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Impact of Water Deficiency, Mycorrhiza Inoculation, and Paclobutrazol on Physiological and Biochemical Parameters in *Dracocephalum moldavica* L.

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ABSTRACT

This study investigates the effects of arbuscular mycorrhizal fungi (AMF) inoculation (with and without) and paclobutrazol foliar application (0 and 200 µg mL^{-1}) on the physiological and biochemical responses of *Dracocephalum moldavica* L. under varying water availability (100%, 75%, and 50% of field capacity). Key parameters evaluated include plant growth, pigment content, antioxidant activity, secondary metabolite levels, and stress-related enzyme activities. Water deficit significantly inhibited plant growth and reduced pigment concentrations, while increasing oxidative stress markers such as malondialdehyde (MDA) and electrolyte leakage (EL). In response to drought, plants exhibited elevated phenolic and flavonoid levels, indicating an enhanced defense mechanism. Total phenolic content (TPC) increased with the severity of water stress, peaking at 47.35 GAEs g^{-1} extract under 75% field capacity (S2), compared to 32.24 GAEs g^{-1} extract under well-watered conditions (100% field capacity, S0). AMF inoculation notably improved growth, chlorophyll content, phenolic and flavonoid accumulation, and antioxidant enzyme activities, while reducing oxidative stress indicators, highlighting its role in enhancing drought resilience. Mycorrhiza-inoculated plants (M1) exhibited a TPC of 43.00 GAEs g^{-1} extract, compared to 36.01 GAEs g^{-1} in non-inoculated plants (M0). Paclobutrazol application promoted a compact growth habit with increased branching, higher chlorophyll content, and enhanced antioxidant capacity. When combined, AMF inoculation and paclobutrazol demonstrated synergistic effects, improving plant tolerance to water stress by stabilizing growth, maintaining pigment levels, and boosting antioxidant defenses. The highest TPC (51.40 GAEs g^{-1} extract) was recorded under 75% field capacity with AMF inoculation (S1M1), indicating a strong interactive effect. These findings suggest that the integrated use of AMF and paclobutrazol offers a promising strategy to mitigate the adverse effects of water deficiency, promoting healthier and more resilient plant growth. Future research should focus on elucidating the underlying molecular mechanisms to further optimize stress management strategies in crops.

Abbreviation: Electrolyte leakage (EL), Malondialdehyde (MDA)

Introduction

Drought stress is a major abiotic factor that significantly limits plant growth and productivity worldwide, especially in arid and semi-arid regions. The increasing frequency and severity of droughts

due to climate change further exacerbate this challenge, highlighting the urgent need for effective strategies to enhance plant tolerance to water scarcity (Gupta et al., 2020). *Dracocephalum moldavica*, commonly known as Moldavian balm, is a medicinal and aromatic plant from the Lamiaceae family, valued for its essential oils and bioactive compounds with antioxidant, antimicrobial, and anti-inflammatory properties (Khan et al., 2017). Despite its economic and therapeutic importance, the productivity of *D. moldavica* is highly sensitive to water deficit conditions. Plants respond to drought stress through a series of complex physiological, biochemical, and molecular mechanisms aimed at conserving water, protecting cellular structures, and ensuring survival. These include stomatal closure, reduced transpiration, osmotic adjustment, and the accumulation of osmoprotectants such as proline and soluble sugars (Ashraf and Akram, 2017). However, prolonged drought stress can lead to oxidative damage, impaired photosynthesis, and reduced growth and yield (Kang et al., 2019).

Various agronomic and biotechnological approaches have been explored to mitigate the adverse effects of drought stress. Among these, the use of plant growth regulators (PGRs) and beneficial soil microorganisms has shown particular promise. Paclobutrazol (PBZ), a triazole-based PGR, inhibits gibberellin biosynthesis, resulting in reduced stem elongation, enhanced root development, and improved stress tolerance (Rademacher, 2016). PBZ has been shown to improve water-use efficiency, increase chlorophyll content, and boost antioxidant enzyme activity in a variety of crops under drought conditions (Almeida et al., 2019; Savvides et al., 2016). It enhances Rubisco activity, thereby increasing stomatal conductance and net photosynthesis through improved carboxylation (Xu et al., 2020). Stomatal conductance plays a key role in regulating photosynthesis by controlling transpiration rates and CO_2 influx into mesophyll tissues (Saykhul et al., 2013). PBZ has also been reported to increase stomatal conductance in several species, including *Paeonia lactiflora* (Xia et al., 2018), *Euonymus japonicus* (Xu et al., 2020), and *Ocimum basilicum* (Santos Filho et al., 2022). The enhanced water-use efficiency associated with PBZ application is partly attributed to increased abscisic acid (ABA) levels, which lead to reduced stomatal aperture, decreased leaf surface area for transpiration, and greater root proliferation for water uptake (Soumya et al., 2017). This is particularly important for medicinal plants, in which secondary metabolites are often sensitive to environmental stress. PBZ's role in mitigating drought effects can help preserve the concentration and bioactivity of these compounds. For instance, it has been shown to enhance the production of key bioactive constituents under drought conditions in plants such as *Aloe vera* and *Withania somnifera* (Gupta et al., 2020).

Similarly, mycorrhizal inoculation, establishing a symbiotic association between plant roots and arbuscular mycorrhizal fungi (AMF), has been widely studied for its beneficial effects on plant nutrient uptake, water relations, and stress resilience (Berruti et al., 2016). AMF associations improve soil structure, expand the effective root surface area, and enhance the absorption of water and essential nutrients, particularly phosphorus. Moreover, AMF symbiosis has been shown to stimulate the production of phytohormones, antioxidants, and secondary metabolites, thereby promoting plant growth and enhancing tolerance to various stresses (Begum et al., 2019). Recent studies suggest that the combined application of AMF and paclobutrazol (PBZ) may offer additive or even synergistic benefits in improving water-use efficiency and mitigating drought-induced damage. While AMF enhances root water uptake and nutrient acquisition, PBZ limits shoot elongation and redirects metabolic resources toward root development. This interaction results in plants with more extensive and efficient root systems capable of accessing deeper soil moisture and nutrients, thereby improving drought resilience (Abd El-Rahman et al., 2020). AMF also supports photosynthetic activity by improving phosphorus uptake, while PBZ contributes to photosynthetic efficiency by preserving chlorophyll content and restricting excessive leaf expansion. Both AMF and PBZ are known to upregulate antioxidant enzyme activities, strengthening the plant's defense mechanisms against oxidative stress caused by environmental challenges such as drought and salinity (Azcon-Aguilar et al., 2018). Together, these mechanisms contribute to enhanced cellular protection and overall plant vigor under stress conditions.

Although the individual effects of paclobutrazol and AMF inoculation are well-documented, their combined influence on *Dracocephalum moldavica* under drought stress remains insufficiently explored. This study hypothesizes that their concurrent application may exert synergistic effects, improving drought tolerance and optimizing plant performance more effectively than either treatment alone. Accordingly, the primary objective of this research is to evaluate the combined effects of paclobutrazol foliar application and AMF inoculation on growth, biochemical responses, and secondary metabolite production in *D. moldavica* under varying levels of water deficiency. By elucidating the interactive mechanisms of these treatments, the study aims to provide a deeper understanding of their synergistic potential. The findings are expected to contribute to the development of integrated, sustainable management practices that enhance the resilience and productivity of *D. moldavica* under drought

conditions, supporting both agricultural sustainability and the effective utilization of this valuable medicinal plant.

Material and methods

Plant matter, growth conditions, and treatments

This pot experiment was conducted in 2023 in the research greenhouse of the Faculty of Agriculture and Natural Resources at Imam Khomeini International University, Qazvin, Iran. The greenhouse environment was maintained at a temperature range of 19–25 °C with relative humidity between 50–60%, under natural light conditions. The study followed a factorial experimental design based on a randomized complete block layout, incorporating three factors: irrigation levels at 100%, 75%, and 50% of field capacity (denoted as S0, S1, and S2, respectively); paclobutrazol foliar application at 0 and 200 µg mL^{-1} (P0 and P1, respectively); and the presence or absence of arbuscular mycorrhizal fungi (AMF) inoculation (M0 and M1, respectively). A total of 12 treatment combinations were tested, each replicated three times. Paclobutrazol was obtained from Kimia Novin (Tetra Chem), while the AMF strain *Glomus mosseae* was provided by Zist Fanavar Turan. Seeds of *Dracocephalum moldavica* were sourced from Pakan Bazr Esfahan Company. The pots were filled with a sterilized soil mixture consisting of sand, black peat, and clay-loam soil in a 3:2:2 ratio by volume. The sterilization process involved autoclaving the soil mixture at 115 °C for 60 minutes over three consecutive days. Seeds were surface-sterilized prior to sowing by rinsing in tap water, soaking in 70% ethanol for 30 seconds, treating with 2% sodium hypochlorite for 15 minutes, and thoroughly rinsing 8–10 times with autoclaved distilled water. The sterilized seeds were then sown at a depth of 2 cm, evenly distributed across five sections in each pot to ensure uniform growth.

Mycorrhizal treatment was carried out at the time of sowing by applying 5 g of Glomus mosseae inoculum directly to each sowing point. Seedling emergence occurred approximately three weeks after planting. Thinning was performed to retain one healthy seedling per sowing point, resulting in a total of five plants per pot. Standard agronomic practices were followed throughout the growing period. Paclobutrazol foliar application was initiated at the 8-leaf stage and repeated at monthly intervals until harvest. Irrigation was administered every five days. To quantify water deficiency, soil moisture levels were regularly monitored, and irrigation volumes were adjusted accordingly. Soil water content was determined by weighing each pot, allowing for accurate assessment of water loss through evaporation and transpiration. Initially, each pot was fully saturated to determine its weight at 100% field capacity (FC). Based on this baseline, water deficiency treatments were imposed by maintaining soil moisture at 100%, 75%, and 50% of FC, corresponding to S0, S1, and S2 levels, respectively. Pot weights were continuously monitored, and the appropriate volume of water was added to sustain the desired moisture level throughout the experimental period. Morphological traits were recorded at the flowering stage. Biochemical analyses, including catalase (CAT), peroxidase (POD), total protein content, electrolyte leakage (EL), and photosynthetic pigment concentration, were conducted at the onset of flowering, approximately one week before harvest. At full flowering, plants were harvested by cutting 3 cm above the soil surface. The fresh weight of each plant was recorded immediately after harvest, after which the samples were transferred to a drying room and air-dried at room temperature in complete shade. Once fully dried, the dry weight of each plant was measured and documented.

Photosynthetic pigments

Before harvesting, plant samples were prepared, and 0.25 g of young leaves were extracted in 10 mL of 80% acetone. Chlorophyll a and b were determined by measuring absorbance at 663 and 645 nm wavelengths using a UNICO 2100 spectrophotometer. Calculations were performed based on mg g^{-1} of fresh leaf (Wellburn, 1994).

$$Chlorophyll\ a = \frac{(19.3 \times A_{663} - 0.86 \times A_{645})\,V}{100W}$$

$$Chlorophyll\ b = \frac{(19.3 \times A_{645} - 3.6 \times A_{663})\,V}{100W}$$

V= volume of the supernatant obtained from centrifugation A= light absorption at 663 and 645 nm wavelengths W= weight of the sample in grams.

The total chlorophyll content was quantified by summing the values of chlorophyll a and chlorophyll b.

Electrolyte Leakage Assay (EL)

To evaluate membrane integrity, 1 g of fresh, undamaged aerial tissue from the plant leaf was rinsed with distilled water to remove surface ions. The tissue was placed in sealed test tubes with 10 mL of deionized water and submerged in a water bath at 32 °C for 2 h. The electrical conductivity of the samples (EC1) was measured using a Winlab Data Windaus EC meter. The tubes were then autoclaved at 121 °C for 20 min. After cooling to 25 °C, the electrical conductivity was measured again (EC2). The percentage of ion leakage was calculated using the following formula:

$$\text{EL}\ (\%) = \frac{\text{EC1}}{\text{EC2}} \times 100$$

Malondialdehyde assay (MDA)

To assess lipid peroxidation in the membrane, the malondialdehyde (MDA) content was measured as a marker. For this assay, 0.2 g of plant leaf material, ground in liquid nitrogen, was transferred to a test tube with 4 mL of 1% trichloroacetic acid. After centrifugation at 10,000 rpm for 10 min at 4 °C, 1 mL of the supernatant was mixed with 4 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid. This mixture was heated at 95 °C for 30 min in a water bath, rapidly cooled in an ice bath, and absorbance was measured at 532 and 600 nm. The MDA content was determined using a molar extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as nanomoles per gram of sample (Yazici et al., 2007).

Antioxidant enzyme activity assay

Enzyme extraction from Dracocephalum moldavica (Moldavian balm) leaves was performed by finely grinding 1 g of fresh leaf tissue in liquid nitrogen. The powdered material was homogenized in 5 mL of 50 mM phosphate buffer (pH 7.8) and kept on ice to preserve enzyme activity. The homogenate was then centrifuged at 15,000 rpm for 30 min at 4 °C, and the resulting supernatant was collected for enzymatic assays. Catalase (CAT) activity was determined by monitoring the decomposition of hydrogen peroxide, measured as the decrease in absorbance at 240 nm over 30 s. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 200 mM hydrogen peroxide (H_2O_2), and 0.1 mL of the enzyme extract, following the method described by Gheshlaghpour et al. (2021). Peroxidase (POD) activity was measured by recording the increase in absorbance at 420 nm, which reflects the oxidation of guaiacol in the presence of H_2O_2. The reaction mixture contained 1 mM guaiacol, 5 mM hydrogen peroxide, and 0.1 mL of the enzyme extract in 50 mM phosphate buffer (pH 7.0), according to the protocol of Upadhyaya et al. (1985).

Total phenolic content assay (TPC)

The phenolic content of plant samples was measured using the Folin-Ciocalteu method and spectrophotometric analysis (Zarrabi et al., 2019). A 10% solution of Folin reagent was prepared, and 100 µL of this solution was mixed with 20 µL of plant extract. After a 10 min incubation in darkness, 80 µL of 5.7% w/v sodium carbonate solution was added. The absorbance was measured at 760 nm. Gallic acid was used as the standard, and the phenolic content was expressed as mg of gallic acid equivalent per gram of extract.

Total flavonoid content assay (TFC)

The total flavonoid content in plant samples was determined using aluminum chloride reagent. Ten microliters of 5% w/v aluminum chloride solution were mixed with 20 µL of plant extract. The mixture was diluted with 60 µL of methanol, and the total reaction volume was brought to 200 µL by adding 10 µL of 0.5 M potassium acetate solution and distilled water. After a 30 min incubation, the absorbance was measured at 415 nm. Quercetin was used as the reference standard, and the results were expressed as mg of quercetin equivalent per gram of dry extract (Asghari et al., 2020).

DPPH radical scavenging activity assay

The antioxidant potential of plant extracts was evaluated using the DPPH assay. Twenty microliters of plant extracts at known concentrations were mixed with 180 µL of 1.0 mM DPPH solution. After a 30 min incubation, the absorbance was measured at 517 nm. The results were expressed as the percentage of DPPH radical inhibition.

Statistical analysis

Data were analyzed using SPSS software version 26, and graphs were plotted using Excel. The normality of the data was verified before analysis, and after confirming a normal distribution, the data were analyzed. Means were compared using Duncan's multiple range test at the 5% significance level.

Results

Plant height

Plant height was significantly affected by water availability, mycorrhizal inoculation, paclobutrazol application, and the interaction between water availability and paclobutrazol (S × P), all at the 1% probability level (Table 1). The tallest plants were observed under full irrigation (S0), with an average height of 44.88 cm. As water availability decreased, plant height declined significantly, with mean values of 42.74 cm under moderate water deficit (S1, 75% field capacity) and 38.56 cm under severe water deficit (S2, 50% field capacity). Mycorrhizal inoculation (M1) resulted in significantly taller plants compared to non-inoculated controls (M0), with average heights of 45.15 cm and 38.96 cm, respectively, highlighting the positive impact of AMF on shoot elongation. In contrast, paclobutrazol application (P1) significantly reduced plant height to an average of 38.31 cm, compared to 45.80 cm in untreated plants (P0) (Table 2), consistent with its known growth-retarding effects. Analysis of the interaction between water availability and paclobutrazol revealed that the tallest plants were recorded under the S0P0 (46.79 cm) and S1P0 (40.41 cm) treatments. No statistically significant differences were observed among the other treatment combinations (Fig. 1), indicating that paclobutrazol mitigated the positive effects of higher water availability on shoot elongation.

Table 1. Variance analysis of effects of paclobutrazol foliar application and mycorrhiza inoculation on morphophysiological traits of *D. moldavica* under water deficiency conditions.

Source	df	M.S.					
		Plant Height	Internode Length	Inflorescence length	Number of Branches per Plant	Fresh weight	Dry weight
S	2	124.078**	0.422**	5.453**	1.950**	113.172**	8.733**
M	1	344.102**	4.723**	5.138**	8.644**	124.441**	9.831**
P	1	504.751**	7.471**	7.934**	38.564**	3.142^{ns}	2.518**
S * M	2	0.204^{ns}	0.034^{ns}	0.060^{ns}	0.099^{ns}	1.227^{ns}	0.155^{ns}
S * P	2	45.768**	0.552**	1.812**	0.369^{ns}	6.237*	0.096^{ns}
M * P	1	8.900^{ns}	0.042^{ns}	1.361*	0.504^{ns}	32.897**	1.814**
S * M * P	2	2.773^{ns}	0.169^{ns}	0.033^{ns}	0.012^{ns}	4.112^{ns}	0.631^{ns}
Error	24	2.854	0.073	0.219	0.326	1.697	0.129
Total	35						

*, **, ^{ns}: Significantly difference at the 5 and 1 of probability level\, and non-significantly difference, respectively. S: Water deficiency; M: Mycorrhiza inoculation; P: Paclobutrazol foliar application.

Table 2. Mean comparison of simple effects of water deficiency, mycorrhiza inoculation and paclobutrazol foliar application on morphophysiological traits of *D. moldavica*.

Treatment	Plant Height (cm)	Internode Length (cm)	Inflorescence length (cm)	Number of Branches per Plant	Fresh weight per Plant (g)	Dry weight per Plant (g)
S0	44.88^{a}	4.08^{a}	8.10^{a}	7.00^{a}	22.89^{a}	5.60^{a}
S1	42.74^{b}	3.84^{b}	7.91^{a}	6.70^{ab}	20.51^{b}	4.90^{b}
S2	38.56^{c}	3.72^{b}	6.85^{b}	6.20^{b}	16.79^{c}	3.90^{c}
M0	39.00^{b}	3.50^{b}	7.11^{b}	6.10^{b}	18.21^{b}	4.27^{b}
M1	45.10^{a}	4.20^{a}	8.00^{a}	7.10^{a}	21.92^{a}	5.32^{a}
P0	45.80^{a}	4.34^{a}	4.34^{a}	5.60^{b}	^{ns}	4.53^{b}
P1	38.31^{b}	3.42^{b}	3.42^{b}	7.60^{a}	^{ns}	5.06^{a}

Common letters within each factor indicate the absence of a significant difference at a 5% probability level, as per the Duncan test. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

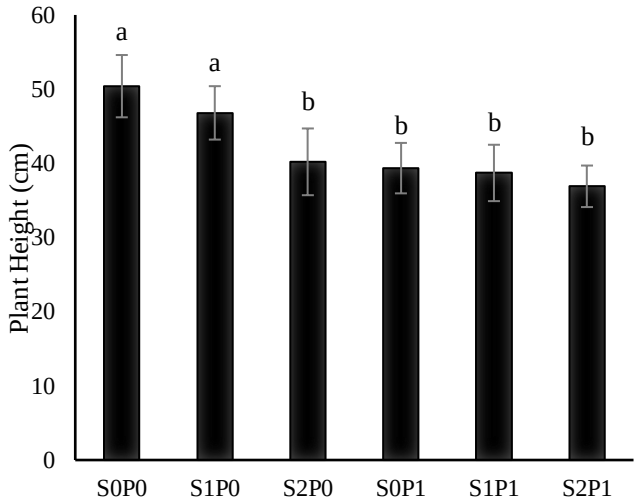

Fig. 1. Interaction effects of water deficiency and Paclobutrazol foliar application on plant height of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. P0 and P1: Paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

Internode length

Internode length was significantly affected by water availability, mycorrhizal inoculation, paclobutrazol application, and the interaction between water availability and paclobutrazol (S × P), all at the 1% probability level (Table 1). The longest internodes

were observed under full irrigation (S0), with an average length of 4.09 cm, followed by 3.84 cm under moderate water deficit (S1) and 3.72 cm under severe deficit (S2). Mycorrhiza-inoculated plants (M1) exhibited significantly greater internode length (4.24 cm) than non-inoculated plants (M0), which averaged 3.52 cm, indicating a positive effect of AMF on shoot elongation. Paclobutrazol application (P1) led to a notable reduction in internode length (3.42 cm), compared to 4.34 cm in untreated plants (P0) (Table 2), consistent with its growth-regulating properties. The interaction between water availability and paclobutrazol revealed that the highest internode lengths were recorded in the S0P0 (4.62 cm) and S1P0 (4.56 cm) treatments. These values were significantly higher than those observed in other treatment combinations (Fig. 2), indicating that the inhibitory effect of paclobutrazol on internode elongation becomes more pronounced under water-limited conditions.

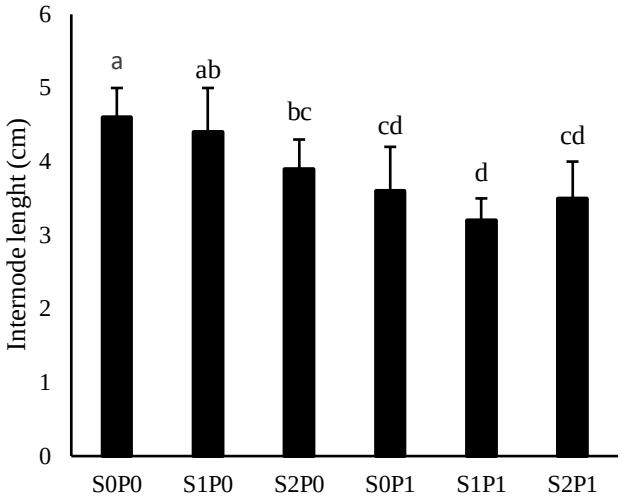

Fig. 2. Interaction effects of water deficiency and Paclobutrazol foliar application on Internode length of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. P0 and P1: Paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

Inflorescences length

Inflorescence length was significantly affected by water availability, paclobutrazol application, and mycorrhizal inoculation, each at the 1% probability level (Table 1). The greatest inflorescence length was recorded under full irrigation (S0), averaging 8.1 cm, followed by 7.9 cm under moderate water deficit (S1) and 6.85 cm under severe deficit (S2), indicating a progressive decline with increasing water stress. Mycorrhiza-inoculated plants (M1) produced significantly longer inflorescences (8.0 cm) compared to non-inoculated plants (M0), which averaged 7.24 cm. Similarly, paclobutrazol-treated plants (P1) showed a significant increase in inflorescence length (7.66 cm) relative to untreated controls (P0), which measured 7.15 cm (Table 2), suggesting a positive effect of PBZ on reproductive development. Interaction effects further revealed that the longest inflorescences occurred in the S0P1 (8.8 cm) and S1P1 (8.6 cm) treatments, both significantly surpassing the other water × paclobutrazol combinations (Fig. 3A). In contrast, the shortest inflorescences were observed in the M0P0 treatment (6.5 cm), highlighting the synergistic benefits of combined mycorrhizal inoculation and PBZ application (Fig. 3B).

Number of branches per plant

The number of branches per plant was significantly affected by water availability (S), mycorrhizal inoculation (M), and paclobutrazol application (P), all at the 1% probability level (Table 1). The highest number of branches was recorded under full irrigation (S0), with an average of 7.01, followed by moderate water deficit (S1, 6.66) and severe water deficit (S2, 6.20), indicating a gradual decline in branching under increasing drought stress. Mycorrhiza-inoculated plants (M1) produced significantly more branches (7.11) than non-inoculated plants (M0), which averaged 6.13 branches per plant. Paclobutrazol treatment (P1) also led to a marked increase in branching (7.66) compared to untreated plants (P0), which produced only 5.59 branches (Table 2). These findings suggest that both AMF inoculation and paclobutrazol application enhance branching, potentially contributing to greater canopy density and reproductive potential under varying moisture conditions.

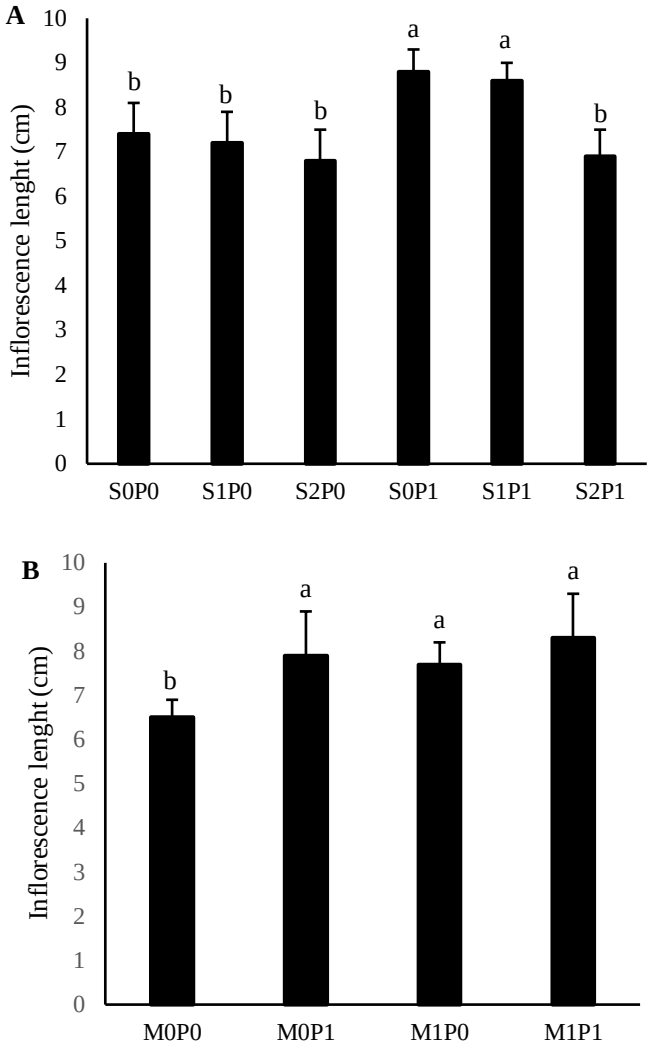

Fig. 3. Interaction effects of water deficiency and paclobutrazol foliar application (A); mycorrhiza inoculation and Paclobutrazol foliar application (B) on inflorescences length of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

Plant fresh weight

Plant fresh weight was significantly affected by water availability and mycorrhizal inoculation at the 1% probability level. Moreover, the interaction effects between water availability and paclobutrazol (S × P), as well as mycorrhiza and paclobutrazol (M × P), were also significant at the same level (Table 1). Plants grown under full irrigation (S0) exhibited the highest fresh weight (22.89 g), followed by moderate (S1: 20.51 g) and severe (S2: 16.79 g) water deficit conditions, indicating a clear reduction in biomass with increasing drought stress. Mycorrhiza-inoculated plants (M1) had a significantly higher fresh weight (21.92 g) than non-inoculated plants (M0: 18.21 g), highlighting the beneficial role of AMF in promoting biomass accumulation. Similarly, paclobutrazol-treated plants (P1) demonstrated a marked increase in fresh weight (30.31 g) compared to untreated controls (P0: 19.77 g) (Table 2). Interaction analysis showed that the highest fresh weights were recorded in M1P1 (22.6 g) and M1P0 (21.3 g) treatments, whereas the lowest values occurred in M0P0 (16.95 g) and M0P1 (19.45 g) (Fig. 4A), emphasizing the synergistic effect of mycorrhizal inoculation and paclobutrazol. Additionally, the S × P interaction revealed that while increasing water deficiency significantly reduced fresh weight, the foliar application of paclobutrazol effectively mitigated these negative effects, contributing to improved biomass production under drought conditions (Fig. 4B).

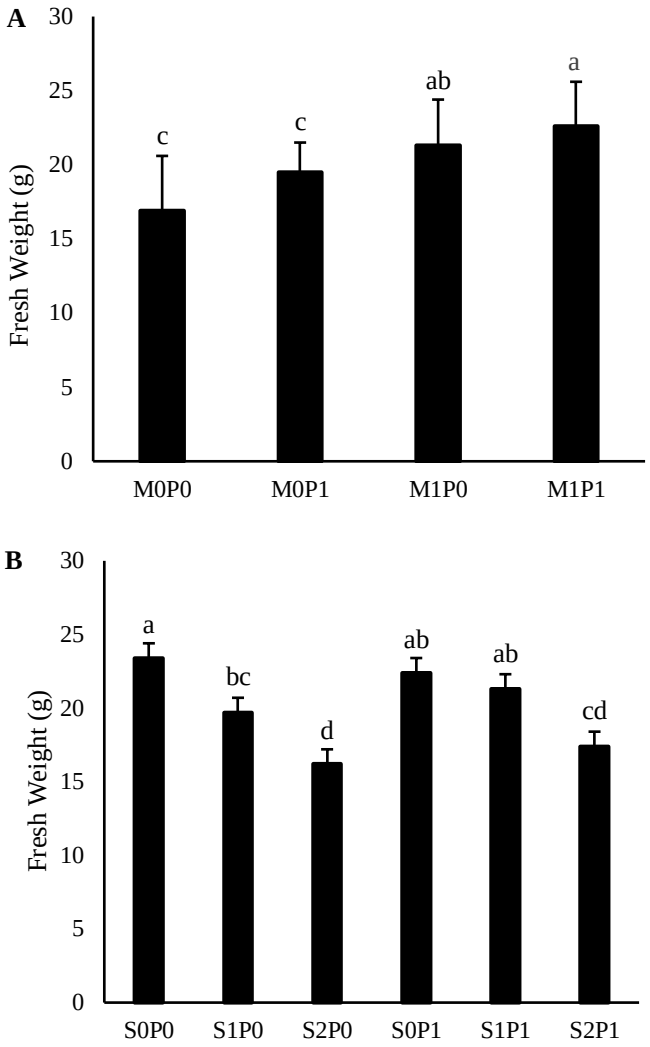

Fig. 4. Interaction effects of water deficiency and Paclobutrazol foliar application (A); Mycorrhiza inoculation and Paclobutrazol foliar application (B) on plant fresh weight of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

Plant dry weight

Plant dry weight was significantly affected by water availability, mycorrhizal inoculation, paclobutrazol application, and the interaction between mycorrhiza and paclobutrazol (M × P), all at the 1% probability level (Table 1). Increasing water deficiency led to a marked reduction in plant dry weight, with mean values of 5.57 g under full irrigation (S0), 4.93 g under moderate drought (S1), and 3.88 g under severe drought conditions (S2). Mycorrhiza-inoculated plants (M1) had significantly higher dry weights (5.32 g) than non-inoculated plants (M0: 4.27 g), highlighting the positive effect of AMF in promoting biomass accumulation under stress. Similarly, paclobutrazol treatment (P1) increased plant dry weight to 5.06 g, compared to 4.53 g in untreated plants (P0) (Table 2). The interaction between mycorrhiza and paclobutrazol revealed that the highest dry weights were recorded in M1P1 (5.36 g) and M1P0 (5.28 g), with no significant difference between them, indicating that mycorrhizal inoculation alone was highly effective. In contrast, the lowest dry weight was observed in the M0P0 treatment (3.78 g), suggesting that the absence of both treatments led to the most pronounced biomass reduction (Fig. 5).

Chlorophyll a (Chl a)

Chlorophyll *a* content was significantly influenced by water deficiency, mycorrhiza inoculation, and their interactions at the 1% probability level (Table 3). The highest Chl *a* content was observed in plants under no water stress (S0), with a value of 22.18 mg g^{-1} fresh weight, followed by S1 with 21.41 mg g^{-1} fresh weight. Mycorrhiza-inoculated plants (M1) exhibited significantly higher Chl *a* content (22.29

mg g^{-1} fresh weight) compared to M0, which had 19.34 mg/g fresh weight (Table 4). The interaction effects revealed that the highest Chl *a* content was found in the S1M1H0 (24.11 mg g^{-1} fresh weight) and S0M1H1 (23.63 mg g^{-1} fresh weight) treatments, while the lowest Chl *a* content was recorded in the S2M0H0 (15.12 mg g^{-1} fresh weight) treatment (Fig. 6).

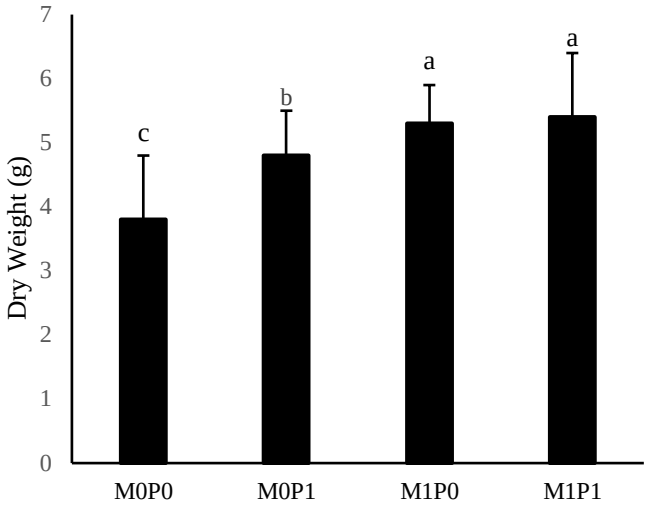

Fig. 5. Interaction effects of water deficiency and Paclobutrazol foliar application on plant dry weight of *Dracocephalum moldavica*. M0 and M1: non-inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

Table 3. Variance analysis of effects of Paclobutrazol foliar application and Mycorrhiza Inoculation on biochemical traits of *Dracocephalum moldavica* under water deficiency conditions.

Source	df	Mean Square									
		Ch *a*	Ch *b*	Carotenoid	EL	MDA	Phenol	Flavonoid	DPPH	CAT	POD
S	2	36.422**	32.500**	21.132**	1389.934**	2.209**	687.720**	88.509**	294.202**	12.207**	3.803**
M	1	78.331**	53.939**	20.229**	87.502**	0.447**	439.979**	283.647**	755.874**	10.075**	3.010**
P	1	4.973^{ns}	0.001^{ns}	5.707**	107.985**	0.214**	1.555^{ns}	73.265**	123.132**	1.363**	0.506**
S * M	2	5.999^{ns}	2.326^{ns}	0.017^{ns}	34.105**	0.117**	24.278**	2.435^{ns}	75.994**	1.051**	0.200**
S * P	2	3.665^{ns}	2.846^{ns}	0.705^{ns}	26.867**	0.041*	19.626**	0.104^{ns}	5.797^{ns}	0.108^{ns}	0.029^{ns}
M * P	1	4.038^{ns}	0.023^{ns}	0.549^{ns}	39.280**	0.166**	15.624*	38.911**	35.699*	0.668**	0.262**
S * M * P	2	10.334**	1.516^{ns}	0.576^{ns}	16.872*	0.071**	2.745^{ns}	2.537^{ns}	0.475^{ns}	0.009^{ns}	0.102**
Error	24	1.783	1.335	0.739	4.833	0.009	2.704	3.015	5.625	0.037	0.018
Total	35										

*, **, ^{ns}: Significantly difference at the 5 and 1 of probability level, and non-significantly difference, respectively. S: Water deficiency; M: Mycorrhiza inoculation; P: Paclobutrazol foliar application.

Table 4. Mean comparison of simple effects of Water deficiency, Mycorrhiza Inoculation and Paclobutrazol foliar application on morphophysiological traits of *Dracocephalum moldavica*.

Treatment	Ch *a* (mg g^{-1} FW)	Ch *b* (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	EL (%)	MDA (nmol g^{-1} FW)	Total Phenol Content (mg GAEs g^{-1} Extract)	Total Flavonoid Content (mg QEs g^{-1} Extract)	DPPH (%)	Catalase (U mg^{-1} Protein)	POD (U mg^{-1} Protein)
S0	22.18^{a}	14.04^{a}	8.35^{a}	14.07^{c}	1.15^{c}	32.24^{c}	19.90^{c}	46.16^{c}	1.42^{c}	0.82^{c}
S1	21.41^{a}	12.31^{b}	7.20^{b}	26.34^{b}	1.46^{b}	38.93^{b}	22.60^{b}	48.68^{b}	2.58^{b}	1.14^{b}
S2	18.85^{b}	10.75^{c}	5.70^{c}	35.51^{a}	2.00^{a}	47.35^{a}	25.30^{a}	55.71^{a}	3.43^{a}	1.91^{a}
M0	19.34^{b}	11.14^{b}	6.30^{b}	26.87^{a}	1.65^{a}	36.01^{b}	19.79^{b}	45.60^{b}	1.95^{b}	1.00^{b}
M1	22.29^{a}	13.59^{a}	7.80^{a}	23.75^{b}	1.43^{b}	43.00^{a}	25.40^{a}	54.76^{a}	3.01^{a}	1.58^{a}
P0	^{ns}	^{ns}	6.70^{b}	27.04^{a}	1.61^{a}	^{ns}	21.17^{b}	48.33^{b}	2.28^{b}	1.17^{b}
P1	^{ns}	^{ns}	7.50^{a}	23.57^{b}	1.46^{b}	^{ns}	24.02^{a}	52.03^{a}	2.67^{a}	1.41^{a}

Common letters within each factor indicate the absence of a significant difference at a 5% probability level, as per the Duncan test. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with Mycorrhiza respectively.

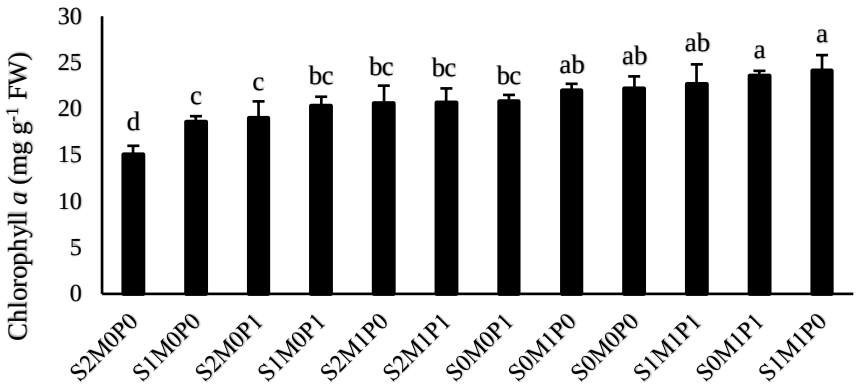

Fig. 6. Interaction effects of water deficiency, Mycorrhiza inoculation and Paclobutrazol foliar application on Ch a of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

Chlorophyll b (Chl b)

Water deficiency and mycorrhiza inoculation significantly influenced Chl *b* content at the 1% probability level, while other factors were not significant (Table 3). The highest Chl *b* content was observed in plants under no water deficiency (S0), with 14.03 mg g^{-1} fresh weight, and the lowest Chl *b* content was in plants under severe water deficiency (S2), with 10.74 mg g^{-1} fresh weight. Mycorrhiza-inoculated plants (M1) had significantly higher Chl *b* content (13.59 mg g^{-1} fresh weight) compared to non-inoculated plants (M0), which had 11.14 mg g^{-1} fresh weight (Table 4).

Carotenoid content

Water deficiency, mycorrhiza inoculation, and paclobutrazol significantly influenced carotenoid content at the 1% probability level (Table 3). Mycorrhiza-inoculated plants (M1) exhibited higher carotenoid content (13.58 mg g^{-1} fresh weight) compared to non-inoculated plants (M0), which had 11.14 mg g^{-1} fresh weight. Carotenoid content decreased significantly with increasing water deficiency, with the highest content observed in plants under no water stress (S0) at 14.04 mg g^{-1} fresh weight, and the lowest content in plants under severe water deficiency (S2) (Table 4).

Electrolyte leakage (EL)

Electrolyte leakage (EL) was significantly affected by water deficiency (S), mycorrhizal inoculation (M), and paclobutrazol application (P) at the 1% probability level. Additionally, the three-way interaction among these factors (S × M × P) was significant at the 5% level (Table 3). EL% increased markedly with escalating water stress, reaching a maximum of 35.52% under severe drought conditions (S2), compared to 14.09% under full irrigation (S0). Mycorrhizal inoculation (M1) significantly reduced EL% to 23.75%, in contrast to 26.87% in non-inoculated plants (M0). Similarly, paclobutrazol treatment (P1) lowered EL% to 23.57%, compared to 27.04% in untreated plants (P0) (Table 4). The combined interaction revealed that the highest EL% (43.69%) occurred in the S2M0P0 treatment, indicating severe membrane damage under drought when neither paclobutrazol nor mycorrhiza was applied. In contrast, the application of either treatment, especially in combination, significantly mitigated EL%, underscoring their role in enhancing membrane stability under water-deficient conditions (Fig. 7).

Malondialdehyde (MDA) content

Malondialdehyde (MDA) content, an indicator of lipid peroxidation and oxidative stress, was significantly influenced by water deficiency, mycorrhizal inoculation, and paclobutrazol application at the 1% probability level, while the interaction between water deficiency and paclobutrazol (S × P) was significant at the 5% level (Table 3). Mycorrhizal inoculation (M1) effectively reduced MDA levels to 1.43 nmol, compared to 1.65 nmol in non-inoculated plants (M0). Similarly, paclobutrazol treatment (P1) lowered MDA content to 1.46 nmol, whereas untreated plants (P0) recorded 1.62 nmol. MDA levels increased in response to water stress, with the highest value observed under severe drought conditions (S2: 2.00 nmol), followed by moderate stress (S1: 1.67 nmol), and the lowest under full irrigation (S0: 1.15 nmol) (Table 4). The three-way interaction (S × M × P) revealed that the highest MDA content occurred in the S2M0P0 treatment, indicating elevated oxidative damage in plants subjected to severe water stress without any mitigating treatments. Conversely, the lowest MDA

levels were observed in S0M1P1 (1.14 nmol), S0M0P1 (1.15 nmol), S0M0P0 (1.15 nmol), and S0M1P0 (1.17 nmol), all under non-stressed conditions. These results demonstrate that both mycorrhizal inoculation and paclobutrazol application significantly attenuated oxidative stress in *D. moldavica*, particularly under drought conditions (Fig. 8).

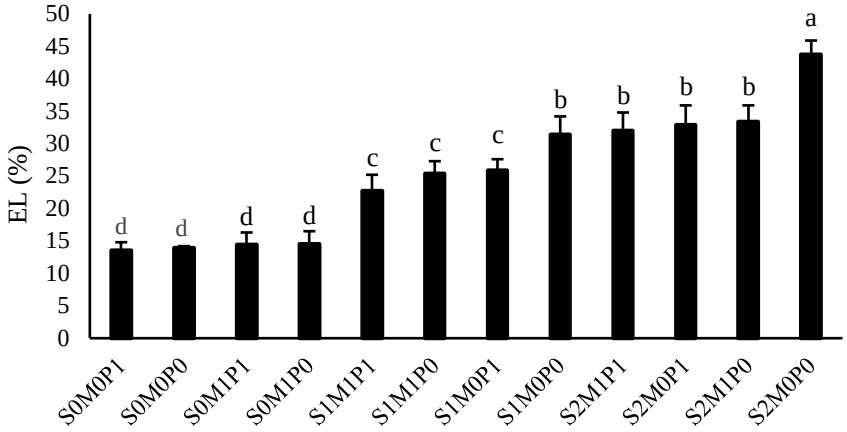

Fig. 7. Interaction effects of water deficiency, mycorrhiza inoculation and paclobutrazol foliar application on EL % of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

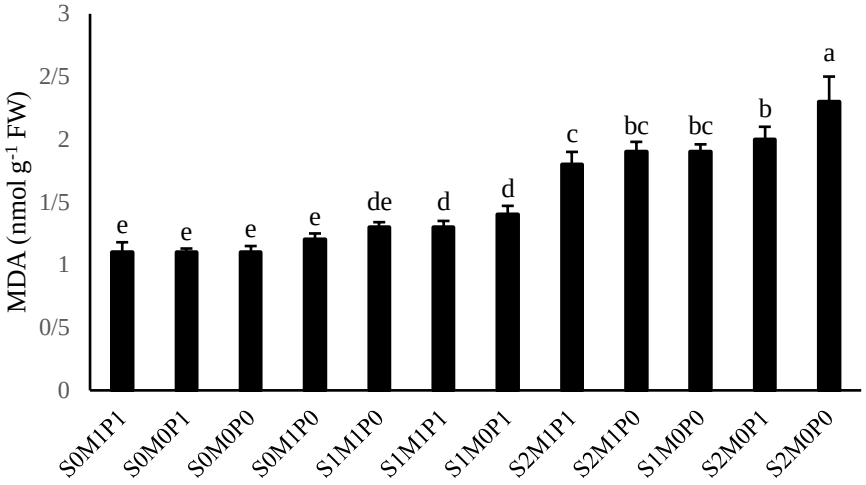

Fig. 8. Interaction effects of water deficiency, mycorrhiza inoculation and paclobutrazol foliar application on MDA of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with mycorrhiza. P0 and P1: paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

Total phenol content (TPC)

Water deficiency, mycorrhiza inoculation, and the interactions between water deficiency and mycorrhiza (S * M) and water deficiency and paclobutrazol (S * P) were significant at the 1% probability level, while the interaction between mycorrhiza and paclobutrazol (M * P) was significant at the 5% level (Table 3). TPC increased significantly with increasing water deficiency, with the highest value recorded in S2 (47.35 GAEs g^{-1} extract) and the lowest in S0 (32.24 GAEs g^{-1} extract). Mycorrhiza-inoculated plants (M1) exhibited higher TPC (43.00 GAEs g^{-1} extract) compared to non-inoculated plants (M0), which had 36.01 GAEs g^{-1} extract (Table 4). The interaction between S and M showed the highest TPC in S1M1 (51.40 GAEs g^{-1} extract) and the lowest in S0M0 (30.36 GAEs g^{-1} extract) (Fig. 9A). The interaction between S and P revealed the highest TPC in S2P1 (48.57 GAEs g^{-1} extract) and S2P0 (46.13 GAEs g^{-1} extract) (Fig. 9B). The interaction between M and P indicated the lowest TPC in M0P1 (35.14 GAEs g^{-1} extract), with other treatments showing comparable levels. The highest TPC overall was observed in the M1P1 treatment, at 43.45 GAEs g^{-1} extract (Fig. 9C).

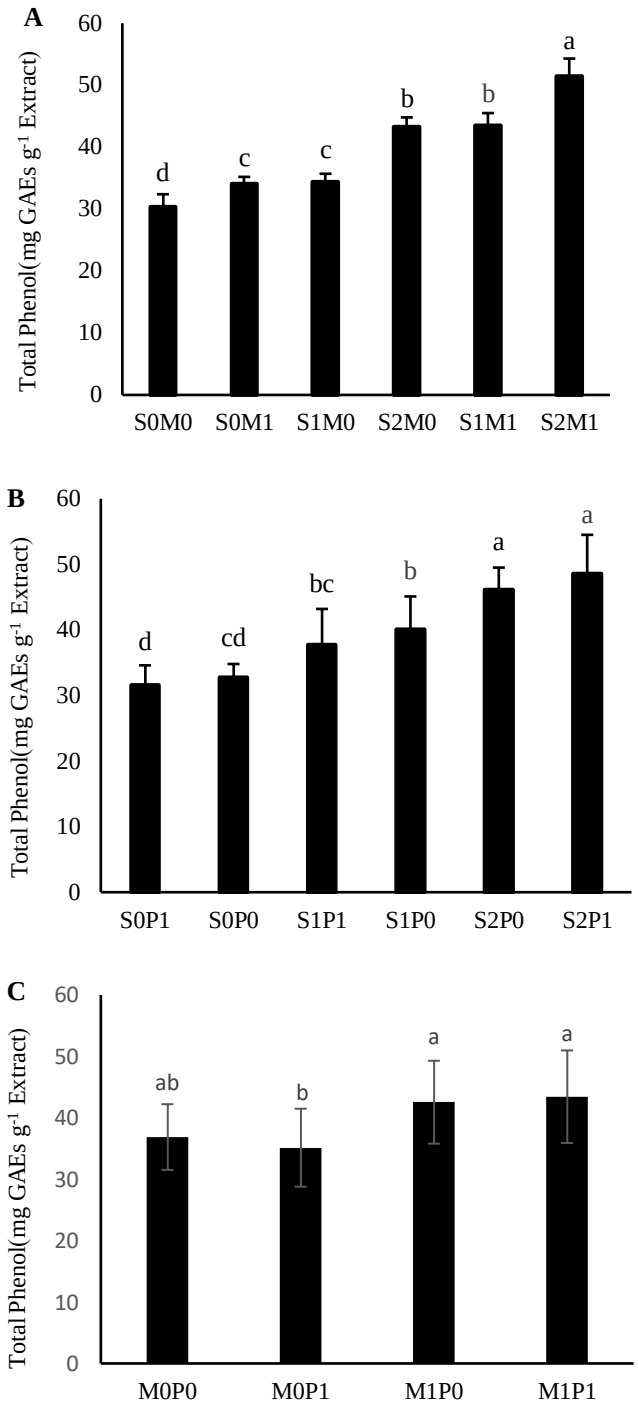

Fig. 9. Interaction effects of water deficiency and mycorrhiza inoculation; water deficiency and paclobutrazol foliar application (B); mycorrhiza inoculation and paclobutrazol foliar application (C) on Total phenol content of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with mycorrhiza. P0 and P1: paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

Total flavonoid content (TFC)

Water deficiency, mycorrhiza inoculation, paclobutrazol, and the interaction between mycorrhiza and paclobutrazol (M * P) were significant at the 1% level (Table 3). TFC increased significantly with increasing water deficiency, with the highest value recorded in S2 (25.32 mg QEs g^{-1} extract). Mycorrhiza-inoculated plants (M1) had higher TFC (25.40 mg QEs g^{-1} extract) compared to non-inoculated plants (M0), which had 19.78 mg QEs g^{-1} extract. Paclobutrazol application (P1) raised TFC to 24.02 mg QEs g^{-1} extract, compared to 21.16

mg QEs g^{-1} extract in P0 (Table 4). The interaction between M and P showed the highest TFC in the M1P1 treatment (27.87 mg QEs g^{-1} extract), while the lowest TFC was observed in M0P0 (19.40 mg QEs g^{-1} extract) and M0P1 with 20.17 mg QEs g^{-1} extract (Fig. 10).

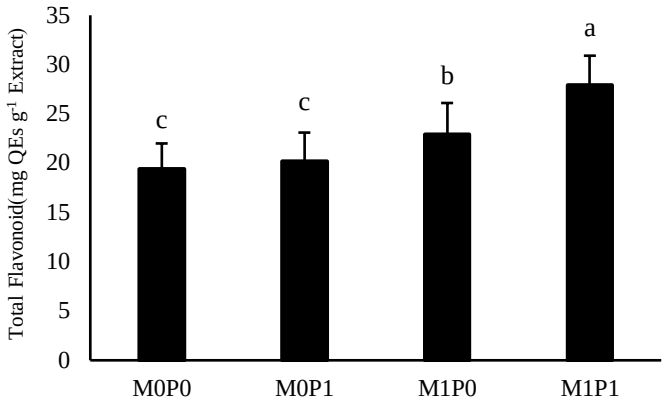

Fig. 10. Interaction effects of mycorrhiza inoculation and paclobutrazol foliar application on total flavonoid content of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with mycorrhiza. P0 and P1: paclobutrazol foliar application with 0 and 200 µg mL^{-1} respectively.

DPPH radical scavenging activity

Water deficiency, mycorrhiza inoculation, paclobutrazol, and the interaction between water deficiency and mycorrhiza (S * M) were significant at the 1% level, while the interaction between mycorrhiza and paclobutrazol (M * P) was significant at the 5% level (Table 3). DPPH radical scavenging activity increased significantly with greater water deficiency, with the highest activity observed in S2 (55.71%). Mycorrhiza-inoculated plants (M1) exhibited higher DPPH activity (54.76%) compared to non-inoculated plants (M0), which had 45.60%. Paclobutrazol application (P1) enhanced DPPH activity to 52.03%, compared to 48.33% in untreated plants (Table 4). The interaction between S and M showed the highest DPPH activity in S2M1 (62.86%), while the lowest was observed in S0M0 (44.04%) and S1M0 (44.20%) (Fig. 11A). The interaction between M and P revealed the highest DPPH activity in the M1P1 treatment (57.61%), with the lowest activity recorded in M0P0 (44.74%) and M0P1 (46.45%) (Fig. 11B).

Catalase (CAT) activity

Water deficiency, mycorrhiza inoculation, paclobutrazol, and the interactions between water deficiency and mycorrhiza (S * M) and mycorrhiza and paclobutrazol (M * P) were significant at the 1% level (Table 3). The highest CAT activity was observed under severe water stress (S2), with 3.43 U mg^{-1}, while the lowest activity was recorded in the no-stress condition (S0) at 1.42 U mg^{-1}. Mycorrhiza-inoculated plants (M1) exhibited higher CAT activity (3.01 U mg^{-1}) compared to non-inoculated plants (M0), which had 1.95 U mg^{-1} (Table 4). The interaction between S and M showed the highest CAT activity in the S2M1 treatment (4.22 U mg^{-1}) and the lowest in S0M0 which had 1.21 U mg^{-1} (Fig. 12A). The interaction between M and P revealed the highest CAT activity in M1P1 (3.34 U mg^{-1}), with no significant differences observed between M0P0, M0P1, and M1P0 (Fig. 12B).

Peroxidase (POD) activity

Water deficiency, mycorrhiza inoculation, paclobutrazol, and the interactions between water deficiency and mycorrhiza (S * M), mycorrhiza and paclobutrazol (M * P), and the combined interaction of water deficiency, mycorrhiza, and paclobutrazol (S * M * P) were significant at the 1% level (Table 3). The highest POD activity was observed under severe water stress (S2), with 1.91 U mg^{-1}, followed by moderate stress (S1) at 1.14 U mg^{-1}, and no stress (S0) at 0.82 U mg^{-1}. Mycorrhiza-inoculated plants (M1) exhibited higher POD activity (1.58 U mg^{-1}) compared to non-inoculated plants (M0), which had 1.00 U mg^{-1}. Paclobutrazol application (P1) increased POD activity to 1.41 U mg^{-1}, compared to 1.17 u/mg in untreated plants (Table 4). The three-way interaction of S, M, and P demonstrated the highest POD activity in S2M1P1 (2.53 U mg^{-1}) and the lowest in S0M0P0 which had 0.58 U mg^{-1} (Fig. 13).

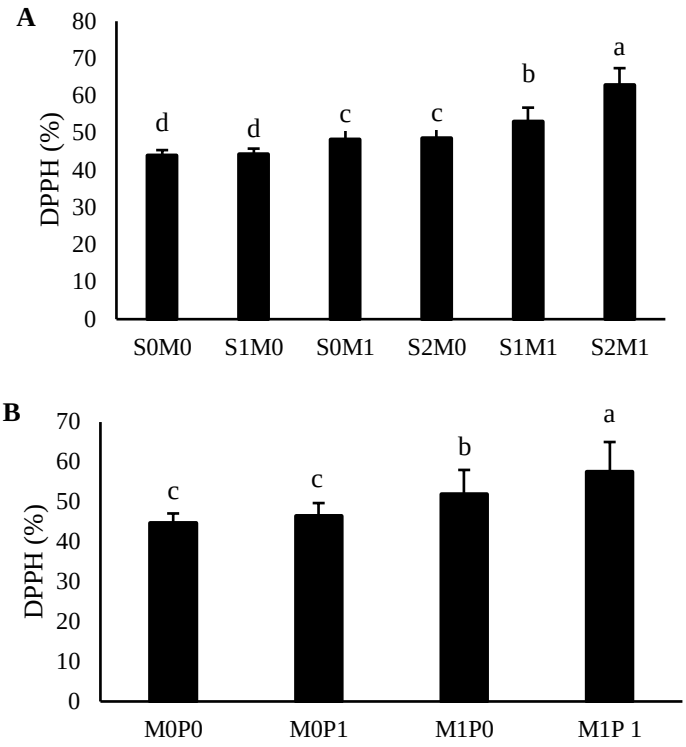

Fig. 11. Interaction effects of water deficiency and mycorrhiza inoculation (A); mycorrhiza inoculation and paclobutrazol foliar application (B) on DPPH% of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 μg mL^{-1}, respectively.

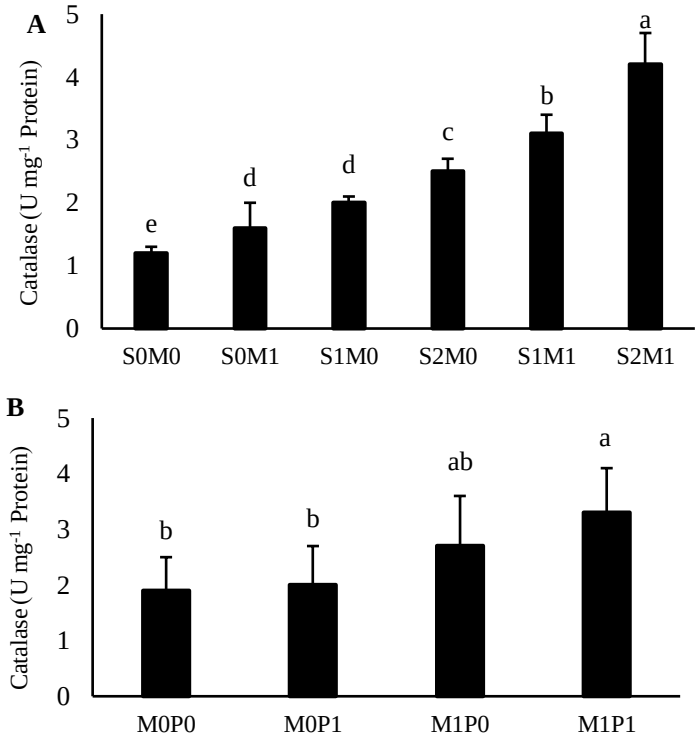

Fig. 12. Interaction effects of water deficiency and Mycorrhiza inoculation (A); Mycorrhiza inoculation and Paclobutrazol foliar application (B) on Catalase of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 μg mL^{-1} respectively.

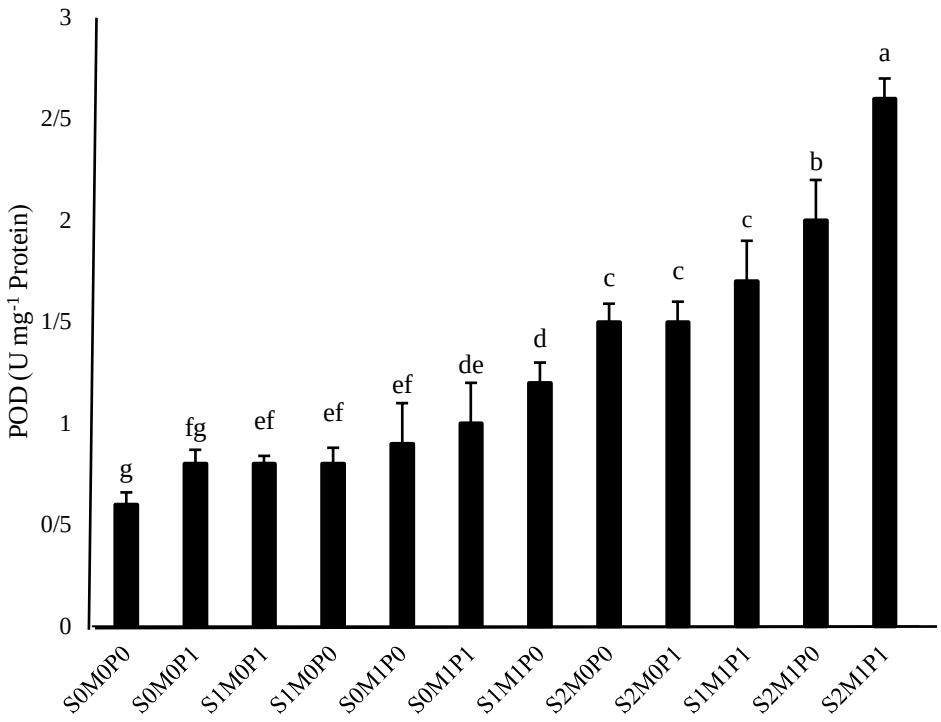

Fig. 13. Interaction effects of water deficiency, Mycorrhiza inoculation and Paclobutrazol foliar application on POD of *Dracocephalum moldavica*. S0, S1, and S2: irrigation levels at 100, 75, and 50% FC respectively. M0 and M1: non-inoculation and inoculation with Mycorrhiza. P0 and P1: Paclobutrazol foliar application with 0 and 200 μg mL^{-1} respectively.

Discussion

This study examined the effects of water deficiency, mycorrhizal inoculation, and paclobutrazol foliar application on a range of physiological and biochemical parameters in *Dracocephalum moldavica*. The results revealed significant interactions among these factors, influencing plant growth, pigment concentration, antioxidant activity, and stress-related enzymatic responses. Water deficiency markedly reduced plant growth, with the highest biomass observed under well-watered conditions (S0). This aligns with earlier studies reporting that drought stress commonly impairs plant development by restricting cell expansion and division (Farooq et al., 2017).

Similar growth-inhibitory effects of water stress have been reported in *D. moldavica*, *Salvia officinalis*, *Ocimum basilicum*, and *Thymus vulgaris* (Khaleghnezhad et al., 2021; Abdelaziz et al., 2019; Bahreininejad et al., 2019; Mohammadi et al., 2018). The primary physiological cause of growth suppression under drought conditions is the loss of turgor pressure, which limits cell elongation and expansion (Schulze et al., 2019).

Mycorrhizal inoculation significantly promoted plant growth by enhancing water and nutrient uptake, emphasizing the beneficial symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and plant roots (Smith and Read, 2017). Ghanbarzadeh et al. (2019) demonstrated that under water deficit conditions, the combined application of AMF and plant growth-promoting rhizobacteria (PGPR) improved nutrient availability, particularly nitrogen, resulting in enhanced growth, development, and photosynthetic capacity in *D. moldavica*. This led to increased plant height, leaf number, and dry matter yield. Similar positive outcomes have been reported in other Lamiaceae species such as *Mentha piperita* and *Thymus vulgaris* (Kapoor et al., 2017; Baslam et al., 2018). Mycorrhizal fungi improve plant performance under stress by increasing root surface area and enhancing the efficiency of water and nutrient absorption (Bi et al., 2018).

Application of paclobutrazol reduced plant height but increased the number of branches, consistent with its role in inhibiting gibberellin biosynthesis and promoting more compact growth (Seyedi et al., 2019). As a growth retardant, paclobutrazol limits cell elongation and enhances lateral development, leading to a denser, bushier plant architecture (Rademacher, 2016; Natarajan et al., 2021). In saffron, for example, paclobutrazol application has been shown to reduce leaf size and overall plant stature (Heidari et al., 2022), a trait particularly

valued in horticulture for producing robust, compact plants. The study also revealed that water deficiency significantly reduced chlorophyll a, chlorophyll b, and carotenoid content. These findings align with those of Shao et al. (2018), who reported that water stress induces chlorophyll degradation and diminishes photosynthetic capacity. A similar decline in pigment concentration under drought conditions has been observed in *Rosmarinus officinalis* (Alvarez et al., 2016), often attributed to oxidative damage to chloroplasts and the disruption of chlorophyll biosynthesis (Razi and Muneer, 2021). However, mycorrhizal inoculation mitigated these negative effects, likely by improving plant water and nutrient status (Baslam et al., 2017). Mycorrhizal symbiosis enhances nutrient acquisition, particularly phosphorus, a key element for chlorophyll synthesis, and helps maintain higher chlorophyll levels under stress (Bhantana et al., 2021).

Paclobutrazol also positively influenced chlorophyll content, potentially by stabilizing chloroplast structure and function under stress (Tesfahun, 2018). It is known to modulate chlorophyll synthesis and retention, which are critical for efficient light absorption and photosynthesis. Studies have shown that paclobutrazol can increase chlorophyll levels, largely by suppressing gibberellin biosynthesis. This redirection of metabolic resources from leaf expansion to pigment accumulation enhances the plant's capacity for light harvesting (Luo et al., 2019). Additionally, by restricting excessive vegetative growth, paclobutrazol reduces self-shading within the canopy, improving light penetration and potentially boosting photosynthetic efficiency, particularly under stress conditions (Hashem et al., 2020). Moreover, paclobutrazol has been reported to delay chlorophyll degradation, preserving chlorophyll a content during environmental stress such as drought, thereby sustaining photosynthetic capacity (Saied et al., 2021). This protective effect has been documented across various crops, underscoring paclobutrazol's potential for alleviating the adverse impacts of abiotic stresses (Heidari et al., 2022; Tesfahun, 2018).

The observed increase in DPPH radical scavenging activity and the activities of catalase (CAT) and peroxidase (POD) under water stress conditions indicates an enhanced antioxidant defense system. This response aligns with the findings of Gill and Tuteja (2016), who reported that plants upregulate their antioxidant machinery to mitigate oxidative stress induced by water deficiency. Similar trends have been reported in *Origanum vulgare* and *Melissa officinalis* under drought stress (Narasimhan et al., 2018; Kalhor et al., 2018). The activation of antioxidant enzymes under such conditions is critical for scavenging reactive oxygen species (ROS), thereby protecting cellular components from oxidative damage (Dumanović et al., 2021).

Mycorrhizal inoculation further enhanced these antioxidant activities, suggesting a synergistic role in strengthening the plant's stress tolerance (Begum et al., 2019). This improvement is likely due to the mycorrhizal symbiosis enhancing nutrient uptake and modulating hormonal balance, both of which contribute to a more robust antioxidant response (Bedini et al., 2018). Similarly, paclobutrazol treatment led to increased CAT and POD activities, reinforcing its role in improving stress resilience by modulating antioxidant defense pathways (Li et al., 2020). The ability of paclobutrazol to stimulate antioxidant enzyme activity is associated with its influence on hormonal regulation and metabolic adjustments essential for stress adaptation (Soumya et al., 2017).

Under severe water stress (S2), the highest levels of malondialdehyde (MDA) and electrolyte leakage (EL) were recorded, indicating significant lipid peroxidation and cell membrane damage (Hasanuzzaman et al., 2019). Similar increases in MDA and EL have been documented in *Lavandula angustifolia* under drought conditions (Naghizadeh et al., 2019). Elevated MDA levels are a hallmark of oxidative stress, reflecting the peroxidation of membrane lipids by ROS and consequent cellular injury (Jadoon and Malik, 2017). The upregulation of antioxidant enzymes under drought plays a vital role in cellular protection and plant survival. Specifically, increased CAT and POD activities are central components of the plant's defense mechanisms (Ahangir et al., 2020). This is consistent with findings by Cham et al. (2022), who observed enhanced CAT and POD activities in *Dracocephalum kotschyi Boiss.* under drought stress, supporting the present results.

Both mycorrhizal inoculation and paclobutrazol application significantly reduced MDA and EL levels, suggesting their protective roles in maintaining membrane integrity under stress conditions (Wu et al., 2020). The decreased levels of oxidative stress markers in treated plants highlight the efficacy of these interventions in enhancing resilience to abiotic stress. Mycorrhizal fungi contribute by improving the plant's water relations and nutrient acquisition, thus alleviating stress-induced damage (Wahab et al., 2023). Likewise, paclobutrazol's ability to stabilize membranes and regulate stress responses is well-established, reinforcing its utility as a stress mitigation agent (Rademacher, 2016).

Water deficiency significantly increased total phenol and flavonoid contents, consistent with the role of these secondary metabolites in plant defense against abiotic stress (Gharibi et al., 2016). Similar responses have been observed in other members of the Lamiaceae family, including *Thymus citriodorus*

and *Salvia miltiorrhiza* (Saeidnejad et al., 2019; Cai et al., 2017). Phenolic compounds and flavonoids are key antioxidants that scavenge reactive oxygen species (ROS), thereby mitigating oxidative damage and contributing to cellular protection under stress (Dumanović et al., 2021).

Both mycorrhizal inoculation and paclobutrazol application further elevated phenol and flavonoid levels, indicating their potential to enhance secondary metabolite production and improve overall stress tolerance (Lio et al., 2022). The increase in these compounds with mycorrhizal colonization is largely attributed to improved nutrient uptake and metabolic reprogramming (Kaur and Suseela, 2020). Supporting this, Ostadi et al. (2022) found that biofertilizer application significantly enhanced phenolic and flavonoid content in *Mentha piperita L.* under drought conditions. Similarly, Rashidi et al. (2022) reported increases of 50%, 55.8%, and 71% in flower phenolic content of *Ipomoea purpurea L.* following colonization by *Funneliformis mosseae*, *Rhizoglomus fasciculatum*, and *Rhizoglomus intraradices*, respectively.

Paclobutrazol also promoted secondary metabolite accumulation, likely through its modulation of hormonal balances that regulate phenolic and flavonoid biosynthesis (Desta and Amare, 2021). These findings highlight the interactive effects of water stress, mycorrhizal symbiosis, and paclobutrazol on enhancing the physiological and biochemical resilience of *Dracocephalum moldavica*. The delicate balance observed between stress mitigation and growth enhancement underscores the value of integrating biostimulants and growth regulators in cultivation strategies to produce more robust and productive plants. Further research exploring these dynamic interactions across species and environmental conditions will provide deeper insights into the mechanisms by which these treatments confer stress tolerance and improve plant health.

Conclusions

This study demonstrated that water deficiency, mycorrhizal inoculation, and paclobutrazol application significantly influence the physiological and biochemical responses of *Dracocephalum moldavica*. Individually and in combination, these treatments enhanced plant resilience to water stress by improving growth parameters, photosynthetic pigment content, antioxidant defense mechanisms, and the accumulation of secondary metabolites. Mycorrhizal inoculation contributed to stress tolerance primarily by enhancing root development, nutrient acquisition, and metabolic activity, while paclobutrazol modulated hormonal balance to promote compact growth and stimulate the synthesis of phenolic and flavonoid compounds. The synergistic effects of these treatments resulted in a more robust antioxidant system, as evidenced by elevated levels of phenols, flavonoids, and antioxidant enzyme activities under water-limited conditions. These findings established a valuable framework for developing integrated management strategies that leverage both biological inoculants and chemical growth regulators to enhance the resilience and productivity of medicinal plants under drought stress. Further research is recommended to explore the consistency of these responses across different environmental conditions and plant species, thereby refining their application in sustainable horticultural and agricultural systems.

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Author Contributions

Conceptualized the study, designed the experiments, conducted data analysis, drafted the original manuscript, and led the revision process, SM; Performed data collection and carried out laboratory experiment, RN; Contributed to experimental design, provided input during revisions, and approved the final manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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