

Organic inputs and L-phenylalanine amino acid impacts on yield and essential oil compounds of sage (*Salvia officinalis* L.) under different soil moisture conditions

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Abstract

To distinguish the impacts of soil moisture conditions and the foliar spraying with an amino acid as affected by two organic inputs on the dry matter yield and volatile oil of sage (*Salvia officinalis* L.) at the early flowering stage, a field experiment was done according to a split-plot in time and place design in West of Iran in two experimental years. Experimental treatments were two irrigation frequency levels [optimum or irrigation at field capacity (F.C.) and reduced irrigation or irrigation at 50% F.C.] as the main plot, and in subplots: organic inputs in three levels including control (without amendment), arbuscular mycorrhizal fungi (AMF), and cow dung manure, and also the foliar spraying of L-phenylalanine amino acid at four levels: negative control (non-spraying), positive control (with distilled water as solvent), and foliar spraying with 25 and 50% L-phenylalanine. Results indicated that the basic compounds of the volatile oil from sage were oxygenated monoterpenes, including 1,8-cineole, camphor, α -thujone, and β -thujone. Generally, the highest values of these oxygenated monoterpenes were obtained from optimum irrigation and foliar spraying with amino acids (50% L-phenylalanine) under an organic farming system, which significantly influenced the quantity and quality of the biologically active compound of sage. In addition, the highest value of dry matter yield (972.97 g m^{-2}) was observed in the second experimental year in the interaction of 50% L-phenylalanine $\times$ 100% F.C. $\times$ cow dung manure, and also 943.52 g m^{-2} , was seen in the first year in the 50% L-phenylalanine $\times$ 100% F.C. $\times$ mycorrhizal symbiosis. Generally, the foliar spray of amino acids under organic inputs (AMF and cow dung manure), and their interaction could ameliorate the dry matter yield and essential oil compounds of sage in environmentally friendly agriculture conditions.

Introduction

Medicinal herbs are a source of many chemical contents that are used as medicinal compounds. Products from the secondary metabolism of the plants are among the most expensive plant chemical compounds. 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; Yusupova et al. 2023; Wang et al. 2020). The above-ground parts of sage (*Salvia officinalis* L.) from the Lamiaceae family, have several medicinal properties, as well as varied vitamins and minerals. Volatile oil from the above-ground parts of sage is one of the biologically active compositions that have various biological effects such as antiseptic, antimicrobial, antioxidant, and antiparasitic properties (Rahmani Samani et al. 2021; Vosoughi et al. 2018).

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 or arid climates. The medicinal herbs, unlike other horticultural crops that are damaged their yield under water deficiency conditions, may be more economically productive in such a situation by producing more phytochemicals (Ansari et al. 2016; Caser et al. 2019; Ilkaee et al. 2011; Khalili et al. 2020, Raza et al. 2013).

Organic and biological sources can be an 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 solutions, 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 (Kumar et al. 2021; Begum et al. 2021; Samarbakhsh et al. 2009). The application of animal manure 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 (Roubik et al. 2018; Scarlat et al. 2018) or even on the seed germination (Mir-Mahmoodi et al. 2011). 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.) (Hamed et al. 2022), and lavender (*Lavandula angustifolia* Mill.) (Mavandi et al. 2021).

Among the amino acid compounds, phenylalanines are organic molecules that affect and enhance physiological activity, growth, and plant developments. 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 targets of this research 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 volatile oil of sage (*S. officinalis*) in a semi-arid climate.

Materials and methods

Plant material and field conditions

Field experiment was administrated in Kangavar district (altitude 1450 m a.s.l, longitude 47° 59' E, latitude 34° 30' N), Kermanshah province, West of Iran during two continuous seasons of (2017–2018). The study region has cold winter and semi-arid climate, where its average annual rainfall 180 mm and temperature is 12.5°C. 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 amberothermic curve for the two experimental years was drawn (Fig. 1).

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

Year	O.C (%)	EC (dS m ⁻¹)	K (mg kg ⁻¹)	P (mg kg ⁻¹)	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
O.C: Organic Carbon, EC: Electrical Conductivity.							

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

Year	EC (dS m ⁻¹)	K (mg kg ⁻¹)	P (mg kg ⁻¹)	N (%)	pH
2017	7.5	645	83	1.14	7.42
2018	8.64	760	90	1.18	7.73
EC: Electrical Conductivity.					

Treatment details

The experimental treatments were ordered as a split plot in time and place in a randomized complete block design (RCBD) with four replications. The experimental treatments consisted Factor A: two irrigation levels [optimum irrigation (irrigation at 100% Field Capacity- FC) and reduced irrigation (irrigation at 50% F.C.)) as the main plot; The sub plots were, Factor B: organic manure and biofertilizer [(control or non-amendment, cow dung manure (20 ton ha⁻¹ or 14.4 kg per experimental plot), and AMF symbiosis; Factor C: the foliar spraying of amino acid [negative control or non-spraying, positive control or spraying of solvent, and L-phenylalanine foliar spraying at 25 and 50% levels.

The arbuscular mycorrhizal fungi (AMF) spores number (~ 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*, *Rhizophagus intraradices* and *Claroideoglomus etunicatum* (former scientific names, respectively: *Glomus mosseae*, *G. intraradices* and *G. etunicatum*) spores and hyphae, each other mycorrhized root fragments applied an stationary bearer (Attarzadeh et al. 2019). Briefly, 10 mg of mycorrhizal spore pendancy consisting 120 spore mg⁻¹, which minimum 50 living spore mg⁻¹ was applied. Rotten cow dung manure added to the soil two days before planting and mixed with soil. In two experimental years, the foliar application of L-phenylalanine solution (adjusted with NaOH to pH 7.0) was sprayed to the above- ground at three times (15 and 25 July, 5 August 2017 and 5, 16 June, and 25 July 2018) before flowering stage.

Measurements

The leaves, stem, and inflorescences of sage were harvested with 2 cm of stem with clippers at more than 50% flowering stage (August 2017 and August 2018). Dried plant (200 g) were powdered and combined to hydro-distillation for three hours applying a Clevenger-type device. The volatile oils were dried with anhydrous Na₂SO₄ and preserved in amber vials at 4°C before to application.

The volatile oils of sage were analyzed with GC-FID and GC/MS. GC-FID decomposition done by Agilent Technologies 7890 GC-FID provided with FID and a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 µm film thicknesses). Helium was the carrier gas at a flow of 0.8 mL min⁻¹. 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 (Shaykh-Samani et al. 2023). The singular compounds were recognized by retention indices and evaluated with compounds recognized from the literature (Adam 2007). The compounds percentage were evaluated from the GC-FID summit zones without applying any modification parameters.

Statistical analysis

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

Results

Results of variance analysis indicated that, the main effect of the year, irrigation, organic inputs, and L-phenylalanine, as well as the interaction between irrigation, organic inputs and L-phenylalanine, also interaction of year and triple interaction of these treatments had significant impacts ($p \leq 0.01$) on essential oil content, oil yield, and dry matter yield of sage (Table 3). Application of cow dung manure and use of mycorrhizal symbiosis increased the essential oil content, oil yield and dry matter yield of sage compared to control. It didn't any observation between negative control or non-spraying, and positive control or spraying of solvent, on essential oil content, oil yield, and dry matter yield. In

addition, 100 F.C. irrigation increased essential oil content, oil yield, and dry matter yield of sage compared to 50% FC irrigation. The highest dry matter yield 972.97 g m^{-2} , was observed in second year in interaction of 50% L-phenylalanine $\times$ 100% F.C. $\times$ cow dung manure, and also 943.52 g m^{-2} , was seen in first year 50% L-phenylalanine $\times$ 100% F.C. $\times$ AMF. The lowest dry matter yield 212.66 g m^{-2} , was seen in first year and negative control (without water) $\times$ 50% F.C. $\times$ control of organic input (Fig. 2).

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

S.O.V	d.f	Mean of Squares		
		Essential oil content	Essential oil yield	Dry matter yield
Replication	2	0.012ns	0.84ns	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 $\times$ O	2	0.922**	3.97**	96255.126**
I $\times$ E	3	2.16**	2.76**	88580.077**
O $\times$ E	6	0.812**	2.73**	19290.023**
I $\times$ O $\times$ E	6	0.825**	1.58**	99959.091**
Replication (I $\times$ O $\times$ E)	44	0.018	0.067	8084.002
Year	1	0.6622**	0.1501**	1019.018**
Year $\times$ I	1	0.123ns	0.079ns	9202.058**
Year $\times$ rep (I)	4	0.038	0.159	2533.0014
Year $\times$ O	2	1.13**	4.75**	95662.153**
Year $\times$ E	3	1.25**	0.123ns	1748.91ns
Year $\times$ I $\times$ O	2	6.79**	0.091ns	3466.38ns
Year $\times$ I $\times$ E	3	0.52ns	0.103ns	881177.30**
Year $\times$ O $\times$ E	6	2.52**	5.79**	155390.81**
Year $\times$ I $\times$ O $\times$ 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 ($p < 0.05$).				

The highest concentration of essential oil content 2.24% was seen in second year in interaction of 50% L-phenylalanine $\times$ 100% F.C. $\times$ cow dung manure, and 2.23% in interaction of 50% L-phenylalanine $\times$ 100% F.C. $\times$ AMF, also 2.21%, was seen in first year in 50% L-phenylalanine $\times$ 100% F.C. $\times$ cow dung manure and 2.22% was related to 50% L-phenylalanine $\times$ 100% F.C. $\times$ AMF. The lowest one 0.38%, was seen in first year and negative control (without water) $\times$ 50% F.C. $\times$

control of organic input (Fig. 3). The highest oil yield 16.79 ml m^{-2} , was found in second year in interaction of 50% L-phenylalanine $\times$ 100% F.C. $\times$ cow dung manure, and 16.36 ml m^{-2} , in interaction of 50% L-phenylalanine $\times$ 100% F.C. $\times$ AMF, also 15.29 ml m^{-2} , was seen in first year in 50% L-phenylalanine $\times$ 100% F.C. $\times$ cow dung manure and 15.34 ml m^{-2} , was found in 50% L-phenylalanine $\times$ 100% F.C. $\times$ AMF. The lowest oil yield 0.808 ml m^{-2} , was observed in first year and negative control (without water) + 50% F.C. $\times$ control of organic input (Fig. 4).

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 oxygenated monoterpenes as the major chemical group were the main constituents in the essential oil. 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). Also, results of ANOVA for the main constituents in the essential oil presented that the irrigation, organic inputs, and L-phenylalanine significantly ($p \leq 0.01$) affected on the amounts of 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. There was not differences between negative control or non-spraying, and positive control or spraying of solvent on the main compounds. Monoterpene hydrocarbons, sesquiterpenes hydrocarbons, and oxygenated sesquiterpenes compounds, the 50% F.C. irrigation improved the compounds compared to the 100 F.C. irrigation, however, oxygenated monoterpenes compounds, the 100 F.C. irrigation promoted essential oil compounds of sage compared to 50% F.C. irrigation. In irrigation regimes treatments, the highest values of camphor (37.61%), 1,8-cineole (28.02%), and α -thujone (21.55%) % were related to 100% F.C, however, the highest α -Caryophyllene, α -Humulene, and Caryophyllene oxide were observed in 50% F.C. (Table 6).

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

S.O.V	d.f	Mean of Squares						
		α -Thujene	α -Pinene	Comphene	β -Pinene	Limonene	Linalool	1,8-Cineole
Replication	2	0.61 ns	1.43 ns	13.05 ns	0.46 ns	4.32 ns	0.21 ns	18.78 ns
Irrigation (I)	1	12.90 **	9.38 **	416.72 **	6.98 **	28.41 **	7.46 **	864.70 **
Replication*I	2	1.43	1.06	28.46	1.05	3.28	1.82	23.82
Organic inputs (O)	2	11.29*	12.21*	321.85*	7.41*	13.64*	8.73*	873.52*
L phenylalanine (L)	3	8.44 **	6.68 **	204.68 **	8.65 **	10.26 **	9.99 **	952.37**
I×O	2	0.57 ns	1.24 ns	11.73 ns	0.52 ns	1.79 ns	0.63 ns	497.61**
I×L	3	0.35 ns	0.95 ns	26.02 ns	0.34 ns	1.16 ns	0.58 ns	752.80**
O×L	6	0.51ns	0.84ns	13.68ns	0.63ns	1.38ns	0.23ns	834.41 **
I×O×L	6	0.49ns	1.05ns	19.71ns	0.77ns	1.11ns	0.69ns	921.35 **
Replication (I×O×L)	44	1.27	1.73	30.11	1.49	3.76	1.35	49.62
Year	1	0.64 ns	1.73 ns	26.73 ns	0.63 ns	1.16 ns	0.59 ns	113.90 ns
Year× I	1	0.71ns	2.59ns	38.97ns	0.51ns	0.91ns	0.83ns	116.37 ns
Year× rep (I)	4	1.85	3.64	52.11	1.86	2.26	1.58	149.52
Year× O	2	0.89ns	2.21ns	26.73ns	0.45ns	1.41ns	1.21ns	134.86 ns
Year×L	3	0.97ns	2.19ns	40.28ns	0.48ns	0.86ns	0.95ns	95.83ns
Year ×I×O	2	1.26 ns	1.82 ns	38.16 ns	0.74 ns	1.55 ns	1.02 ns	176.37 ns
Year ×I×L	3	1.13 ns	1.79 ns	31.72 ns	0.62 ns	1.39ns	1.24 ns	143.51 ns
Year× O×L	6	1.04 ns	1.83 ns	18.32 ns	0.34 ns	1.28 ns	0.96 ns	102.12 ns
Year× I×O×L	6	0.79 ns	2.16 ns	28.11 ns	0.28 ns	1.65 ns	0.98 ns	98.24 ns
Error	43	2.85	5.73	47.12	2.97	4.53	3.21	205.83
C.V		8.43	11.54	14.73	9.73	8.56	10.32	13.93
**Significant at $p < 0.01$. *Significant at $p < 0.05$. ns, no significance ($p < 0.05$).								

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

S.O.V	d.f	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**	2.61ns	0.81 ns	0.62 ns	3.02 ns	1.62 ns	0.38 ns
I×L	3	521.65**	5.72ns	0.76 ns	0.55 ns	2.49 ns	1.41 ns	0.21 ns
O×L	6	411.53 **	3.04 ns	0.67ns	0.81ns	1.79ns	1.11ns	0.65ns
I×O×L	6	386.28 **	1.96 ns	0.88ns	0.73ns	1.23ns	1.79ns	0.79ns
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	24.68ns	6.81ns	0.64ns	0.65ns	6.05ns	0.93ns	0.29ns
Year×L	3	10.46ns	4.56ns	0.52ns	0.74ns	5.21ns	0.81ns	0.35ns
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 6
Effect of irrigation on oil compounds of Sage (*Salvia officinalis* L.).

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 C5–C24 <i>n</i> -alkanes; RI _{Lit} = Retention indices according to literature. Dissimilar letters demonstrate significant differences at the 5% level according to Duncan’s test.					

In L-phenylalanine treatments, the highest values of monoterpene hydrocarbons (α -thujene, α -pinene, comphene, β -pinene, and limonene) and oxygenated sesquiterpenes (caryophyllene oxide) compounds were seen in negative control, the highest value of oxygenated monoterpenes compounds (1,8-cineole, linalool, α -thujone, β -thujone, camphor, and borneol) were found in L-phenylalanine 50% and the highest content of sesquiterpenes hydrocarbons (α -caryophyllene and α -humulene) were seen in positive control or solvent (Table 7).

Table 7
Effect of L-phenylalanine on oil compounds of Sage (*Salvia officinalis* L.).

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 C5–C24 <i>n</i> -alkanes; RI _{Lit} = Retention indices according to literature. Dissimilar letters demonstrate significant differences at the 5% level according to Duncan’s test.							

In organic input treatments, the highest values monoterpene hydrocarbons and oxygenated monoterpenes compounds were found in usage of organic input (cow dung manure and mycorrhizal symbiosis) and the highest contents of esquiterpenes hydrocarbons and oxygenated sesquiterpenes compounds were seen in control (without organic inputs) (Table 8).

Table 8
Effect of organic inputs on oil compounds of Sage (*Salvia officinalis* L.).

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 C5–C24 <i>n</i> -alkanes; RI _{Lit} = Retention indices according to literature. Dissimilar letters demonstrate significant differences at the 5% level according to Duncan's test.						

Total dry matter yield (DMY) had significant positive correlations ($p \leq 0.01$) with essential oil content, oil yield, 1,8-cineole and β-thujone of sage, other essential oil compounds did not have effective correlation with DMY. The greatest correlation was found between the DMY 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).

Interaction effect of treatments on 1,8-cineole indicated that the highest 1,8-cineole 32.93% was seen in interaction of 50% L-phenylalanine × 100% F.C. × cow dung manure, and also 32.76% was found in interaction of 50% L-phenylalanine × 100% F.C. × AMF. The lowest of the percentage of 1,8-cineole (26.07%) was related to negative control (without water) × 50% F.C. × control of organic input (Fig. 5).

Interaction effect of the experimental treatments that the highest α-thujone (23.67%) was observed in interaction of 50% L-phenylalanine × 100% F.C. × cow dung manure, and also 23.5% was found in interaction of 50% L-phenylalanine × 100% F.C. × AMF. The lowest 1,8-cineole (17.81%) was related to negative control (without water) × 50% F.C. × control of organic inputs (Fig. 6).

Discussion

Organic inputs (AMF and cow dung manure) treatments, increased the sage dry matter yield, with the availability of high-consumption and low-consumption elements. Also, by helping the rooting process in plants, it has increased the attraction of elements like nitrogen and phosphorus, which have a great role in chlorophyll and proteins synthesis. 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 by plants (by the application of organic inputs), 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; Gurmanpreet et al. 2019). 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 (Wang et al. 2021; Adnan et al. 2020).

On the other hand, 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. 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; Mirjalili et al. 2022). The existence of these nutrients in the soil and their attraction by the roots promotes the plant's growth and leaf production, which causes increased light absorption, and improved photosynthesis. With increasing photosynthesis in the plant, the accumulation of dry matter yield in the plant increases too (Wang et al. 2021).

Mycorrhizal symbiosis enhanced host plant development with increased plant nutrition such as phosphors (Iggehon and Babalola 2018; Teotia et al. 2017), and improved water and nutrient uptake (Zhang et al. 2018; Sun et al. 2018). 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. As a result of that, nitrogen densities in plants. Arginase and urease are substantial enzymes in the transfer of nitrogen from mycorrhizal treatments. 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; Gholami Ganjeh and Salehi 2015; Zakhikhani et al. 2012). Other researchers reported that AMF evaluated the yield and essential oil of aromatic plants such as *Salvia officinalis* L. (Tarraf et al. 2015) and also *Ocimum tenuiflorum* L (Thokchom et al. 2020). The results of another study presented that AMF inoculation increased *A. dracunculus*, *L. angustifolia* and *H. officinalis*, yield in stress conditions (Golubkina et al. 2020).

In addition, the promoted sage essential oil yield with the use of organic inputs is perhaps owing to the improvement of nutrients availability in the soil, such as nitrogen, which can raise the agglomeration of plant nutrients, caused to more 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 production of essential oil content such as isopentenyl pyrophosphate, dimethyl allyl pyrophosphate and geranyl pyrophosphate and increased the amount of the mentioned precursor compounds. On the other hand, considering the role of this element in ATP and NADPH, it can be argued that the raise in the amount of essential oil was in response to the increase in the amount of precursors and energy-carrying molecules (Eshaghi Gorgi et al. 2021; Degenhardt et al. 2009). Bajeli et al. (2016) presented that the use

of organic inputs gained essential oil content, oil yield and menthol content of peppermint. Other studies reported that the use of AMF meliorated the yield of peppermint (*Mentha piperita*) (Ostadi et al. 2020).

It can be said that, one of the reasons for promoting the essential oil yield in biofertilizer 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. There are many reports that the amount of essential oil increases with the increase of nitrogen in the soil, and the direct relationship between nitrogen and the amount of essential oil is also mentioned. Therefore, the application of cow dung manure and AMF has increased the essential oil and the yield of essential oil in sage. Since essential oils are terpenoid compounds, and their constituents (isoprenoids) require ATP and NADPH, and as a matter of fact, the presence of elements such as nitrogen and phosphorus is necessary for the formation of good compounds, therefore, the use of biological fertilizers increased the essential oil. 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 (Chahal et al. 2017; Fang et al. 2018; Romero-Munar et al. 2017).

In these results, the substantial essential oil compounds are 1,8-cineole, camphor, α -thujone, and β -thujone. Other researchers' reports have similar chemical groups and major contents of *S. officinalis* essential oil (Rahmani Samani et al. 2019; Vosoughi et al. 2018; Tarraf et al. 2017). Phenolic compounds function effectively as hydrogen donors; for this reason, they can act as an effective antioxidant. Accordingly, there is a positive and direct relationship between phenolic compounds and antioxidants acting in essential oils and plant extracts. In addition, it seems that the differences in essential oil compositions in other researches depend on factors such as light, water, food elements, and oil extraction methods. Phenolic and flavonoids compounds synthesis is related to plants photosynthetic rates and also the phenylpropanoid pathway (Dawood et al. 2021; Verma et al. 2013; Oliveira et al. 2013; Thokchom et al. 2020). Begum et al. (2021), reported that AMF boosted all over growth pattern and secondary metabolism in tobacco (*Nicotiana tabacum* L.) in drought stress conditions.

When the essential oil compounds of medicinal plants changed with amino acids, it has been assigned to a change in the 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 (Rezakhani and Haj Seyed Hadi 2017).

L-Phenylalanine is a component of phenylalanine ammonia lyase, which is the first phase of the biosynthesis of plant phenolic contents, and it is a significant ingredient in the signal transduction pathway metabolic. The metabolites constructed from phenylalanine across phenylalanine ammonia lyase activity, like essential oils, flavonoids, phenolics, tannins and lignin, are related to the plant growth effects and exogenous motivation. 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 (Govindaraju and Arulselvi 2018; Li et al. 2020). Similarly, Poorghadir et al. (2020) suggested that application of phenylalanine improved the 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).

Drought stress due to a lack of water in the plant, reduces the essential oil content and oil yield in medicinal plants by decreasing leaf area, which cuts down on the efficiency of the light received, and this is the main reason for decreasing photosynthetic pigments, especially chlorophyll content. But the interaction of biofertilizers 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. For this reason, in order to produce products of appropriate quantity and quality, soil moisture during the growing season of plants should be equal to 80% (Begum et al. 2021). In our results, dry matter yield, volatile oil content and volatile oil yield increased with 100 FC irrigation, confirming earlier reports that the highest oil yield of basil (*Ocimum basilicum*) was related to the irrigation treatment of 100 FC (Nejatzadeh-Barandozi 2020). In the same direction, reported that mycorrhizal symbiosis improved essential oil productivity and quality of sage in drought stress (Ostadi et al. 2022).

Results indicated that there was a positive correlation between dry matter yield and essential oil; our results were in agreement with other studies. Other researchers reported that there was a strong correlation between the essential oil yield and the essential oil of *Tanacetum species* (Alizadeh et al. 2017) and purple basil (*Ocimum basilicum*) (Tursun 2022).

Conclusions

L-phenylalanine application with organic inputs (cow dung manure and AMF) improves nutrients availability for the plants, increased dry matter yield, essential oil content and also the quality of essential oil compared to the control and decrease drought stress negative effects. L-phenylalanine foliar application with a level of 50% was more effective than 25%. There was no substantial difference between the positive control (distilled water) and the negative control (no water) treatments. The highest amount of sage essential oil compositions was found in the four compounds such as 1,8-cineol, α -thujene, β -thujene, and camphor, which increase the antibacterial effect of this plant. Results showed that, there was a positive correlation between volatile oil content, volatile oil yield and dry matter yield in two experimental years. Finally, for improving dry matter yield and the essential oil of sage in organic farming, we suggested 100% F.C. $\times$ cow manure $\times$ 50% L-phenylalanine, as well as, 100% F.C. $\times$ AMF $\times$ 50% L-phenylalanine.

Declarations

Conflict of interest

The authors declare that they have no competing interests.

Author Contribution

SH field and lab works writing the article. MRA performed the research concept and design, writing and critical revision of the article. AGP statistical analysis, writing the article. FP& DH contributed in lab works. All authors reviewed the manuscript.

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Table 9

Table 9 is available in the Supplementary Files section.

Figures

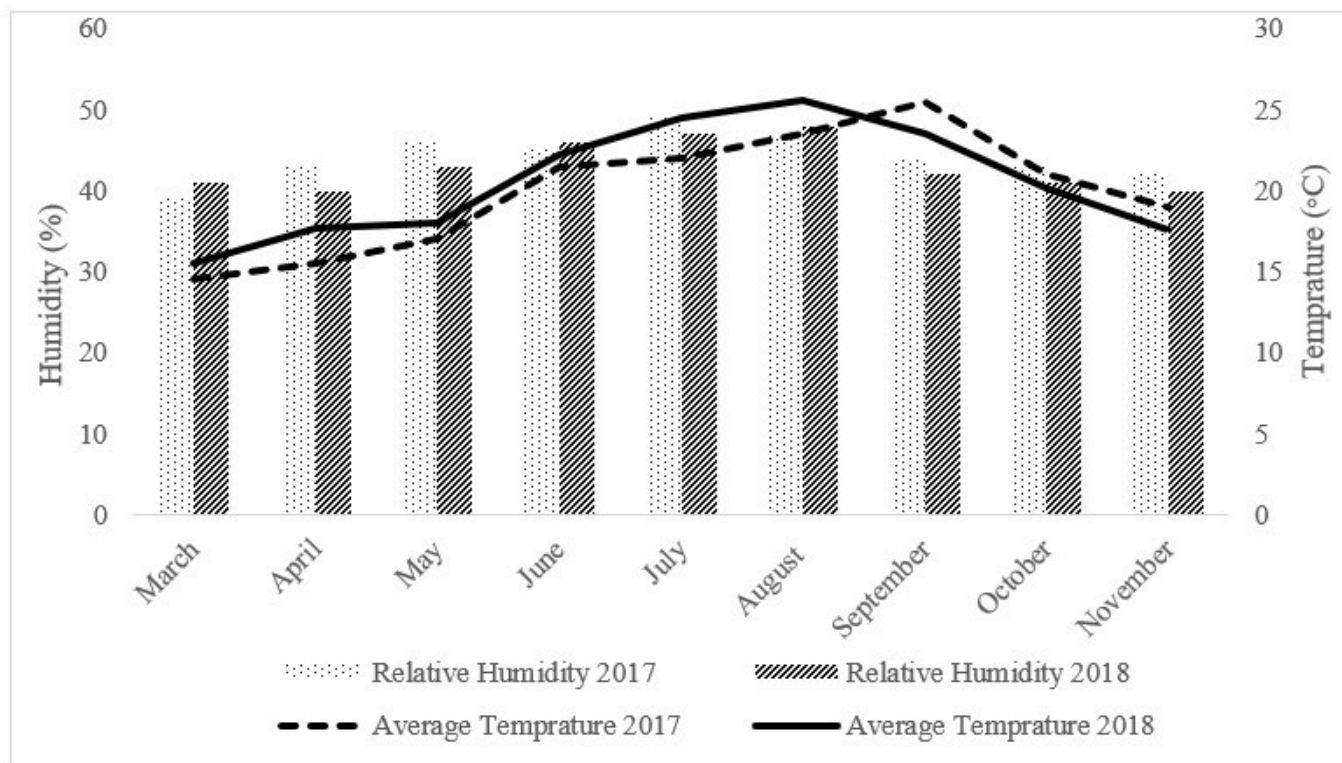


Figure 1

Climatology (amperothermic) for Kangavar city from March to November (2017-2018).

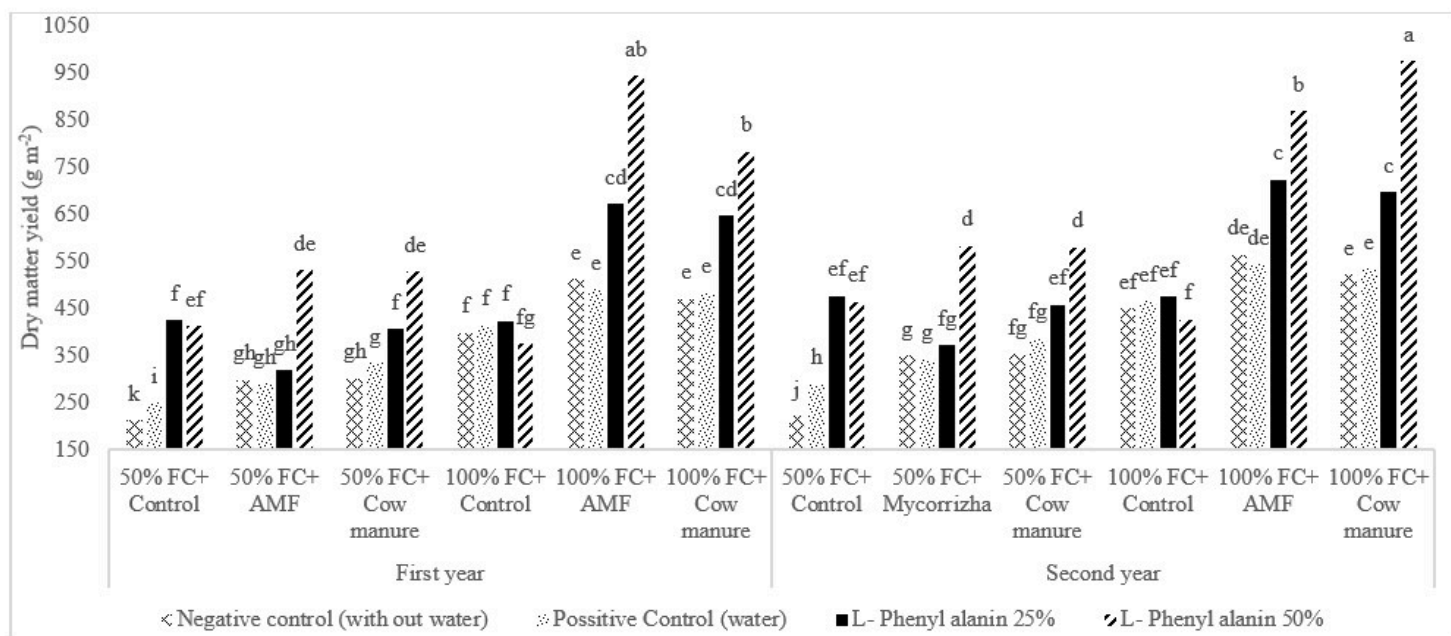


Figure 2

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.

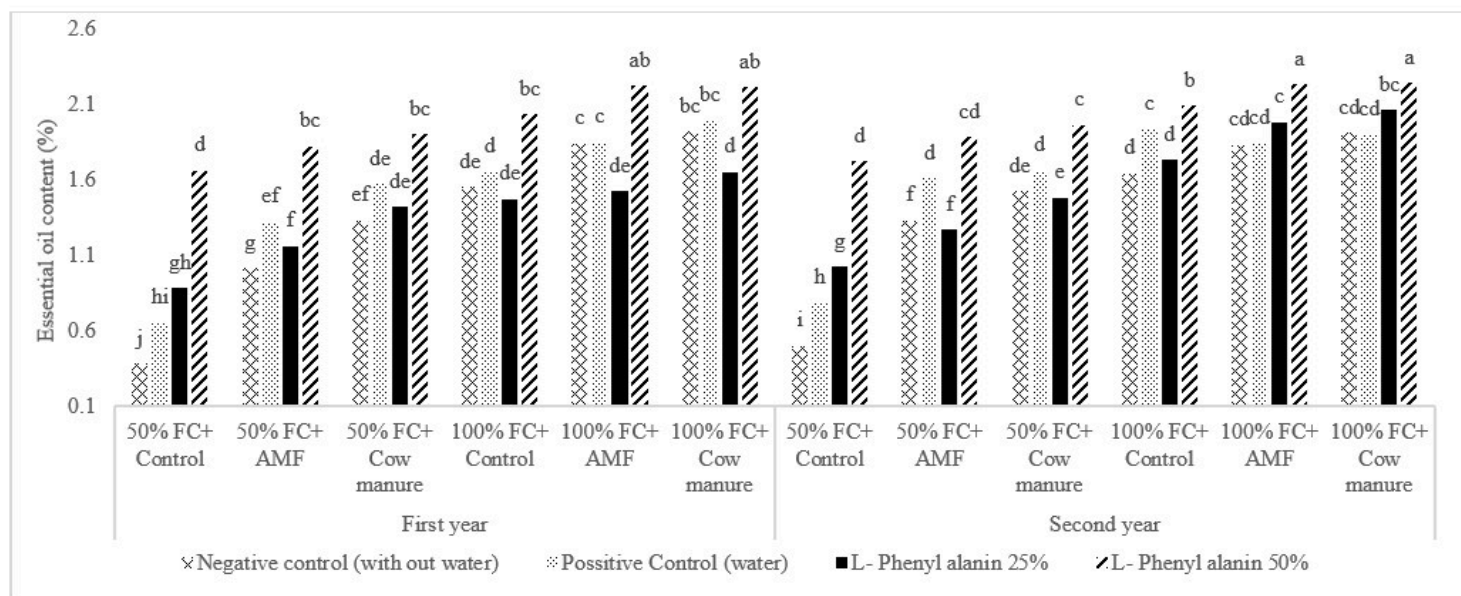


Figure 3

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.

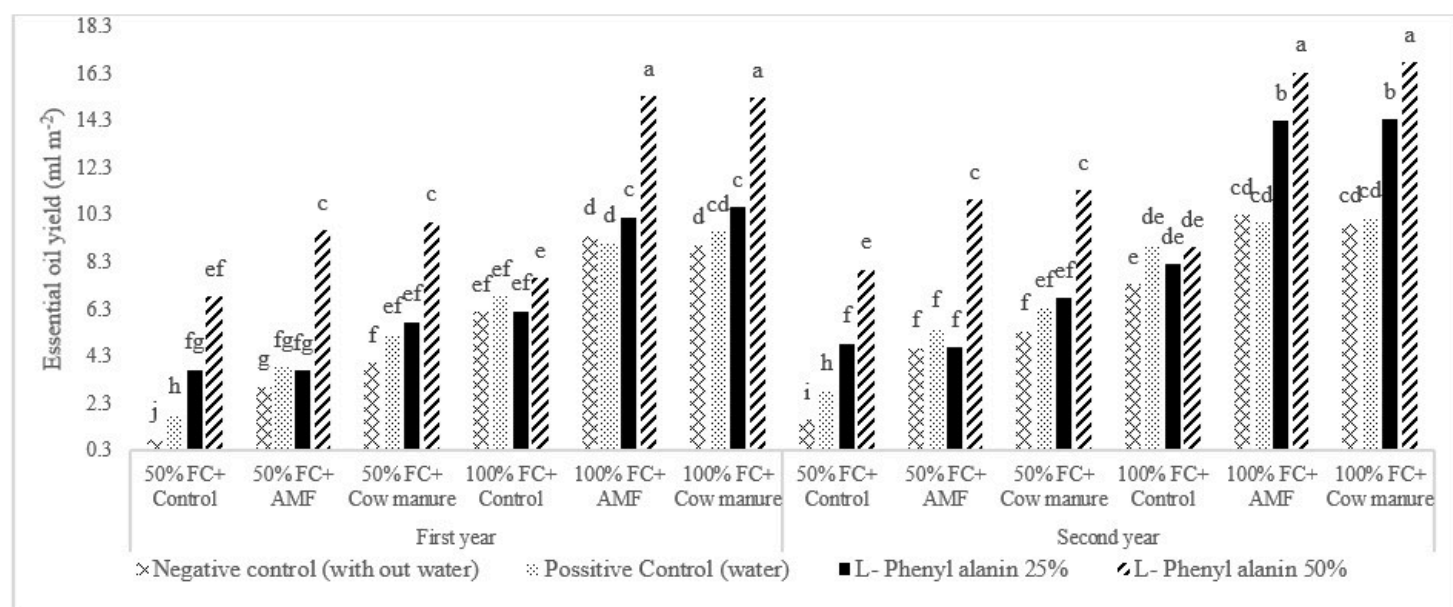


Figure 4

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

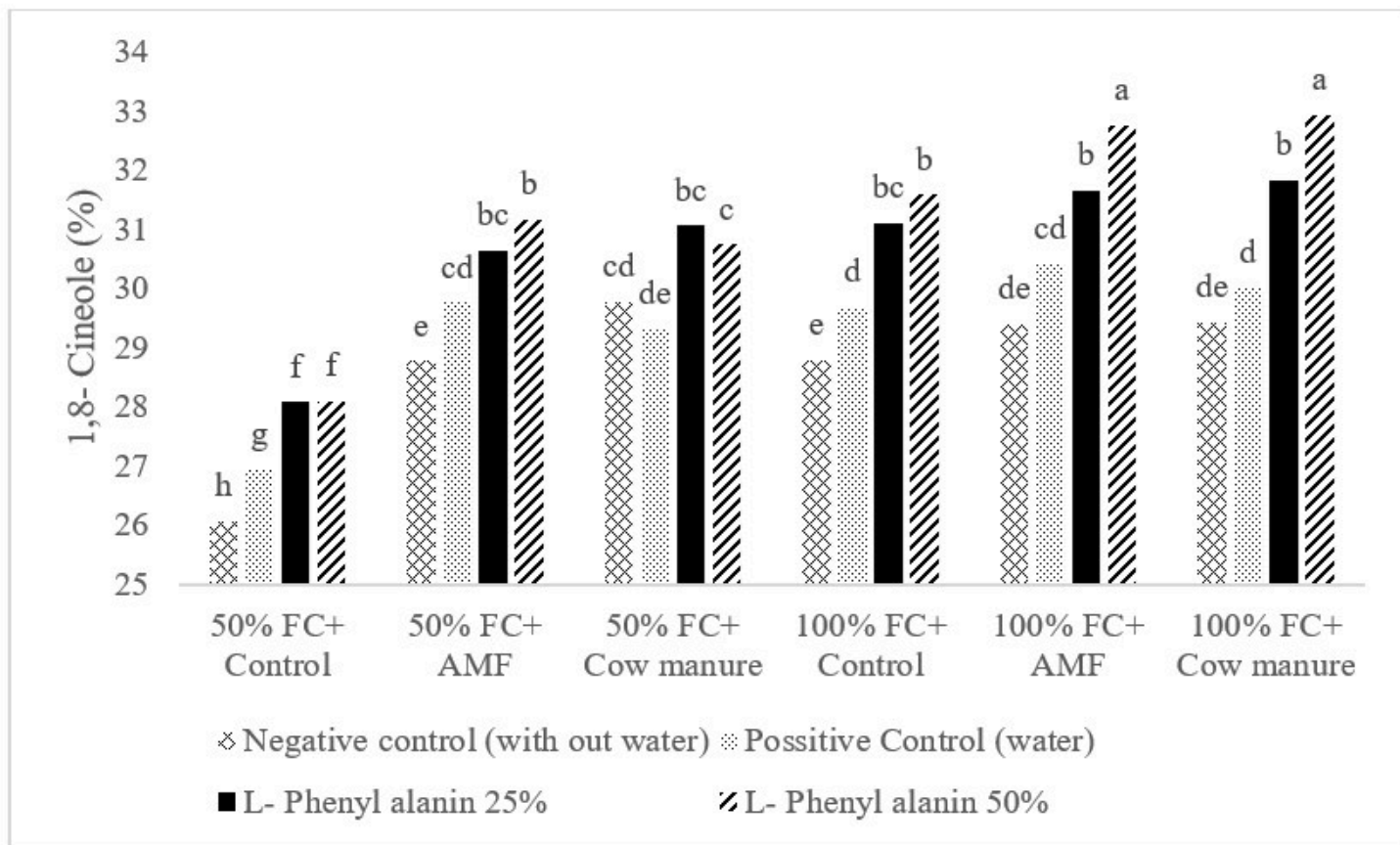


Figure 5

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.

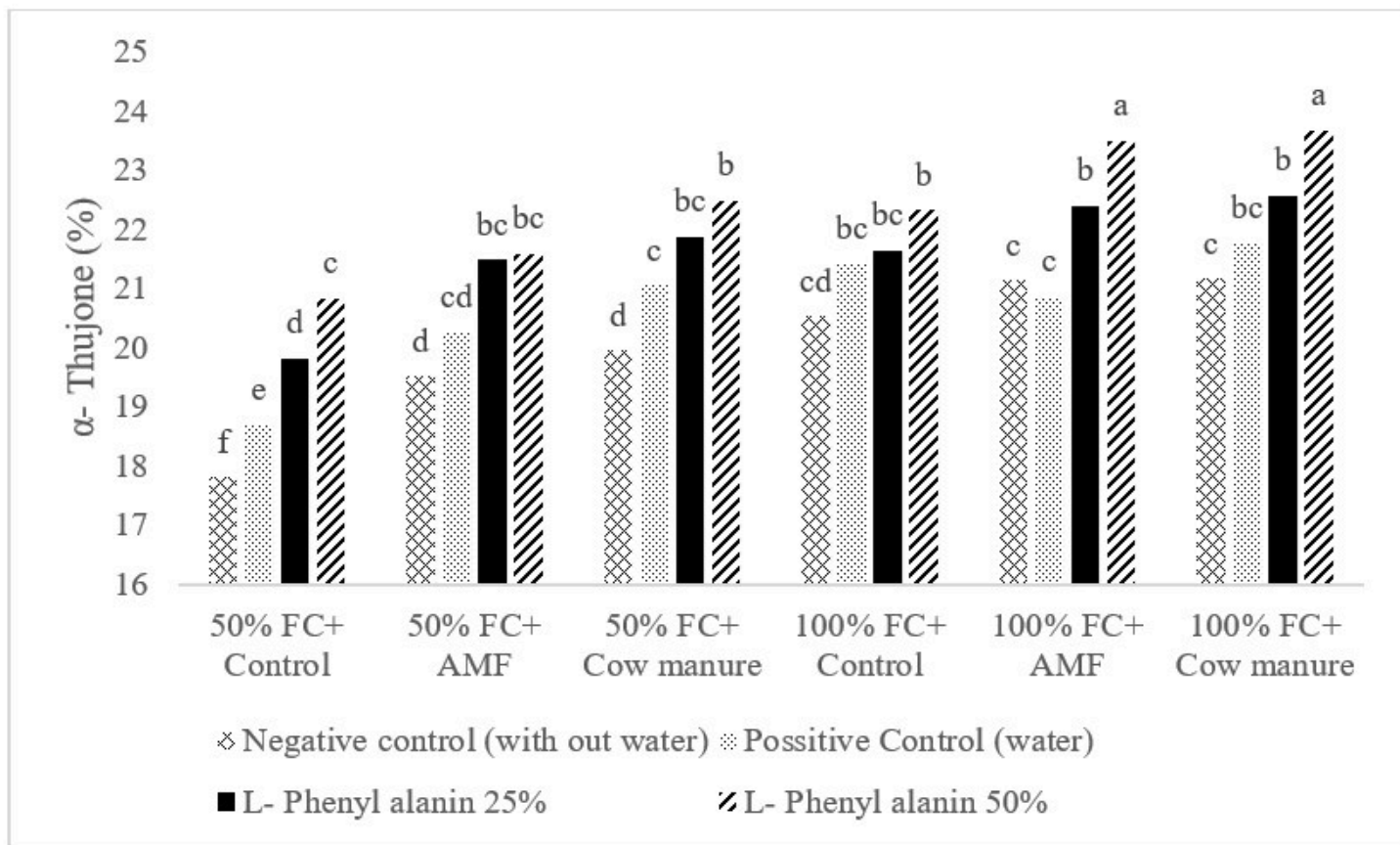


Figure 6

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table9Pearsoncorrelationcoefficientsfortotaldrymatteryield.docx](#)