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Adoption of arbuscular mycorrhizal fungi and biochar for alleviating the agro-physiological response of lavander (*Lavandula angustifolia* L.) subjected to drought stress

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ABSTRACT

Increasing the productivity of crops in water deficit circumstances is of great significance in order to face the challenge of securing global food production. Nowadays, the use of biochar (BC) and arbuscular mycorrhizal fungi (AMF) are considered as one of the most effective methods for empowering plants to deal with drought stress. However, the literature regarding the effects of the simultaneous use of AMF+BC on plants under drought stress is scarce. Hence, the present study was conducted to consider the combined application of AMF and biochar on the physiological and biochemical properties of lavender under various levels of irrigation. The first factor includes three irrigation regimes of 90 % field capacity (FC), 60 % FC, and 30 % FC, respectively, corresponding to normal irrigation, mild and severe drought stress. The second factor included a fertilizer or microbial inoculant source, which were respectively: no inoculation (control), inoculation with AMF, BC, and co-application of AMF along with BC. The highest nutrient, chlorophyll and carotenoid content, and fresh, and dry weight of lavender were recorded under normal irrigation conditions with combined application of AMF+BC. The combined application of AMF+BC was significantly effective in increasing the activity of antioxidant enzymes against oxidative damage caused by drought stress and in reducing the accumulation of proline and malondialdehyde. An increase in phenolic, flavonoid, and concentration of essential oil was observed under mild stress and using AMF+BC, while the highest essential oil yield was recorded under a normal irrigation regime and treated with AMF+BC. The highest content of linalool, camphor, borneol, and linalyl acetate was obtained in mild stress by adding AMF+BC. It can be concluded that the use of AMF+BC combination treatment may be a useful strategy to improve the quantity and quality of lavender EO in drought-stress circumstances.

1. Introduction

Climate change, including long, severe, and frequent droughts, has become one of the most important challenges in agriculture to assure food security for the global rising inhabitants in the current century (Roy et al., 2022; Wang et al., 2023). Drought stress reduces crop productivity and compromises the quality of the products, compromising the morphological, physiological, and biochemical characteristics of plants (Babaei et al., 2021; Kausar et al., 2023). Furthermore, drought stress leads to excessive accumulation of ROS, which causes damage to the structural and functional integrity of the plant (Haghaninia et al., 2024; Zhanassova et al., 2021; Kausar et al., 2023). Also, excessive accumulation of ROS may cause the oxidation of lipids and the destruction of proteins. In addition, ROS accumulation in plant cells plays a notable function in reducing photosynthesis and plant growth by inhibiting mineral absorption (Farouk and Al-Huqail, 2020; Alotaibi et al., 2023; Begum et al., 2023). Therefore, drought stress is considered one of the main threats to the production of agricultural products and the global economy (Haghaninia et al., 2023; Das et al., 2023; Mehralian et al., 2023).

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In addition to the adverse effects on yield, drought stress causes the concomitant intensification of other stresses, such as nutrient deficiency (Hamedani et al., 2022; Aslani et al., 2023; Xiao et al., 2023). Various researches have shown that drought stress can significantly reduce plant access to nutrients by reducing soil diffusion and mass flow, as well as affecting solubility (Ahanger et al., 2021; Ostadi et al., 2022). In this situation, sufficient access to nutrients can help to reduce the harmful influence of drought stress by activating biochemical, physiological, and metabolic processes in plants (Arpanahi et al., 2020; Beiranvandi et al., 2022; Hamedani et al., 2022). Consequently, innovative, sustainable, and environmentally friendly solutions are necessary to increase the absorption of nutrients, particularly in arid and semi-arid areas. In this regard, the use of arbuscular mycorrhizal fungi and biochar has many potential applications to modulate the impact of biotic and abiotic stresses (Darakeh et al., 2021; Hosseinzadeh et al., 2021; Langeroodi et al., 2021).

Arbuscular mycorrhizal fungi (AMF) are important microorganisms that act as bio-fortifiers and their application reduces the use of mineral fertilizers (Arpanahi et al., 2020; Azizi et al., 2021). These fungi are the main part of rhizosphere microflora in natural ecosystems and create a two-way symbiotic relationship with the roots of over 80 % of plants (Fattahi et al., 2021; Alotaibi et al., 2023). In addition, these fungi enter soil pores inaccessible to root hairs through mycorrhizal hyphal networks, and by increasing the level of access and absorption of the roots, they have a noteworthy role in supplying water and nutrients to the inoculated plant (Darakeh et al., 2021; Jabborova et al., 2021). The symbiotic connection established between plants and AMF can improve the physiological condition by growing the leaf area and phosphorus content, regulating the opening of stomata, improving photosynthesis, and increasing the efficiency of water consumption (Hassena et al., 2022; Begum et al., 2023; Neto et al., 2023). Similarly, several studies have reported that inoculation of plant roots with mycorrhizal fungi increases the uptake of several plant's nutrient such as nitrogen, potassium, phosphorus, calcium, and magnesium, which can improve drought tolerance by affecting various physiological and biochemical processes (Arpanahi et al., 2020; Darakeh et al., 2021; Aslani et al., 2023).

Biochar, which is also called black gold in agriculture, is an environmentally friendly soil conditioner that is obtained from the pyrolysis process, thermal decomposition of biomass or plant residues exposed to anaerobic conditions at temperatures between 300 °C and 1000 °C (Farhangi-Abriz and Torabian, 2017; Khan et al., 2021; Ghassemi-Golezani and Farhangi-Abriz, 2023). Recently, the use of biochar in agricultural systems has been considered an efficient tool for carbon sequestration and for increasing soil fertility and stability of agro-ecosystems (Beiranvandi et al., 2022; Védère et al., 2023). The application of biochar improves soil water holding capacity, activity of microorganisms, and mineral absorption for plants (Ibrahim et al., 2019; Langeroodi et al., 2021). Among the other beneficial effects of biochar application in agricultural soils, it increases cation exchange capacity and interaction with the soil nutrient cycle by regulating soil pH, improving the synthesis of plant hormones, and reducing the leaching of nutrients (Fascella et al., 2020; Mannan et al., 2021; Yildirim et al., 2021). Similarly, Khan et al. (2021) reported that the application of biochar can prevent oxidative damage caused by drought stress and increase product productivity by reducing the content of MDA, H_2O_2, and EL and regulating the activity of antioxidant enzymes. However, its effectiveness can vary due to factors like the source of biomass used for its production, the conditions of its creation (such as temperature and duration), soil characteristics, application practices, interactions with other soil additives, and environmental considerations (Kamali et al., 2022). Therefore, while biochar shows promise as a sustainable soil amendment, its application requires careful consideration and management to ensure positive outcomes and minimize potential drawbacks (Kuppusamy et al., 2016).

According to the statistics of the World Health Organization, currently, about 80 % of the population of developing countries and 60 % of the world's inhabitants depend on medicinal plants to meet their health and medical needs. Lavender (*Lavandula angustifolia* L.) is a valuable medicinal plant belonging to the *Lamiaceae* family (Fascella et al., 2020; Pirsarandib et al., 2022). It has 45 species and 400 varieties that are native to the Mediterranean basin, from southern Europe to northern and eastern Africa and the Middle East to southwestern Asia and southeastern India (Basch et al., 2004; Pistelli et al., 2017; Khatami et al., 2022). Lavender is one of the most well-known medicinal plants that is cultivated all over the world due to its high economic and ornamental potential, but 80 % of the world market is reserved for Europe, which is the largest producer and consumer of lavender essential oil (Fascella et al., 2020; Ciocarlan et al., 2021; Sharafabad et al., 2022). Due to its antimicrobial, analgesic, antifungal, sedative, anti-depressant, anti-inflammatory, healing, regenerating, tonic, and antiseptic properties, lavender performs an outstanding role in the pharmaceutical and food industries and human health (Basch et al., 2004; Rafii et al., 2020; Ebrahimi et al., 2022; Khatami et al., 2022).

In contemporary agriculture, there is a pressing demand for the implementation of innovative and sustainable methodologies to foster the development of robust agro-ecosystems. These systems should facilitate a reduction in the reliance on chemical fertilizers while concurrently mitigating the harmful impacts of water deficit stress, a paramount challenge in modern agriculture. While numerous investigations have assessed the individual applications of biochar (BC) and arbuscular mycorrhizal fungi (AMF) in enhancing crop productivity, a noteworthy research gap exists. Specifically, there is no literature on the simultaneous influence of AMF and BC on antioxidant activity and phytochemical properties of lavender subjected to drought stress conditions. This study hypothesizes that the combination of BC and AMF can be a promising agricultural strategy to support lavender production and its essential oil quality under drought conditions in arid environments. The principal aim of this research is: (i) to assess the impact of separate and combined applications of AMF and BC on the agronomical and physiological performances of lavender plants under drought conditions, and (ii) to calculate the lavender essential oil quantity and quality exposed to normal and deficit of water.

2. Materials and methods

2.1. Experimental growth conditions, treatments, and design

The morphological, physiological, and biochemical responses of lavender plants subjected to the application of biochar (hereafter called BC) and arbuscular mycorrhizal fungi (hereafter called AFM) under different drought levels were studied under controlled environmental conditions. The research was carried out in the form of pots in the spring and summer of 2022 in the research greenhouse of Maragheh University, East Azerbaijan, Iran (37°23′ N latitude, 46°16′ E longitude, and 1486 m a.s.l. altitude). Optimal growth conditions of 18–25 °C temperature, 60–70 % humidity, 8–16 h, light intensity, 180 μmol m^{-2} s^{-1} photoperiod were provided to the lavender. The following trial treatments were utilized: (i) three irrigation regimes corresponding to 90 %, 60 %, and 30 % of field capacity (FC), respectively, corresponding to normal irrigation, mild drought stress, and severe drought stress, and (ii) four levels of fertilization or microbial inoculant application: (1) no fertilizer (Control), (2) biochar (BC), (3) inoculation with (AMF), and (4) combined application of AMF along with BC (BC + AMF), respectively. It was adopted a randomized complete block design that involved a factorial combination of irrigation regime and fertilization management. Each experimental treatment was replicated five times, resulting in 60 pots.

2.2. Soil, biochar, AMF, and planting seeds

2.2.1. Soil

The soil employed in this research was provided from the upper soil layer (0 to 20 cm) of the Maragheh University agricultural research farm, and soil samples were randomly taken from a depth of 0–2 cm indicating the soil texture as sandy clay loam with an electrical conductivity of 1.13 dS m^{-1}, pH of 8.17, organic matter of 1.17 %, total N concentration of 0.82 mg.kg^{-1}, and available phosphorous of 8.8 mg.kg^{-1}. Before lavender planting, the collected soil was sterilized for one hour at a temperature of 100 °C and a pressure of 1 atm.

2.2.2. Arbuscular mycorrhizal fungi (AMF) and biochar (BC) characteristics

The commercial AMF (Mykoroot), which was a combination of three species, *Funneliformis mosseae, Rhizophagus intraradices*, and *Claroideoglomus etunicatum*, in equal amounts were purchased from Zist Fanavar Sabz Company, Iran. A total of 20 g in each pot of AMF was applied in AMF and BC + AMF treatments, respectively, which had 10^7–10^8 CFU g^{-1}. The AMF amount was applied based on previous studies (Javanmard et al., 2022).

The biochar used in this experiment was procured from a local company (Fasle Panjom Company, Shiraz, Iran) and was used at the rate of 10 % of soil weight. Biochar was obtained from a natural mine in the Koohbanan region of Kerman Province, Iran. According to the Fasle Panjom company method, the used biochar in this experiment is a natural product that is obtained from a mine, after that, it is grounded for any activity including agricultural employment (Nasiri et al., 2023). Here are the chemical properties of the biochar: nitrogen: 1.38 %, soluble phosphorus: 0.15 %, potassium: 0.14 %, organic carbon: 13.85 %, potential hydrogen: 6.4, electrical conductivity: 1.23 mS/cm, cadmium: 0.02 mg/kg, copper: 0.56 mg/kg, zinc: 1.74 mg/kg, magnesium: 3.42 mg/kg, and iron: 4.15 mg/kg.

2.2.3. Planting seeds

The local variety seeds used in this research were prepared by Pakan Bazar Company in Isfahan. The seeds were sterilized using a 10 % sodium hypochlorite solution for 10 min, washed, and planted in cocopeat-filled trays. After growth, the 3-month-old seedlings were transferred to 5-liter plastic pots filled with soil, sand, and animal manure (pH: 7.8, EC (ds.m^{-1}): 5.1, OC%: 26.3, N%: 2.8, P%: 0.99, K: 1.7), and perlite.

2.4. Implementation of irrigation regimes

Irrigation of the pots was done regularly up to two weeks after transplanting the seedlings, according to the plant's needs, and up to 90 % FC. Water stress was precisely implemented by monitoring the weight of the pots daily throughout the experimental period, until full flowering was achieved. To control the field capacity (FC), the pots were irrigated to saturation point after being filled with the same weight of soil. Then plastic was drawn on the pots and after 24 h when the soil moisture (excess water) was removed from the holes at the bottom of the pots, the soil of the pots was weighed and oven dried at 100 °C for 24 h and thus FC was calculated from the following equation (Javanmard et al., 2022):

$$FC(\%) = \frac{\mathbf{Wf} - \mathbf{Wd}}{\mathbf{Wd}} \times 100$$

Where FC is the percentage of field capacity, Wf is soil weight in the field capacity, and Wd weight of soil dried in the oven.

2.5. Measurements

2.5.1. AMF root colonization

To determine the percentage of mycorrhizal symbiosis, first, the root samples were randomly collected in each pot and washed with water to clean the excess soil. The roots were cut to a size of one centimeter, then they were heated for 10 min in a 10 % KOH solution to clean them. Root pieces were washed three times with distilled water. Then, the root samples underwent an acidification process using a 2 % HCl solution for 15 min at room temperature (Fig. 1a). Subsequently, the samples were stained with 0.05 % trypan blue, followed by a measurement of the colonization percentage. The measurement was carried out using the grid line intersection method, as detailed by Giovannetti and Mosse (1980).

2.5.2. Morphological traits

A ruler was used to measure plant height, and plant fresh weight (FW) was determined with an analytical scale (A&D Weighing, Tokyo, Japan). To measure the dry weight, the plant samples were dried at 75 °C in an oven until they reached a constant weight, and the dry matter (DW) was then recorded (Pirsarandib et al., 2022).

2.5.3. Mineral content

The aboveground biomass of lavender plants was employed to measure N, P, and K content. The N content was estimated based on the Kjeldahl method (Jones et al., 1972), the P content according to flame photometry (Tandon et al., 1968), and the K content using the yellow method (Tandon et al., 1968).

2.5.4. Essential oil analysis

2.5.4.1. Essential oil extraction. The lavender essential oil (EO) was extracted from 40 gr of dried organs ground for 3 h by a British Pharmacopoeia model Clevenger machine. The extracted samples were sealed in dark vials and kept at 4°C until chemical analysis. Finally, the percentage and yield of EO were determined (Ostadi et al., 2022).

2.5.4.2. GC/MS and GC-FID analysis. To thoroughly analyze the various components of lavender essential oil, two high-precision techniques were employed - gas chromatography-mass spectrometry (GC–MS) and gas chromatography with flame ionization detection (GC-FID). The Agilent 7990 B instrument from the USA was utilized, which was equipped with a 5988A mass spectrometer and an HP 5MS column. The column dimensions were 0.25 mm in diameter, 30 m in length, and 0.25 μm in film thickness, with 5 % phenylmethylpolysiloxane. The oven temperature was programmed to reach 60 °C for 5 min at a rate of 3 °C per minute. The carrier gas flow rate was set at 1 ml/min, and the injector split ratio was set to 1:30. The injector temperature was maintained at 230 °C, and the detector at 240 °C. The mass range and electron impact were set to 40–400 *m/z* and 70 eV, respectively. To identify the constituents of the oil with utmost accuracy, linear retention indices (RIs) were calculated against a homologous series of n-alkanes (C8-C40, Supelco, Bellefonte, CA, USA), and the mass spectra were matched with libraries (ADAMS, WILEY 275 and NIST 17). It is important to note that this method was described by Adams in 2017, and has since been widely accepted as a highly reliable means of identifying the various components of essential oils. For quantification, the VF 5MS capillary column (30 m, 0.25 mm/d, 0.50 μm f.t., 5 % phenylmethylpolysiloxane) was utilized. The injection volume of essential oil samples (1:100) was 1 microliter, dissolved in hexane (oil: hexane). The peak level normalization method was used to quantify the oil components, as described by Ostadi et al. (2020). This method is known for its accuracy and precision and is widely used in the field of essential oil analysis.

2.5.5. Chlorophylls (Chl) and carotenoids (CAR)

To measure Chl_a, Chl_b, and CAR, 0.5 g of fresh lavender leaves were ground using liquid nitrogen and mixed with 10 ml of acetone. Then the samples were centrifuged at 10,000 × *g* and transferred to a tube after 10 min. The absorbance at wavelengths 645, 663, and 470 nm was read spectrophotometrically and photosynthetic pigments were determined

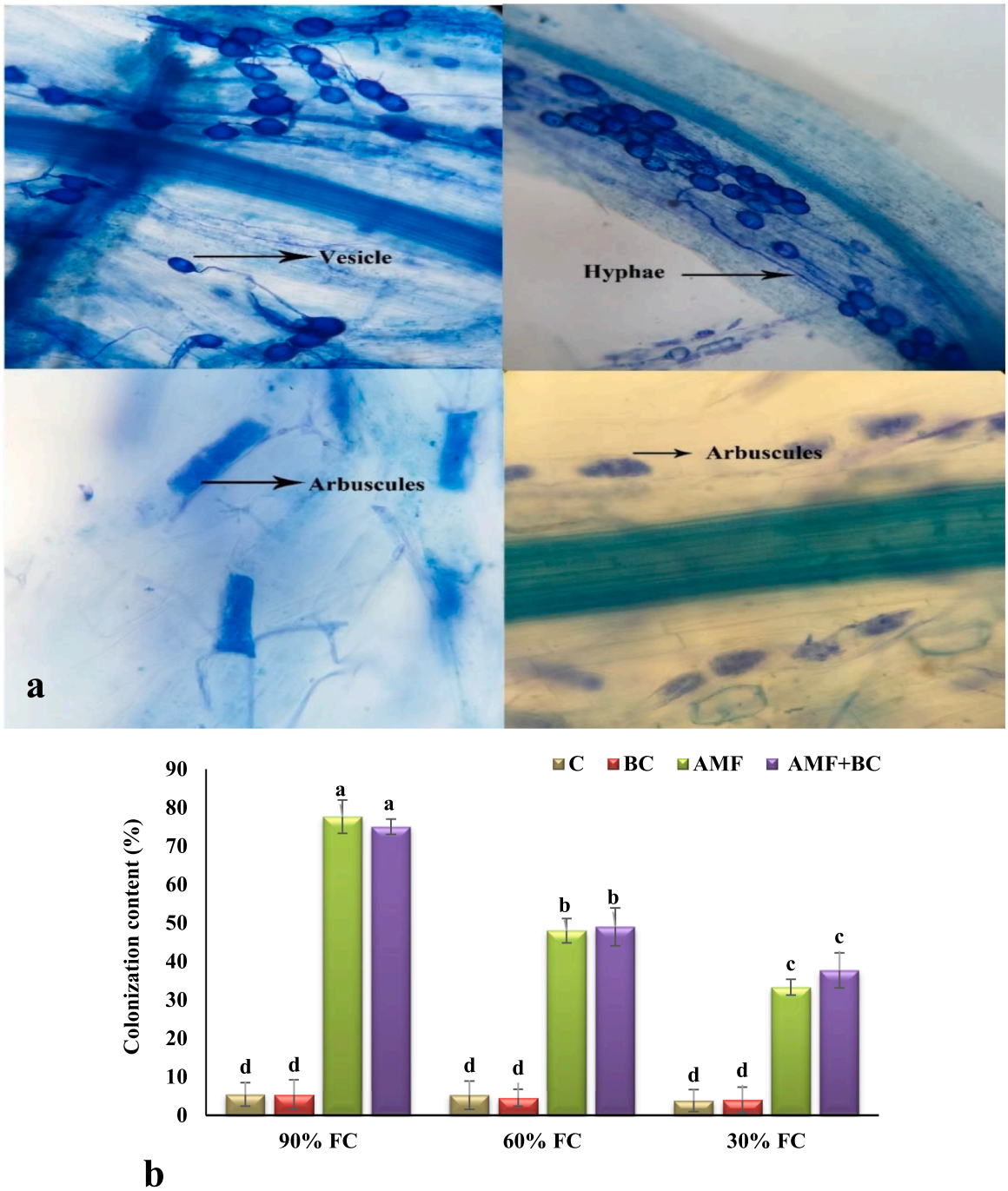

Fig. 1. Microscopic images of the stained lavender roots to detect the arbuscular mycorrhiza (*Funneliformis mosseae*) colonization (a); Percentage of mycorrhizal root colonization of *Lavandula angustifolia* L. plants under different fertilizer/microbial inoculant sources and at different irrigation levels. C: Control, AMF: Arbuscular mycorrhizal fungi, BC: Biochar. 90 % FC, 60 % FC, and 30 % FC, corresponding to normal irrigation, mild and severe drought stress, respectively. Different letters indicate significant differences at the 5 % level according to the LSD test (b).

as described by (Armon, 1949)

2.5.6. Total phenolic (TPC) and flavonoid content (TFC)

The (Singleton and Rossi, 1965) methods were used to measure total phenolic TPC and flavonoid content TFC of lavender (Nagy and Grancai, 1996).

2.5.7. Malondialdehyde (MDA) content

To conduct Malondialdehyde (MDA) analyses, we took 0.1 g of a fresh lavender leaf sample, which was then extracted using 1 ml of 0.1 % TCA. The mixture was centrifuged at 12,000 rpm, and after 15 min, the supernatant was collected. We then mixed it with 4 ml of reaction mixture consisting of 20 % TCA and 0.67 % 2-TBA. The samples were then placed in a water bath at 95 °C for 15 min. Then, they were cooled in an ice bath for 10 min. In the next step, the samples were centrifuged for 5 min at 4 °Celsius at a speed of 10,000 rpm. In the end, the absorbance at 532 and 600 nm was read by a spectrophotometer (Heath and Packer, 1968).

2.5.8. Proline (Pro)

To measure the proline concentration in lavender leaves, firstly, 0.5 gs of the leaves were extracted using 10 ml of 3 % sulfosalicylic acid and

centrifuged. Within 10 min, 2 ml of the resulting liquid was collected in a tube and mixed with acid ninhydrin and glacial acetic acid. The mixture was then kept in a water bath at 100 °C for an hour and transferred to an ice bath. After adding 4 ml of toluene to the samples, they were vigorously vortexed for 20 s. Finally, the absorbance was precisely measured spectrophotometrically at a wavelength of 520 nm (Bates, 1973).

2.5.9. Antioxidant enzymes activity

For the determination of ascorbate peroxidase (APX) content, was used the method of Nakano and Asada (1981), instead for superoxide dismutase (SOD) was used the method proposed by Beauchamp and Fridovich (1971). For the determination of peroxidase (POX) is was used the method of Kumar and Khan (1982), while for catalase (CAT) activity, the method proposed by Liu et al. (2009) was used.

2.6. Statistical analysis

To analyze the collected data, we used the ANOVA method with SAS version 9.4 (SAS Institute, Cary, NC, USA) software. Before the analysis, we transformed the percentage data using the arcsine transformation method to ensure that the variance was homogenized (Gomez and Gomez, 1984). We then conducted a two-way factorial analysis of the measured parameters by considering drought stress conditions and fertilization management as independent variables. The adopted approach allows us to make comparisons between means using the least significant difference test (LSD) at 1 % and 5 % probability levels. We also created graphs using the Excel program to visualize the results. The cluster dendrogram heatmap analysis was done by R software (version 4.3.1), Iran (2021) performed (URL https://cran.um.ac.ir/, accessed on 10 September 2023).

3. Results

3.1. AMF colonization

The percentage of AMF colonization was significantly influenced using irrigation regimes, fertilizer, or microbial sources (Table 1). The uppermost percentage of AM root colonization was recorded under normal irrigation conditions (with values over 75 % of mycorrhizal root length). The root colonization decreased by 36.42 % under mild stress (with values about 48 % of mycorrhizal root length) and by 53.4 % under severe stress (with values between 35 and 40 % of mycorrhizal root length) compared to normal irrigation (Fig. 1b).

3.2. Plant height

The lavender plant height was affected by drought stress, and fertilization/microbial inoculant (Table 1). The highest height of the lavender plant was measured under non-stressed conditions (90 % FC) and with the application of AMF + BC. The height of the lavender under water stress at 60 % FC and 30 % FC was reduced by 46.8 and 42.2 %, respectively, compared to non-stressed conditions. In addition, the application of AMF + BC increased the height of lavender under normal irrigation by 22.4 %, under mild stress by 33.9 %, and under severe stress by 41 % compared to the control treatment (Fig. 2a).

3.3. Plant fresh weight (FW) and dry weight (DW)

The plant FW and DW were significantly affected by drought stress, fertilization, and the interaction effect of drought stress × fertilizer/microbial inoculant (Table 1). The Maximum FW and DW of lavender plants were recorded in 90 % FC treated with AMF + BC, while the minimum FW and DW were observed in severe drought stress for control plants. Compared to normal irrigation, the amount of FW decreased by 14.7 % and 30.5 % in conditions of mild stress and severe stress, respectively (Fig. 2b). Furthermore, the DW value decreased by 14.3 % in mild stress and 40.9 % in severe stress (Fig. 2c). In return, the utilization of AMF + BC compared to the control increased the amount of FW under the condition of normal irrigation by 23.5 %, under moderate stress by 26 %, and under severe stress by 36.7 %. In addition, the application of AMF + BC increased the DW value by 43.6 % under normal irrigation, by 31.2 % in mild stress, and by 39.2 % in severe stress, compared to the control treatment (Fig. 2b and 2c).

3.4. Essential oil content (EO) and essential oil yield (EOY)

Different sources of fertilization/microbial inoculant, drought stress, and interaction effect of fertilization/microbial inoculant × drought stress had a significant effect on EO and EOY content of lavender (Table 1). The findings showed that the highest EO content of lavender was obtained under mild stress (60 % FC) treated with AMF + BC. However, the highest EOY was obtained in normal irrigation conditions with the application of AMF + BC (Fig. 2d). The amount of EO under moderate stress increased by 2.8 and 19.3 % compared to normal irrigation and severe stress, respectively. Interestingly, by using AMF + BC, the amount of essential oil increased by 43.2 % compared to the control (Fig. 2d). In addition, due to the positive effects of the simultaneous using of AMF + BC on DW as well as EO content, the EOY of lavender plants increased by 100.5 % with the combined utilization of AMF + BC compared to the control (Fig. 2e).

3.5. Essential oil constituents

Based on GC–MS and GC-FID analysis, 26 compounds were identified in lavender EO, which constitute 90.2–97.5 % of the total EO (Table 3). Its main components are borneol (15.7–21.8 %), linalyl acetate (6.7–9.1 %), linalool (5.2–7.7 %), camphor (4.7- 6.8 %), 1.8 Cineole (4.8–6.5 %), caryophyllene oxide (3.8–5.3 %), O—Cymene (3.2–3.9 %), and β-pinene (2.8–3.9 %). The maximum content of linalool, camphor, borneol, and linalyl acetate was obtained in mild stress by using AMF + BC. On average, the combined applying of AMF + BC increased the content of linalool, camphor, borneol, and linalyl acetate by 20.9, 10.3, 13.7, and 19.1 % compared to the control (Table 3).

3.6. Nutrient content

Based on the results obtained from this research, nitrogen, phosphorus, and potassium concentrations were affected by drought stress, fertilizer/microbial inoculant, and the interaction of the two mentioned factors (Table 1). The maximum concentrations of nitrogen, phosphorus, and potassium were recorded under the 90 % FC conditions and inoculation/application of AMF + BC, and the minimum values were

Table 1
ANOVA results of root colonization, height, nutrient content, fresh weight, dry yield, essential oil content and yield, of *Lavandula angustifolia* L. affected by experimental factors.

Sources of Variation	df	Colonization	Height	Fresh weight	Dry weight	Essential oil content	Essential oil yield	Nitrogen	Phosphorus	Potassium
Irrigation (I)	2	0.02**	1057.91**	350.82**	0.06**	0.06**	344.85**	5.61**	127.01**	2.24**
Fertilizer (F)	5	2.71**	125.52**	82.03**	0.09**	0.038**	24.23**	0.41**	18.55**	0.20**
$I \times F$	10	2256.66**	269.68**	539.06**	203.07**	0.07**	0.05**	168.97**	2.48**	68.61**

ns, * and ** indicated no significant difference, significant at 5 % probability level, and significant at 1 % probability level, respectively.

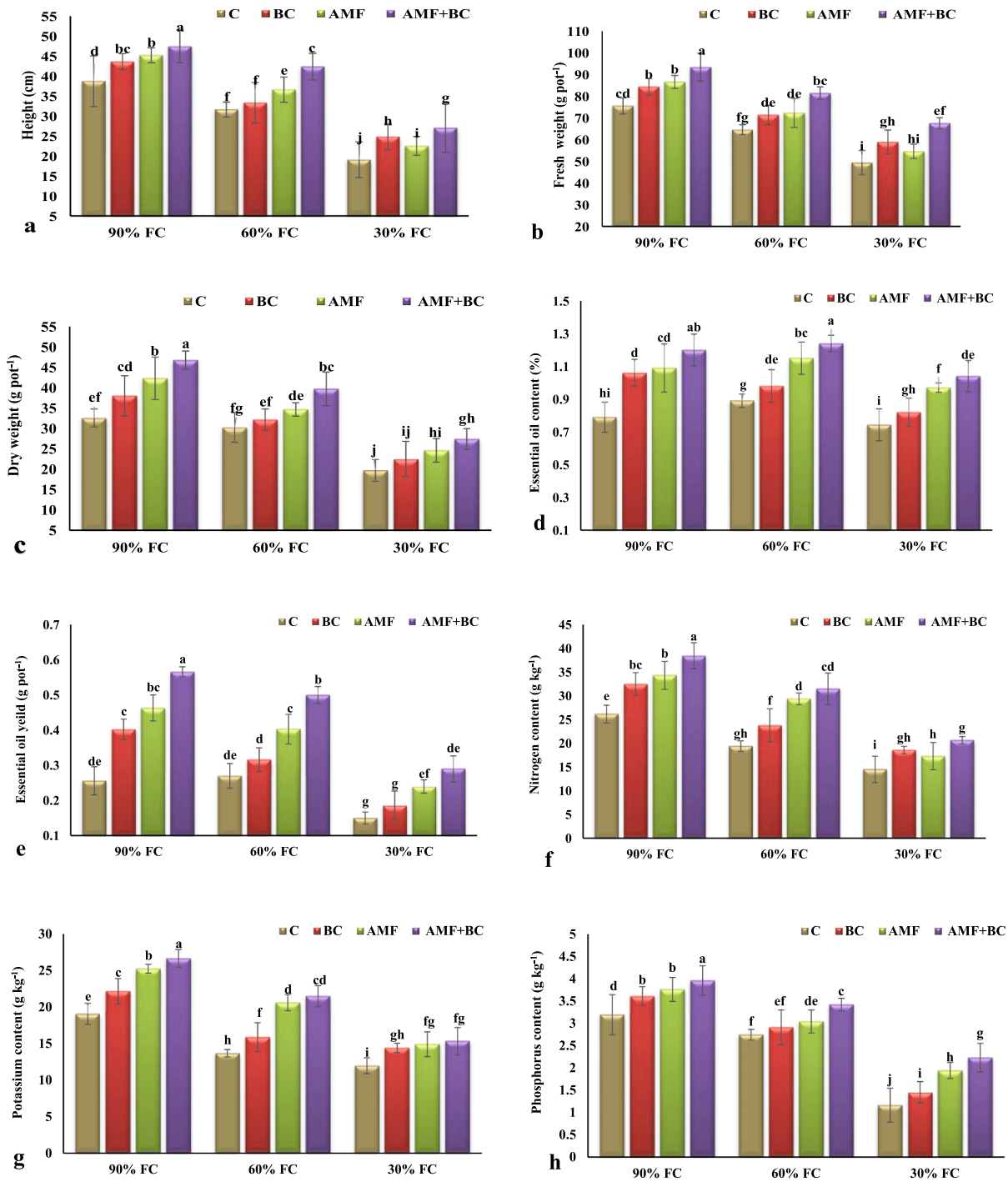

Fig. 2. Plant dry, fresh weight, essential oil content, essential oil yield, height, nitrogen, phosphorus, and potassium content of *Lavandula angustifolia* L. under different fertilizer/microbial inoculant sources and at different irrigation levels. C: Control, AMF: Arbuscular mycorrhizal fungi, BC: Biochar. 90 % FC, 60 % FC, and 30 % FC indicating normal irrigation, mild, and severe drought stress, respectively. Different letters indicate significant differences at the 5 % level according to the LSD test.

obtained in control plants under severe stress (Fig. 2f, 2g, and 2h). The content of nitrogen, phosphorus, and potassium decreased by 20.1, 16.5, and 23 % in moderate stress conditions, and 45.5, 53.3, and 39.1 % in severe drought stress compared to normal irrigation. In addition, on average, the content of the mentioned above nutrients increased by 50.1, 35.5, and 42 %, respectively, using AMF + BC compared to the control treatment (Fig. 2f, 2g, and 2h).

3.7. Chlorophyll (Chl) a, b, and car

In this research, the use of fertilizer/microbial inoculant, drought stress, and the interaction effect of fertilizer/microbial inoculant and drought stress on the amount of Chla, Chlb, and CAR had a significant effect (Table 2). Water stress of mild and severe stress reduced the photosynthetic parameters of lavender compared to normal irrigation

Table 2
Chlorophyll (Chl) a, Chl b, carotenoid (CAR), Proline (Pro), malondialdehyde (MDA), superoxide dismutase activity (SOD), catalase activity (CAT), ascorbate peroxidase activity (APX), peroxidase activity (POX), phenolic (TPC) and flavonoid (TFC) content of *Lavandula angustifolia* L. as affected by different fertilizer/microbial inoculant resources and irrigation levels.

Treatments		Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	CAR (mg g^{-1} FW)	Pro (μmol g^{-1} FW)	MDA (nmol g^{-1} FW)	TPC (mg.g^{-1})	TFC (mg.g^{-1})	SOD (μmol min $^{-1}$ mg^{-1} Protein)	CAT (μmol min^{-1} mg^{-1} protein)	APX (μmol min^{-1} mg^{-1} Protein)	POX (μmol min^{-1} mg^{-1} protein)
90 % FC	Control	5.29 ef	2.43 f	3.29 e	2.30 hi	4.06 f	11.95 i	12.76 j	3.39 j	0.916h	0.86 j	0.138 i
	BC	5.62 c	3.08 c	3.70 c	2.21 i	3.79 f	14.12 h	16.21 ij	3.74 i	1.15 g	1.09 i	0.162 h
	AMF	5.81 b	3.24 b	3.92 b	2.08 j	3.22 g	16.22 g	19.36 hi	3.98 h	1.27 f	1.47 h	0.183 g
	AMF+BC	6.17 a	3.41 a	4.23 a	1.74 k	2.76 h	17.19 g	20.19 gh	4.11 h	1.37 f	1.75 g	0.201 g
60 % FC	Control	4.70h	1.96 i	3.12 f	2.53 de	7.99 c	24.30 d	29.41 cd	5.81 d	2.06 c	2.91 c	0.362 c
	BC	5.26 f	2.36 fg	3.18 ef	2.48 ef	5.87 d	30.44 bc	32.60 bc	6.05 c	2.13 bc	3.10 bc	0.379 bc
	AMF	5.42 de	2.58 e	3.30 de	2.41 fg	5.62 de	31.59 b	36.02 ab	6.39 b	2.19 ab	3.28 ab	0.393 b
	AMF+BC	5.51 cd	2.90 d	3.45 d	2.35 gh	5.52 de	34.44 a	39.49 a	6.59 a	2.27 a	3.43 a	0.420 a
30 % FC	Control	3.95 j	1.68 j	2.31 i	3.20 a	9.05 a	19.36 f	23.65 fg	4.76 g	1.65 e	2.15 f	0.263 f
	BC	4.88 g	2.25 h	2.85 h	2.75 c	8.28 bc	22.11 e	25.48 ef	5.07 f	1.72 de	2.38 e	0.283 e
	AMF	4.31 i	1.88 i	2.95 gh	2.93 b	8.63 b	20.11 f	26.78 def	4.94 f	1.73 de	2.51 de	0.274 ef
	AMF+BC	5.32 ef	2.29 gh	3.05 fg	2.60 d	5.35 e	29.01 c	29.21 cde	5.24 e	1.82 d	2.66 d	0.312 d
LSD		0.153	0.10	0.163	0.999	0.392	1.58	3.86	0.147	0.117	0.198	0.02
Source of Variation	Significance Levels											
Irrigation (I)		1.89**	1.19**	0.77**	32.87**	340.16**	409.13**	7.56**	1.24**	4.80**	0.06**	935.14**
Fertilizer sources (F)		0.06**	0.15**	0.12**	0.45**	16.94**	34.26**	0.28**	0.03**	0.28**	0.002**	2602.95**
$I \times F$		1.19**	0.91**	0.77**	0.45**	14.40**	163.27**	191.82**	3.35**	0.57**	2.17**	0.02**

C: Control, AMF: Arbuscular mycorrhizal fungi, BC: Biochar. 90 % FC, 60 % FC, and 30 % FC indicating normal irrigation, mild, and severe drought stress, respectively. Different letters indicate significant differences at the 5 % level according to the LSD test.

Table 3
The essential oil constituents of *Lavandula angustifolia* L. as affected by different levels of drought stress and fertilizer/microbial inoculant sources.

			90 % FC				60 % FC				30 % FC			
	Compounds	RI	C	AMF	BC	AM+BC	C	AMF	BC	AMF+BC	C	AMF	BC	AMF+ BC
1	α-Pinene	929.9	1.41	1.33	1.18	1.77	1.63	0.95	1.14	1.77	1.51	1.23	0.95	1.83
2	Camphene	944.1	1.96	2.08	1.13	1.69	0.89	1.7	1.32	1.79	2.76	1.88	1.13	1.71
3	Sabinene	969.2	2.75	2.35	1.21	3.11	3.06	3.26	1.7	0.68	2.5	2.25	1.21	3.17
4	***β*-Pinene**	**972**	**2.83**	**3.99**	**3.6**	**3.57**	**2.85**	**3.24**	**3.09**	**3.61**	**2.03**	**3.42**	**3.11**	**3.55**
5	*β*-myrcene	988.8	1.56	0.89	2.04	2.76	0.92	0.421	1.73	1.34	0.87	0.98	0.94	0.82
6	*n*-Decane	998.7	1.61	1.69	2.3	1.76	1.93	2.41	1.29	1.68	1.81	1.59	1.54	2.34
7	*δ*-3-Carene	1005.2	3.51	3.37	2.52	2.86	2.64	1.38	1.68	1.91	2.59	2.99	2.81	3.05
8	**O-Cymene**	**1019.1**	**3.71**	**3.63**	**3.55**	**3.25**	**3.78**	**3.92**	**3.73**	**3.75**	**3.61**	**3.77**	**3.65**	**3.71**
9	**1.8-Cineole**	**1025.8**	**4.86**	**5.1**	**4.75**	**5.16**	**5.36**	**5.24**	**5.66**	**5.63**	**5.53**	**6.08**	**5.56**	**6.54**
10	(Z)-β-Ocimene	1033.7	1.36	1.07	1.77	0.76	1.58	0.89	0.77	0.84	2.76	0.97	0.85	1.19
11	**Linalool**	**1098.3**	**6.03**	**6.55**	**6.28**	**6.78**	**6.52**	**7.23**	**7.4**	**7.76**	**5.24**	**6.55**	**6.53**	**6.97**
12	**Camphor**	**1139.6**	**4.93**	**4.79**	**5.22**	**5.04**	**5.84**	**5.96**	**5.29**	**6.84**	**5.26**	**5.44**	**5.67**	**5.81**
13	**Borneol**	**1160.3**	**15.7**	**18.08**	**17.37**	**18.19**	**18.26**	**19.81**	**19.46**	**21.81**	**16.55**	**18.44**	**18.66**	**17.47**
14	Terpinen-4-ol	1170.4	2.23	1.79	1.47	1.81	2.31	1.31	2.56	1.51	2.69	1.59	1.84	1.07
15	P-Cymene-8-ol	1185.6	2.34	1.78	1.64	2.16	2.66	1.71	2.53	2.28	2.74	1.68	2.34	2.92
16	Cryptone	1188.6	2.93	2.75	3.64	3.06	2.72	2.87	2.25	1.06	2.7	3.45	2.46	1.87
17	Bornyl formate	1219.8	1.93	2.44	2.68	2.56	1.85	1.86	2.89	2.06	2.13	2.94	2.7	2.62
18	**Linalyl acetate**	**1251.6**	**6.73**	**7.44**	**7.81**	**7.78**	**7.69**	**7.45**	**7.86**	**9.13**	**6.53**	**7.79**	**7.48**	**8.05**
19	Bornyl acetate	1284	1.92	2.12	2.16	1.89	1.74	1.74	1.45	1.23	2.32	2.82	2.96	2.27
20	Lavandulyl acetate	1286.8	3.16	2.17	2.84	2.88	3.48	1.79	1.53	3.9	3.76	2.87	1.94	2.02
21	Geranyl acetate	1380.6	2.11	1.48	1.94	0.89	0.83	1.74	1.55	1.1	0.91	2.32	2.83	2.28
22	Santalene	1415	3.57	3.29	3.55	3.74	3.53	3.41	3.44	4.82	3.46	3.59	2.66	3.68
23	Bergamotene	1431	1.92	1.83	1.79	2.22	1.84	2.12	2.35	2.02	1.72	1.98	2.26	2.18
24	α-Amorphene	1482.7	2.78	2.68	2.86	3.73	3.28	3.35	3.64	2.6	3.25	3.41	3.55	3.69
25	**Caryophyllene oxide**	**1580**	**3.86**	**4.01**	**3.21**	**2.96**	**4.07**	**4.15**	**5.31**	**4.39**	**4.17**	**4.54**	**4.15**	**4.87**
26	α-Cadinol	1649	2.57	3.56	2.66	2.06	2.19	3.18	2.85	2.06	2.47	0.46	2.66	1.12
Total (%)			90.27	92.26	91.17	94.44	93.45	93.09	94.47	97.53	91.87	95.03	92.44	96.8

RI, linear retention indices on DB-5 MS column, C: Control, AMF: Arbuscular mycorrhizal fungi, BC: Biochar. 90 % FC, 60 % FC, and 30 % FC indicating normal irrigation, mild, and severe drought stress, respective.

(Table 2). However, the integrative use of AMF + BC increased the mentioned parameters. The highest and lowest contents of Chla, Chlb, and CAR were recorded under 90 % FC with the integrative use of AMF + BC and under 30 % FC for control plants, respectively. In comparison with normal irrigation, the content of Chla, Chlb, and CAR under severe water stress decreased by 19.3, 33.3, and 26.2 %, under moderate water stress, and by 8.7, 19.4, and 13.8 %, respectively. Interestingly, after the integrative use of AMF + BC, the concentration of chlorophyll a, b, and carotenoids increased by 21.9, 41.6, and 23.1 %, respectively (Table 2).

3.8. Total phenolic (TPC) and flavonoid content (TFC)

The ANOVA results revealed that the TPC and TFC content of lavender was significantly influenced by fertilization/microbial inoculant, drought stress, and the interaction effect of both factors (Table 2). The highest and lowest amounts of phenols and flavonoids were noted respectively under mild drought stress treated with AMF + BC and under normal irrigation conditions for control plants. In comparison with 90 % FC, the TPC and TFC content were heightened by 101.53, 100.7 % in 60 % FC, and enhanced by 52.3, and 53.4 % in 30 % FC, respectively. In addition, the application of AMF + BC improved the phenolic content by 45 % and the flavonoid content by 35 % compared to no fertilizer (Table 2).

3.9. Proline (Pro) content

The proline content of lavender was considerably impacted with irrigation regimes, fertilizer/microbial inoculant, and the interactions between both factors (Table 2). The highest and lowest amount of proline was recorded for control plants under the treatment of severe drought and under the treatment of 90 % FC treated with AMF + BC, respectively (Table 2). Pro-content increased by 17.2 % and 37.8 % under mild stress and severe drought conditions, respectively (Table 2). In addition, the use of AMF + BC treatment compared to the control reduced the content of Pro-by 24.3 % in severe stress, 7.1 % in mild stress, and 18.7 % in normal irrigation (Table 2).

3.10. Malondialdehyde (MDA)

Fertilization/microbial inoculant, drought stress, and the interaction between both factors significantly affected lavender MDA content (Table 2). The maximum concentration of MDA was obtained in control plants under severe drought stress, while the lowest amount of MDA was recorded under the condition of 90 % FC and with the combined treatment of AMF + BC. The MDA content increased by 126.3 and 80.7 % under severe and mild drought stress, respectively. In addition, with the application of AMF + BC treatment, MDA content decreased by 32 % in severe stress, 30.1 % in mild stress, and 38.9 % in normal irrigation compared to without fertilizer (Table 2).

3.11. Antioxidant enzymes activity

Based on the obtained results, the activity of antioxidant enzymes were significantly affected by fertilization/microbial inoculant, drought stress, and the interaction between both factors (Table 2). The highest activities of SOD, CAT, APX, and POX were observed under 60 % FC and after the application of AMF + BC. The activities of SOD, CAT, APX, and POX increased by 63.2, 83.8, 146.1, and 126.9 % under mild stress and by 31.5, 46.6, 87.6, and 64.9 % under severe stress, respectively, compared to normal irrigation. Interestingly, over to the control lavenders, the use of AMF + BC improved the activity of the mentioned enzymes up to 14.2, 18.2, 32.4, and 22.1 %, respectively (Table 2).

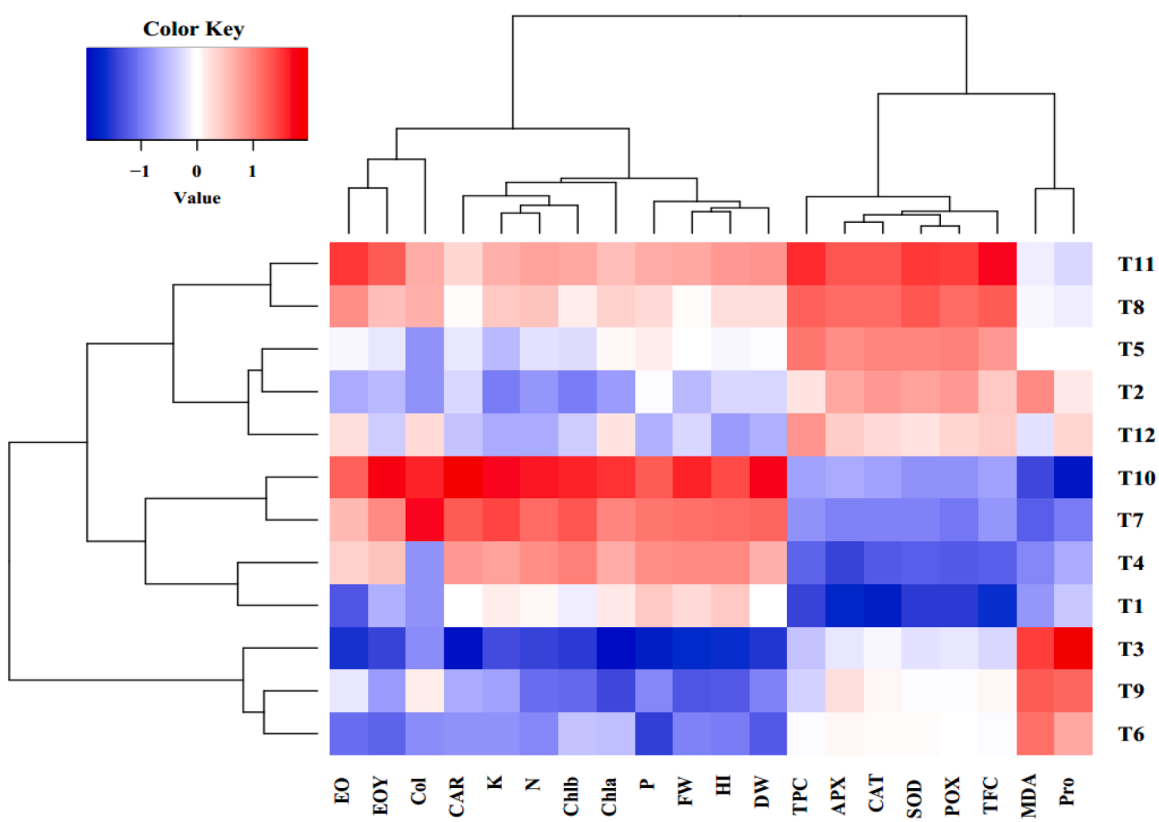

Fig. 3. Heat map of the enzymatic and non-enzymatic antioxidants pool, the morphological and the biochemical changes in Lavander to drought stress by biochar (BC) and Arbuscular mycorrhizal fungi (AMF) application. Heat map representing colonization (col), fresh and dry weight (FW and DW), essential oil content (EO) and essential oil yield (EOY), height (HI), nitrogen (N), phosphorus (P), potassium (K), chlorophyll a (Chl a), chlorophyll *b* (Chl b), carotenoids (CAR), proline (pro), malondialdehyde (MDA), phenol (TPC), flavonoid (TFC), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX). T1, T2, and T3 refer to the lavender plants grown under 90, 60, and 30 % FC along with no fertilizers, respectively; T4, T5, and T6 refer to the plants under 90, 60, and 30 % FC along with biochar application, respectively; T7, T8, and T9 refer to the plants subjected to 90, 60, and 30 % FC with arbuscular mycorrhiza application; T10, T11, and T12 refer to the plants exposed to 90, 60, and 30 % FC with simultaneously application of arbuscular mycorrhiza and biochar.

3.12. Multivariate analysis of lavendula angustifolia exposed to drought stress treated with amf and biochar applications

Heat map analysis according to the reaction of lavender plants exposed to drought stress treated with AMF and biochar applications revealed that the features, comprising EO, EOY, colonization rate, carotenoids, K, P, N, Chl a, Chl b, shoot FW, HI, shoot DW had a positive reaction to biochar and AMF application, while TPC, APX, CAT, SOD, POX, TFC, MDA, and proline showed negative response to biochar and AMF utilization and increasing response to drought stress (Fig. 3). Cluster analysis and dendrograms in the heat map (Fig. 3) presented two main groups in the measured characteristics of lavender plants applied with AMF and biochar under drought stress. Group I was EO, EOY, colonization rate, carotenoids, K, P, N, Chl a, Chl b, shoot FW, HI, shoot DW; and group II contained shoot DW, Chl b, protein content, APX, and TPC, and class III contained TPC, APX, CAT, SOD, POX, TFC, MDA (Fig. 3). Moreover, the heat map showed that the treatments that were applied on the lavender plants were classified into five groups. So, group I was the plants treated with AMF and AMF + BC under moderate drought stress; group 2 included the lavender under moderate drought stress without any fertilizers and BC, as well as the plants under severe drought stress along with AMF + BC; group 3 contained the plants grown in normal irrigation along with AMF and AMF + BC; group 4 was the lavenders grown under normal irrigation along with BC and without fertilizers; group 5 included the plants grown under severe drought stress without fertilizers, also with BC and AMF application (Fig. 3).

4. Discussion

The results of this study reported that drought stress reduces AMF root colonization by decreasing soil population and microbial activities and negatively affecting spore density and germination (Ghanbarzadeh et al., 2019; Arpanahi et al., 2020). Ostadi et al. (2022) found that drought stress reduces AMF inoculation due to reduced moisture and limited carbon supply by host plants. However, the combined application of BC plus AMF improved the colonization rate in drought stress conditions.

Plant height is an important growth indicator that shows crop growth and nitrogen absorption, and it is vital for crop yield (Haghaninia et al., 2024; Tashakorizadeh et al., 2022). Drought stress can reduce plant height through its effect on cell division and elongation, reduction of stomatal conductivity, and CO_2 absorption as a result of stomatal closure (Ostadi et al., 2022; Das et al., 2023). AMF + BC treatment improved lavender plant height. AMF increases the abscisic acid concentration in roots, which enhances growth by reducing water loss and maintaining cell turgor (Hashem et al., 2019; Lahbouki et al., 2022). In line with our results, Hashem et al. (2019) concluded that integrative use of biochar and bio-fertilizers changes root morphology, improving water balance and nutrient absorption. This leads to increased plant growth and height.

Water shortage stress led to decreased fresh and dry weight of lavender plants, which affected their productivity due to lack of available nutrients caused by drought effects on nitrogen mass flow and phosphorus and potassium release (Soltanbeigi et al., 2021; Kausar et al., 2023). Drought stress reduces lavender yield by causing stomatal closure, inhibiting the Rubisco enzyme, and reducing chlorophyll content and photosynthetic capacity (Khodadadi et al., 2022). AMF + BC treatment improved fresh and dry yield by enhancing plant access to water and nutrients, regulating cell osmotic pressure, and increasing antioxidant activities (Lahbouki et al., 2022; Kausar et al., 2023). Also, biochar improves plant growth and productivity by supplying nutrients and enhancing soil properties like density, permeability, and water storage capacity while reducing nutrient leaching (Jabborova et al., 2021; Khan et al., 2021).

In this experiment, drought stress decreases nutrient absorption in lavender. This is because reduced microbial activity limits the decomposition of organic matter, leading to a decrease in plant access to nutrients (Haghaninia et al., 2023; Shah et al., 2023). Our results agree with Aslani et al. (2023) that drought stress decreases nutrient content in sage. However, the combination of AMF and BC effectively improved the nutrient content. Integrative use of fertilizers increases nutrients, attributed to biochar's nutrient release and soil priming caused by the decomposition of native organic matter (Védère et al., 2023). AMF improves nutrient content in drought stress by enhancing root surface area and releasing phosphate or organic acids (Wu et al., 2013; Ma et al., 2022). Similarly, Arpanahi et al. (2020) stated that the application of AMF improves the availability of nutrients by increasing the acidity around the rhizosphere and by releasing H^+ ions.

Plants produce secondary metabolites to adapt to adverse environmental factors and balance critical activities (Arpanahi et al., 2020). Our results showed that Lavender oil content is highest under mild stress, while severe stress reduces oil content due to the plant's allocation of resources towards osmotic regulation and sugar production, and using AMF + BC treatment increased essential oil content and essential oil yield. Biochar's porous structure and oxygen functional groups help conserve water, improve nutrient absorption, and promote plant growth (Beiranvandi et al., 2022). AMF inoculation leads to increased absorption of nutrients, resulting in higher production of carbohydrates and expansion of glandular trichomes, EO channels, and secretory ducts, which in turn explains the increase in EO and EOY of lavender (Ghanbarzadeh et al., 2019; Neto et al., 2023).

Water stress hinders photosynthesis in plants by reducing the absorption of carbon dioxide through the closing of stomata. As a result, NADPH+H+ levels increase in plant cells, inhibiting photosynthesis (Farouk and Al-Huqail, 2020; Khodadadi et al., 2022). Our findings are in agreement with Ostadi et al. (2020) reported that the percentage of main components and compounds of Mentha x piperita essential oil increased in mild drying. AMF + BC fertilizers improved the content and quality of lavender essential oil by providing better access to nutrients that play a significant role in EO precursor and intermediary compounds (Aghaei et al., 2019; Hosseinzadeh et al., 2021; Aslani et al., 2023).

Drought stress reduces lavender's photosynthetic pigments in this experiment. Lack of essential nutrients can disturb pigment synthesis (Abd Elbar et al., 2019; Babaei et al., 2021). Drought stress reduces chlorophyll content due to the destruction of chloroplast thylakoid membranes, and the photooxidation of chlorophyll from ROS increases (Ahanger et al., 2021; Zhanassova et al., 2021; Tashakorizadeh et al., 2022; Begum et al., 2023). The study found that using AMF + BC treatment improves photosynthetic pigments. Biochar enhances soil microbial carbon utilization and nutrient content, preventing nitrogen leaching and making it readily available to the plant. Nitrogen improves chlorophyll structure, thereby enhancing photosynthetic pigments (Fascella et al., 2020; Farooq et al., 2021; Bornø et al., 2022). AMF inoculation can increase lavender chlorophyll *by* improving phosphorus nutrition, nitrogen, and magnesium content, which play a crucial role in CO_2 absorption (Azizi et al., 2021; Darakeh et al., 2022).

Phenolic and flavonoid contents help plants cope with adverse conditions to reduce the harmful effects of ROS (Haghaninia et al., 2023; Ahanger et al., 2021; Azizi et al., 2021; Hassena et al., 2022). AMF and biochar help increase these compounds by improving nutrient availability and boosting enzyme activity in flavonoid and phenolic compound biosynthesis. The combination of AMF and BC increased the production of these useful plant compounds (Hashem et al., 2019; Azizi et al., 2021; Hassena et al., 2022) Similarly, the results of Begum et al. (2021) reported that inoculation with AMF led to the improvement of phenol and flavonoid content.

Proline is an osmotic protector that accumulates under stress conditions (Javanmard et al., 2022), and MDA is an essential and reliable indicator to evaluate the extent of plasma membrane damage and the ability of plants to withstand stress resulting from lipid peroxidation (Das et al., 2023; Mehralian et al., 2023). Furthermore, drought stress causes the excessive increase of reactive oxygen species and destroys the

balance between ROS production and quenching (Ahanger et al., 2021; Tashakorizadeh et al., 2022). This result is consistent with other reports that MDA and proline content increased in response to ROS under drought stress conditions (Farouk and Al-Huqail, 2020; Babaei et al., 2021). AMF and BC treatment reduces MDA and proline content in plants by improving water access, osmotic regulation, and antioxidant defense systems (Hashem et al., 2019; Ibrahim et al., 2019; Fascella et al., 2020). Therefore, these results confirmed the positive role of AMF and BC in protecting host plants and modulating adverse effects under drought stress conditions.

Based on our findings, the activity of antioxidant enzymes enhanced in deficiency water stress conditions, especially at 60 % FC, however, the integrative application of AMF + BC improved the activity of antioxidant enzymes. Drought stress by increasing ROS compounds causes oxidative and membrane damage and ultimately cell death (Haghaninia et al., 2024; Zhanassova et al., 2021). However, plants lead to the reduction or elimination of ROS and stabilization of cell structure through the improvement of defense systems such as SOD, CAT, APX, and POX (Begum et al., 2023). Drought stress harms plants by causing lipid peroxidation of membrane cells. However, increased antioxidant activity can reduce the harmful effects of stress and preserve the cells' structural and functional integrity (Mehralian et al., 2023). AMF inoculation enhances nutrient absorption by increasing root hydraulic conductivity, decomposing organic matter, and improving the efficiency of fungal hyphae, which leads to reduce the negative effects of water stress (Ghanbarzadeh et al., 2019; Arpanahi et al., 2020). Furthermore, the improvement of antioxidant activity with the use of biochar is attributed to the increase in soil water holding capacity, porous structure, and high levels of biochar (Yildirim et al., 2021).

Based on the heat cluster analysis, the lavender plants under moderate drought stress exposed to AMF and the combined AMF + BC, as well as in severe water deficit with AMF + BC the best essential oil quality and quantity, and then the plants in mild drought stress without any treatment and with BC was placed at the second group in EO yield, the third and fourth group of the plants were in normal irrigation with AMF and AMF + BC and the lavenders without any treatments and BC, respectively. Finally, the lavenders under severe drought stress without any treatment and also with BC and AMF have the lowest EO yield.

5. Conclusion

The present study reported that the combined application of AMF + BC has a high potential to improve the growth characteristics of lavender under dry conditions. The use of AMF + BC can improve the absorption of nutrients that play a key role in increasing growth under drought stress conditions. In addition, AMF + BC treatment decreased malondialdehyde and proline content by improving the activity of antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, and guaiacol peroxidases. Importantly, the use of AMF + BC combination treatment significantly improved the quantity and quality of lavender essential oil, especially under mild stress conditions. Overall, the results obtained from this study support the role of the simultaneous addition of AMF + BC as an environmentally compatible strategy for the quantitative and qualitative improvement of lavender essential oil under drought stress conditions. Nevertheless, it is recommended that more long-term studies in greenhouse and field conditions are needed to explore the role of simultaneous application of AMF + BC in a broader sense under different stressful conditions.

CRediT authorship contribution statement

Mohammad Haghaninia: Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Abdollah Javanmard:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Emanuele Radicetti:** Writing – review & editing, Data curation, Supervision, Validation, Visualization, Writing – original draft. **Farzad Rasouli:** Validation, Methodology, Data curation, Conceptualization, Writing – original draft. **Juan Manuel Ruiz-Lozano:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision. **Paolo Sabbatini:** Writing – review & editing, Visualization, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100461.

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