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Assessing the effects of water stress and bioorganic fertilizers on English and French Lavandula species in different locations

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Abstract. A study was conducted utilizing a factorial split-plot design within complete randomized blocks to evaluate the effects of water stress (at 95±5, 75±5, 50±5, and 25±5% of the field capacity (FC)) and various fertilizer sources (Azotobacter-Psedomonas Bacterial (APB), arbuscular mycorrhizal fungi (AMFs), and biochar (BI), applied individually and in combination, along with a control treatment on the two species of lavender (French and English) in two distinct climates (Fars and Hamedan) in Iran. The results revealed that severe water deficit stress reduced the growth, yield, and essential oil content in both lavender species. APB+AMFs+BI and APB+BI led to the highest flower yield for French lavender in Fars and Hamedan while subjected to well-watered conditions. The essential oil yield significantly decreased in both experimental regions under 25% FC. Employing APB+BI under 75 and 50% FC treatments led to the highest essential oil content (4.23 and 1.56%, respectively) in English lavender. Linalool showed the highest average (22.46 to 30.06%) in both species. French and English lavender in Fars showed significant differences in fatty acid compositions, but no such differences were reported in Hamedan. APB+BI emerged as the most effective fertilizer combination in terms of total fatty acids and rank index. Simultaneous use of bio-organic fertilizers yielded appropriate results under water-deficient conditions. Finally, the study underscored the strong correlation between climatic parameters such as rainfall and temperature, as well as the efficacy of different fertilizer sources, emphasizing that climatic condition and plant species can significantly impact the effectiveness of fertilizer use.

Keywords: Azotobacter-Psedomonas Bacterial, biochar, essential oil content, fatty acid compositions, irrigation regimes.

HIGHLIGHT

- English and French lavender species varied in biochemistry and quality traits across climates.
- Water stress conditions (25% FC) reduced yield attributes, including flower yield and total biomass.
- Linalool, a fatty acid composition in the essential oil, had the highest average in both species.
- Azotobacter-Psedomonas Bacterial + Biochar proved most effective in total fatty acid content.

INTRODUCTION

Lavandula spp., as a member of the Lamiaceae family, is considered as a distinguished genus of flowering plants commonly related to aromatic herbs such as mint, basil, and thyme. The above-mentioned perennial plant, originally native to the Mediterranean region, has garnered global recognition due to its diverse applications in horticulture, medicine, and gastronomy (Pokajewicz *et al*. 2021; Özsevinç and Alkan 2023). *Lavandula angustifolia* (English lavender) and *L. stoechas* (French lavender) are regarded as two notably cultivated and commercially significant varieties of lavender species (Crișan *et al*. 2023). *L. angustifolia*, which is native to the Mediterranean region, is extensively grown for its aromatic flowers and essential oil. In addition, *L. stoechas*, known as Spanish lavender or butterfly lavender, is found in specific regions of Southern Europe and North Africa. "French lavender", which is cultivated in French gardens, is widely used in French perfumes and soaps, despite its nomenclature. It is worth noting that French lavender differs from the lavender species native to France such as *L. angustifolia* (Du and Rennenberg 2018; Peçanha *et al*. 2023). Studies conducted in various climatic conditions can provide valuable insights due to the significance of this medicinal plant and distinct characteristics of its two main species in terms of essential oil quality.

Water scarcity significantly impacts the growth and yield of agricultural, horticultural, and medicinal plants, particularly in arid and semi-arid regions (Nouri *et al*. 2023). It directly affects processes like leaf reduction, stomatal closure, diminished stomatal conductance, and decreased chloroplast and protoplasmic components, leading to reduced photosynthetic efficiency (Wahab *et al*. 2022; Nouri *et al*. 2023). Water stress also affects biochemical processes related to photosynthesis and indirectly reduces carbon dioxide entry into closed stomata (Afshari *et al*. 2022). Furthermore, it hampers the transport of photosynthetic materials, saturating leaves and limiting photosynthesis, ultimately impairing plant growth and yield (Li *et al*. 2024).

Various strategies are used including employing arbuscular mycorrhizal fungi (AMFs) and plant growthpromoting rhizobacteria (PGPRs) to mitigate the adverse effects of drought stress on plants (Begum *et al*. 2022). Biofertilizers, which contain beneficial microorgan-

isms, are generated for specific objectives such as nitrogen fixation (Soumare *et al*. 2020), phosphate ion release (Kumar *et al*. 2022), mobilization of potassium and iron from their insoluble compounds, and the like (Jiao *et al*., 2024). AMFs, which can increase plant growth hormones such as cytokinins, gibberellins, and auxins, play a crucial role in stimulating plant growth (Liu *et al*. 2023). Such enhancement is achieved through increased water conductivity in the roots, leading to elevated rates of transpiration and evaporation (Liu *et al*. 2023), improved plant nutritional conditions by enhancing water and nutrient absorption, especially for less mobile elements such as phosphorus (Wahab *et al*. 2023), better plant establishment (Mitra *et al*., 2021), and raised water use efficiency (Sun and Shahrajabian 2023). Biological fertilizers, which contain beneficial bacteria and fungi, are applied for specific objectives such as nitrogen fixation, phosphate ion release, and mobilization of iron and potassium from their insoluble compounds (Singh *et al*. 2021). Such bacteria, which are typically established in the root environment, contribute to nutrient absorption by the plant (Singh *et al*. 2021). Some sources indicate the positive impact of biological fertilizers on the growth of medicinal plants such as *Thymus vulgaris* L. (Nadjafi *et al*. 2014), *Catharanthus roseus* L. (Sharma *et al*. 2023), and *Mentha aquatic* L. (Javanmard *et al*. 2022). Furthermore, the synergistic combination of AMFs and PGPRs enhances the quantity and quality of various plants such as *Melissa officinalis* L. (Eshaghi Gorgi *et al*. 2022), tobacco (Begum *et al*. 2022), soybean (Sheteiwy *et al*. 2021), and common myrtle (Azizi *et al*., 2021) under environmentally stressful conditions such as drought.

Biochar (BI), which is considered as a soil organic amendment, improves the physical and chemical traits of soil, increases nutrient availability, diminishes leaching of elements, and enhances agricultural production (Allohverdi *et al*. 2021; Saha *et al*. 2022). BI can potentially enhance plant growth by improving the chemical, physical, and biological traits of soil such as increasing soil moisture availability, leading to higher crop yields (Diatta *et al*. 2020). Adding BI can increase water retention capacity in the soil. In other words, soil amendment with BI can improve crop production by conserving more rainwater in arid regions (Allohverdi *et al*. 2021), as well as reducing the frequency or amount of irrigation water required (Zhang *et al*. 2020).

Lavender, valued for its medicinal essential oil, is grown for pharmaceutical, perfumery, and cosmetic purposes. Research on cultivating and comparing English and French varieties, of interest to farmers and producers, is limited. Additionally, there's a lack of studies on lavender's growth and physiology under different irri-

gation regimes in field cultivation, crucial due to water crises worldwide and the importance of medicinal plant cultivation. Organic fertilizers, known for their environmental friendliness, are gaining traction. They improve plant stress tolerance and alter metabolites and physiological processes positively. This study aims to explore the morphological and biochemical responses of English and French lavender to AMFs, PGPRs, and BI under varied irrigation and climate conditions in Hamedan and Fars.

MATERIALS AND METHODS

Experimental design and study area

This study followed a split-plot factorial scheme implemented in randomized complete block design layouts with three replications in two regions including Firuzabad (Fars) and Hamedan (Hamedan). The present study was conducted during the 2022-23. The maximum, minimum, and average air temperature in Hamedan equaled 36.8, 3.6, and 9.6 °C, respectively. The annual rainfall totaled 317.7 mm, indicating variability across different months. Hamedan with geographical coordinates of 34.80°N and 48.52°E is situated at an altitude of 1741 m above sea level. Firuzabad in Fars, which is located at an altitude of 1600 m above sea level, has an annual average temperature and precipitation equal to 21.78°C and 522 mm, respectively. The location of Fars is specified by coordinates 36.85°N and 54.44°E (Table 1).

Experimental treatments and setup

The initial factor in this experiment involved inducing irrigation regimes at four levels, especially at 95 ± 5 , 75 \pm 5, 50 \pm 5, and 25 \pm 5% of the field capacity (FC). The second factor comprised diverse combinations of Azotobacter-Psedomonas Bacterial (APB), AMFs (*Funneliformis mosseae* and *Funneliformis intraradices*), and BI, applied individually and in combinations, along with a control treatment (no application), resulting in forming a total of 96 experimental plots. The third factor assessed the responses of English (*L. angustifolia*) and French lavender (*L. angustifolia*) which serve as the focal plant species under investigation.

The soil structure and concentration of macro- and micro-nutrients were reviewed in both study areas prior to planting (Table 2). The procedure proposed by Carter and Gregorich (2007) was applied to ascertain the concentration of soil elements and various soil characteristics. All of the essential micro- and macro-nutrients **Table 1.** Geographical and climatic characteristics of investigated experimental sites (Fars and Hamedan) in Iran.

$\begin{tabular}{ll} Experimental\\ \end{tabular} Latitude & Longi-\\ \end{tabular}$			Eleva- Avg. Avg. tion Precipitation Temperature $(m a.s.l.)$ (mm)	$(^{\circ}C)$
Fars	36.85° N 54.44° E 1600		522	21.78
Hamedan		34.80°N 48.52°E 1741	317.7	9.6

Table 2. Soil characteristics in experimental sites (Fars and Hamedan, Iran) - 0-30 cm depth,

were present at levels deemed adequate to support the growth of plants.

The APB and AMFs isolated from the rhizosphere of maize and wheat plants were obtained from the Soil and Water Research Institute in Karaj, Iran. The fungal inoculum and roots, comprising spores, and colonized root fragments were incorporated into the sandy substrate at a rate of 50 g $kg⁻¹$ of soil around the root zone (Agnihotri *et al*., 2021). The root colonization percentage was measured employing the method proposed by Campo *et al*. (2020), indicating that the colonization level in fungal treatments was above 50%. The aforementioned value was below 10% in other treatments, indicating proper inoculation of fungi with plant roots. Concurrently, a bacterial inoculum with an approximate population of 107 colony-forming units (CFU/ml) at the time of planting was employed (Yadav *et al*. 2020). A combination of *Azotobacter* sp. strain 5 and *Pseudomonas fluorescens* strain 168 was used here. Biochar application as a BI fertilizer was achieved from the Fifth Season Company and utilized at a rate of 3 tons per ha (wood BI) following the recommendations provided by the manufacturer. All of the fertilizers were applied simultaneously with planting and inoculated into the soil.

Seedlings for both lavender species were cultivated in seedling trays and transplanted to the field after selecting nearly uniform seedlings (approximately 5 cm in height). Each experimental plot, measuring 6×3 m (18 m^2) , had six planting rows with 50-cm spacing and 40-cm intervals between plants. Plot and block spacing were designated as one and two meters, respectively (Hadipour *et al*. 2013). Planting was conducted in Fars and Hamedan during late March and early April, respectively.

It is noteworthy that the irrigation treatments commenced two weeks after the seedlings were fully established in the field. The irrigation was performed employing a drip irrigation system with regulated water output. For each irrigation cycle, the soil moisture samples were taken from the root development depth, and its content was computed based on dry weight. The soil moisture was measured using a weight-based method, and the relationship between water potential and soil moisture at specific potential points was determined utilizing the soil moisture characteristic curve. The required water amount was calculated applying the Optiwat software and the irrigation process was executed after determining the soil moisture levels and identifying the optimal irrigation timing (Siahbidi and Rrezaizad 2018). The number of irrigations employed in all of the irrigation treatments was 7 times, and no rainfall occurred during the entire growth period. Totally, 7500, 5842, 3910, and 3050 m³ ha⁻¹ of water were used for irrigation treatments at 95, 75, 50, and 25% of FC, respectively.

Assessing the growth and yield characteristics

Totally, three plants were randomly selected to mitigate marginal effects after four months of cultivation following the completion of vegetative and generative growth, along with achieving 100% flowering. Diverse characteristics including plant height, number of leaves per plant, root volume, dry root weight, and root length were documented (Aghighi Shahverdi *et al*. 2020). Then, a half-square meter from each plot was harvested to assess the biomass and yield. Measurements and records were taken for the total leaf biomass, flower yield, and total biomass (or biological yield calculated by summing leaf, flower, and root weight) (Aghighi Shahverdi *et al*. 2018).

Evaluating the essential oil content and components

Totally, 100 g samples (leaf and flower) were collected from each replicate of each treatment. The essential oil was extracted from the whole plant using a Clevenger apparatus (steam distillation method). The yield of the essential oil was determined utilizing biomass calculation.

Essential oil components were identified via Gas Chromatography-Mass Spectrometry (GC-MS) using a ThermoQuest-Finnigan TRACE MS model equipped with a 30-meter HP-5MS column (0.25 mL inner diameter, 0.25 µm layer thickness). The temperature regimen included an initial oven temperature of 60 °C for five min, a thermal gradient of 3 °C per min to 250 °C, held for five min. Injection column temperature was 290 °C, and helium gas flowed at 1.1 mm per min. Mass spectrometer utilized electron ionization (EI) at 220 °C with 70 electron volts. Spectra were compared with standards and mass spectra of standard compounds, further confirmed via computerized libraries (Pokajewicz *et al*. 2021). Essential oil components were measured in one trial by combining samples from three replicates (Fig. 1). Rank indices were assigned based on the highest and lowest mean for each compound in each treatment. Sum of ranks for each treatment level determined the rank index.

Statistical analysis

The Statistical Analysis Software, 9.2 (SAS software) was utilized following the assessment of data distribution normality applying Kolmogorov-Smirnov and Shapiro-Wilk tests to examine all of the gathered data from Fars and Hamedan. A three-factor factorial ANOVA was independently conducted for each experimental location employing a split-plot design to investigate the variance components related to the impacts of irrigation regimes, fertilizer sources, species, and their interactions. Treatment differences were studied using Duncan's Multiple Range Test (DMRT) when the ANOVA F-test showed a significance level of 0.05. Pearson correlation between characteristics and Stepwise regression (where essential oil content was considered as a dependent variable and other growth and yield traits as independent variables) was performed utilizing Microsoft Excel 2013 and Minitab 18 software.

RESULTS

Effect of experimental treatments on plant height

Severe water deficit stress decreased the plant height by 37.9 and 40.5% in Fars and Hamedan compared to

Figure 1. GC-Mass chromatogram of the essential oil of English species (A) and French species (B) of lavender.

well-watered conditions. Additionally, using various fertilizer sources increased the average plant height. Utilizing AMFs+BI and all of the fertilizer sources (APB+AMFs+BI) demonstrated the tallest plant height with average heights of 36.06 and 30.5 cm for Fars and Hamedan, respectively. The average plant height in the French species exceeded that of the English ones in both climatic conditions, showing 17.9 and 37.2% higher averages in Fars and Hamedan, respectively (Table 3).

Effect of experimental treatments on number of leaves

As indicated in Table 3, the number of leaves per plant is significantly influenced by irrigation regimes and fertilizer sources in both experimental regions. An increase in the severity of water deficit stress decreased the number of leaves per plant significantly. The reduction was 50 and 48.9% in Fars and Hamedan compared to well-watered conditions. Applying various fertilizer sources increased the number of leaves per plant compared to the no-fertilizer treatment. Employing APB+BI in Fars and APB+AMFs in Hamedan led to the highest number of leaves per plant (22.76 and 29.23, respectively). No significant difference was reported in the number of leaves per plant between the two lavender species in Fars. However, the French species exhibited a 38.54% higher number of leaves in Hamedan compared to the English ones.

Effect of experimental treatments on total leaves biomass

A significant decrease in total leaf biomass was observed with increasing severity of water deficit stress similar to other growth and morphological traits (plant height and leaf number). At the 25% FC level, the total leaf biomass reduced in Fars and Hamedan by 29.8 and 43.0% compared to well-watered conditions. Using AMFs+BI in Fars and APB+AMFs+BI in Hamedan led to the highest total leaf biomass, reaching 2588.1 and 2401.2 kg ha⁻¹, respectively. The lowest mean for the aforementioned trait was related to the non-application of fertilizer in both regions (1526.5 and 1284.6 kg ha-1, respectively). The French species demonstrated a significantly higher total leaf biomass compared to the English ones with a 9.33 and 14.7% average superiority in Fars and Hamedan, respectively (Table 3).

Effect of experimental treatments on flower yield

Based on the results, the irrigation regimes, fertilizer sources, plant species, and their interactions affected the flower yield considerably at a 1% significance level. Severe water deficit stress reduced the flower yield 2.17 and 1.93 times in Fars and Hamedan compared to wellirrigated conditions. The flower yield in both regions was higher for the French species than the English ones, reaching more than 2.5 times in Hamedan. Utilizing all of the fertilizer sources in the French species under non-stress conditions in Fars showed the highest flower yield (1402 kg ha-1). In addition, applying ABP+BI in the French species under non-stress conditions led to the highest flower yield with an average of 1991.3 kg ha⁻¹. The lowest flower yield was related to the non-application of fertilizer, as well as the use of ABP AMFs in both plant species under severe water deficit stress conditions in Fars. The absence of fertilizer application in the English species under severe water deficit stress conditions in Hamedan showed the lowest flower yield with an average of 252.1 kg ha⁻¹ (Fig. 2A).

Effect of experimental treatments on total biomass yield

Severe water deficit stress reduced the total biomass yield in Fars and Hamedan by 36.62 and 44.4% com-

Figure 2. The interaction effect of irrigation regimes and fertilizer treatments on the total flower yield (A) and total biomass yield (B) of two Lavandula species (English and French) was investigated in two different climates, Fars and Hamadan, Iran. The averages presented in each column, sharing similar letters, do not have a statistically significant difference based on the Duncan's multiple range test at the 1% significance level. The two experimental sites were analyzed separately. Non-application (CK); Azotobacter-Psedomonas Bacterial (APB); Arbuscular mycorrhiza fungi (AMF); Biochar (BI).

pared to well-irrigated conditions. Employing various fertilizer sources increased the average total biomass yield. The French species consistently exhibited a higher average total biomass yield compared to the English ones in both experimental sites (Table 3). As illustrated in Fig. 2B, the highest total biomass yield in Fars is related to using BI in the French species under 75% FC irrigation (5686 kg ha-1). Utilizing ABP+BI and AMFs+BI in the French species under non-stress conditions in Hamedan led to the highest total biomass yield (8770.6 and 7752.5 kg ha-1, respectively). The non-application of fertilizer in both experimental sites led to the lowest total biomass yield in the French species under severe water deficit stress conditions (1196.6 and 1175.9 kg ha⁻¹, respectively).

Effect of experimental treatments on root traits

The results indicated that the 75% FC irrigation treatment reduced the root-related traits significantly

compared to the control with a substantial decrease in root volume (36.93 and 31.7%), root dry weight (60.5 and 39.7%), and root length (50.3 and 30.5%) in Fars and Hamedan, respectively. All of the fertilizer sources increased the average of all of the measured parameters in both experimental sites compared to the absence of fertilizer application. Generally, applying fertilizer sources demonstrated superior root traits performance compared to their individual use. ABP+BI and AMFs+BI were considered as the most appropriate fertilizer sources based on the overall three root traits in both experimental sites. English species exhibited higher averages in most cases, except for root volume in Fars (Table 4).

The highest root dry weight was related to employing all of the fertilizer sources in the English species under 50% FC irrigation treatment with an average of 32.88 g plant⁻¹ in Fars. Meanwhile, using ABP+BI in the English species under 75% FC treatment in Hamedan showed the highest average for the above-mentioned trait (41 g plant⁻¹). The non-application of fertilizer under severe

Figure 3. The interaction effect of irrigation regimes and fertilizer treatments on the root dry weight (A) and root length (B) of two Lavandula species (English and French) was investigated in two different climates, Fars and Hamadan, Iran. The averages presented in each column, sharing similar letters, do not have a statistically significant difference based on the Duncan's multiple range test at the 1% significance level. The two experimental sites were analyzed separately. Non-application (CK); Azotobacter-Psedomonas Bacterial (APB); Arbuscular mycorrhiza fungi (AMF); Biochar (BI).

water deficit stress conditions in the French species led to the lowest average root dry weight $(4.13 \text{ and } 5.76 \text{ g plant}^{-1})$ for Fars and Hamedan, respectively) (Fig. 3A).

Using all of the fertilizer sources in English species under 50% FC irrigation treatment demonstrated the highest root length in Fars and Hamedan with lengths of 43.78 and 28.38 cm, respectively. The lowest root length in both experimental sites was related to the non-application of fertilizer in the French species under severe water deficit stress treatment with average lengths of 6.15 and 3.56 cm, respectively (Fig. 3B).

Effect of experimental treatments on essential oil content and yield

The results represented that the irrigation regime and fertilizer treatments influenced the essential oil content and yield significantly. Partial water deficit stress (75 and 50% FC) increased the essential oil content compared to the control irrigation in both experimental regions. Severe water deficit stress led to an increase in essential oil content in Fars and a decrease in Hamedan compared to the control irrigation treatment. However, the essential oil yield in both experimental regions significantly decreased under severe water deficit stress (1.44- and 3.33-times reduction, respectively). Some fertilizer treatments decreased the essential oil content significantly compared to the non-fertilized treatment, while most of the fertilizer treatments increased the average content.

Applying AMFs and BI in Fars, as well as APB+AMFs and APB+BI in Hamedan led to the highest average essential oil yield $(62.71, 38.18,$ and 38.82 g ha⁻¹, respectively). The lowest essential oil yield was related to the non-fertilized treatment in both experimental sites $(30.6 \text{ and } 19.41 \text{ g ha}^{-1})$. The English species exhibited a higher average than the French ones in both experimental regions with this difference being statistically non-

Figure 4. The interaction effect of irrigation regimes and fertilizer treatments on the essential oil content of two Lavandula species (English and French) was investigated in two different climates, Fars and Hamadan, Iran. The averages presented in each column, sharing similar letters, do not have a statistically significant difference based on the Duncan's multiple range test at the 1% significance level. The two experimental sites were analyzed separately. Non-application (CK); Azotobacter-Psedomonas Bacterial (APB); Arbuscular mycorrhiza fungi (AMF); Biochar (BI).

significant in Hamedan. The essential oil yield varied based on the climatic conditions of the experiment site in two lavender species. The highest essential oil yield in Fars $(48.97 \text{ g ha}^{-1})$ was related to the English species, while the French species exhibited the highest yield in Hamedan (35.09 g ha⁻¹) (Table 4).

The essential oil content in employing APB+BI in the English species under 75% FC irrigation treatment showed the highest average (4.23%) in Fars. Meanwhile, the highest average of this trait in Hamedan was related to using APB+BI under 50% FC irrigation treatment in the English species with an average of 1.56%. All of the fertilizer levels in both lavender species under 25% FC irrigation treatment demonstrated the lowest essential oil content in Hamedan. Utilizing APB, fertilizer sources in the French species under control irrigation treatment, and APB+AMFs in the French species under 50% FC irrigation treatment exhibited the lowest essential oil content (0.57, 0.59, and 0.54%, respectively) (Fig. 4).

Effect of experimental treatments on fatty acids composition

Analyzing GC-MS on the essential oil indicated that eight compounds were identified in all of the treatment levels, plant species, and experimental sites. The aforementioned compounds included α-Pinene, B-Pinene, Linalool, Camphor, Borneol, Terpinen, Linalyl acetate, and Caryophyllene Oxide. As demonstrated in Fig. 5, Linalool shows the highest average in both experimental sites and plant species (22.46 to 30.06%). Linalyl acetate and Caryophyllene Oxide exhibited the highest averages in Fars after the above-mentioned compound, while Borneol and Terpinen showed the highest averages in Hamedan. No significant difference was reported in the measured compounds in Hamedan. However, significant differences were observed in Fars in the levels of Linalool, Terpinen, and Caryophyllene Oxid with the English species excelling in the first and the French ones in the second and third compounds.

Table 5 shows the results related to the effect of irrigation treatments and fertilizer sources in Fars on the composition of essential oil fatty acids. As represented, severe water stress (except for Terpinen) exhibits the lowest average in most of the identified compounds, meaning a reduction in the average compounds. Meanwhile, irrigation levels of 75 and 50% FC allocated the highest averages in most of the compounds. The control irrigation treatment showed superiority in terms of the Borneol compound. The 75% FC treatment demonstrated the highest average (91.83%), while the lowest average was related to severe water stress (76.91%) in terms of the total compounds. Similar results were obtained with the highest and lowest indices reported in the 75 and 25% FC treatment, respectively. Different fertilizer sources affected the amount of compounds differently. Utilizing BI led to the highest averages in α-Pinene, Camphor, and Terpinen (2.93, 6.88, and 8.94%, respectively). Applying APB+BI led to the highest averages for Linalool and Linalyl acetate (29.8 and 26.88%, respectively). In addition, employing all of the fertilizer sources showed the highest averages for B-Pinene and Caryophyllene Oxide (5.75 and 13%). The fertilizer sources APB+BI and APB+AMFs+BI exhibited the highest averages (89.14 and 88.97%, respectively),

Figure 5. The variations in the essential oil fatty acid content of two lavender species (French and English) in the climates of Fars and Hamedan, Iran.

while the lowest total compounds were observed in the non-application and AMFs application treatments (76.87 and 76.77%, respectively). Non-application of fertilizer and combined use of the fertilizer sources demonstrated the lowest and highest-ranking indices, respectively.

As presented in Table 6, the highest average of α-Pinene and Linalool is reported in the 95% FC irrigation treatment in Hamedan. In addition, the highest averages of Camphor, Borneol, Terpinen, and Linalyl acetate are observed in the 75 and 50% FC irrigation levels, while the highest averages of β-Pinene and Caryophyllene Oxide are reported in the 25% FC irrigation level. Irrigation at 50 and 25% FC exhibited the highest averages, while irrigation at 75% FC showed the lowest average. Such classification was observed in the ranking index. Using the sources led to the highest averages of β-Pinene (8.1%), Linalool (33.23%), Borneol (19.91%), and Caryophyllene Oxide (2.85%). Further, the highest averages of α-Pinene and Linalyl acetate were reported in the treatment without fertilizer (2.44 and 8.47%, respectively). Utilizing AMFs and APB+BI showed the highest total compound averages (90.39 and 89.79%, respectively). However, the lowest total compound average was related to applying all of the fertilizer sources (87.11%). The highest index was related to APB, and the lowest one was observed in employing all of the fertilizer sources.

Correlation analysis

Table 7 and 8 indicate the results related to simple correlations between measured traits for Fars and

Hamedan, respectively. No significant correlation was observed between the physiological, biochemical, growth, morphological, and yield parameters with essential oil content in Fars. However, a positive correlation was reported between the percentage of essential oil with traits such as RWC, chlorophyll b content, root dry weight, and root length in Hamedan. Additionally, a negative and significant correlation was observed between the aforementioned traits with proline content and activities of antioxidant enzymes such as CAT, POX, SOD, and APX. Further, no significant correlation was reported between Linalool, as the most indicative fatty acid compound, with the growth, yield, physiological, biochemical, or morphological traits in Hamedan. However, a positive correlation was observed between the above-mentioned traits with root dry weight and length at a 5% significance level in Fars.

DISCUSSION

This study seeks to review the efficiency of different fertilizer sources under various irrigation regimes. The results indicated that severe water scarcity (25% FC) decreased the average plant height, leaf number, leaf biomass, flower yield, and total biomass compared to the control irrigation treatment. In addition, employing different fertilizer sources increased the aforementioned traits. Water scarcity reduces the growth parameters such as leaf area and number, as well as alterations in physiological parameters (Afshari *et al*. 2022), resulting in

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		$\overline{2}$	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		\ast	\ast	$**$	\ast	\ast	\ast	\ast	ns	ns	ns	ns.	ns	ns	ns	ns	ns	ns
2	0.5		\ast	$**$	\ast	∗	\ast	\ast	ns	∗	ns	ns	ns	ns	ns	ns	ns	ns
3	0.37	0.35		*	$**$	ns	ns	ns	ns	$**$	ns	ns.	ns	ns	ns	ns	ns	ns
4	0.52	0.7	0.5		$**$	$**$	\ast	\ast	ns	∗	ns	ns	ns	ns	ns	_{ns}	_{ns}	ns
5	0.46	0.5	0.96	0.72		\ast	ns	ns	ns	$**$	ns	ns	ns	ns	ns	ns	ns	ns
6	0.43	0.5	0.29	0.53	0.4		$**$	$**$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
7	0.35	0.31	0.22	0.32	0.28	0.68		$**$	ns	\ast	*	$**$	*	$*$	ns	*	ns	ns
8	0.35	0.31	0.22	0.32	0.28	0.68	0.99		ns	\ast	*	$**$	*	⊁	ns	∗	ns	ns
9	-0.2	0.09	-0.07	0.07	-0.03	0.01	0.2	0.2		$***$	ns	ns	ns	ns	ns	ns	ns	ns
10	0.09	0.37	0.52	0.43	0.56	0.26	0.34	0.34	0.76		ns	ns	ns	ns	ns	ns	ns	ns
11	-0.23	0.01	0.06	-0.03	0.04	0.21	0.47	0.47	0.05	0.07		$**$	$**$	$**$	ns	$***$	ns	ns
12	-0.08	0.09	0.13	-0.01	0.1	0.29	0.54	0.54	0.06	0.13	0.64		\ast	$**$	ns	$**$	ns.	ns
13	-0.22	0.06	-0.04	-0.06	-0.05	0.09	0.36	0.36	0.17	0.15	0.6	0.44		\ast	ns	$**$	ns	\ast
14	-0.19	-0.02	0.06	-0.13	0.01	0.22	0.37	0.37	0.05	0.07	0.71	0.65	0.41		ns	$**$	ns	$**$
15	0.15	-0.03	0.13	0.09	0.13	0.08	0.19	0.19	-0.16	-0.04	0.26	0.15	0.1	0.03		ns	ns	ns
16	0.25	-0.08	-0.04	0.09	-0.01	-0.16	-0.46	-0.46	-0.26	-0.29	-0.57	-0.67	-0.67	-0.61	0.06		ns	\ast
17	0.24	0.21	-0.03	0.26	0.06	0.23	0.28	0.28	0.14	0.13	0.01	-0.09	-0.06	-0.2	0.05	0.08		ns
18	0.14	0.18	0.15	0.22	0.19	0.04	-0.07	-0.07	-0.07	0.04	-0.28	-0.27	-0.31	-0.57	0.11	0.44	0.24	

Table 7. The simple correlation coefficients between growth and yield attributes as well as essential oil content and components of two lavender species (English and French) under effect of irrigation regimes and different fertilizers sources in Fars, Iran.

Table 8. The simple correlation coefficients between growth and yield attributes as well as essential oil content and components of two lavender species (English and French) under effect of irrigation regimes and different fertilizers sources in Hamedan, Iran.

ns: non-significant; * and **: significant at 5 and 1% probably level, respectively.

1. Plant height, 2. Number of leaves, 3. Total leaves biomass, 7. Total flower yield, 8. Total biomass yield, 9. Root volume, 7. Root dry weight, 8. Root length, 9. EOC, 10. EOY, 11. α-Pinene, 12. B-Pinene, 13. Linalool, 14. Camphor, 15. Borneol, 16. Tripinen, 17. Linalyl acetate, 18. Caryophyllene Oxide.

declining the yield-related traits such as flower and biomass yield. Further, water scarcity diminishes solar energy absorption and nutrient uptake by reducing leaf number and area, as well as shortening the plant growth period, resulting in affecting the quantitative and qualitative aspects of plant yield negatively (Benadjaoud *et al*. 2022). For example, Chrysargyris *et al* (2016) argued that water stress declined the growth and chemical composition of lavender (*L. angustifolia*) compared to plants subjected to regular irrigation. In addition, Zollinger *et al* (2006) observed a reduction in leaf number and total dry weight under water stress conditions in *L. stoechas*, as another lavender species. The impact of water deficit on growth inhibition has been extensively documented in various plant species including *Stellaria dichotoma* L. (Zhang *et al*. 2017), *Stevia rebaudiana* (Afshari *et al*. 2022), and *Foeniculum vulgare* Mill (Peymaei *et al*. 2024). Some others (e.g. García-Caparrós *et al*. 2019; Salata *et al*. 2020) claimed that the exposure of medicinal plants including *L. latifolia*, *M. piperita*, and *T. capitatus* to drought stress decreased their aerial fresh weight and yield.

The plant responds by increasing the activity of antioxidant enzymes, as well as the synthesis of osmolytes under severe water deficit conditions, which leads to a higher consumption of photosynthetic materials by the plant to cope with drought stress, resulting in decreasing the growth and yield parameters (Mahajan and Pal 2023).

The distribution of biomass in plants, specifically root and shoot dry weights, was significantly affected under water stress conditions. Water stress altered the allocation of biomass to roots, resulting in impacting photosynthetic efficiency, nutrient absorption, and overall growth parameters (Begum *et al*. 2022). The alterations in biomass composition and structure were related to varying water availability, resulting in influencing plant adaptation strategies and productivity. Severe water stress induced modifications in water use efficiency, root-shoot ratios, and physiological processes, indicating the ability of plant to adapt to limited water conditions (Rodríguez-Pérez *et al*. 2017; Afshari *et al*. 2022; Peymaei *et al*. 2024).

The results represented that using fertilizers including APB, AMFs, and BI increased the average yield traits of the plant under well-irrigated and low irrigation conditions. Apparently, utilizing BOFs enhanced the growth and yield parameters by reducing soil acidity and providing appropriate conditions for absorbing essential nutrients, especially nitrogen, phosphorus, and trace elements such as iron, zinc, and copper (El-Attar *et al*. 2023). Sharma *et al* (2023) reported that the highest mean values for plant height, number of branches, pod length and weight, seed number, and total plant yield of

Utilizing APB and BI in both regions under irrigation treatments of 75 and 50% FC showed the highest essential oil content, indicating that partial water stress increases the essential oil content compared to normal irrigation conditions. However, severe water stress or drought reduced the essential oil content significantly. The essential oil content reduces under severe water stress due to the decrease in the activity of enzymes and biochemical pathways related to the synthesis of essential oils in plants, which diminishes by water stress (Mohammadi *et al*. 2018). Additionally, severe water deficit declines the quality and quantity of the essential oil by weakening the plant to absorb and transport water and essential nutrients (Asghari *et al*. 2020). According to other researchers, moderate water stress in *L. angustifolia* increases the essential oil content compared to the control treatment (Sałata *et al*. 2020). The stress induced by drought decreases the production of secondary metabolites significantly, resulting in impacting the accumulation of essential oils in plants (Kleinwächter *et al*., 2015). Comparable results were reported in cultivating *L. latifolia* (García-Caparrós *et al*. 2019), and *Thymus vulgaris* (Kleinwächter *et al*. 2015).

Applying APB + BI increased the essential oil content in both species of lavender. Employing the aforementioned fertilizers elevates the synthesis of secondary metabolites by enhancing plant growth and yield (Ullah *et al*. 2020), improving nutrient uptake (Phares *et al*., 2022), and increasing resistance to environmental stresses (Lalay *et al*. 2022). In addition, enhancing the nutrient balance using the above-mentioned fertilizers empowers the plant to absorb elements, resulting in augmenting enzyme activity and biochemical pathways involved in essential oil synthesis (Kari *et al*. 2021). No report was found on employing APB + BI regarding the essential oils of medicinal plants. However, several studies have been conducted on using the aforementioned substances in enhancing the quantitative and qualitative yield of maize plants under low-water irrigation stress (Ullah *et al*. 2020) and increasing the microbial population in acidic soils (Kari *et al*. 2021). Utilizing organic fertilizer with nitrogen-fixing bacteria (Azetobacter) in a similar experiment was regarded as the most effective fertilizer combination in enhancing the quality of lavender essential oil under conditions of moderate salinity stress (Khatami *et al*. 2023).

Applying Bi and APB improved the root characteristics significantly, resulting in increasing nutrient absorption and enhancements in quantitative and qualitative parameters. Ethylene, as a well-known stress hormone, is recognized for its inhibitory effect on root growth

(Ullah *et al*. 2020). APB promotes root growth through the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which aids in ethylene reduction within the root zone (Bechtaoui *et al*. 2021). The bacteria involved in the above-mentioned process possess ACC deaminase, resulting in enabling the conversion of ACC, as an ethylene precursor, into ammonia and α-ketobutyrate. The aforementioned enzymatic activity reduces ethylene-induced stress, resulting in fostering root growth (Ahmad *et al*. 2024). The synergistic effect of rhizobacteria and BI application was further pronounced because BI served as a nutrient source for bacteria and enhanced the physicochemical characteristics of soil. Therefore, bacterial growth was amplified, resulting in augmenting the root parameters (Ullah *et al*. 2020).

The results represented that employing BI, especially in combination with APB and AMFs, increased physiological parameters, resulting in enhancing the quantitative and qualitative yield in both lavender species. Thus, BI increases the growth parameters, as well as the quantitative and qualitative yield by improving the physical, chemical, and biological characteristics of the soil such as moisture availability (Allohverdi *et al*. 2021). Fascella *et al* (2020) asserted that the highest plant height, leaf area, leaf, and flower yield in lavender were recorded in substrates with 25-75% BI content. In addition, the increase in BI content improved the several chemical and physical characteristics such as electrical conductivity, pH, apparent density, and total porosity, as well as reducing the water holding capacity and increasing the availability of nutrients such as phosphorus, magnesium, and calcium (Fascella *et al*. 2020).

BOFs can elicit various responses in various crops under different environmental conditions (Januškaitienė *et al*. 2022). This phenomenon was observed in the present experiment. The flower and total biomass yield were observed in Fars by using APB, AFMs, and BI, while utilizing APB and AFMs demonstrated the highest average for such traits in Hamedan. Even the fertilizer treatments showed differences in both regions while combined with other factors such as irrigation and plant species. For instance, the total biomass yield in the English species under the treatment with BI alone under 75% FC irrigation exhibited the highest average in Fars. However, applying fertilizers such as APB+BI under control irrigation in the French species showed the highest average in Hamedan. Such variations can be ascribed to discrepancies in climatic factors including geographical dimensions, elevation, mean temperature, and precipitation. The climatic data underscores the lower average temperatures and precipitation levels in Hamedan unlike Fars. The less appropriate conditions

concerning the above-mentioned parameters necessitate an augmentation in metabolite production to counteract the imposed stress conditions. In addition, the overall inhospitable conditions influence the growth and performance by modifying physiological and biochemical parameters compared to Fars (Aghighi Shahverdi *et al*. 2018; Farrokhi *et al*. 2021). Farrokhi *et al* (2021) found that temperature variations and precipitation levels are among the key climatic factors, which can influence the quantitative and qualitative performance of plants. The microbial activity of BOFs is highly influenced by temperature and soil moisture levels. Similarly, the presence of appropriate environmental conditions in terms of precipitation and elevated temperatures can enhance their activity, resulting in increasing efficiency in fertilizer utilization (Cui *et al*. 2016; Kumar *et al*. 2022). Therefore, employing fewer fertilizer sources can be highly effective under appropriate environmental conditions. However, multiple fertilizer sources should be used to meet the nutritional requirements of plant in inappropriate environmental conditions (Kumar *et al*. 2022). Thus, utilizing all of the fertilizer sources showed the highest average performance in flower-related traits and total biomass under the climatic conditions in Hamedan. The efficiency of fertilizer use is significantly impacted by the type and frequency of fertilizer application, with organic fertilizer and single applications generally exhibiting low values (Zhu *et al*. 2023). According to Yu *et al* (2022), the rise in the growing season temperature for rice and wheat increased nitrogen use efficiency significantly.

The physical and chemical condition of the fields is among the reasons for the difference in fertilizer compositions between Fars and Hamedan. As presented in Table 2, the levels of nitrogen, potassium, iron, and magnesium in Fars are higher than those in Hamedan. Therefore, higher concentrations of resources should be applied compared to Fars to meet the intended plant nutrient requirements in Hamedan. The results indicate the influence of soil physicochemical characteristics on the quantity and efficiency of fertilizer utilization (Yu *et al*. 2022).

Based on the results, linalool was considered as the predominant compound in both species at both experimental sites. Others (e.g. Crișan *et al*. 2023; Khatami *et al*. 2023) reported similar results regarding the lavender plant. No significant difference was reported between the English and French lavender species in terms of fatty acid content in Hamedan. However, some variations were observed in compounds such as Linalool, Terpinen, and Caryophyllene Oxide in Hamedan. The English species exhibited higher levels of the first compound, while the French ones excelled in the second and third compounds. Differences in the GC-MS composition of

the essential oil of English and French lavender varieties may be attributed to various factors. Environmental factors such as diverse climatic conditions in Hamadan and Fars, distinct soil characteristics in these areas, and genetic variations between the two varieties could contribute to differences in the essential oil composition (Détár *et al*. 2020). Environmental factors such as precipitation, temperature, sunlight, and soil characteristics can influence physiological processes in plants, resulting in altering the essential oil composition (Özsevinç and Alkan 2023). Additionally, genetic differences between the species play a crucial role in the aforementioned variations. Further studies should be conducted in the fields of plant physiology, genetics, and ecology for a more comprehensive understanding of the above-mentioned differences (Détár *et al*. 2020).

The results indicated that severe water deficit stress (25% FC) reduced the average content of most fatty acid compounds in the essential oil of both species, while moderate stress levels (75 and 50% FC) increased the average compound content compared to the wellwatered treatment. The levels of borneol, camphor, linalyl acetate, gamma-cadinene, caryophyllene oxide, and muurolol identified in the essential oil of Lavandula under water stress conditions increased compared to the control, which differed from the obtained results (Sałata *et al*. 2020). The variation in irrigation treatment levels in the aforementioned study and this experiment appears to be the main reason for the above-mentioned difference since two irrigation treatment levels were employed in the aforementioned study, while four levels were implemented here, and the highest stress level reduced the average content. The results indicated that utilizing APB+BI and APB+AMFs+BI in Fars led to the highest total fatty acids and rank index. However, the non-application of fertilizer led to the lowest total fatty acids and rank index. In addition, applying three fertilizer sources in Hamedan led to the lowest rank index and total fatty acids. Employing the combined fertilizer APB+BI in both experimental regions affected the fatty acid content positively. Finally, the composition of essential oils in aromatic-medicinal plants may vary based on the genotype, ecological conditions, and fertilizer treatments (Giannoulis *et al*. 2020).

CONCLUSION

The following conclusions emerge from the conducted experiment in line with the predefined objectives: Severe water stress (25% FC) resulted in diminished growth and yield characteristics, consequently reducing

the quantity and quality of essential oil in both French and English lavender varieties. Application of various fertilizers, especially in combination (AMFs, APB, BI), demonstrated a notable enhancement in growth, yield, and essential oil content compared to non-fertilized plants. Essential oil content, fatty acid quality, and yield exhibited variations among lavender species and experimental sites. Notably, Fars exhibited the highest total fatty acids when treated with combined fertilizers, while Hamedan showcased the lowest. The combination of APB + BI emerged as the most effective fertilizer blend. French lavender displayed a higher average yield, whereas English lavender exhibited superior essential oil content, particularly in the Fars region. Parameters associated with root development exhibited a positive correlation with essential oil content. The composition of essential oils encompassed α-Pinene, β-Pinene, Linalool, Camphor, Borneol, Terpinen, Linalyl acetate, and Caryophyllene Oxide, with Linalool being particularly prominent across both species and sites. Climatic factors and soil characteristics significantly influenced the efficacy of fertilizers and the responses of Lavandula species, particularly in the climates of Fars and Hamedan. Future investigations should delve into these influences concerning qualitative traits in both species.

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