-cineole, and borneol, and these increases were even more pronounced with the combined treatments. Conversely, the levels of geraniol and geranial decreased under drought stress and further with treatment. Overall, this research demonstrates that melatonin and *Glomus intraradices* inoculation can effectively enhance drought tolerance in *Dracocephalum moldavica* by improving its physiological characteristics and biochemical composition.

Keywords Abiotic stress, Moldavian balm, Essential oil, Antioxidant activity, Secondary metabolites

Abbreviations

AMF	Arbuscular mycorrhizal fungi
FC	Field capacity
CAT	Catalase
POD	Peroxidase
EL	Electron leakage
MDA	Malondialdehyde assay
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	2,2-Diphenyl-1-picrylhydrazyl
Eos	Essential oils
ROS	Reactive oxygen species

[1]Department of Horticultural Sciences Engineering, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran. [2]Department of Chemistry, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran. ✉email: behvar.asghari@gmail.com; asghari@eng.ikiu.ac.ir

Global climate anomalies along with destruction of ecological balance, have led to serious ecological problems like water deprivation that significantly limit plants' production by impeding their growth and development[1,2]. Plants employ various mechanisms, involve osmotic adjustment[3], balancing of photosynthesis[4,5], creation of antioxidant and scavenger compounds[6], as well as regulation of water absorption and flow[7] in various tissues to tolerate drought stress. Additionally, the accumulation of secondary metabolites serves as a defense mechanism in plants coping with water deficit stress[8,9]. Based on previous researches, the symbiosis of arbuscular mycorrhizal fungi (AMF) with plant roots has been enhanced the ability of different plant species to tolerate and overcome water stress episodes[10,11]. Mycorrhizae refers to the mutualistic association between soil fungi and plant roots[12]. In this symbiosis, the fungus receives carbohydrate materials, mainly in the form of sucrose, from the plant and provides nutrients such as phosphorus for plant[13–15]. The fungus actively transfers nutrients to the plant through membrane carriers that act with protein gradients[16]. Furthermore, the fungus converts carbohydrate materials in the plant phloem into glucose and fructose, which are then absorbed by carriers[17]. AMF has been shown to enhance and regulate water and nutrient absorption, photosynthesis, root structure, antioxidant defense systems, polyamine and fatty acid homeostasis, osmotic adjustment, aquaporin expression, soil structure, and hormone balance, thus enabling plants to resist water deficit stress[18–20]. Different AMF genotypes exhibit varying functionalities in affecting host plants, including the spread of extraradical mycelia, nutrient absorption efficiency, and mycorrhizae-specific gene expression, leading to different growth responses in host plants[21]. Mycorrhizal fungi are particularly effective in plants with unbranched roots, as they can alter the speed of water movement outside and inside the host plant, thereby influencing tissue dehydration and leaf physiology[22].

Melatonin is a novel plant growth regulator that is increasingly being used in various crops to enhance their resistance to environmental stress[23]. Its accumulation in plants may indicate a mechanism for the preservation of tissues against oxidative damage, caused by water stress[24]. Previous studies have demonstrated the protective role of melatonin against biotic and abiotic stresses, including drought[25], salinity[26], extreme temperatures[27], excess heavy metals[28], pathogen attacks[29], and senescence[30]. Additionally, melatonin regulates physiological processes such as ion homeostasis[31]. Moreover, melatonin acts as an antioxidant and effectively scavenges free radicals in plants[32]. Among all plant growth regulators, melatonin possesses the highest antioxidant capacity[33] and is recognized as the molecule that affects the countenance of stress-responsive genes and promotes water conservation in plants[34]. The positive effect of melatonin in reducing oxidative injury caused by water deficiency has been demonstrated in *Cucumis sativus*[35]. The antioxidant activity of melatonin is attributed to its direct scavenging of free radicals[36], stimulation of enzymatic and non-enzymatic antioxidant systems activity[37], and enhancement of mitochondrial electron transfer chains[38], which reduces the production of free radicals and ion leakage. So, the application of exogenous melatonin enables plants to survive under abiotic stresses by improving their recovery potential and resilience[39].

Interest in medicinal plants and their natural compounds has been steadily increasing, particularly for their use in both medicinal and cosmetic applications. As a result, these plants are being utilized more frequently across various industries, including medical, cosmetic, sanitary, and food sectors. This surge in demand underscores the importance of conducting comprehensive basic and applied research to ensure their effective and sustainable use. Proper research and responsible exploitation of these pharmaceutical plants are essential for maximizing their potential and ensuring their long-term availability[40,41].

Dracocephalum moldavica L., commonly known as Moldavian dragonhead or Moldavian balm, is a native plant to central Asia belonging to the Lamiaceae family[42]. *Dracocephalum* is a genus of approximately 70 species of flowering plants and the essential oil composition of this genus has been investigated[43]. *Dracocephalum moldavica* is an upright, thick, branching herb that is frequently used as a food additive or oral antibacterial agent[44]. The essential oil and extracts of this plant exhibit antimicrobial[45], antitumor[46], antigenotoxic[47], anti-cardiovascular[48] antioxidant and anti-inflammatory[49] properties. The secondary metabolites extracted from the aerial parts of the plant, such as monoterpene glycosides, lignans, rosmarinic acid, and flavonoids, have great economic and medicinal significance[50–52]. *Dracocephalum moldavica* is traditionally used as a heart tonic, tranquilizer, flatulence remedy, sudorific agent, treatment for snakebites, and remedy for nausea, gastroenteritis, stomatitis, and fungal infections[53,54].

In this research, we investigated the impact of low irrigation stress, foliar application of melatonin and presence or absence of mycorrhizal fungi (*Glumus intraradices*) on *Dracocephalum moldavica*. We assessed various growth indicators, effective substances, and the plant's essential oil composition.

Material and methods

Plant matter, growth condition and treatments

This pot experiment was undertaken during March to June of 2023 at the research greenhouse of the Faculty of Agriculture and Natural Resources, International Imam Khomeini University in Qazvin, Iran. The greenhouse was maintained at a temperature range of 18–25 °C with 50–60% relative humidity under natural light conditions.

This study implemented a factorial experimental design, incorporating three factors: irrigation levels at 100%, 75%, and 50% of field capacity (FC), melatonin treatment at 0 and 150 μM and arbuscular mycorrhizal fungi inoculation (present or absent). The experiment was arranged in a randomized complete block design with 12 treatments replicated three times. Pakan Bazr Company, located in Isfahan, Iran, provided the Moldavian balm seeds for the experiment. Melatonin, sourced from Kimia Novin (Tetra Chem), and the *Glomus intraradices* mycorrhiza strain from Turan Biotechnology Company were utilized. A total of 20 seeds were planted in each pot, distributed evenly in 5 parts of the pot, and sown at a depth of 2 cm in the soil. The plastic pots containing field soil and vermicompost in a ratio of 3:1 had a depth of 20 cm and a diameter of 22 cm. The physical and chemical properties of the soil are presented in Table 1. After thorough washing in running tap water, the seeds were briefly soaked in 70% ethanol for 30 s. Following this, they underwent surface sterilization using 2% sodium hypochlorite solution for 15 min and were then rinsed extensively with autoclaved distilled water

Soil texture	Available K^+ (ppm)	Available P (ppm)	Total N (%)	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	Electrical conductivity ($dS\ m^{-1}$)	pH
Loamy-Sandy	283	9.7	0.08	35	38	27	0.75	1.8	7.4

Table 1. Soil physical and chemical properties.

eight to ten times. Mycorrhizal treatment was concurrently applied during sowing via seed inoculation, with 5 g of mycorrhizal fungi powder applied per sowing point. Seedling emergence occurred approximately 3 weeks after planting. Thinning occurred after seed germination, retaining one plant per sowing point, resulting in five plants per pot. Standard agronomic practices were followed throughout the growth phase. At the 6-leaf stage, which occurred two months after planting, the first foliar spraying of melatonin was conducted. Subsequent foliar sprayings were performed once every ten days until harvest. Additionally, irrigation with tension was carried out once every 5 days until harvest. Morphological features were measured during the flowering stage. Analyses, including catalase (CAT), peroxidase (POD), electron leakage (EL), and photosynthetic pigments, were performed at the beginning of the flowering stage, approximately one week before harvest.

To investigate the effects of experimental treatments on various growth parameters four plants were randomly selected from each pot and evaluated. Internode length, leaf length and width, and plant height were measured using a ruler. The number of lateral branches and the number of inflorescences per plant were also carefully counted and recorded before harvesting. Then, at the flowering stage, the plants were harvested 3 cm above the soil surface. The harvested plants were measured for fresh weight and then transferred to a drying room for desiccation. Drying was carried out at room temperature under complete shade, and subsequently, the dry weight of each plant was determined and recorded.

Photosynthetic pigments
Leaf samples weighing 0.1 g underwent extraction using 80% acetone. The obtained extracts were centrifuged at 5000 rpm for 15 min. Absorbance readings of the resultant supernatants were taken at specific wavelengths: 663, 645, and 470 nm, utilizing a UV-1800 Shimadzu spectrophotometer from Japan. Subsequently, the concentrations of chlorophyll a, b and carotenoids pigments were calculated based on established formula[55].

Electrolyte leakage (EL) assay
To evaluate membrane integrity (EL), 1 g of fresh and undamaged aerial tissue from the plant leaf was rinsed with distilled water to eliminate any surface ions. This tissue was then placed in sealed test tubes, and 10 ml of deionized water was added to each tube to extract ions. The tubes were submerged in water bath at 32 °C for 2 h, during which we measured the electrical conductivity of the samples (EC1) using a Winlab Data Windaus EC meter. Subsequently, the tubes were autoclaved at 121 °C for 20 min. After cooling the contents to 25 °C, the electrical conductivity of the samples was measured again (EC2). Finally, we calculated the percentage of ion leakage using the following formula[56]:

$$\mathrm{EL\%} = \frac{\mathrm{EC1}}{\mathrm{EC2}} \times 100$$

Malondialdehyde assay (MDA)
To assess the degree of lipid peroxidation in the membrane, we utilized the measurement of malondialdehyde (MDA) as a marker compound. In this process, 0.2 g of plant leaf material, previously ground in liquid nitrogen, was transferred to a test tube. Subsequently, 4 ml of a 1% trichloroacetic acid solution was added to the tube. Following centrifugation at 10,000 rpm for 10 min at 4 °C, 1 ml of the resulting supernatant was mixed with 4 ml of a 20% trichloroacetic acid solution containing 0.5% thiobarbituric acid. This mixture was then heated at 95 °C for 30 min using a water bath. Finally, the solutions were rapidly cooled in an ice bath, and their absorbance was measured at wavelengths of 532 and 600 nm. The quantity of MDA was determined by applying a molar extinction coefficient of 155 $mM^{-1}cm^{-1}$, expressed as nanomoles per g of sample[57].

Antioxidant enzymes activity assay
The enzymes found in Moldavian Balm samples were extracted from finely grinding 1 g of leaves in liquid nitrogen. Subsequently, 5 ml of 50 mM phosphate buffer solution (pH 7.8) was added on the ground leaves, and the mixture was placed in an ice bath. Afterward, the mixture was centrifuged at 15,000 *rpm* for 30 min at 4 °C. The resulting supernatant was then utilized for subsequent analyses.

Catalase (CAT) enzyme activity was assessed by monitoring changes in absorbance over a 30 s period at a wavelength of 240 nm. The reaction solution consisted of phosphate buffer with a pH of 7.8 and a concentration of 50 mM, 200 mM hydrogen peroxide, and 0.1 ml of enzyme solution obtained from plant samples[56].

Peroxidase (POD) enzyme activity was determined by measuring the increase in absorbance of a solution containing 1 mM guaiacol, 5.0 mM hydrogen peroxide, and 0.1 ml of the enzyme extract obtained from the plant in a 50 mM phosphate buffer (pH 7.8) at a wavelength of 420 nm[58].

Total phenolic content (TPC) assay
The phenolic composition of plant samples was assessed using the Folin-Ciocalteu technique and spectrophotometric analysis. To accomplish this, a 10% volumetric solution of Folin reagent was prepared. Subsequently, 100 µl of this solution were mixed with 20 µl of plant extract, followed by a 10 min incubation period in darkness. Then, 80 µl of a 5.7% w/v sodium carbonate solution was added and incubated for 90 min

in dark at room temperature. The absorbance of the reaction mixture was ultimately measured at 760 nm. Gallic acid served as the standard for calibration, and the phenolic content of the samples was expressed as mg of gallic acid equivalent per g of extract[59].

Total flavonoid content (TFC) assay

The determination of total flavonoid compounds in plant samples was conducted using aluminum chloride reagent. In this procedure, 10 µl of a 5% w/v solution of the reagent were mixed with 20 µl of the plant extract. Following dilution with 60 µl of methanol, the total reaction volume was adjusted to 200 µl by addition of 10 µl of a 0.5 M potassium acetate solution and an appropriate volume of distilled water. After 30 min incubation period, the absorbance of the resulting solution was measured at 415 nm. Quercetin served as the reference standard for constructing the calibration curve, and the findings were expressed as mg of quercetin equivalent per g of dry extract[60].

DPPH radical scavenging activity assay

The assessment of the antioxidant potential of the plant extracts was conducted via the estimation of their radical scavenging capacity using the DPPH assay. In this procedure, 20 µl of plant extracts of known concentrations were combined with 180 µl of DPPH solution at a concentration of 1.0 mM. Subsequently, after a 30 min incubation period, the absorbance of the resulting mixture was measured at 517 nm. The outcomes are delineated as the percentage of DPPH radical inhibition[61].

Essential oil extraction and analysis

The aerial parts of the treated plants were collected at the peak flowering stage and dried in the shade at room temperature. The dried plant samples were then ground into powder, and their essential oils (EOs) were extracted through hydro-distillation for three hours using a Clevenger-type apparatus. Quantitative and qualitative analysis of the EOs were subsequently carried out by gas chromatography with a flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC–MS). For the GC analysis, an Agilent 7890A (USA) gas chromatograph equipped with an FID was utilized. The analysis employed a fused silica capillary DB-5 column (30 m × 0.25 mm internal diameter; film thickness 0.25 µm). The injector and detector temperatures were maintained at 280°C and 300°C, respectively. Helium was used as carrier gas with a linear velocity of 32 cm^3s^{-1}; the oven temperature was programmed to be held at 50°C for 5 min and then increased to 280°C at a rate of 10°C/min.; the split ratio was 1:60. The GC–MS analysis was conducted using a Varian 3400 GC/MS system fitted with a fused silica capillary DB-5 column (60 m × 0.25 mm internal diameter; film thickness 0.25 µm). Helium was used as carrier gas with an ionization voltage of 70 eV. The ion source and interface temperatures were set at 200°C and 250°C, respectively. The mass range spanned from 40 to 300 AMU. The oven temperature program for the GC–MS was the same as described above for the GC analysis.

The constituents of the essential oils (EOs) were identified by calculating their retention indices under temperature-programmed conditions for n-alkanes (C_6–C_{24}) and comparing these with the oils on a DB-5 column using the same chromatographic parameters. Each compound was then identified by comparing their mass spectra with those in the internal reference mass spectra library (Adams and Wiley 7.0) or with known standards. The identification was further confirmed by comparing their retention indices with those of authentic compounds or values reported in the literature[62].

Statistical analysis

A two-way analysis of variance (ANOVA) was performed using SAS software 26 to examine the differences among the various employed treatments. Multiple mean comparisons were conducted using Duncan's Multiple Range Test at a significant level of $p \leq 0.05\%$.

Results

Morphological traits

Based on the results of analysis of variance, the effect of drought stress on all morphological traits of Moldavian balm plant was significant at 1% level. Inoculation with mycorrhizal fungi also significantly affected all morphological traits at 1% level, except for leaf length, which was significant at 5% level. Foliar application of melatonin had a significant effect only on certain traits such as plant height, number of lateral branches, fresh weight, and dry weight of the plant at the 1% level, and on leaf width at the 5% level. Overall, by examining the results obtained for various morphological traits studied in plants (Table 2), the negative effects of drought stress on all these traits are clearly evidenced. For instance, in some of the most important morphological traits such as plant height, fresh weight, and dry weight, it can be observed that applying drought at 75% of FC, resulted in decreases of 4.74%, 21.48%, and 24.8%, respectively. Meanwhile, applying irrigation restrictions at 50% of FC led to further reductions in these traits by 17.52%, 38.91%, and 42.94%, respectively. The application of melatonin and inoculation with mycorrhizal fungi (Fig. 1) demonstrated beneficial effects on plant growth traits not only under normal irrigation conditions but also in alleviating the adverse impacts of drought stress on morphological characteristics. Detailed analysis of the data indicates that the most favorable outcomes in morphological traits were observed in plants subjected to normal irrigation combined with mycorrhizal fungi inoculation and melatonin spraying. For instance, the simultaneous application of mycorrhizal fungi inoculation and external melatonin solution spray under 50% FC drought conditions compensated for the damage to plant height, fresh weight, and dry weight by 18.39%, 34.31%, and 40.28%, respectively.

		Plant height (cm)	Internode length (cm)	Length of inflorescences (cm)	Number of branches	Fresh weight (g)	Dry weight (g)	Leaf length (cm)	Leaf width (cm)
100% FC	Mela (0)—FnI	46.80 ± 1.77 de	4.33 ± 0.29 b-e	6.83 ± 0.29 de	5.25 ± 0.25 cd	21.23 ± 1.62 cd	4.96 ± 0.14 c	5.25 ± 0.25 a-d	2.33 ± 0.15 a-c
	Mela (0)—FI	53.35 ± 0.49 a	4.92 ± 0.14 a	9.33 ± 0.28 a	6.17 ± 0.25 b	24.26 ± 0.81 b	5.34 ± 0.25 bc	5.70 ± 0.36 ab	2.60 ± 0.30 ab
	Mela (150)—FnI	51.63 ± 2.95 a-c	4.50 ± 0.25 a-d	7.03 ± 0.21 de	5.80 ± 0.26 bc	23.10 ± 2.49 bc	5.03 ± 0.20 c	5.33 ± 0.38 a-d	2.33 ± 0.21 a-c
	Mela (150)—FI	54.33 ± 0.76 a	4.83 ± 0.15 a	8.58 ± 0.52 ab	6.80 ± 0.27 a	28.27 ± 0.44 a	6.20 ± 0.37 a	5.93 ± 0.40 a	2.67 ± 0.14 a
75% FC	Mela (0)—FnI	44.58 ± 2.12 e	4.02 ± 0.36 ef	6.67 ± 0.58 de	4.83 ± 0.58 d	16.67 ± 0.85 f.	3.73 ± 0.25 d	5.13 ± 0.15 a-d	1.70 ± 0.10 e
	Mela (0)—FI	50.04 ± 0.66 bc	4.67 ± 0.14 ab	7.93 ± 0.12 bc	5.73 ± 0.22 bc	19.30 ± 0.64 de	4.95 ± 0.34 c	5.53 ± 0.55 a-c	2.32 ± 0.26 a-d
	Mela (150)—FnI	48.93 ± 1.40 cd	4.32 ± 0.20 b-e	7.07 ± 0.15 de	5.63 ± 0.15 bc	18.87 ± 0.55 ef	4.23 ± 0.09 d	5.25 ± 0.43 a-d	2.20 ± 0.35 b-c
	Mela (150)—FI	52.71 ± 2.28 ab	4.58 ± 0.14 a-c	8.17 ± 1.12 bc	5.76 ± 0.21 bc	24.43 ± 2.45 b	5.64 ± 0.59 b	5.43 ± 0.51 a-d	2.22 ± 0.10 a-d
50% FC	Mela (0)—FnI	38.60 ± 1.85 f.	3.68 ± 0.35 f.	6.25 ± 0.25 e	4.87 ± 0.35 d	12.97 ± 0.64 g	2.83 ± 0.30 e	4.53 ± 0.54 d	1.26 ± 0.22 f.
	Mela (0)—FI	45.83 ± 2.05 de	4.00 ± 0.25 ef	7.42 ± 0.52 cd	5.25 ± 0.66 cd	17.40 ± 0.66 ef	3.99 ± 0.21 d	4.67 ± 0.80 cd	2.05 ± 0.23 c-d
	Mela (150)—FnI	43.83 ± 1.63 e	4.10 ± 0.10 de	6.33 ± 0.29 e	5.30 ± 0.20 cd	16.68 ± 0.59 f.	3.75 ± 0.15 d	4.83 ± 0.38 b-d	1.87 ± 0.25 de
	Mela (150)—FI	45.70 ± 1.42 e	4.17 ± 0.15 c-e	7.08 ± 0.38 de	5.40 ± 0.52 cd	17.42 ± 0.88 ef	3.97 ± 0.35 d	5.01 ± 0.50 b-d	2.08 ± 0.38 c-d

Table 2. Effect of mycorrhizal fungi (*Glomus intraradices*) inoculation and foliar application of melatonin on morphological characteristics of *Dracocephalum moldavica* under different irrigation regimes (100, 75 and 50% FC). Different letters are significantly different according to Duncan's multiple-range test at $p \leq 0.05$. Mela (0) and (150): spray with melatonin solution with 0 and 150 µM concentrations, respectively; FnI and FI: mycorrhizal fungi non-inoculated and inoculated plants, respectively.

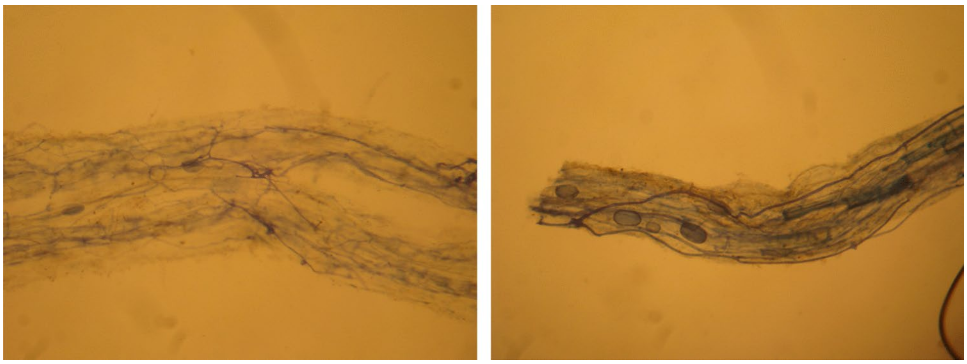

Fig. 1. Mycelium and vesicle of *Glomus intraradices* in the root of *Dracocephalum moldavica*.

Photosynthetic pigments content

According to the analysis of variance, drought stress and mycorrhizal inoculation had statistically significant effects on chlorophyll a and b contents ($p \leq 0.01$). Additionally, melatonin application significantly influenced chlorophyll a and b levels at a 5% significance level. However, while these factors were independently significant, their interactive effects were not statistically meaningful. Drought stress negatively affects chlorophyll a and b content in Moldavian Balm, as evidenced by the reduced levels of both pigments under 75% and 50% field capacity (FC) irrigation conditions. However, the application of mycorrhizal inoculation and melatonin played a crucial role in mitigating these adverse effects. Plants treated with both mycorrhiza and melatonin showed significantly higher chlorophyll a and b levels compared to non-treated plants under similar drought conditions. The highest chlorophyll a content (31.36 mg/g FW) was observed in plants receiving full irrigation (100% FC) combined with mycorrhizal inoculation and melatonin application. Under 75% and 50% FC drought conditions, the simultaneous application of mycorrhizal inoculation and melatonin increased chlorophyll a level by approximately 28.1% and 26.3%, respectively (Fig. 2A). The effectiveness of mycorrhizal treatment in increasing chlorophyll b content was superior to that of melatonin application. Furthermore, the combined application of these treatments improved the plant's resilience against drought-induced reductions in chlorophyll b levels (Fig. 2B). Notably, the protective effect of combined mycorrhizal inoculation and melatonin application was more pronounced under severe drought stress (50% FC), where it led to a 35.5% increase in chlorophyll b content, compared to a 21% increase under moderate drought stress (75% FC).

The results of the analysis of variance (ANOVA) indicated that the effects of drought stress, mycorrhizal inoculation, and melatonin foliar spray were individually significant on leaf cell carotenoid level ($p \leq 0.01$). However, no significant differences were observed in their interactive effects on carotenoid amount. Application of drought stress at 75% and 50% FC led to a decrease in leaf carotenoid content in the plant by 16.9 and 37.3%, respectively (Fig. 2C). Inoculation with mycorrhiza fungi and application of melatonin treatment have elicited an augmentation in carotenoid levels under both applied drought conditions. Nevertheless, under non-stress

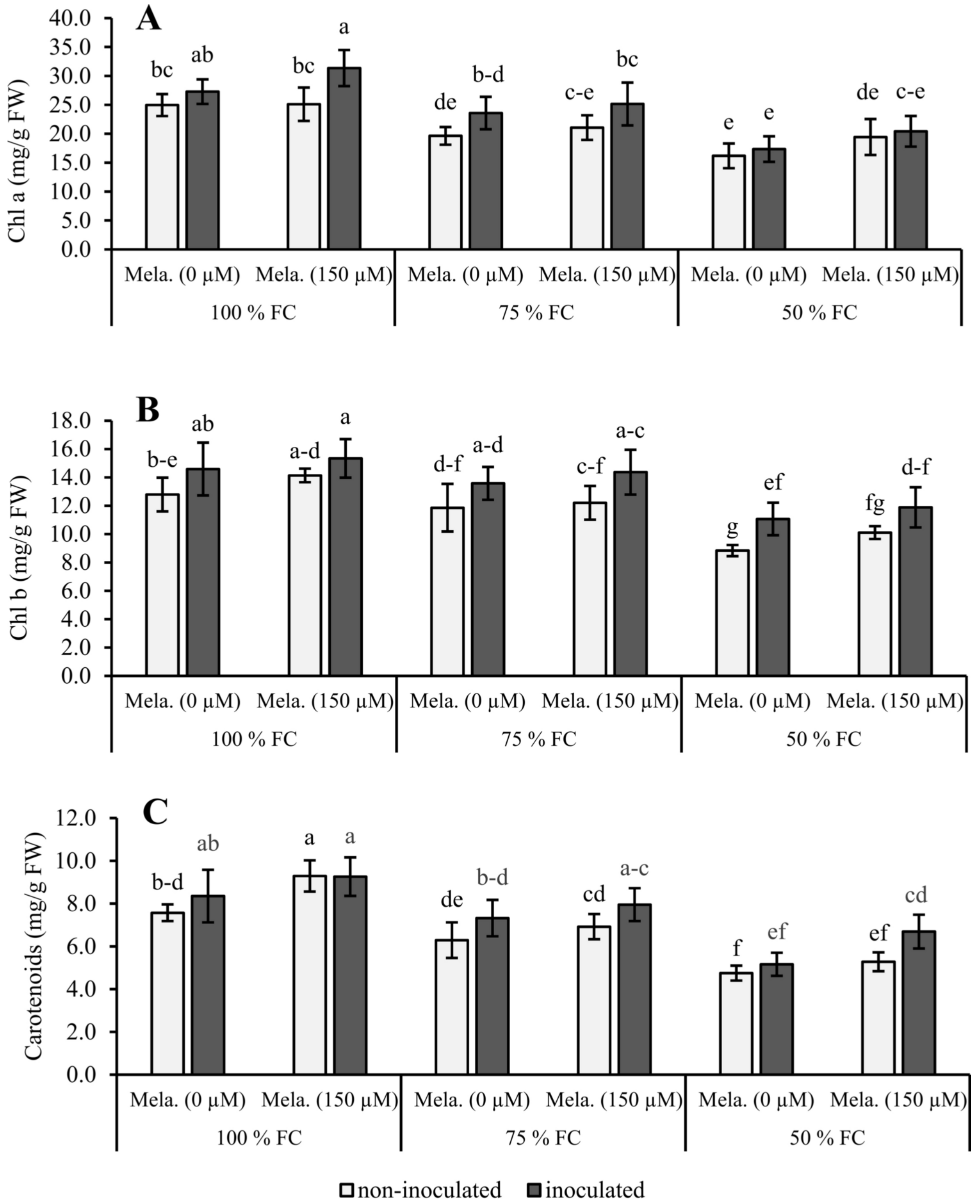

Fig. 2. Effect of mycorrhizal fungi (*Glomus intraradices*) inoculation and foliar application of melatonin on chlorophyll a (**A**), chlorophyll b (**B**) and carotenoid (**C**) contents of *Dracocephalum moldavica* under different irrigation regimes (100, 75 and 50% FC). Bars labeled by different letters are significantly different according to Duncan's multiple-range test at $p \leq 0.05$.

conditions, mycorrhizal inoculation failed to induce a statistically significant disparity in the carotenoid content of Moldavian balm plants.

EL and MDA

The outcomes of the analysis of variance (ANOVA) elucidated drought stress, mycorrhizal inoculation, melatonin spray, and their duplicate interactive effects significantly influenced the level of leaf cells EL at 1% level ($p \leq 0.01$). Additionally, a noteworthy triple interaction effect of the mentioned treatments and drought stress was observed to exert a significant influence on EL levels, with a significance level set at 5% ($p \leq 0.05$).

Based on the obtained results (Fig. 3A), the imposition of drought stress significantly increased EL and with escalation in stress severity from 75 to 50% of field capacity (FC), a significant augmentation of EL within plant cells has been resulted. The highest level of EL was observed in samples subjected to the most severe drought condition (50% FC), devoid of mycorrhizal inoculation and melatonin treatment (68.2%). The application of mycorrhizal inoculation and melatonin spray distinctly reduced EL level in both drought scenarios, leading to a respective decrease of 12.4% and 20.02%, when concurrently administered under 75% and 50% FC drought conditions. However, mycorrhizal inoculation and melatonin treatment did not induce a substantial change in EL levels of *D. moldavica* under unstressed conditions.

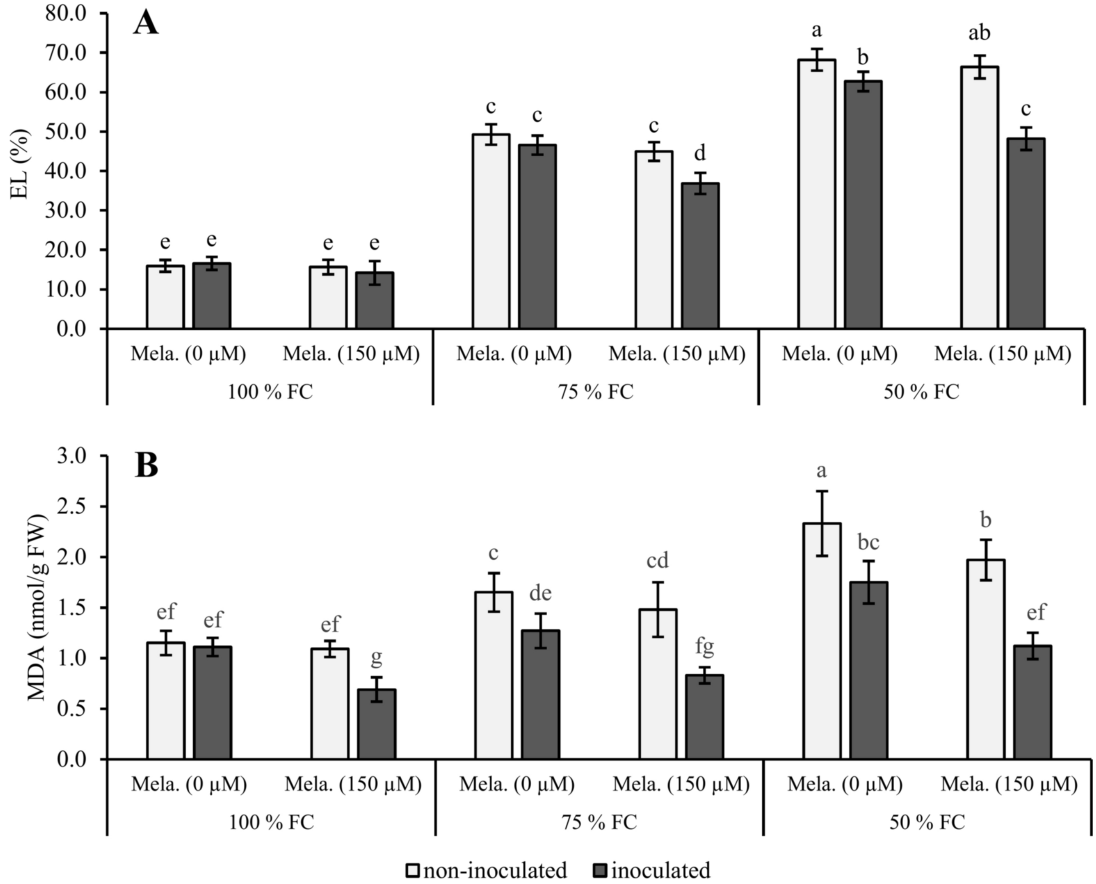

Fig. 3. Effect of mycorrhizal fungi (*Glomus intraradices*) inoculation and foliar application of melatonin on electrolyte leakage (EL) (**A**) and malondialdehyde (MDA) (**B**) content of *Dracocephalum moldavica* under different irrigation regimes (100, 75 and 50% FC). Bars labeled by different letters are significantly different according to Duncan's multiple-range test at $p \leq 0.05$.

The effects of drought stress, mycorrhizal inoculation, melatonin foliar spray, and the interaction between drought stress and mycorrhizal inoculation on MDA levels were found to be statistically significant at 1% level ($p \leq 0.01$). Moreover, the interaction between mycorrhizal inoculation and melatonin treatment on MDA levels showed significance at a 5% level ($p \leq 0.05$). However, the triple interaction effects of these treatments did not exhibit statistically significant differences. In a similar trend to the electrolyte leakage (EL) the malondialdehyde (MDA) levels in Moldavian Balm plants exhibited an increase under drought stress conditions. The highest recorded MDA level, reaching 2.33 nmol/g FW, was observed in plants subjected to the most severe drought stress level without any additional treatments. Simultaneous inoculation with mycorrhiza and spraying with melatonin solution in drought stress conditions reduced the MDA content by approximately half (Fig. 3B).

Antioxidant enzymes activity (CAT and POD)

This study investigated the activity levels of two antioxidant enzymes including catalase and peroxidase, in response to the application of melatonin treatments and mycorrhizal inoculation under drought stress in Moldavian Balm plants. The findings from the analysis of variance revealed statistically significant effects of drought stress, mycorrhizal inoculation and melatonin spray application on both antioxidant enzymes ($p \leq 0.01$).

Drought stress individually imposition, notably augmented the activities of both antioxidant enzymes (Fig. 4). Notably, subjecting the plants to 50% field capacity (FC) drought stress sans additional treatments amplified catalase and peroxidase activities by approximately 2.2 and 2.7 times, respectively. Moreover, mycorrhizal inoculation and melatonin application under drought stress conditions elicited heightened activities of these antioxidant enzymes. Remarkably, the pinnacle of catalase activity (4.7 U/mg protein) was recorded in Moldavian balm plants subjected to the most severe drought stress (50% FC), concurrently treated with mycorrhizal inoculation and melatonin. Meanwhile, the co-application of mycorrhizal inoculation and melatonin spray at both 75% and 50% FC levels yielded the highest peroxidase activity (2.64 and 2.68 U/mg protein). It's noteworthy that under non-stress conditions, catalase activity showed no significant disparity with mycorrhizal inoculation alone, whereas the combined application of this treatment with melatonin spray notably augmented catalase enzyme activity.

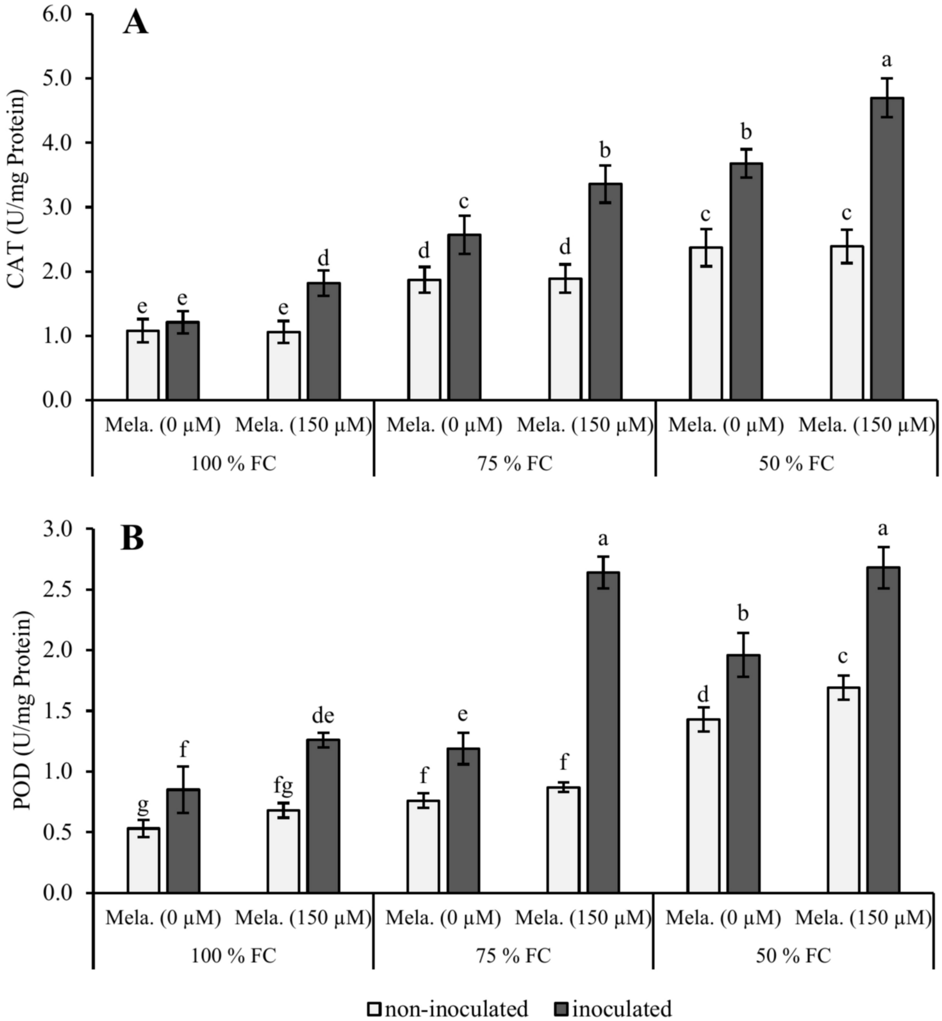

Fig. 4. Effect of mycorrhizal fungi (*Glomus intraradices*) inoculation and foliar application of melatonin on catalase (CAT) (**A**) and peroxidase (POD) (**B**) activities of *Dracocephalum moldavica* under different irrigation regimes (100, 75 and 50% FC). Bars labeled by different letters are significantly different according to Duncan's multiple-range test at $p \leq 0.05$.

Total phenolic and flavonoid contents

The results of the analysis of variance indicate that the effect of drought stress, mycorrhizal inoculation, melatonin spray, and their duplicate interactions were significant on the phenolic content of Moldavian balm at 1% level. Moreover, the triple interaction of the treatments at 5% level was also found to be significant on TPC. As depicted in Fig. 5A, the imposition of drought stress exhibited an increasing trend in the phenolic compound levels. The highest amount of these compounds, equivalent to 73.4 mg GAEs per gram dry weight of extract, was measured in samples subjected to the highest level of drought stress, along with mycorrhizal inoculation and melatonin spray. The second highest phenolic content position was attributed to the samples treated with 75% drought stress and simultaneous application of mycorrhizal inoculation and melatonin exogenous spray (62.6 mg GAEs/g DW).

Based on the analysis of variance results, the effects of drought stress, mycorrhizal inoculation, and melatonin spray, individually, were statistically significant on the flavonoid content of Moldavian balm plants at 1% level. However, no significant differences were observed in their interaction effects. The results illustrated in Fig. 5B indicate that the effect of drought stress and applied treatments on the flavonoid content of Moldavian balm plants followed a similar trend to that observed for the phenolic content. In other words, under different levels of drought stress, as well as mycorrhizal inoculation and melatonin spray treatments, all led to a positive impact on the flavonoid content of these plants. Consequently, the highest total flavonoid content, equivalent to 29.3 mg of quercetin per gram of dry weight of extract, was observed in samples subjected to all applied treatments simultaneously, showing an almost twofold increase compared to the control samples. Moreover, plant samples grown under 75% of field capacity irrigation and treated with mycorrhizal inoculation and melatonin showed high levels of flavonoid content, ranging from 25 to 55 mg quercetin per gram of extract, which did not significantly differ from samples containing the highest amounts of flavonoids.

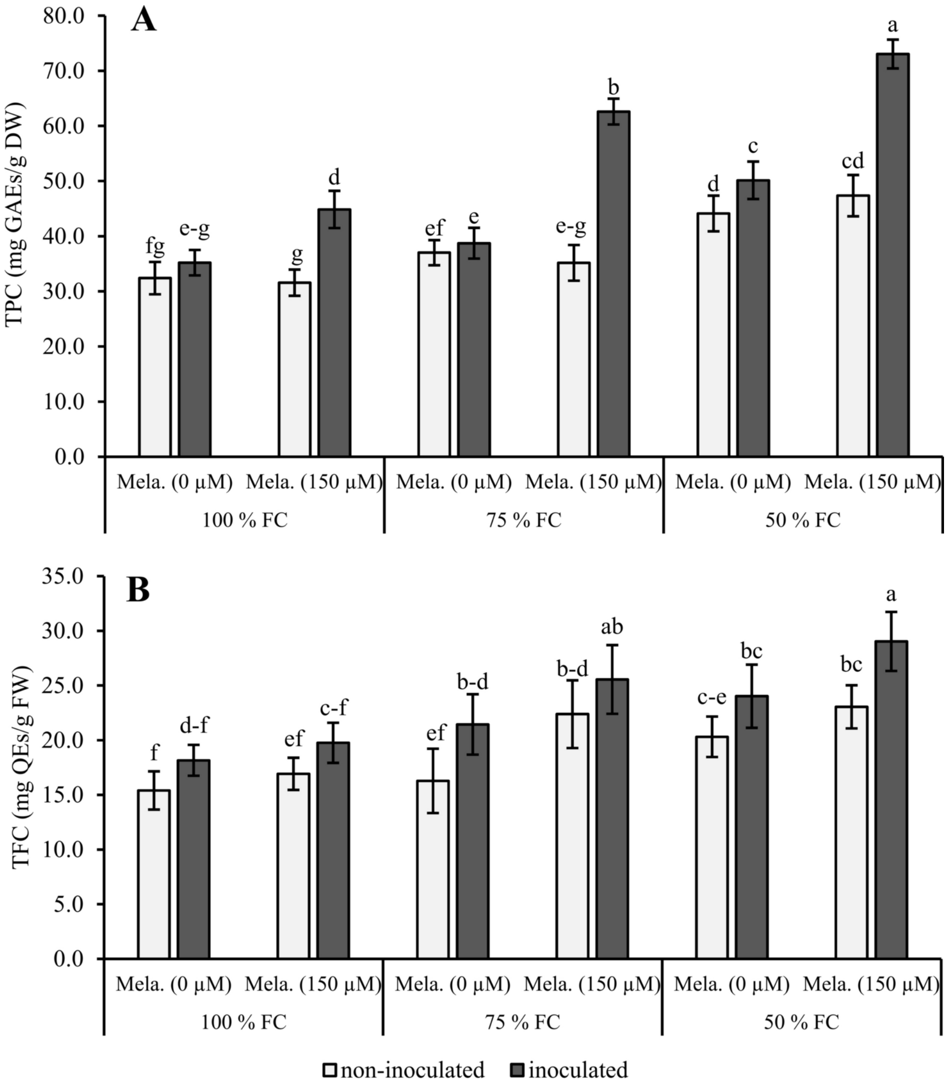

Fig. 5. Effect of mycorrhizal fungi (*Glomus intraradices*) inoculation and foliar application of melatonin on Phenolic (TPC) (**A**) and flavonoid (TFC) (**B**) contents of *Dracocephalum moldavica* under different irrigation regimes (100, 75 and 50% FC). Bars labeled by different letters are significantly different according to Duncan's multiple-range test at $p \le 0.05$.

DPPH radical scavenging effect

According to the results of the analysis of variance while, the individual effects of each applied treatment on DPPH free radical scavenging capacity of Moldavian balm plants were significant at 1% level, among the interaction effects of the treatments, only the dual interactions between drought stress and mycorrhizal fungus inoculation, as well as between mycorrhizal inoculation and melatonin spray, were significant at the 5% level. Based on the findings depicted in Fig. 6, While the concurrent application of mycorrhizal fungus inoculation and exogenous melatonin solution spraying under non-drought stress conditions led to a 24.9% enhancement in DPPH free radical scavenging potential, these treatments exhibited a substantially greater positive impact on this biological property under drought stress conditions. The pinnacle of DPPH radical scavenging efficacy was observed in the Moldavian balm plants cultivated under the most severe drought stress level, i.e., 50% FC, concurrently subjected to mycorrhizal inoculation and melatonin application (72.02%). The positive effect of mycorrhizal inoculation and melatonin treatment is such that it resulted in a 53.2% and 45.8% enhancement in the antioxidant capacity at 50% and 75% FC, respectively.

Essential oil constituents

According to the results from GC and GC–MS analyses (Fig. 7), the main constituents of Moldavian balm essential oil (Fig. 8), are hydrocarbon monoterpenes and oxygenated monoterpenes, including α-pinene, 1,8-cineol, borneol, geraniol, geranial, bornyl acetate, α-citral and geranyl acetate (Table 3). These first eight major ingredients collectively comprised between 70.03% to 78.86% of the essential oils extracted from Moldavian balm plants in this study. The concentrations of each of these compounds were altered by drought stress and

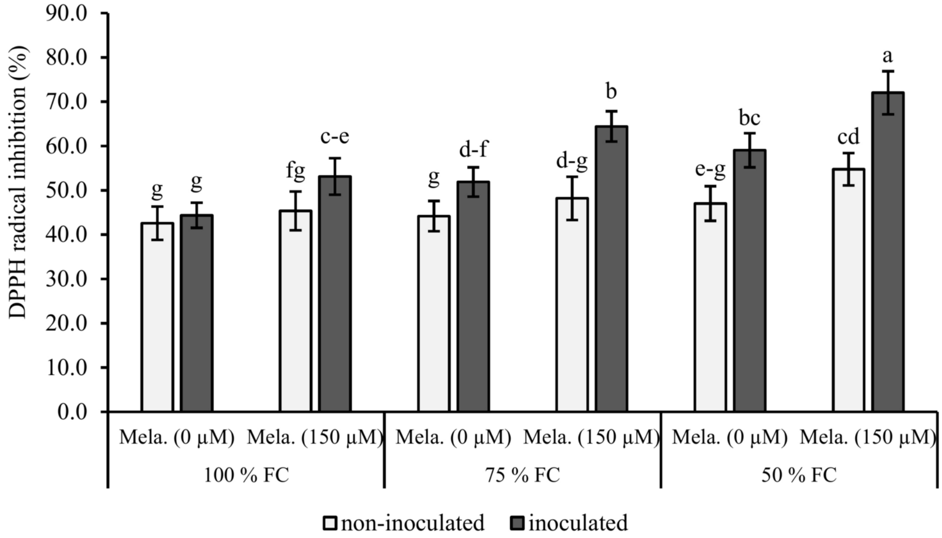

Fig. 6. Effect of mycorrhizal fungi (*Glomus intraradices*) inoculation and foliar application of melatonin on DPPH radical scavenging activity of *Dracocephalum moldavica* extracts under different irrigation regimes (100, 75 and 50% FC). Bars labeled by different letters are significantly different according to Duncan's multiple-range test at $p \leq 0.05$.

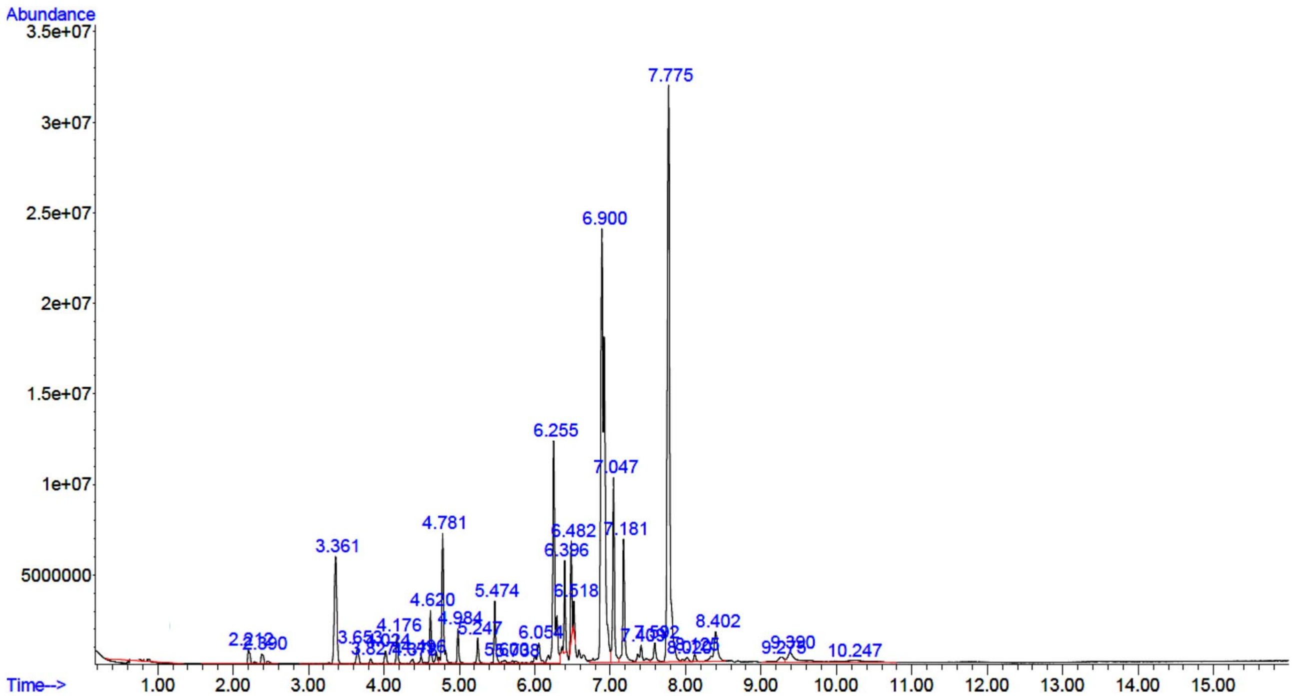

Fig. 7. GC–Ms chromatogram of Moldavian balm essential oil.

the applied treatments. The findings indicated that drought stress increased α-pinene content compared to the control samples. Additionally, the application of melatonin and mycorrhizal symbiosis affected the levels of this compound. The highest amount of α-pinene, the predominant hydrocarbon monoterpene in Moldavian balm essential oil (7.21%), was observed in plants subjected to the lowest irrigation level (50% FC) combined with simultaneous melatonin and mycorrhizal fungi treatments. Among the seven main oxygenated monoterpenes of Moldavian balm essential oil, 1,8-cineole and borneol exhibited an increase in response to drought stress. The highest levels of both compounds were detected across all irrigation regimes when melatonin treatment and mycorrhizal symbiosis with *G. intaradices* were applied concurrently to the plant. The peak concentrations of 1,8-cineole and borneol were 5.02% and 10.16%, respectively, representing increases of approximately 27% and 37% compared to the control samples.

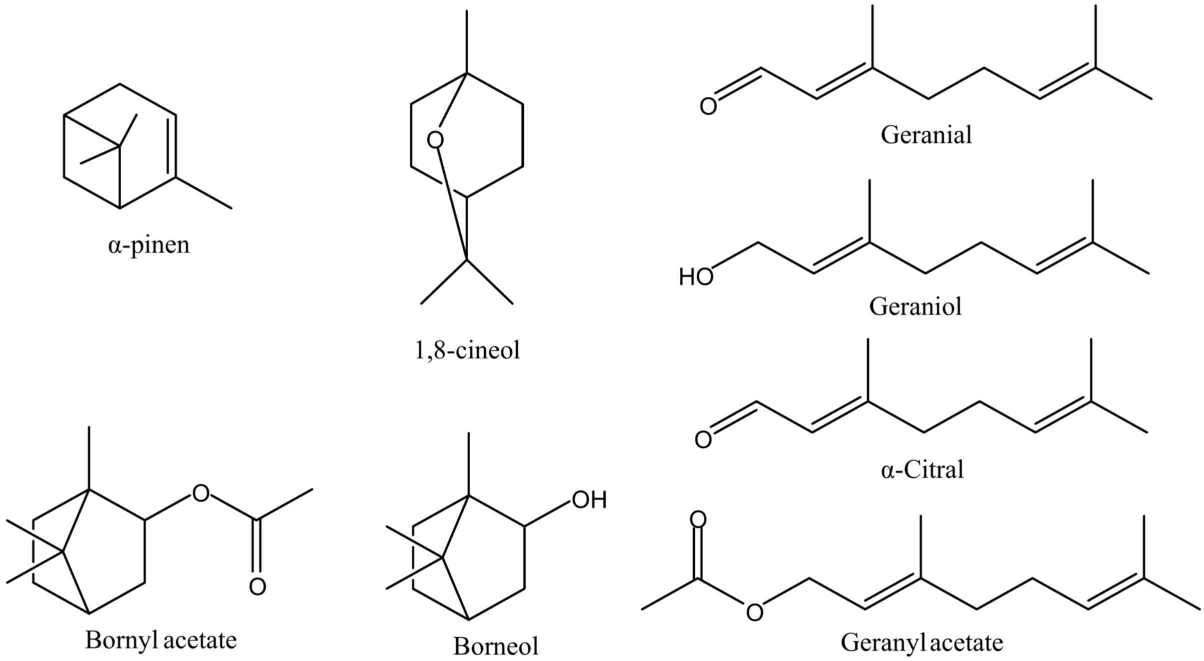

Fig. 8. Chemical structure of main constituents of Moldavian balm essential oil.

		α-Pinene	1,8-Cineol	Borneol	Geraniol	Geranial	Bornyl acetate*	α-Citra*	Geranyl acetate
100% FC	Mela (0)—FnI	4.70 ± 0.24 e	3.95 ± 0.41 bcde	7.40 ± 0.37 e	14.71 ± 1.06 bc	12.06 ± 0.54 ab	5.18 ± 0.72	4.06 ± 0.25	25.48 ± 0.33 de
	Mela (0)—FI	4.82 ± 0.16 e	3.39 ± 0.29 e	8.26 ± 0.33 d	13.02 ± 0.41 c	12.46 ± 0.74 a	5.49 ± 0.32	3.98 ± 0.33	25.13 ± 0.64 def
	Mela (150)—FnI	4.91 ± 0.27 e	3.65 ± 0.38 de	7.59 ± 0.71 de	13.39 ± 0.31 c	11.60 ± 0.65 abc	4.96 ± 0.21	4.56 ± 0.66	25.91 ± 081 cd
	Mela (150)—FI	5.55 ± 0.33 d	4.53 ± 0.39 ab	9.51 ± 0.29 ab	14.98 ± 0.56 ab	12.29 ± 1.32 a	5.52 ± 0.76	4.47 ± 0.94	26.47 ± 0.84 cd
75% FC	Mela (0)—FnI	6.08 ± 0.21 cd	4.23 ± 0.28 bcd	8.32 ± 0.39 d	13.19 ± 0.86 c	11.85 ± 0.95 ab	4.50 ± 0.65	4.60 ± 0.40	27.16 ± 0.58 bc
	Mela (0)—FI	5.98 ± 0.39 cd	3.65 ± 0.19 de	8.47 ± 0.34 cd	13.08 ± 1.33 c	11.21 ± 0.87 abc	4.92 ± 0.61	5.14 ± 0.51	26.94 ± 1.22 bc
	Mela (150)—FnI	6.37 ± 0.43 bc	4.37 ± 0.32 bc	8.29 ± 0.25 d	11.41 ± 0.93 d	10.82 ± 0.17 bcd	5.10 ± 0.39	4.79 ± 0.78	24.48 ± 0.96 ef
	Mela (150)—FI	6.96 ± 0.36 ab	4.97 ± 0.17 a	9.42 ± 0.44 ab	13.07 ± 0.94 c	10.40 ± 0.37 cde	5.57 ± 0.83	5.35 ± 0.12	23.77 ± 0.56 fg
50% FC	Mela (0)—FnI	6.82 ± 0.28 ab	3.88 ± 0.26 cde	9.15 ± 0.69 bc	11.25 ± 1.37 d	10.92 ± 0.49 bcd	5.86 ± 0.43	4.92 ± 0.64	29.13 ± 0.83 a
	Mela (0)—FI	6.63 ± 0.56 abc	4.14 ± 0.39 bcd	9.53 ± 0.27 ab	13.28 ± 0.41 c	10.70 ± 0.86 bcd	5.39 ± 0.95	4.84 ± 0.39	28.04 ± 0.78 ab
	Mela (150)—FnI	6.40 ± 0.18 bc	4.23 ± 0.26 bcd	9.23 ± 0.71 bc	10.42 ± 0.56 d	9.78 ± 0.38 de	4.71 ± 0.39	5.09 ± 0.25	25.95 ± 0.55 cd
	Mela (150)—FI	7.21 ± 0.55 a	5.02 ± 0.35 a	10.16 ± 0.49 a	10.62 ± 0.87 d	9.24 ± 0.27 e	5.17 ± 0.67	4.45 ± 0.61	22.60 ± 0.85 g

Table 3. Effect of mycorrhizal fungi (*Glomus intraradices*) inoculation and foliar application of melatonin on main constituents of *Dracocephalum moldavica* essential oil under different irrigation regimes (100, 75 and 50% FC). Different letters are significantly different according to Duncan's multiple-range test at $p \leq 0.05$. Mela (0) and (150): spray with melatonin solution with 0 and 150 μM concentrations, respectively; FnI and FI: mycorrhizal fungi non-inoculated and inoculated plants. *Based on the analysis of variance (ANOVA) drought stress, mycorrhizal inoculation, melatonin spray, and their duplicate and triplicate interactive effects didn't show significant effects on the compound percentage.

Conversely, the variations in the levels of geraniol and geranial in Moldavian balm essential oil due to drought stress and the applied treatments were distinct from those observed for 1,8-cineol and borneol. Reducing irrigation to 75% and 50% FC resulted in a decrease in the geraniol percentage from 14.71% to 13.19% and 11.25%, respectively. Similarly, the geranial content in control samples was 12.06%, which declined to 11.85% and 10.92% with reduced irrigation to 75% and 50% FC, respectively. Overall, the assessment of changes in geraniol and geranial levels suggests that under drought conditions, the application of melatonin and mycorrhizal fungi treatments had a diminishing effect on these compounds. Regarding the compounds bornyl acetate and α-citral, no significant variations in their concentrations were observed as a result of drought stress or the applied treatments. Chromatographic analysis identified geranyl acetate as the predominant constituent of the essential oil in Moldavian balm. In the control plants, geranyl acetate accounted for 25.48% of the total essential oil composition. The data clearly indicate that drought stress positively influenced the concentration of this compound, with levels rising to 27.16% and 29.13% under irrigation regimes of 75% and 50% field capacity, respectively. Conversely, the introduction of melatonin treatment and mycorrhizal fungi symbiosis under drought conditions resulted in a decrease in geranyl acetate levels. When these treatments were applied concurrently, the geranyl acetate content was reduced to 23.77% and 22.60% at 75% and 50% FC, respectively.

Discussion

Given the escalating problem of water scarcity worldwide, enhancing the ability of plants to survive under drought conditions and elucidating the mechanisms involved, have become crucial research topics[63]. According to published studies, fostering symbiotic relationships between plants and mycorrhizal fungi, as well as the use of plant growth regulators such as melatonin, can be considered strategies to cope with environmental stresses like drought[64,65]. In this research, the dual use of the mycorrhizal fungus *Glomus intraradices* and melatonin solution spray simultaneously, on Moldavian balm plants was investigated aiming to improve their drought tolerance and to assess their effects on the morphological, physiological, and biochemical properties of the plant. An in-depth analysis of the results, regarding the measured morphological factors of Moldavian balm clearly demonstrated the negative impact of reduced irrigation on this plant. This negative effect on factors such as plant height, internode length, number of branches, and leaf length and width can certainly be attributed to the impaired growth, expansion, and mitosis cell division processes caused by water shortage[66]. In fact, the reduction in overall size, especially in organs like leaves, is one of the plants, key adaptive mechanisms to drought stress[67]. This shrinkage, which is due to disruptions in metabolic processes and a consequent decrease in production of biomass-building materials, occurs predominantly in tissues that are the main sites of transpiration and water loss[68]. Therefore, water deficiency leads to a notable decline in both fresh and dry weight of the plant. It is also essential to consider that part of the reduction in fresh weight could also be due to a decrease in the relative water content within the plant tissues[69]. Various studies have been conducted to investigate the use of various substances to enhance plant resistance to drought[70,71]. Additionally, fostering symbiosis between plants and various fungal and bacterial microorganisms is a long-established method to increase plants' ability to survive under water-scarce conditions[72]. All these treatments assist in improving plant tolerance to harsh conditions by inducing changes in their physiological and phytochemical characteristics[73,74].

Photosynthesis is one of the most important physiological processes in plants, reliably reflecting the amount of growth and biomass production. Chlorophyll a and b and carotenoids are the main pigments responsible for absorbing the energy needed for the photosynthesis process[75]. Our results clearly indicate that in Moldavian balm, the levels of these pigments significantly decrease as the available moisture diminishes. One of the mechanisms involved in the reduction of chlorophyll in plants is the increased activity of key enzymes involved in the catabolism of these pigments[76]. These enzymes include chlorophyllase and pheophytinase, which show higher activity under stress conditions[77]. These enzymes catalyze the hydrolysis of chlorophyll molecules into phytol and porphyrin moieties[78]. Moreover, the oxidative burst resulting from drought stress generates high levels of reactive oxygen species (ROS), which predominantly impact chloroplasts, the organelles where photosynthesis occurs[79]. This increase in ROS further exacerbates stress by damaging cellular structures, particularly chloroplasts, thus hindering the photosynthetic efficiency and overall health of the plant[80].

In plant cells, electrolytes are confined within compartments enclosed by membranes. However, when the cells are subjected to stress conditions like water deficit, the constructive proteins and lipids that make up these membranes can degrade or undergo oxidation. These changes can result in structural alterations that compromise membrane integrity and increase permeability so, the electrolytes can leak into the surrounding apoplast[81]. In other side, the oxidation of lipids triggers a process known as lipid peroxidation, leading to the production of compounds such as malondialdehyde (MDA)[82]. In any event, the findings of this study demonstrated that the levels of EL and MDA were significantly reduced when melatonin treatments were applied, and symbiosis between fungus *Glomus intraradices* and the roots of the Moldavian balm plant was established. Since these two parameters indicate the stress level imposed on the plant, it can be concluded that they effectively mitigated the impact of drought conditions on the plant.

Careful analysis of the activity levels of two antioxidant enzymes, catalase and peroxidase, in this study clearly shows that while plants attempt to counter the increased production of ROS during drought stress by enhancing the activity of these enzymes, the application of melatonin treatments and the establishment of mycorrhizal symbiosis further boost the activity of these enzymes. Moreover, the combined use of these two treatments results in an even greater increase in enzyme activity. Thus, it can be concluded that the enhancement of antioxidant enzyme activity is one of the key mechanisms through which melatonin treatment and mycorrhizal symbiosis[11] enable plants to better withstand severe drought conditions. In other plants, such as *Chenopodium quinoa*[83] and *Vaccinium corymbosum* L.[24] melatonin treatment has similarly led to alterations and increases in the activity of antioxidant enzymes like peroxidase (POD) and catalase (CAT). Additionally, melatonin itself has a strong capacity to neutralize ROS free radicals, which in turn affects the expression of genes and the activity of antioxidant enzymes[84]. On the basis of previous studies, arbuscular mycorrhizal fungi can boost the antioxidant capacity of host plants when faced with various stress conditions, including heavy metals[85], low light and cold[86], salinity[87] and drought[88] indicating a reduced accumulation of ROS.

Phenolic and flavonoid compounds are among the most vital secondary metabolites in plants, known for their various biological and medicinal properties[89,90]. These compounds are essential components of the plant's mechanism for scavenging free radicals, playing a crucial role in defending against oxidative stress induced by drought[91].

Exogenous melatonin has been shown to affect secondary metabolite levels in various plants. For example, it was exhibited that melatonin treatments at concentrations of 100 and 200 μM increased the total phenolic content in basil[92]. Other studies have also reported increased phenolic and flavonoid levels in different plants due to melatonin treatment[93], likely due to the upregulation of genes involved in the biosynthesis of these compounds triggered by melatonin[94]. Upon reviewing studies on the effects of mycorrhizal fungi symbiosis with various plants, it is evident that this symbiosis significantly enhances the phenolic and flavonoid content in host plants[95]. Carbohydrate metabolism is one of the most crucial metabolic processes effectively influenced by mycorrhizal symbiosis[96]. Sucrose invertases, which break down sucrose into glucose and fructose, show increased activity in roots colonized by mycorrhizal fungi[13]. Additionally, the expression of genes related to these enzymes is higher in these roots, leading to elevated levels of hexose monosaccharides, especially glucose, in the host plant roots[97]. Since glucose plays a key role in the synthesis of most phenolic compounds in plants[98], the content of these compounds increases with the rise in glucose levels[99]. The primary biological characteristic associated with phenolic and flavonoid compounds is their antioxidant and free radical scavenging properties[41]. This increase in metabolites suggests improved plant properties. The investigation of the antioxidant properties of Moldavian balm extracts in this study confirmed this enhancement.

Given that Moldavian balm is renowned as an aromatic plant, its essential oil is widely used in various industries[100]. This study also examined the effect of drought stress and applied treatments on the components of the plant's essential oil. According to previous studies, various environmental factors such as water scarcity can influence the quantity and composition of plant essential oils. However, it should be noted that this impact can vary depending on the plant species[101].

Beyond its well-established influence on plant growth and development, exogenous melatonin has been shown to play a critical role in the synthesis of both primary and secondary metabolites[102]. Research indicates that melatonin can regulate the biosynthetic pathways of secondary metabolites such as saponins[103], flavonoids[104], alkaloids[105], anthocyanins[106] and carotenoids[107]. Terpenes, which are vital secondary metabolites and the major constituents of plant essential oils, may also have their biosynthetic pathways regulated by melatonin[108]. Glandular trichomes are crucial plant structures that serve as the primary sites for the production and storage of essential oils[109]. One mechanism by which melatonin can impact the quantity and quality of plant essential oils is by increasing the density of glandular trichomes[110], which in turn is linked to changes in the levels of terpenoids that constitute these oils.

Studies emphasize the significant role of mycorrhizal fungi symbiosis in altering the quantitative and qualitative properties of plant essential oils. For instance, research by Akachoud et al[111] demonstrated that mycorrhizal symbiosis induced notable changes in the composition of essential oils in three medicinal plant species: *Thymus satureioides*, *Thymus pallidus*, and *Lavandula dentata*. Research indicates that one of the primary mechanisms through which arbuscular mycorrhizal fungi (AMF) aid plants in coping with stress, particularly under drought conditions, is by modulating the expression of aquaporin genes[112]. Aquaporins proteins that are ubiquitously present in cellular membranes of plants, constitute a highly diverse protein family with at least 30 isoforms in most, higher plants[113]. While their primary function is the transport of water, some aquaporins also facilitate the membrane diffusion of other molecules, such as CO_2, silicon, boron, urea, or ammonia[114]. It is crucial to understand that the regulation of aquaporin gene expression is influenced by the availability of moisture, and this regulation can exhibit divergent or even opposite responses depending on the specific conditions. For instance, under certain circumstances, the expression of these genes in host plants may be down regulated, resulting in reduced membrane permeability and enhanced water retention within the cells. Conversely, in other scenarios, particularly within root cells, the expression of aquaporin genes may be up-regulated, thereby increasing water absorption by the host plants[115,116].

While several studies have explored strategies to alleviate drought stress in Moldavian balm (*Dracocephalum moldavica*), our comprehensive literature review reveals a lack of research specifically addressing the combined use of melatonin and the mycorrhizal fungus *Glomus intraradices* on this plant. Ghanbarzadeh et al[117] conducted an in-depth study on the effects of two microorganisms on Moldavian balm under drought stress conditions. The microorganisms used were *Claroideoglomus etunicatum*, an arbuscular mycorrhizal fungus, and *Micrococcus yunnanensis*, a bacterium known for promoting plant growth. The findings revealed that both microorganisms positively impacted the growth, physiological processes, and biochemical properties of Moldavian balm under drought stress. However, unlike our results, their study found that while drought stress alone led to an increase in the antioxidant capacity and phenolic content of Moldavian balm, these levels decreased in plants inoculated with microorganisms. Notably, when both microorganisms were applied together, there was a significant increase in the production of secondary metabolites within the plant[118]. Further metabolomic analysis conducted under similar experimental conditions indicated that carbohydrate metabolism is the most susceptible metabolic pathway under drought conditions. In addition, the study identified several other pathways that contribute to drought tolerance in Moldavian balm, particularly in non-inoculated plants. These include the elevated levels of specific amino acids, compounds involved in the Krebs cycle, and phenolic acids. The study also highlighted that inoculating plants with bacterial and fungal microorganisms enhanced the accumulation of metabolites that function as osmolytes, which are crucial for helping Moldavian balm mitigate the detrimental effects of drought stress[119].

Previous studies indicate that the foliar application of melatonin on *Dracocephalum moldavica* has successfully contributed to the induction of drought resistance in the plant to some extent[120]. These findings are consistent with the results of the current research, which highlights melatonin's role in stimulating the antioxidant defense system in Moldavian balm. Moreover, the positive effects of melatonin treatment under drought stress have been demonstrated through its enhancement of photosynthetic pigment content and the increased accumulation of secondary metabolites, including flavonoids, polyphenols, and anthocyanins. The research also confirms that melatonin treatment boosts the activity of key enzymes, such as phenylalanine ammonia-lyase and polyphenol oxidase, further supporting the plant's resilience to drought conditions[121].

Conclusion

Based on the findings of this research, it can be concluded that the combined use of melatonin and mycorrhizal fungi can significantly enhance drought resistance in Moldavian balm (*Dracocephalum moldavica*). This treatment can be considered a viable strategy for mitigating stress conditions in this plant. The application of these treatments helps the plant to better withstand harsh environmental conditions by boosting the activity of both enzymatic and non-enzymatic antioxidant defense systems, as well as improving the uptake of nutrients and water. An examination of the physiological and morphological characteristics of Moldavian balm revealed that the application of melatonin and mycorrhizal fungi (*Glomus intraradices*) treatments effectively counteracts the negative impacts of drought stress. Furthermore, these treatments influence the production of various secondary metabolites in Moldavian balm, including essential oils and aromatic compounds, which are among the most important metabolites. Ultimately, the findings of this study support our hypothesis regarding the positive and protective effects of melatonin and mycorrhizal fungi treatments in enhancing the resilience of Moldavian balm against drought conditions.

Data availability
The data used or analysed in the current study is available from the corresponding author on reasonable request.

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Author contributions
B. A.: Supervision, Conceptualization, Methodology, Writing—original draft, Writing -review & editing. M. H.: Methodology, Resources. S. M.: Conceptualization, Writing—review & editing.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to B.A.

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