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Chemical compositions and yield of essential oil from sage (*Salvia officinalis* L.) under organic inputs, L-phenylalanine, and varying moisture levels

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Abstract:
The effects of the foliar application of L-phenylalanine, organic inputs, and different soil moisture conditions on the phytochemistry of the essential oil of sage (*Salvia officinalis* L.) were investigated. This field study was done according to a split-plot in time and place design in West of Iran in two experimental years. According to essential oils analysis by GC-FID and GC/MS, the major compounds of the sage essential oil were oxygenated monoterpenes, including camphor (32-39%), 1,8-cineole (25-31%), α-thujone (19-23%), and β-thujone (3-4%). The highest values of these oxygenated monoterpenes were obtained from the optimum soil moisture and foliar spraying with 50% L-phenylalanine under an organic farming system, which significantly influenced the quantity and quality of the biologically active compounds of sage. In conclusion, the foliar spray of L-phenylalanine under organic inputs, and their interaction could ameliorate the dry matter yield and essential oil compounds of sage in environmentally friendly agriculture conditions.

Keywords: Amino acid; Camphor; 1,8-Cineole; Essential oil; Environmentally friendly; GC/MS; Sage; α-Thujone

1. Introduction

Medicinal herbs are an invaluable source of many chemical compounds that are used as medicinal products. The secondary metabolites of the plants are utilized in various industries such as pharmaceutical, medicine, food, cosmetic, and health care. The value of global markets for herbal medicines, such as medicinal plants and their products, has always been growing significantly. Due to the fact that most of the world market for medicinal plants is related to the output and supply of secondary metabolites derived from these plants, biologically active compounds from herbs have very high economic values (Shabani et al., 2015; Wang et al., 2021; Farhadi et al., 2025). Sage (*Salvia officinalis* L.) belonging to the Lamiaceae family is the an important herb. Essential oil or volatile oil extracted from the sage leaves has valuable biologically active compounds that these compositions have various biological effects such as antiseptic, antimicrobial, antioxidant, and antiparasitic properties (Vosoughi et al., 2018; Rahmani-Samani et al., 2019; Rahmani-Samani et al., 2021).

Evaluation of the growth, yield, and secondary metabolites of the medicinal plants under water shortage conditions can be a guide for the cultivation of resistant medicinal plants in semiarid and arid regions (Danesh-Shahraki et al., 2023). The medicinal herbs may be more economically productive by producing more phytochemicals under water deficiency conditions compared to other horticultural crops (Ilkaee et al., 2011; Raza et al., 2013; Khalili et al., 2020; Shaykh-Samani et al., 2023; Rezaei-Adl et al., 2025; Asghari-Lalami et al., 2025).

Organic and biological sources can be acceptable option rather chemical fertilizers due to their efficiencies and reduction of environmental impacts. Among environmentally

friendly methods for plants growth and production, the use of arbuscular mycorrhizal fungi (AMF) is another superlatively appropriate solution, specifically in organic farming systems. In fact, the AMF ameliorates plant growth nutrients and water uptake; thereby further expanding the root system and optimizing protection against biotic and abiotic tensions (Begum et al., 2021; Kumar et al., 2021; Kaviani-Darani et al., 2025). The application of animal manures may promote the growth and yield of the plants by enhancing the soil organic matter and then increasing the solubility and absorption of low-consumption and high-consumption elements in the plant root system, especially in drought conditions (Hamedi et al., 2022a, 2022b). Some studies presented that the usage of cow dung manure increased essential oil content and yield of Bakhtiari savory (*Satureja bachtiarica* Bunge.) (Mirjalili et al., 2022), damask rose (*Rosa damascena* Mill.) (Hamedi et al., 2022a), and lavender (*Lavandula angustifolia* Mill.) (Mavandi et al., 2021).
Phenylalanine compounds as an amino acid are organic molecules that can enhance physiological activity, growth and developments as well as secondary metabolites. L-Phenylalanine is one of the most significant superlative amino acids in antioxidants activity and also in the production of aromatic compounds. In addition, it has a special role in promoting the production of active ingredients in medicinal plants (Li et al., 2020). The results of a previous investigation by Aghaei et al. (2019) on hyssop reported that the usage of biological and organic inputs (AMF, vermicompost, and animal manure) along with the foliar application of amino acids play a useful and effective role in ameliorating the essential oil quality. Furthermore, Rahmani-Samani et al. (2021) confirmed that the spraying of L-phenylalanine with *Mycorrhizal symbiosis* increased the yields of specific metabolites of medicinal penchant from the sage leaves.
The aims of this investigation were to figure out of the foliar application of L-phenylalanine in two soil moisture conditions under effect of biological and organic inputs (cow dung manure and *Mycorrhizal symbiosis*), and their interplay impacts on the yield, quantity and quality of the essential oil of sage (*S. officinalis* L.) in a semi-arid climate.

2. Experimental

2.1 Chemicals and reagents

L-phenylalanine, sodium sulphate, and *n*-alkanes were purchased from Merck Co. (Darmstadt, Germany).

2.2 Plant material

The aerial parts particularly the leaves of sage (*Salvia officinalis* L.) at the early flowering were collected from the field experiment in Kangavar district (altitude 1450 m above sea level, longitude 47°59′ E, latitude 34°30′ N), Kermanshah province, West of Iran. Plant identify was confirmed by H.A. Shirmardi, Ph.D. (Research Center for Agriculture and Natural Resources, Iran). A voucher specimen of *S. officinalis* was preserved in the herbarium of the Research Center of Medicinal Plants, I.A.U., Shahrekord Branch, Iran. After washing, the specimens were air-dried at 25 °C with 25-28% moisture and kept out of direct sunlight to maintain their aroma and fragrance.

2.3 Field conditions and experimental treatments

Field experiment was administrated during two continuous experimental years (2017-2019). The study region has cold winter and semi-arid climate, where its average annual rainfall is 180 mm and temperature is 12.5 °C (Fig. 1). Before planting the seedlings of sage (*Salvia officinalis* L.), the soil was sampled from a depth of 0-30 cm (Table 1), soil physical and chemical properties as well as cow dung manure was analyzed (Table 2).
The experimental treatments were arranged in a split-plot design in time and space within a randomized complete block design (RCBD) with four replications. The experimental treatments included: Factor A—two irrigation levels (optimum soil moisture and reduced soil moisture) as the main plot; Factor B—the subplots, consisting of organic manure and biofertilizer treatments, including a control (no amendment), cow dung manure (20 ton/ha or 14.4 kg per experimental plot), and AMF symbiosis; and Factor C—foliar spraying of amino acids, including a negative control (no spraying), a positive control (spraying with sol-

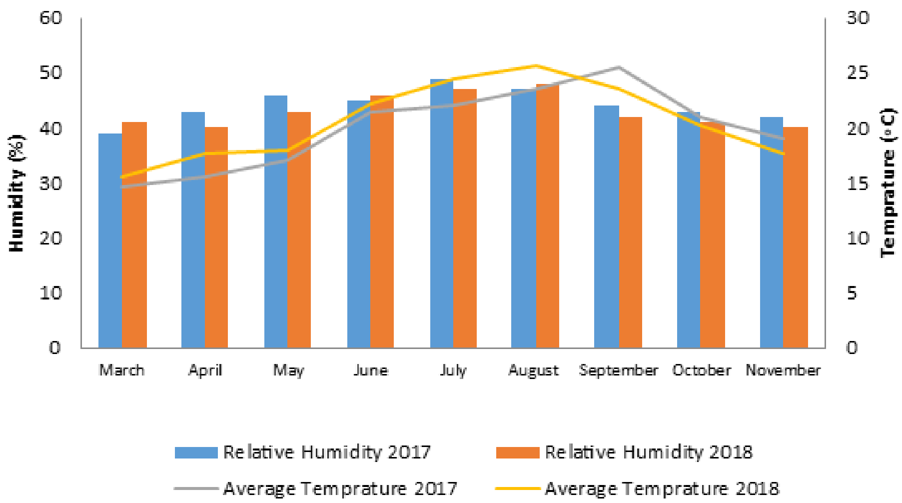

Figure 1. Climatology of Kangavar city from March to November (2017-2018).

Table 1. Physicochemical properties of the soil in the field before planting (0-30 cm depth), in two experimental years (2017-2018).

Year	OC[1](%)	EC[2](dS/m)	K(mg/kg)	P(mg/kg)	N(%)	pH	Soil texture
2017	1.5	0.75	360	16.2	0.16	7.5	Sandy clay
2018	1.42	0.90	375	18.4	0.17	7.7	Sandy clay

[1]OC: Organic Carbon, [2]EC: Electrical Conductivity.

Table 2. Physicochemical properties of the cow dung manure in two experimental years (2017-2018).

Year	EC[1](dS/m)	K(mg/kg)	P(mg/kg)	N(%)	pH
2017	7.5	645	83	1.14	7.42
2018	8.64	760	90	1.18	7.73

[1]EC: Electrical Conductivity.

vent), and L-phenylalanine foliar spraying at 25% and 50% levels. The arbuscular mycorrhizal fungi (AMF) spores number ($\sim$ 20 per 10 g soil) which was produced by Pishtaz Varian biotechnology company under the license and direct supervision of the Soil and Water Institute of Iran, in equal proportions of the three genus included *Funeliformis mossea*, *Rhizaphagus intraradices* and *Claroideoglomus etunicatum* (former scientific names, respectively: *Glomus mosseae*, *G. intraradices* and *G. etunicatum*) spores and hyphea, each other mycorrhized root fragments applied an stationary bearer (Attarzadeh et al., 2019). Briefly, 10 mg of mycorrhizal spore pendency consisting 120 spore/mg, minimum 50 living spore/mg, was applied. Rotten cow dung manure added to the soil two days before planting and mixed with soil. The foliar application of L-phenylalanine solution (adjusted with NaOH to pH 7.0) was sprayed to the aerial parts of plants before flowering stage at three times (15 July, 25 July, and 5 August in first experimental year and 5 July, 16 June, and 25 July in second experimental year) at dew point ($\sim$ 200 mL/plant) with an atomizer onto the leaves until it completely ran off.

2.4 Essential oil extraction

To extract the essential oil present in the aerial parts of sage at early flowering, the hydro-distillation method was used. For this purpose, 100 g of the samples were transferred into a flask containing 1000 mL of water in a Clevenger apparatus (made by Glass Fabricating of Ashk-e-Shisheh Co., Tehran, Iran). The herbs and water mixture was boiled for two hours, after which the volume of the extracted essential oil was recorded. By opening the apparatus valve, the essential oil was transferred into dark-colored bottles, and anhydrous sodium sulfate was added to the extracted essential oil.

2.5 Essential oil analysis

To separate and identify the components of the volatile oil of sage, the essential oil obtained from each treatment was injected into GC-FID and GC/MS devices with the following specifications. GC was a Younglin Acme6000 with a column length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 µm of type BP5. Primary temperature of column was 60 °C and programmed to enhancement at 4 °C min to 280 °C. Initial oven temperature was 50 °C and programmed to increase at 3 °C/min to 240 °C and then increased to 300 °C at a rate of 15 °C/min. The FID detector temperature was set at 300 °C, and helium gas was used as the carrier gas with a flow rate of 1 mL/min.

GC/MS analyses of the samples were performed on an Agilent Technologies 6890 gas chromatograph coupled to an Agilent 5973 C mass selective detector (MSD) and quadrupole EI mass analyzer (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS 5.0% capillary column (30.00 m $\times$ 0.25 mm, 0.25 µm film thicknesses). Mass spectra was recorded at 70 eV. Mass range was from *m/z* 40-500. Helium was the carrier gas at a flow of 0.8 mL/min. Primary temperature of column was 60 °C and programmed to enhancement at 4 °C min to 280 °C. The split ratio was 1:100. The injector temperature was arranged at 300 °C. The purity of helium gas was 99.999% and 0.1 µL samples were controlled handy in the split mode.

The chemical constituents of the sage volatile oil were identified by comparison of their KI (Kovats index) relative to C_5-C_{24} *n*-alkanes obtained on a nonpolar HP-5MS column with those reported in the literature (Adam, 2017) and by comparison of the obtained mass spectra with those recorded in the NIST 08 and Willey MS libraries. The percentage composition (average of three independent analyses) was computed from the GC peak areas without using any correction factors.

2.6 Statistical analysis

Compound data analysis covering the two-year experiment was completed using SAS (version 9.4). Mean values were evaluated using Duncan's multiple range test at $p \leq 0.05$.

3. Results and discussion

3.1 Dry matter yield

Results of indicated that the experimental treatments and their interaction had significant impacts ($p \leq 0.01$) on the dry matter yield of sage (Table 3). Application of cow dung manure and use of *Mycorrhizal symbiosis* increased the dry matter yield of sage compared to control. The highest dry matter yield 972.97 g/m was obtained from the application of 50% L-phenylalanine $\times$ optimum soil moisture $\times$ cow dung manure in the second experimental year, and 943.52 g/m, was observed in 50% L-phenylalanine $\times$ AMF $\times$ opti-

Table 3. Analysis of variance of essential oil content, oil yield and dry matter yield of sage (*Salvia officinalis* L.).

S.O.V	df	Mean of squares		
		Essential oil content	Essential oil yield	Dry matter yield
Replication	2	0.012 ns	0.84 ns	917.53 ns
Irrigation (I)	1	1.695**	3.75**	10032.27**
Replication*I	2	0.021	0.97	1067.99
Organic inputs (O)	2	2.27**	3.76**	55937.17**
L-Phenylalanine (E)	3	2.40**	4.61**	122284.61**
I×O	2	0.922**	3.97**	96255.126**
I×E	3	2.16**	2.76**	88580.077**
O×E	6	0.812**	2.73**	19290.023**
I×O×E	6	0.825**	1.58**	99959.091**
Replication (I×O×E)	44	0.018	0.067	8084.002
Year	1	0.6622**	0.1501**	1019.018**
Year×I	1	0.123 ns	0.079 ns	9202.058**
Year×rep (I)	4	0.038	0.159	2533.0014
Year×O	2	1.13**	4.75**	95662.153**
Year×E	3	1.25**	0.123 ns	1748.91 ns
Year×I×O	2	6.79**	0.091 ns	3466.38 ns
Year×I×E	3	0.52 ns	0.103 ns	881177.30**
Year×O×E	6	2.52**	5.79**	155390.81**
Year×I×O×E	6	2.02**	4.21**	22890.53**
Error	43	0.156	0.173	8718.01
C.V		6.97	10.28	13.48

**Significant at $p < 0.01$. ns, no significance ($p < 0.05$).

mum soil moisture in the first experimental year. The lowest dry matter yield (212.66 g/m) was achieved from reduced soil moisture × application of negative control × control of organic input (Fig. 2).

Organic inputs (AMF and cow dung manure) treatments improved the sage dry matter yield, with the availability of high-consumption and low-consumption elements. Organic inputs increase the absorption of elements, improve photosynthesis, and increase dry matter production. Another reason for promoting yields of sage is improved nitrogen uptake, which has also led to more cell division, increased water content of plant tissues, and more weight gain of the shoots (Kumar et al., 2021; Rahmani-Samani et al., 2021). The use of cow dung manure improves the sage dry matter yield by preparing nitrogen and other important elements like phosphorus, potassium, calcium and magnesium, which are fundamental minerals for plants and cow dung manure can make them available for plants easily. Cow dung manure produces auxins and gibberellins hormones in plants that help plants grow better (Adnan et al., 2020; Wang et al., 2021; Hamedi et al., 2022b, 2022a).

Moreover, the cow dung manure can help soil fertility, because cow dung manure will provide most of the plant's nutritional needs and boost the efficiency of absorption of nutrients, which increases the agglomeration of dry matter in the plants (Hamedi et al., 2022b). Due to the presence of micronutrient elements, cow dung manure elevates the organic matter of the soil and increases its potency to absorb iron, copper, zinc, potassium, phosphorus, and nitrogen. Iron and manganese are the elements in the structure of cytochrome, which has a basic effect on the biosynthesis of chlorophyll, and the use of cow dung manure increases these micronutrients and increases their absorption by the plant. More absorption of these elements as well as more chlorophyll production cause an enhancement in photosynthesis, and finally, the accumulation of dry matter in the plant increases (Geng et al., 2019; Hamedi et al., 2022b, 2022a; Mirjalili et al., 2022).

Mycorrhizal symbiosis enhanced host plant development with increased plant nutrition such as phosphors (Goudarzian et al., 2020; Kaviani-Darani et al., 2025), and improved water and nutrient uptake (Zhang et al., 2018; Sun et al., 2018; Kaviani-Darani et al., 2025). In mycorrhizal treatments, surface areas of the root extended and absorbed better nutrients for the host plants, boosting nutrient uptake (Kumar et al., 2021). *Mycorrhizal symbiosis* has a positive effect on root physiology, characteristics, and urease. Nitrogen in the form of nitrate or ammonium is absorbed by external mycelium and altered to organic matter by glutamine synthetase. Thus, it can help host plants grow better and have more dry matter yield (Kumar et al., 2021). Other researchers reported that AMF evaluated the yield and essential oil of aromatic plants such as *Salvia officinalis* L. (Tarraf et al., 2015) and *Ocimum tenuiflorum* L (Thokchom et al., 2020). The results of a previous study (Golubkina et al., 2020) indicated that *mycorrhizal symbiosis* increased

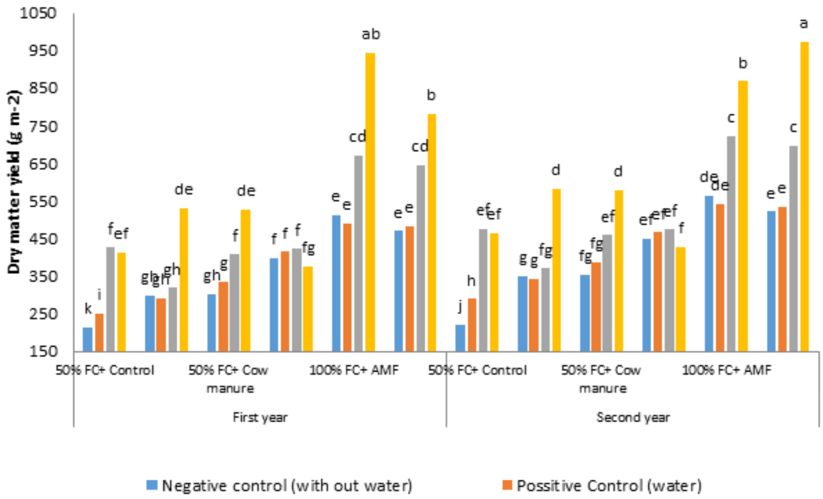

Figure 2. Interaction of year × irrigation × organic inputs × L-phenylalanine on the dry matter yield of sage (*Salvia officinalis* L.). Dissimilar letters demonstrate significant differences at the 5% level according to Duncan's test.

the dry matter yields of three herbs under drought stress condition.

3.2 Essential oil content and yield

Results of this study illustrated that the experimental treatments and their interaction had significant effects ($p \leq 0.01$) on essential oil content and yield of sage (Table 3). The greatest essential oil contents (2.23-2.24%) were obtained from the application of 50% L-phenylalanine × cow dung manure × optimum soil moisture in the first experimental year and treatment of the application of 50% L-phenylalanine × AMF × optimum soil moisture condition. The lowest essential oil content was observed in negative control (without water) × control of organic input × reduced soil moisture condition (Fig. 3). Moreover, the highest oil yield were found in 50% L-phenylalanine × cow dung manure × optimum soil moisture in the second experimental year (16.79 mL/m^2) and 50% L-phenylalanine × AMF × optimum soil moisture 16.36 mL/m^2 in the second experimental year. The lowest essential oil yield (0.808 mL/m^2) was in negative control (without water) × control of organic input × reduced soil moisture condition in the first experimental year (Fig. 4).

In general, the application of cow dung manure and use of *Mycorrhizal symbiosis* increased the essential oil content and yield of sage compared to control. The promoted sage essential oil yield with the use of organic inputs is perhaps owing to the improvement of nutrients availability in the soil like nitrogen, which can raise the agglomeration of plant nutrients, the production of biomass, ameliorate the production of secondary metabolites, and enhancement the essential oil glands number (Emami-Bistgani et al., 2018). Phosphorus plays a structural and vital role in the biosynthesis of essential oil. Isopentenyl pyrophosphate, dimethyl

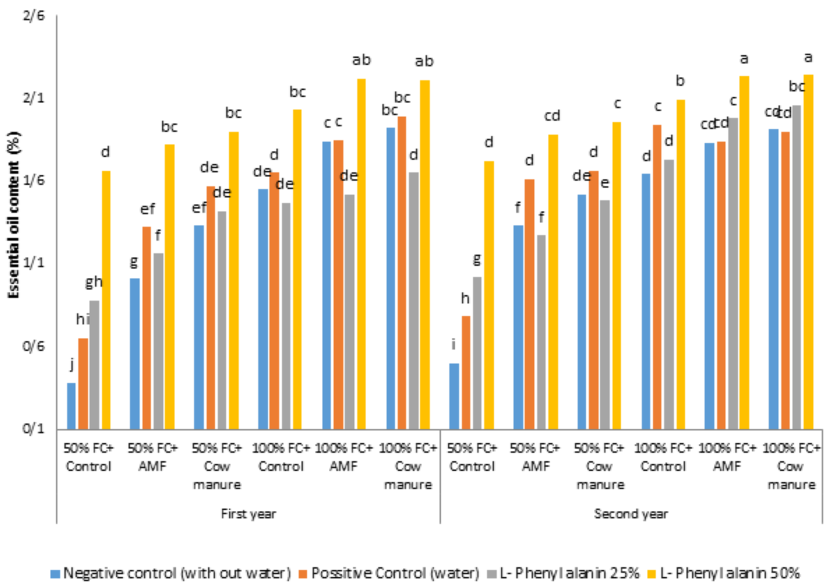

Figure 3. Interaction of year × irrigation × organic inputs × L-phenylalanine on the essential oil content of sage (*Salvia officinalis* L.). Dissimilar letters demonstrate significant differences at the 5% level according to Duncan's test.

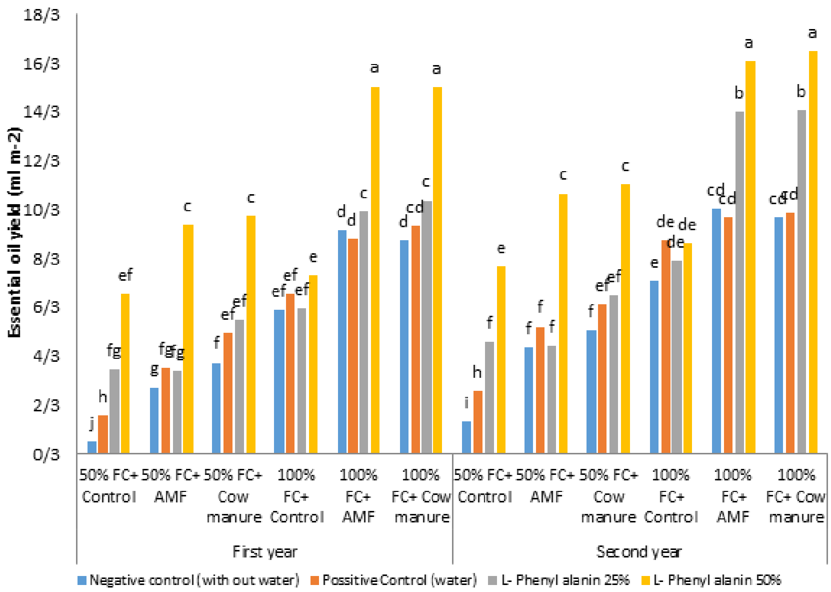

Figure 4. Interaction of year × irrigation × organic inputs × L-phenylalanine on the essetial oil yield of sage (*Salvia officinalis* L.). Dissimilar letters demonstrate significant differences at the 5% level according to Duncan's test.

allyl pyrophosphate, and geranyl pyrophosphate with the phosphorus structural boost the amount of the essential oil. Additionally, considering the role of phosphorus in ATP and NADPH, it can be argued that the raise in the amount of essential oil in response to the increase in the amount of precursors and energy-carrying molecules (Degenhardt et al., 2009; Eshaghi-Gorgi et al., 2021). Kaviani-Darani et al. (2025) reported that the use of organic inputs enhanced the essential oil content, essential oil yield and menthol content of spearmint (*Mentha spicata* L.). It can be said that one of the reasons for promoting the essential oil yield in bio-fertilizer treatments is the positive efficacy of these inputs on the plant growth medium, and developing the root surface, thus expanding the absorption of elements and enhancing the photosynthetic activities that directly affect physiological activities such as the biosynthesis of essential oil. Absorption of nutrients, by influencing the metabolic activities of the plants, has a direct effect on the essential oil of the plants, and improved essential oil yield and content. Results of previous studies indicated that there is positive correlation between enhancement essential oil with the concentration of nitrogen in the soil. In addition, probably, the abundance of nitrogen in cow dung manure and AMF, by increasing the number and surface of leaves, provides a suitable basis for receiving more solar energy, as well as the participation of nitrogen in the structure of chlorophyll and enzymes, which are contained in photosynthetic carbon metabolism. Therefore, it increased the efficiency of photosynthesis and, in this way, played a specific role in raising the essential oil amount (Kaviani-Darani et al., 2025).

3.3 Essential oil composition

According to the results of sage essential oil analysis by GC-FID and GC/MS, 1,8-cineole, camphor, α-thujone (*cis*-thujone), and β-thujone (*trans*-thujone) belonging to oxygenated monoterpenes as the major chemical group were the main constituents in the essential oil (Fig. 5). Similarity, researcher reported that 1,8-cineole, camphor, α-thujone, and β-thujone were the major compounds in the *S. officinalis* essential oil (Tarraf et al., 2017; Vosoughi et al., 2018; Rahmani-Samani et al., 2019). The productivity of the biologically-active compounds practically essential oil plays an important role in plant performance. Changes and improvement the quantity and quality essential oil depend on genetic factor, ecological conditions (Ghasemi-Pirbalouti et al., 2013), agronomic practices (Moghaddam et al., 2017), and their interaction effects can be influence on the herbal yield and the quantity and quality essential oil from sage (Karimi et al., 2020).

Variance analysis for these compounds indicated that the simple effect of the irrigation, organic inputs, and L-phenylalanine had significant impacts ($p \leq 0.01$) on the amounts of α-thujene, α-pinene, comphene, β-pinene, limonene, linalool, and 1,8-cineole in the sage essential oil. In addition, the interaction effects of irrigation, organic inputs, and L-phenylalanine had significant impacts ($p \leq 0.01$) on the percentage of 1,8-cineole (Table 4).

Results of ANOVA illustrated that the experimental treatments had significantly ($p \leq 0.01$) effects on the main constituents in the essential oil such as α-thujone, β-thujone, camphor, borneol, α-caryophyllene, α-humulene, and caryophyllene oxide. The interaction effects of irrigation, organic inputs, and L-phenylalanine had significant impacts ($p \leq 0.01$) on the percentage of α-thujone (Table 5). Usage of cow dung manure and *Mycorrhizal symbiosis* raised the major compounds compared to control. Reduced soil moisture increased the percentages of monoterpene hydrocarbons, sesquiterpenes hydrocarbons, and oxygenated sesquiterpenes compared to the optimum soil moisture condition, while, the highest the oxygenated monoterpenes amount was obtained from the optimum soil moisture promoted. Indeed, the highest values of camphor (37.61%), 1,8-cineole (28.02%), and α-thujone (21.55%) were achieved from the optimum soil moisture condition.

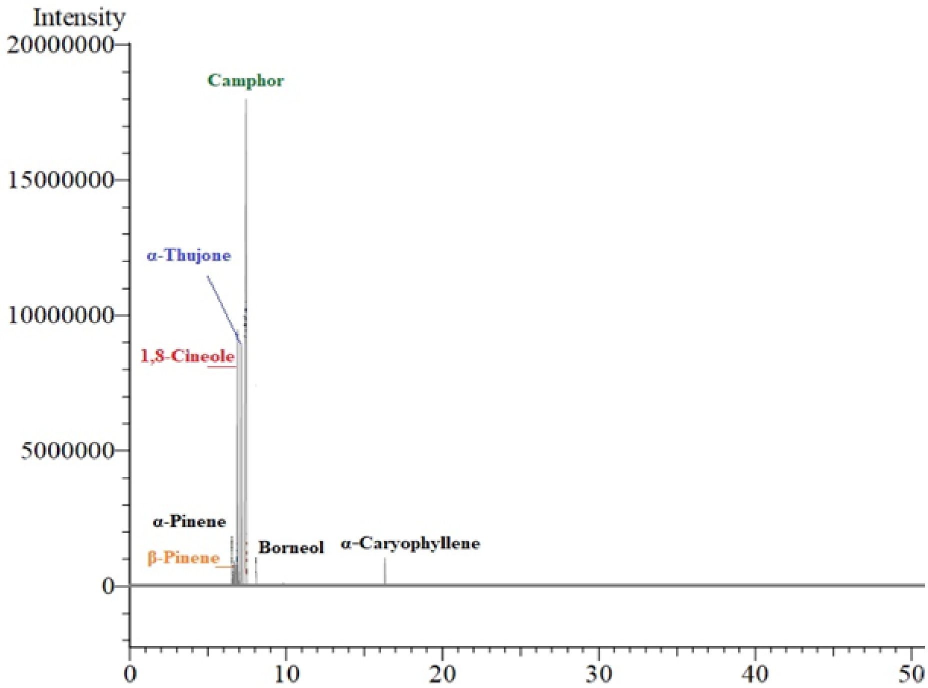

Figure 5. The chromatogram obtained from the aerial parts of sage (*Salvia officinalis* L.) at the early flowering stage, using gas chromatography combined with a flame ionization detector (FID).

Table 4. Analysis variance of some chemical compounds of the sage (*Salvia officinalis* L.) essential oil.

S.O.V	df	Mean of Squares						
		α-Thujone	β-Thujone	Camphor	Borneol	α-Caryophyllene	α-Humulene	Caryophyllene oxide
Replication	2	5.82 ns	1.69 ns	0.95 ns	0.41 ns	3.75 ns	0.32 ns	1.73 ns
Irrigation (I)	1	765.25 **	16.82 **	3.06 **	2.13 **	42.58 **	10.35 **	5.21 **
Replication*I	2	19.92	3.75	0.89	1.24	2.35	0.91	0.49
Organic inputs (O)	2	613.28*	27.43*	4.21*	3.56*	26.73*	12.54*	4.36*
L-Phenylalanine (L)	3	365.73**	31.29**	5.48 **	4.22 **	34.95 **	13.81 **	3.79 **
I×O	2	298.34**	2.61 ns	0.81 ns	0.62 ns	3.02 ns	1.62 ns	0.38 ns
I×L	3	521.65**	5.72 ns	0.76 ns	0.55 ns	2.49 ns	1.41 ns	0.21 ns
O×L	6	411.53 **	3.04 ns	0.67 ns	0.81 ns	1.79 ns	1.11ns	0.65 ns
I×O×L	6	386.28 **	1.96 ns	0.88 ns	0.73 ns	1.23 ns	1.79 ns	0.79 ns
Replication (I×O×L)	44	18.73	9.83	3.80	7.64	6.42	5.73	4.28
Year	1	4.61 ns	3.45 ns	0.71 ns	1.07 ns	1.95 ns	1.74 ns	0.82 ns
Year×I	1	8.52 ns	2.26 ns	0.42 ns	0.86 ns	3.79 ns	1.13 ns	0.53 ns
Year×rep (I)	4	10.36	8.21	1.75	2.35	10.45	3.28	6.73
Year×O	2	24.68 ns	6.81 ns	0.64 ns	0.65 ns	6.05 ns	0.93 ns	0.29 ns
Year×L	3	10.46 ns	4.56 ns	0.52 ns	0.74 ns	5.21 ns	0.81 ns	0.35 ns
Year×I×O	2	19.62 ns	5.79 ns	0.21 ns	0.53 ns	2.97 ns	1.52 ns	0.82 ns
Year×I×L	3	35.90 ns	10.48 ns	0.38 ns	0.81 ns	3.45 ns	1.03 ns	0.46 ns
Year×O×L	6	24.42 ns	9.02 ns	0.42 ns	0.69 ns	2.63 ns	0.38 ns	0.59 ns
Year×I×O×L	6	31.18 ns	11.61 ns	0.56 ns	0.76 ns	1.24 ns	0.91 ns	0.85 ns
Error	43	38.56	26.73	3.79	4.28	13.57	4.32	5.96
C.V		14.73	12.41	13.29	8.49	11.45	10.11	9.84

**Significant at $p < 0.01$. *Significant at $p < 0.05$. ns, no significance ($p < 0.05$).

Table 5. Analysis variance of other chemical compounds of the sage (*Salvia officinalis* L.) essential oil.

S.O.V	df	Mean of Squares						
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L-Phenylalanine (L)	3	365.73**	31.29**	5.48 **	4.22 **	34.95 **	13.81 **	3.79 **
I×O	2	298.34**	2.61 ns	0.81 ns	0.62 ns	3.02 ns	1.62 ns	0.38 ns
I×L	3	521.65**	5.72 ns	0.76 ns	0.55 ns	2.49 ns	1.41 ns	0.21 ns
O×L	6	411.53 **	3.04 ns	0.67 ns	0.81 ns	1.79 ns	1.11 ns	0.65 ns
I×O×L	6	386.28 **	1.96 ns	0.88 ns	0.73 ns	1.23 ns	1.79 ns	0.79 ns
Replication (I×O×L)	44	18.73	9.83	3.80	7.64	6.42	5.73	4.28
Year	1	4.61 ns	3.45 ns	0.71 ns	1.07 ns	1.95 ns	1.74 ns	0.82 ns
Year×I	1	8.52 ns	2.26 ns	0.42 ns	0.86 ns	3.79 ns	1.13 ns	0.53 ns
Year×rep (I)	4	10.36	8.21	1.75	2.35	10.45	3.28	6.73
Year×O	2	24.68 ns	6.81 ns	0.64 ns	0.65 ns	6.05 ns	0.93 ns	0.29 ns
Year×L	3	10.46 ns	4.56 ns	0.52 ns	0.74 ns	5.21ns	0.81 ns	0.35 ns
Year×I×O	2	19.62 ns	5.79 ns	0.21 ns	0.53 ns	2.97 ns	1.52 ns	0.82 ns
Year×I×L	3	35.90 ns	10.48 ns	0.38 ns	0.81 ns	3.45 ns	1.03 ns	0.46 ns
Year×O×L	6	24.42 ns	9.02 ns	0.42 ns	0.69 ns	2.63 ns	0.38 ns	0.59 ns
Year×I×O×L	6	31.18 ns	11.61 ns	0.56 ns	0.76 ns	1.24 ns	0.91 ns	0.85 ns
Error	43	38.56	26.73	3.79	4.28	13.57	4.32	5.96
C.V		14.73	12.41	13.29	8.49	11.45	10.11	9.84

**Significant at $p < 0.01$. *Significant at $p < 0.05$. ns, no significance ($p < 0.05$).

However, the highest α-caryophyllene, α-humulene, and caryophyllene oxide were observed under reduced soil moisture condition (Table 6).
In L-phenylalanine treatment, the highest values of oxygenated monoterpenes compounds such as 1,8-cineole, linalool, α-thujone, β–thujone, camphor, and borneol were found in L-phenylalanine 50% (Table 7). In contrast, L-phenylalanine had not impacts on other constituents. For organic input treatment, the highest values monoterpene hydrocarbons and oxygenated monoterpenes compounds

Table 6. Effect of soil moisture on the chemical compounds of the sage (*Salvia officinalis* L.) essential oil.

Row	Compounds	RI_{cal}	RI_{Lit}	Irrigation regime	
				Optimal Irrigation (100% FC)	Reduced Irrigation (50% FC)
Monoterpene hydrocarbons					
1	α-Thujene	923	922	0.67^b	0.87^a
2	α-Pinene	931	929	1.18^b	1.43^a
3	Comphene	945	944	0.95^b	1.12^a
4	β-Pinene	974	973	1.12^b	1.34^a
5	Limonene	1029	1031	0.03^b	0.74^a
Oxygenated monoterpenes					
6	1,8-Cineole	1031	1028	28.02^a	25.15^b
7	Linalool	1097	1098	1.84^a	0.65^b
8	α-Thujone	1107	1107	21.55^a	19.14^b
9	β-Thujone	1114	1115	3.18^a	3.02^b
10	Camphor	1143	1145	37.61^a	35.30^b
11	Borneol	1165	1163	0.98^a	0.58^b
Sesquiterpenes hydrocarbons					
12	α-Caryophyllene	1416	1417	1.01^b	1.19^a
13	α-Humulene	1453	1452	0.22^b	0.37^a
Oxygenated Sesquiterpenes					
14	Caryophyllene oxide	1587	1588	0.04^b	0.74^a

RI_{cal} = Retention indices relative to C_5-C_{24} *n*-alkanes; RI_{Lit}: Retention indices according to literature. Dissimilar letters demonstrate significant differences at the 5.0% level according to Duncan's test.

Table 7. Effect of L-phenylalanine on the chemical compounds of the sage (*Salvia officinalis* L.) essential oil.

Row	Compounds	RI_{cal}	RI_{Lit}	L-Phenylalanine			
				Negative control or without water	Positive control or solvent	L-Phenylalanine (25%)	L-Phenylalanine (50%)
Monoterpene hydrocarbons							
1	α-Thujene	923	922	0.76^{a}	0.61^{b}	0.41^{c}	0.42^{c}
2	α-Pinene	931	929	1.09^{a}	0.92^{b}	0.84^{c}	0.68^{d}
3	Comphene	945	944	0.78^{a}	0.63^{b}	0.54^{c}	0.55^{c}
4	β-Pinene	974	973	1.23^{a}	1.07^{b}	0.95^{c}	1.01^{c}
5	Limonene	1029	1031	0.79^{a}	0.45^{b}	0.46^{b}	0.23^{c}
Oxygenated monoterpenes							
6	1,8-Cineole	1031	1028	26.14^{d}	28.09^{c}	29.34^{b}	31.51^{a}
7	Linalool	1097	1098	0.52^{c}	0.51^{c}	0.64^{b}	0.78^{a}
8	α-Thujone	1107	1107	18.82^{d}	20.04^{c}	21.45^{b}	22.95^{a}
9	β-Thujone	1114	1115	3.01^{d}	3.21^{c}	3.58^{b}	3.91^{a}
10	Camphor	1143	1145	32.24^{d}	35.01^{c}	36.60^{b}	38.93^{a}
11	Borneol	1165	1163	0.59^{b}	0.51^{b}	0.46^{c}	0.71^{a}
Sesquiterpenes hydrocarbons							
12	α-Caryophyllene	1416	1417	1.14^{b}	1.27^{a}	0.98^{c}	0.81^{d}
13	α-Humulene	1453	1452	0.23^{b}	0.31^{a}	0.14^{c}	0.11^{c}
Oxygenated sesquiterpenes							
14	Caryophyllene oxide	1587	1588	0.31^{a}	0.18^{b}	0.18^{b}	0.09^{c}

RI_{cal} = Retention indices relative to C_5-C_{24} *n*-alkanes; RI_{Lit} = Retention indices according to literature. Dissimilar letters demonstrate significant differences at the 5% level according to Duncan's test.

were found in the application of cow dung manure and *Mycorrhizal symbiosis* (Table 8).

Interaction effect of treatments on the amount of 1,8-cineole indicated that the highest 1,8-cineole 32.93% was seen in the applications of 50% L-phenylalanine and cow dung manure under optimum soil moisture condition, and also 32.76% was found in the applications of 50% L-phenylalanine and AMF under optimum soil moisture condition. The lowest of the percentage of 1,8-cineole (26.07%) was related to negative control (without water) and control of organic input under reduced soil moisture condition (Fig. 6).

Results of interaction effect of the experimental treatments showed that the greatest values of α-thujone (23.67 and

Table 8. Effect of organic inputs on the chemical compositions of the sage (*Salvia officinalis* L.) essential oil.

Row	Compounds	RI_{cal}	RI_{Lit}	Organic inputs		
				Control	AMF	Cow dung manure
Monoterpene hydrocarbons						
1	α-Thujene	923	922	0.45^{b}	0.69^{a}	0.71^{a}
2	α-Pinene	931	929	0.97^{b}	1.24^{a}	1.23^{a}
3	Comphene	945	944	0.54^{b}	0.80^{a}	0.81^{a}
4	β-Pinene	974	973	1.01^{b}	1.19^{a}	1.20^{a}
5	Limonene	1029	1031	0.23^{b}	0.68^{a}	0.70^{a}
Oxygenated monoterpenes						
6	1,8-Cineole	1031	1028	27.45^{b}	29.58^{a}	30.15^{a}
7	Linalool	1097	1098	0.47^{b}	0.75^{a}	0.79^{a}
8	α-Thujone	1107	1107	20.01^{b}	21.44^{a}	22.08^{a}
9	β-Thujone	1114	1115	3.01^{b}	3.15^{a}	3.20^{a}
10	Camphor	1143	1145	35.21^{b}	37.52^{a}	37.64^{a}
11	Borneol	1165	1163	0.47^{b}	0.58^{a}	0.60^{a}
Sesquiterpenes hydrocarbons						
12	α-Caryophyllene	1416	1417	0.66^{a}	0.59^{b}	0.51^{b}
13	α-Humulene	1453	1452	0.19^{a}	0.10^{b}	0.12^{b}
Oxygenated sesquiterpenes						
14	Caryophyllene oxide	1587	1588	0.23^{a}	0.16^{b}	0.15^{b}

RI_{cal} = Retention indices relative to C_5-C_{24} *n*-alkanes; RI_{Lit} = Retention indices according to literature. Dissimilar letters demonstrate significant differences at the 5% level according to Duncan's test.

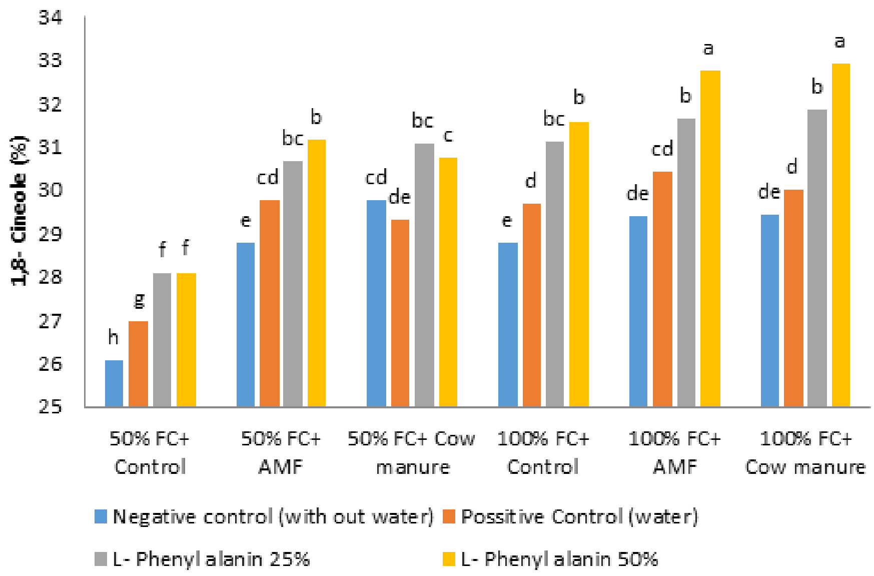

Figure 6. Interaction of irrigation × organic inputs × L-phenylalanine on 1,8-cineole of sage (*Salvia officinalis* L.). Dissimilar letters indicate significant differences at the 5% level according to Duncan's test.

23.50%) were observed in 50% L-phenylalanine × cow dung manure × optimum soil moisture condition and 50% L-phenylalanine × AMF × optimum soil moisture condition (Fig. 7).

In these results, the substantial essential oil compounds are 1,8-cineole, camphor, α-thujone, and β-thujone. The antioxidant activity of the essential oil compositions can mitigate the drought stress in the medicinal plants like sage (Ghasemi-Pirbalouti et al., 2017; Thokchom et al., 2020; Farhadi et al., 2025). Begum et al. (2021) reported that AMF boosted all over growth pattern and secondary metabolism in tobacco (*Nicotiana tabacum* L.) in drought stress conditions.

The essential oil compounds of medicinal plants changed with amino acids throughput phyto-hormonal equivalency like the contents of auxins, cytokinins and gibberellins, or to rectifications in the structural tissues (Tarraf et al., 2017). Use of amino acids like L-phenylalanine, absorbs more nutrients and minerals like nitrogen, phosphorus, and these elements can increase essential oil content and oil content of medicinal plants by synthesis of ATP and NADPH (Rahmani-Samani et al., 2019).

L-Phenylalanine foliar application can improve the absorption of nutrients such as phosphors and nitrogen, which have a direct and positive effect on the essential oil and oil yield of medicinal plants (Aghaei et al., 2019; Li et al., 2020; Rajabzadeh et al., 2024). Similarly, Poorghadir et al. (2020) suggested that application of phenylalanine improved the

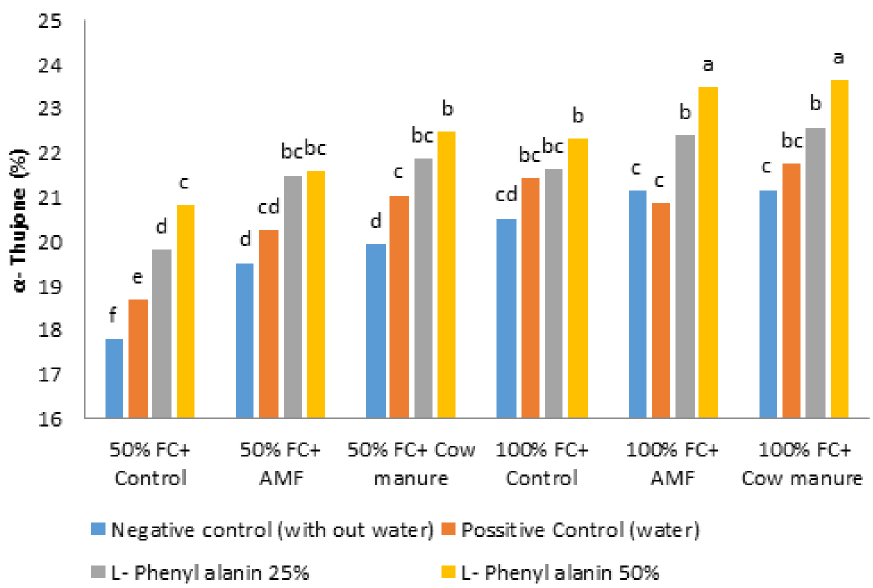

Figure 7. Interaction of irrigation × organic inputs × L-phenylalanine on α-thujone of sage (*Salvia officinalis* L.). Dissimilar letters demonstrate significant differences at the 5% level according to Duncan's test.

Table 9. Pearson correlation coefficients for total dry matter yield, essential oil content, essential oil yield and essential oil compounds of Sage (*Salvia officinalis* L.).

	n=48																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Dry matter yield	1	0.78**	0.972**	0.44 ns	0.36 ns	0.35ns	0.42ns	0.35ns	0.16ns	0.737**	0.16ns	0.83**	0.03 ns	0.06 ns	0.05 ns	0.03 ns	0.06 ns
2. Essential oil content		1	0.899**	0.75 ns	0.47 ns	0.28 ns	0.24 ns	0.28 ns	0.21 ns	0.777**	0.21 ns	0.88 **	0.11ns	0.10ns	0.13ns	0.10ns	0.07
3. Essential oil yield			1	0.41 ns	0.32 ns	0.31 ns	0.11ns	0.11 ns	0.11 ns	0.736**	0.11 ns	0.880**	0.12 ns	0.13 ns	0.10 ns	0.11 ns	0.09 ns
4. α-Thujene				1	0.43 ns	0.12 ns	0.14 ns	0.22 ns	0.10ns	0.745**	0.12ns	0.927**	0.10 ns	0.14 ns	0.11 ns	0.10 ns	0.08 ns
5. α-Pinene					1	0.14 ns	0.13 ns	0.12 ns	0.02 ns	0.712**	0.02 ns	0.798**	0.08ns	0.10ns	0.09ns	0.09ns	0.13ns
6. Comphene						1	0.18 ns	0.19 ns	0.03 ns	0.757**	0.06 ns	0.825**	0.09 ns	0.09 ns	0.06 ns	0.09 ns	0.12 ns
7. β-Pinene							1	0.23 ns	0.05 ns	0.749**	0.05 ns	0.842**	0.06 ns	0.07 ns	0.03 ns	0.08 ns	0.13 ns
8. Limonene								1	0.08 ns	0.763**	0.04 ns	0.811**	0.06 ns	0.08 ns	0.05 ns	0.08 ns	0.11ns
9. Linalool									1	0.711**	0.09 ns	0.746**	0.05 ns	0.04 ns	0.04 ns	0.05 ns	0.09 ns
10. 1,8-Cineole										1	0.07 ns	0.798**	0.03 ns	0.05 ns	0.06 ns	0.04 ns	0.07 ns
11. α-Thujone											1	0.802**	0.02 ns	0.02 ns	0.02 ns	0.02 ns	0.06 ns
12. β-Thujone												1	0.06 ns	0.03 ns	0.04 ns	0.04 ns	0.04 ns
13. Camphor													1	0.09 ns	0.06 ns	0.05 ns	0.05 ns
14. Borneol														1	0.07 ns	0.02 ns	0.02 ns
15. α-Caryophyllene															1	0.03 ns	0.06 ns
16. α-Humulene																1	0.03 ns
17. Caryophyllene oxide																	1

Significant at $**p < 0.01$. ns, no significance.

yield and essential oil compound of savory (*Satureja hortensis* L.) by providing nutrient absorption and uptake. Other researchers demonstrated that the usage of L-phenylalanine raised the yield and secondary metabolites of mint (*Mentha spp.*) (Velička et al., 2022) and chili pepper (*Capsicum anuum*) (Asghari-Lalami et al., 2023).

Drought stress reduces the essential oil content and oil yield in medicinal plants by decreasing the leaf area, use radiation efficiency, and photosynthetic pigments, especially chlorophyll content (Samany et al., 2022). Nevertheless, the interaction of bio-fertilizers and L-phenylalanine with drought stress can help plants improve their essential oil content, and dry matter yield. Because cow dung manure and AMF can hold water, enhance the water-holding capacity of soil, and prepare water for the roots of plants, they can reduce drought conditions for plants. Many researchers have stated that there is a direct relationship between the amount of moisture in the soil and the amount of essential oil produced in the vegetative bodies of medicinal plants (Begum et al., 2021). In our results, dry matter yield, essential oil content and yield increased with optimum soil moisture condition, confirming earlier reports that the highest oil yield of basil (*Ocimum basilicum*) was related to the optimum irrigation treatment (Nejatzadeh-Barandozi, 2020). Similarity, results of a previous study (Ostadi et al., 2022) indicated that *Mycorrhizal symbiosis* improved essential oil productivity and quality of sage in drought stress.

Total dry matter yield had significant positive correlations ($p \leq 0.01$) with essential oil content, oil yield, and the percentages of 1,8-cineole and β-thujone, however, other essential oil compounds did not have effective correlation with dry matter yield. The greatest correlation was found between the dry matter yield and essential oil yield (0.962) and between other traits the strongest correlation was seen between essential oil content and oil yield (0.899) (Table 9). Results indicated that there was a positive correlation between dry matter yield and essential oil; our results were in agreement with other studies (Tursun, 2022).

4. Concluding remarks

L-Phenylalanine application (50%) with organic inputs (cow dung manure and AMF) thought improvement nutrients availability for the plants boosted dry matter yield, essential oil content, and the quality of essential oil compared to the control as well as decreased drought stress negative effects. There was also no substantial difference between the positive control (distilled water) and the negative control (no water) treatments. The highest amounts of sage essential oil compositions was found in the four compounds such as 1,8-cineole, α-thujene, β-thujene, and camphor, which increase the antibacterial effect of the herb. In conclusion, it appears that the utilization of 50% L-phenylalanine along organic inputs (cow dung manure and AMF) can be effective in improving the qualitative and quantitative features of the essential oil of sage in organic farming system in normal condition and experiments related to abiotic stresses in the future.

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Abbreviations

F.C.: Field capacity; **RCBD:** Randomized Complete Block Design; **AMF:** Arbuscular Mycorrhizal Fungi; **FID:** Flame Ionization Detector; **GC:** Gas Chromatography; **GC/FID:** Gas Chromatography-Flame Ionization Detector; **GC/MS:** Gas Chromatography/Mass Spectrometry; **ATP:** Adenosine Triphosphate; **NADPH:** Nicotinamide Adenine Dinucleotide Phosphate.

Authors contributions
All authors contributed to data analysis and interpretation, revised the manuscript, provided final approval for the version to be published, and agreed to be accountable for all aspects of the work. Safoura Hasanabadi carried out field and lab works and wrote the initial draft manuscripts. M. Reza Ardakani and Abdolah Ghasemi Pirbalouti conceived and supervised the study, performed the research concept and design, wrote and critical revision of the manuscript. Farzad Paknejad analyzed data. Davoud Habibi contributed in lab works.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests
The authors declare that there is no conflict of interest.

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