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Evaluation of Essential Oil in *Satureja spicigera* (c. Koch) Boiss. in Dry Farming Under the Effect of Different Organic Fertilizers and Plant Densities

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ABSTRACT

A decrease in agricultural water resources in recent years has led to the cultivation of plants with low water requirements. Creeping savory (*Satureja spicigera*) is a native medicinal species that grows on rock walls in the north of Iran. To evaluate the effects of different planting densities and different treatments of organic fertilizer (O.F.) on creeping savory essential oil (EO) under rainfed cultivation, an experiment was conducted by a split-plot design based on RCBD with three fertilizer treatments (rotten cow manure, enriched straw, and farm soil), and three planting densities (2.66, 4 and 8 plant/m^2). The experiment was carried out in two crop years (2017-2019) in Mehreg -an Research Station of Kermanshah Agricultural and Natural Resour c-es Research and Education Center. After extraction by water distillation, the EO content and EO yield were calculated and the chemical compounds were analyzed by GC and GC/MS. A total of 13 compounds were identified in the EO, comprising more than 97% of the total compounds. The effects of fertilizer treatments had statistical significance (P≤0.01) in terms of EO content, EO yield, and several chemical compounds. The effects of planting density were significant on the EO yield and chemical compounds (P≤0.01). The highest EO content was obtained in the treatment of rotten cow manure×high density (3.75%) and the lowest EO content occurred from the effect of farm soil×high density (2.78%). The highest EO yield was caused by enriched straw×high density (72.59 kg ha^{-1}). The use of O.F. increased the EO content, EO yield, and thymol content, but decreased the carvacrol content.

Introduction

As a matter of limited water resources that affect the cultivation of crops in many regions of Iran and the world, it is important to study the cultivation of plants that can be grown in dryland conditions. *S. Spicigera* is a procumbent soft shrublet, which usually has a diffuse growth, with inflorescences that arise from trailing sinuous stems. The stems are usually recurved-pubescent on the two opposite sides. The leaves are linear-oblanceolate, 8-20(-25) x 2-3(-5) mm, mucronate, glabrous, green, and bear short axillary shoots. The inflorescence is usually oblong to linear. Verticillasters are usually approximate, pedunculate, laxly (2-) 6-flowered. The calyx is sub-bilabiate, 3-4(-5) mm, pubescent, with two lower teeth 1-1.5 x tube, and upper teeth that are only 1/2-3/4 x the lower pair. The corolla is 6-8 mm, white, exserted from the calyx, and the limb is ample. Stamens are exserted long from the

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corolla. Nutlets are 1.2 mm on average and appear as broadly oblong-ovoid. They can be found in eroded dry banks and rocky places, ledges, and screes, with an altitude range of 20-1500 m (Davis, 1982).

Creeping savory is a medicinal plant, native to Iran, and is mostly distributed in the north and north-west of Iran, the Caucasus region, and Turkey. It is reportedly drought-resistant in several pharmacopeias. *S. spicigera* has been introduced as a medicinal plant that is rich in volatile oils, the main ingredient of which is carvacrol. Carvacrol has anticonvulsant and antiflatulent therapeutic effects (Omidbigi, 2008). *S. spicigera* is used for food consumption, preparation of beverages, and production of sanitary materials (Faker Baher et al., 2001).

So far, this wild plant has not been cultivated commercially. In recent years, however, it has been cultivated only in pilot form and in research projects in Iran and Turkey. It seems that many parts of different regions of Iran, including the mountainous slopes of the west, north, north-west, and middle Zagros regions are suitable for irrigated and rainfed cultivations of this species.

For optimal growth and development, plants need appropriate and balanced values of essential elements (both micro- and macronutrients). Chemical fertilizers increase plant growth and yield by answering the nutritional needs of plants (Chaudhry et al., 1999). However, their long-term use can cause environmental pollution and degradation of soil structure (Nasir Khan et al., 2017; Chandini et al., 2019). Organic fertilizers (O.F.) contain large amounts of organic matter, as important sources of NPK nutrients (Fernandez et al., 1993).

Also, manure contains trace elements such as iron which improves plant growth. Of course, O.F. (manures) cannot meet all the nutritional needs of plants (Mallanagouda, 1995). Organic fertilizers improve the physical structure of soils and can balance the chemical properties of the soil so that plants could better absorb nutrients (Chaudhry et al., 1999). Soil health and dynamics, as well as plant access to micronutrients (Zn, Cu, Fe, Mn, B, and Mo), are strongly associated with the accumulation of organic matter (Dhaliwal et al., 2019). The combination or replacement of chemical fertilizers with a variety of organic fertilizers is important for the achievement of sustainable production in agriculture (Roussos et al., 2017).

Planting density affects the absorption of radiation, water, and nutrients in plants by changing the structures of the crown and root systems (Hammer et al., 2009; Du et al., 2021). Plant spacing is an important factor in determining the microenvironment in the field. Having a higher planting density increases competition among plants for light, water, and nutrients (Ciampitti and Vyn, 2011; Rossini et al., 2011) and, subsequently, causes abiotic stress in plants and decreases biomass (Osakabe et al., 2014). The optimization of plant spacing can lead to a higher yield in the crop by favorably affecting the absorption of nutrients and exposure of the plant to light. Although Nigussie et al. (2015) and Aflatuni et al. (2005) reported that the EO content and composition were not influenced by density, Callan et al. (2007) reported that planting density affects the yield and quality of aromatic herbs. In relevant research, the EO content in fennel was significantly affected by different planting densities (Khorshidi et al., 2009). Also, row spacing significantly affected EO yield in mint (Kizil and Toner, 2005). Given the knowledge gap in the available literature on *Satureja spicigera* and its cultivation, the present study aimed to evaluate the effects of different organic fertilizers and planting densities on essential oil content and components of this plant in rainfed conditions. The ultimate objective was to obtain the most effective O.F. treatment and a proper planting density which could be recommended for its commercial cultivation in rainfed conditions.

Materials and Methods

Experimental designs and treatments

This experiment was performed as a split-plot and was based on a randomized complete block design (RCB), with three organic fertilizers as the main factor and three planting densities as the sub-factor. Each treatment group had 3 replications and the experiment was carried out in the cropping seasons of 2017-2018 and 2018-2019. There were three main treatment groups, i.e. rotten cow manure 30 tons ha^{-1}, straw enriched with ammonium sulfate 10 tons ha^{-1}, and the control without fertilizer (farm soil). In the second treatment group, two kg of ammonium sulfate were used per 100 kg of straw. The sub-factor treatments included three densities (2.66, 4, and 8 plants per m^2).

In total, there were 27 plots as experimental units. The area of each sub-plot was $3 \times 4 = 12$ m^2, each main plot was equal to 36 m2 and the net area of each replicate was 108 m^2. The area of each plot, without the distances between the plots and replications, was equal to 324 m^2. When including the distances between the plots and replications, the area was equal to 690 m^2. In autumn, the field was plowed and leveled. Then, heaps were piled at 50-cm intervals. To implement the treatments, 36 kg of rotten cow manure or 12 kg of enriched wheat straw, enriched by sulfate ammonium, were evenly distributed on the furrows in each subplot. Then, the fertilizers were covered with farm soil. Before the main sowing and in early autumn, 12 kg of crushed wheat straw was distributed on the furrows in each subplot and was enriched with 240 g of ammonium sulfate (dissolved in 20 liters of water). Subsequently, the fertilizer was covered with farm soil.

The seeds were provided by the Forests and Rangelands Research Institute of Iran. The seeds were disinfected with 5% sodium hypochlorite for 2 minutes and dried. They were planted in trays and a peat moss bed in a greenhouse at temperatures of 18-24 C° and humidity of 35%. In early April, and before any effective rain, the seedlings were transferred to the field.

Experimental conditions and plant materials

In early April, and before any effective rain, the seedlings of creeping savory were transferred to the field in Mehregan Research Station of Kermanshah Agricultural and Natural Resources Research and Education Center. The field was situated at 34°.9؛ latitude, 47°.9؛ longitude, at 1270 m altitude. The results of soil and treatment analyses are presented in Table 1.

The long-term meteorological statistics included an average annual rainfall of 470.7 mm, an absolute minimum temperature of -13 °C, an absolute maximum temperature of 40.5 °C, an average annual temperature of 13.8 °C, and a semi-arid steppe climate class that dominated the region. For two crop years, data were used from the Kermanshah weather station to provide meteorological statistics (Table 2) about monthly and annual rainfall (Table 3). No herbicides or pesticides were used during the project, and mechanical methods were used for weed control.

Essential oil extraction

After 50% of flowering, in late August, savory plants were collected, dried, and weighed for EO extraction. Seventy grams of crushed plant powder were used for EO extraction in each subplot. The EO was extracted by water distillation using the Clevenger system according to British Pharmacopoeia (1993). The Clevenger apparatus operated for 3 hours in total. The essential oil samples were dehydrated with dry sodium sulfate (Na_2So_4) and were kept in a refrigerator (4 °C) until injection into a chromatographic apparatus. The EO content was calculated by the W/W method from the following formula: EO% = EO weight (g) / plant dry weight (g) ×100 (Khademi Doozakhdarreh et al., 2022). EO yield was obtained by multiplying the EO percentage by dry plant yield per hectare (Yousefi et al., 2021).

GC and GC/MS analysis

After diluting 1 μL of essential oil in 2 μL of dichloromethane, the samples were analyzed by a gas chromatograph with relevant specifications (Ultra Fast Model) Thermo-UFM, Chrom-Card A/D data processor, cap-column Ph-5 (non-polar), with a length of 10m, an inner diameter of 0.1, with 0.4 μm thickness. The inner surface of the device was coated with a stationary phase of 5% dimethyl siloxane phenyl (Thermo Company, Italy). The column temperature was programmed to an initial temperature of 60 °C and an ultimate temperature of 285 °C, which increased at a rate of 80 °C per minute. Then, it stopped at 285 °C for 3 minutes, while the detector type was FID at 290 °C. The temperature of the injection chamber was 280°C, the carrier gas was helium and the inlet pressure on the column was set at 0.5 kg cm-2 (Zakerian et al., 2020). The GC/MS device was Varian 3400, connected to a mass spectrometer (Saturn II), with an ion telephoto system and ionization energy of 70 electron volts. It had a DB-5 column as a semi-polar column (with a length of 30 m, an inner diameter of 0.25 mm, and the static phase layer was 0.25 microns thick). The column head gas pressure was set at 35 pounds per square inch, and the temperature increased from 40 °C to 250 °C, at a rate of 4 °C per minute. The injection chamber temperature was 260 °C and the line transfer temperature was 270 °C. The retention indexes were calculated by the injection of normal hydrocarbons (C7-C25) under the same conditions as essential oil injection. Chemical compounds in the EO were identified by comparing the spectra with those of different sources in the available literature (Adams, 2017).

Data analysis

Data were analyzed by Microsoft Excel. The analysis of variance and Duncan's mean values were compared by SPSS (ver.16).

Table 1. Physical and chemical characteristics of fertilizer treatments and control

Fertilizer treatment	Soil Texture	EC (ds/m)	pH	Absorbable P (ppm)	Absorbable K (ppm)	O.C. (%)
Farm soil	Silty-Clay	0.70	7.03	12.20	520	1.13
Enriched straw	-	-	-	26	860	22.03
Rotten cow manure	-	-	-	138	6800	1.75

Table 2. Metrological statistics in Kermanshah weather station during two crop years of project implementation

Crop years	Absolute maximum temperature (˚C)	Absolute minimum temperature (˚C)	Average maximum temperature (˚C)	Average of minimum temperature (˚C)	Annual average of temperature (˚C)	Average humidity (%)	Annual evaporation (mm)	Average of long time annual precipitation	Average of long time annual temperature
2016-2017	40.8	8.2	24.8	7.7	16.6	37	1931	470.7	12.39
2017-2018	43.5	10.6	23.6	8.3	16.74	45	162.6	470.7	12.39

Table 3. Monthly and annual rainfall statistics (mm) in Kermanshah weather station during two crop years of project implementation

Crop years	October	November	December	January	February	March	April	May	June	July	August	September
2016-2017	0	1	14.7	75.4	808	68	132.5	22.1	0	0	0	0
2017-2018	0	33.8	10.2	27.2	95.1	30.1	63.4	169	5.2	0	0	0

Results

Chemical compounds of EO

A total of 13 compounds were identified in the EO, which comprised more than 97% of the EO compounds. In identifying the chemical components and presenting them through their abbreviations (Table 4), the main compounds of the EO were thymol (29.13%), carvacrol (23.84%), ρ-cymene (21.39%), and γ-terpinene (15.74%). In 6 of the samples, methyl ether carvacrol was detected only in the second year and in negligible amounts, so statistical analysis was not necessary for this particular compound. Methyl ether carvacrol occurred in different amounts in plants that grew on farm soil at medium plant density (1.08-1.27%), on field soil at low plant density (0.36-1.1%), and on enriched straw treatment at medium plant density (0.2-0.39%).

Table 4. Specification of EO chemical compounds identified *in S. spicigera* under the impact of organic fertilizers and different plant densities in dry farming *

Chemical classification	Chemical name	RT	RI	Formula
Monoterpene	Terpinene-4-ol	2.58	1146.53	$C_{10}H_{18}O$
	Myrcene	1.16	986.46	$C_{10}H_{16}$
	Terpinolene	1.65	1054.11	$C_{10}H_{16}$
Phenol monoterpenoids	Carvacrol	4.07	1289.96	$C_{10}H_{14}O$
	Thymol	3.96	1281.22	$C_{10}H_{14}O$
	Methyl ether thymol	3.32	1224.97	$C_{11}H_{16}O$
	Methyl ether carvacrol	3.60	1250.81	$C_{11}H_{16}O$
Bicyclic monoterpene	α-pinene	0.87	931.13	$C_{10}H_{16}$
	Trans-caryophyllene	5.33	1376.02	$C_{15}H_{24}$
Ketonic monoterpene	α-thujone	0.84	924.38	$C_{10}H_{16}O$
Isometric monoterpene	γ-terpinene	1.63	1051.59	$C_{10}H_{16}$
Sesquiterpenes.	β-bisabolene	6.42	1493.57	$C_{15}H_{24}$
Benzene alkyl	ρ-cymene	1.36	1014.15	$C_{10}H_{14}$

* RT = retention time; RI = retention index

Analysis of variance

When compared to each other, the two years caused no significant difference in EO content, whereas significant differences were observed in EO yield between the two years ($P \leq 0.01$). The fertilizer treatments caused significant differences in EO content and EO yield ($P \leq 0.01$). Also, the fertilizer treatments showed significant effects on several EO chemical compounds such as myrcene, γ-terpinene, terpinene-4-ol, trans-caryophyllene, and methyl ether thymol ($P \leq 0.01$), as well as α-pinene ($P \leq 0.05$). Significant differences were observed between the different planting densities that affected EO yield, α-pinene, terpinolene, terpinene-4-ol, and thymol ($P \leq 0.01$). This also applied to α-thujone, methyl ether thymol, and carvacrol ($P \leq 0.05$). The interaction between year and fertilizer had a significant effect on γ-terpinene and trans-caryophyllene ($P \leq 0.01$). The interaction effect between year and planting density was significant only on α-pinene ($P \leq 0.05$). The interaction between fertilizer and planting density was significant on α-thujone, γ-terpinene, terpinolene, trans-caryophyllene, methyl ether thymol, and terpinene-4-ol ($P \leq 0.01$), as well as on myrcene and thymol ($P \leq 0.05$). The interaction effects of year, fertilizers, and planting density were significant only on α-pinene ($P \leq 0.05$) (Table 5a and Table 5b).

Mean comparison of EO content, EO yield, and EO chemical compounds

According to Duncan's comparison of means

(Table 6a and Table 6b), the highest EO content was caused by the rotten cow manure treatment, along with high planting density (3.75%), followed by the effect of enriched straw with a low planting density (3.73%). The lowest EO content was observed in plants that grew on the farm soil with high planting density (2.78%). The highest EO yield was observed in plants that grew on the enriched straw with a high planting density (72.59 kg/ha), whereas the lowest EO yield (16.76 kg/ha) was obtained from plants that grew on farm soil with a low planting density. The highest amount of thymol (33.68%) was observed in plants that grew on rotten cow manure with a low planting density, whereas the lowest amount of carvacrol (26.55%) occurred in plants with a high planting density on farm soil.

Principal component analysis (PCA)

In principal component analysis, the first four components had values higher than one. Also, 75% of the variance was explained by the first two components. The results of PCA showed that carvacrol (CARVA), terpinolene (TRPNOLN), ρ-cymene (PCYM), α-thujone (ATHUJ), terpinene-4-ol (TRP4OL), carvacrol methyl ether (MECRVA), methyl ether thymol (METHYM) and α-pinene (APIN) had the most positive contribution to the first component. Meanwhile, γ-terpinene (GTERP), β-bisabolone (BBISA), α-thujone, trans-caryophyllene, myrcene (MYRCE), ρ-cymene, terpinene-4-ol, and methyl ether carvacrol (MECRVA) had the most positive contribution to the second component. Also, thymol (THYM) had the most negative contribution to the first and second components.

The treatments of enriched straw and high planting density (SD1), enriched straw and low planting density (SD3), and rotten cow manure with medium planting density (MD2) caused high levels of trans-caryophyllene, γ-terpinene, β-bisabolene, and myrcene. Enriched straw and medium density (SD2), as well as field soil and medium planting density (CD2), caused high amounts of α-thujone, terpinolene, ρ-cymene, and carvacrol methyl ether. Rotten cow manure and high planting density (MD1) and farm soil with high planting density (CD1) caused the highest values of α-pinene, terpinene-4-ol, carvacrol, and methyl ether thymol. Finally, field soil and low planting density (CD3), as well as rotten cow manure and low planting density (MD3) caused higher levels of thymol (Fig. 1).

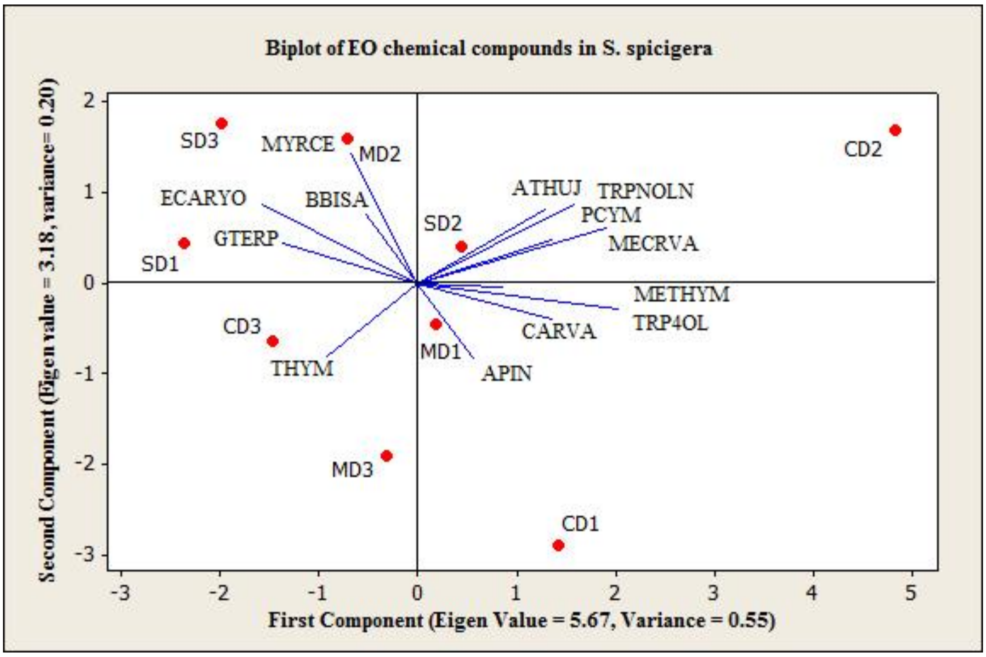

Fig. 1. Biplot diagram of EO chemical compounds based on different organic fertilizers × planting density treatments in *S. spicigera* under rainfed cultivation*

* (CD1 = farm soil / high density; CD2 = farm soil / medium density; CD3 = farm soil / low density; MD1 = rotten cow manure / high density; MD2 = rotten cow manure / medium density; MD3 = rotten cow manure / low density; SD1 = enriched straw / high density; SD2 = enriched straw / medium density; SD3 = enriched straw / low density; CARVA = carvacrol; TRPNOLN = terpinolene; PCYM = ρ-cymene; ATHUJ = α-thujone; TRP4OL = terpinene-4-ol; MECRVA = methyl ether carvacrol; METHYM = methyl ether thymol; APIN = α-pinene; GTERP = γ-terpinene; BBISA = β-bisabolene; MYRCE = myrcene; THYM = thymol)

Table 5a. Variance analysis for EO content (%), EO yield (kg ha^{-1}), and EO chemical compounds (%) in *S. Spicigera* under dry farming, with different O.F. values and planting densities

Source of Variations	df	Mean Squares						
		EO content (%)	EO yield (kg ha^{-1})	E- caryophyllene (%)	Carvacrol (%)	Thymol (%)	α- thujone (%)	γ- terpinene (%)
Year (Y)	1	0.01^{ns}	20640**	8.51**	12.91^{ns}	64.82^{ns}	0.06^{ns}	58.72^{ns}
Y/ rep (R)	4	0.07	32.16	0.02	45.60	10.74	0.08	20.01
Fertilizer (F)	2	3.3**	2419**	0.6**	0.03^{ns}	16.12^{ns}	0.03^{ns}	38.3**
Y/ F	2	0.94^{ns}	674.2*	0.4**	0.43^{ns}	11.90^{ns}	0.01^{ns}	45.1**
Y/ F /R.	8	0.25^{ns}	97.34	0.04^{ns}	12.20^{ns}	10.62^{ns}	0.06^{ns}	2.97^{ns}
Density (D)	2	0.11^{ns}	3896**	0.03^{ns}	43.90*	142**	0.10*	12.11^{ns}
Y/ D	2	0.06^{ns}	1348**	0.07^{ns}	7.00^{ns}	15.50^{ns}	0.01^{ns}	2.72^{ns}
F/ D	4	0.23^{ns}	444**	0.4**	28.4*	57.92*	0.03^{ns}	13.4**
Y/ F/ D	4	0.05^{ns}	154.21	0.05^{ns}	4.62^{ns}	4.09^{ns}	0.04^{ns}	10.71^{ns}
Error	24	0.10	75.45	0.04	8.61	48.73	0.04	4.13
CV (%)		9.66	23.04	18.50	12.32	14.9	4.88	12.91

* and **, significant differences at the level of 5% and 1% respectively; ns = non-significant; EO = essential oil; O.F. = organic fertilizers; CV = coefficient of variation

Table 5b. Variance analysis of EO chemical compounds (%) in *S. Spicigera* under dry farming, with different O.F. values and planting densities

Sources of variation	df	Mean Squares						
		ρ- cymene (%)	Methyl ether thymol (%)	β- bisabolene (%)	Terpinene- 4- ol (%)	Myrcene (%)	Terpinolene (%)	α- pinene (%)
Year (Y.)	1	81.6^{ns}	0.31^{ns}	0.97**	0.002*	0.02^{ns}	0.01^{ns}	3.1*
Y/rep (R.)	4	119.7	0.37	0.01	0.00	0.05	0.03	0.06
Fertilizer (F.)	2	31.98^{ns}	0.50**	0.00^{ns}	0.18**	0.3**	0.01^{ns}	0.6*
Y. / F.	2	3.92^{ns}	0.01^{ns}	0.00^{ns}	0.00^{ns}	0.02^{ns}	0.00^{ns}	0.05^{ns}
Y. / F. / R.	8	31.4^{ns}	0.05^{ns}	0.00^{ns}	0.00^{ns}	0.02^{ns}	0.01^{ns}	0.07^{ns}
Density (D.)	2	67.59^{ns}	0.40*	0.01^{ns}	0.16**	0.06^{ns}	0.09*	0.2**
Y. / D.	2	5.12^{ns}	0.06^{ns}	0.00^{ns}	0.00^{ns}	0.01^{ns}	0.00^{ns}	0.12*
F. / D.	4	26.22^{ns}	0.8**	0.00^{ns}	0.14**	0.25*	0.06**	0.1**
Y. / F. / D.	4	3.98^{ns}	0.04^{ns}	0.00^{ns}	0.00^{ns}	0.02^{ns}	0.00^{ns}	0.3**
Error	24	20.20	0.12	0.01	0.02	0.07	0.01	0.03
CV (%)		21.02	26.2	13.91	26.07	14.78	24.60	18.69

* and **, significant differences at the level of 5% and 1% respectively; ns = non-significant; EO = essential oil; O.F. = organic fertilizers; CV = coefficient of variation

Table 6a. Mean comparison of interaction effects of fertilizers and planting densities for the EO content (%), EO yield (Kg ha-1), and EO compounds (%) in *S. Spicigera* under dry farming, with different O.F. values and different planting densities. *,**, ***

Treatments and abbreviations	Means±SE						
	EO content (%)	EO yield (Kg ha^{-1})	*α*-thujone (%)	*α*-pinene (%)	Myrcene (%)	*P*- cymene (%)	*γ*- terpinene (%)
Farm soil (F.S.) × High density (H.D.)	2.78±0.08c	32.02±10.30c	0.49±0.11bc	1.20±0.26a	1.38±0.07d	21.16±3.02b	14.86±1.45b
F.S. × medium density (M.D.)	2.80±0.17c	25.81±6.45cd	1.09±0.10a	0.90±0.09bc	1.82±0.09abc	27.47±1.50a	14.55±1.20b
F.S. × low density (L.D.)	2.82±0.21c	16.76±6.16d	0.60±0.10bc	1.09±0.16ab	1.65±0.08bcd	19.26±2.02b	18.01±1.31a
Rotten cow manure (R.M.) × H.D.	3.75±0.12a	58.81±11.26b	0.48±0.04bc	0.79±0.08c	1.78±0.07abc	21.36±2.45b	14.39±1.35b
R.M. × M.D.	3.60±0.15ab	36.04±6.043c	0.78±0.12b	0.83±0.07c	1.90±0.03ab	22.80±2.78ab	14.66±1.24b
R.M. × L.D.	3.43±0.16ab	27.63±6.34d	0.55±0.14bc	0.69±0.20cd	1.58±0.05cd	20.55±2.06b	13.68±0.75b
Enriched straw (E.S.) × H.D.	3.49±0.12ab	72.59±19.99a	0.44±0.02c	0.93±0.14bc	2.00±0.08a	19.52±0.53b	18.39±0.34a
E.S.× M.D.	3.20±0.19bc	33.09±8.618c	0.58±0.14bc	0.82±0.16c	1.73±0.10abc	20.49±2.36b	15.35±1.32b
E.S. × L.D.	3.73±0.17a	36.54±8.42c	0.55±0.05bc	0.51±0.05d	1.92±0.17ab	19.92±2.26b	17.73±1.45a

* The common letters in each column indicate no significant difference; ** α= 0.05; *** (EO = essential oil; O.F. = organic fertilizers; CV= coefficient of variation; F.S. = farm soil; R.M. = rotten cow manure; E.S. = enriched straw; H.D. = high density; M.D. = medium density; L.D. = low density)

Table 6b. Mean comparisons of the interaction effects between fertilizers and planting densities for the EO chemical compounds (%) in *S. Spicigera* under dry farming, with different O.F. values and different planting densities *,**, ***

Treatments and abbreviations	Means ± SE						
	Terpinolene (%)	Terpinene-4-ol (%)	Methyl ether thymol (%)	Thymol (%)	Carvacrol (%)	*E*- caryophyllene (%)	*β*- bisabolene (%)
Farm soil (F.S.) × High density (H.D.)	0.36±0.05c	0.67±0.08bc	1.43±0.08b	29.04±3.33ab	26.54±0.76a	0.94±0.18cd	0.21±0.10ab
F.S × Medium density (M.D.)	0.54±0.02a	0.75±0.03a	1.55±0.11a	23.66±1.34cb	25.29±0.58a	0.83±0.13d	0.21±0.09ab
F.S × Low density (L.D.)	0.36±0.03c	0.35±0.04bc	1.10±0.17b	33.64±0.88a	19.65±1.47b	1.08±0.06cd	0.24±0.11ab
Rotten cow (R.M.) manure × H.D.	0.48±0.05ab	0.44±0.04ab	1.12±0.09b	28.58±2.10abc	25.02±1.05a	1.15±0.21bcd	0.18±0.10b
R. M × M.D.	0.40±0.02b	0.35±0.02c	1.15±0.13b	28.33±1.02abc	22.77±2.12ab	1.52±0.26a	0.26±0.12ab
R.M × L.D.	0.29±0.02d	0.44±0.06c	1.41±0.18ab	33.69±0.585a	23.84±1.92ab	1.01±0.23cd	0.20±0.09ab
Enriched straw (E.S.) × H.D.	0.27±0.02d	0.32±0.01c	0.72±0.01c	24.96±1.31bc	23.34±0.72ab	1.31±0.23ab	0.21±0.09ab
E.S × M.D.	0.53±0.05a	0.60±0.04a	0.74±0.03c	30.45±1.29a	25.41±1.50a	1.10±0.22bcd	0.29±0.13ab
E.S × L.D.	0.44±0.07b	0.35±0.03ab	1.61±0.23a	29.81±0.99ab	22.66±1.61ab	1.52±0.22a	0.26±0.12ab

* The common letters in each column indicate no significant difference; ** α = 0.05; *** (EO = essential oil; O.F. = organic fertilizers; CV = coefficient of variation; F.S. = farm soil; R.M.= rotten cow manure; E.S. = enriched straw; H.D. = high density; M.D. = medium density; L.D. = low density)

Discussion

The main compounds of essential oil were thymol, carvacrol, ρ-cymene, and γ-terpinene. The results of previous research (Gokturk, 2021) were precisely in line with the current results, but some other results obtained herein were almost similar to those reported in the literature, for instance, in the case of carvacrol and carvacrol methyl ether (Farzaneh et al., 2015), carvacrol, γ-terpinene, carvacrol methyl ether, ρ-cymene, and β-caryophyllene (Bahtiyarca Bagda, 2010).

The fertilizer treatments (i.e. rotten cow manure and enriched straw) had significant effects on EO content, EO yield, and several chemical compounds in the EO. These results were rather expected because of the greater availability of organic carbon, some nutrients such as N, P, K, and more moisture in the organic fertilizer treatments which led to higher biomass yield and EO accumulation, thereby confirming previous results. Skubij and Dzida (2019) reported that the availability of nutrients significantly affected the process of EO accumulation in savory plants. In previous research, the use of cow manure led to a significant increase in EO yield in summer savory (Gholami Sharafkhanah et al., 2015) and had a positive effect on improving the quantitative and qualitative yield of purple basil (Tehrani Sharif et al., 2015). EO content and EO chemical compounds significantly affected savory plants through the application of organic fertilizers (Esmaielpour et al., 2018). Also, organic fertilizers affected EO content and EO yield in some other medicinal plants (Keshavarz et al., 2019; Giovannini Costa et al., 2013; Anwar et al., 2005), which were in agreement with our results. The effects of organic fertilizers on the production and changes in EO chemical compounds were found to be variable. In the case of *Satureja hortensis* L., Alizadeh et al. (2009) reported that the amounts of carvacrol and terpinene increased by the effect of soil and chicken manure as a mixture. The organic manure increased the percentage of α-phellandrene and ρ-cymene in *Anethum graveolens* (Rostaei et al., 2018). In geranium EO, however, citronellol and geraniol were not affected by organic fertilizers (Ram et al., 2003). In dragonhead, the percentages of geranial and neral increased by the effect of chemical fertilizers, whereas piperitone content decreased by the effect of organic manure (Fallaha et al., 2018).

There is a wide range of differences in the response of medicinal plant species to the application of organic fertilizers in terms of EO production and their chemical compounds. These differences are mainly related to plant genetics and species (Tawfeeq, 2017), the type of chemical compounds in the EO (Nejatzadeh, 2015), as well as environmental and ecological conditions (Yang et al., 2018).

The availability or deficiency of low- or high-consumption nutrients can be very effective in determining enzyme activity (Seif Sahandi et al., 2019), especially in producing chemical compounds or shifts in parallel reactions. Nonetheless, they may be ineffective or less effective in forming other compounds. For example, the availability of nitrogen reportedly caused increases in ATP and NADPH, which subsequently led to an increase in terpenoid compounds (Nejatzadeh, 2015) but reduced the sesquiterpenes content (Said-Al Ahl et al., 2010; Poshtdar et al., 2016).

In the current study, the plant density affected EO yield and some chemical compounds but did not affect EO yield (%). Callan et al. (2007) reported that planting density affected the EO yield and EO compounds of aromatic herbs. Also, there have been several reports on how planting density affected EO quantity and quality in different types of medicinal plants (Berimavandi et al., 2011; Morteza et al., 2009; Okoh et al., 2008). In *Satureja hortensis*, the EO content and EO yield significantly increased in response to higher planting densities (Karimi et al., 2021). In cumin seeds (Hashemi et al., 2008) and *Anethum graveolens* L. (El-Zaeddi et al., 2017), EO compounds were affected by plant densities. Nonetheless, in *Origanum vulgare*, the planting density did not affect EO compounds (Tuttolomondo et al., 2016). The response of plants to density in terms of EO compounds is variable, however, depending on the plant species and ecological conditions such as duration, intensity, direction, and frequency of light (Zoratti et al., 2014).

Conclusion

The use of rotten cow manure (30 tons ha^{-1}) and enriched straw by ammonium sulfate (12 tons ha^{-1}) was found to be suitable for the cultivation of creeping savory. The treatments assisted in the increase of EO production and made the cultivation economical in dryland farming. A high amount of EO yield (72.59 kg ha^{-1}) was obtained from enriched straw and by the planting density of 8 plants per m^2. A high percentage of EO (3.75 g/100 g) was obtained from the treatment of rotten cow manure and by the planting density of 8 plants per m^2.

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Conflict of interest
The authors declare that they have no conflict of interest.

References

Adams RP. 2017. Identification of essential oils by ion trap mass spectroscopy. Academic Press, San Diego, CA., USA.

Aflatuni A, Uusitalo J, Hohtola A. 2005. Variation in the amount of yield and in the extract composition between conventionally produced and micropropagated peppermint and spearmint. Journal of Essential Oil Research 17(1), 66-70. https://DOI:10.1080/10412905.2005.9698833

Alizadeh A, Khoshkhui M, Javidnia K, Firuzi O, Tafazoli E, Khalighi A. 2012. Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (*Lamiaceae*) cultivated in Iran. Journal of Medicinal Plant Research 4(1), 33-40. https://doi.org/10.5897/JMPR09.361.

Anwar M, Patra D.D, Chand S, Khanuja S.P.S. 2005. Effect of organic manures and inorganic fertilizer on growth, herb and oil yield, nutrient accumulation, and oil quality of French basil. Communications in Soil Science and Plant Analysis 36(13-14), 1737-1746. https://doi.org/10.1081/CSS-200062434.

Bahtiyarca Bagda R. 2010. Essential oil composition of culture materials of *Satureja spicigera* (c. Koch) Boiss. from Turkey. Sixth Conference on Aromatic and Medicinal Plants of Southeast European Countries, Antalya, Turkey.

Berimavandi AM, Hashemabadi D, Facouri-Ghaziani MV, Kavian B. 2011. Effects of plant density and sowing date on the growth, flowering and quantity of essential oil of *Calendula officinalis* L. Journal of Medicinal Plants Research 5(20), 5110-5115. https://doi.org/10.5897/JMPR.9000534.

Callan NW, Johnson LD, Westcott Welty LE. 2007. Herb and oil composition of dill (*Anethum graveolens* L.): effects of crop maturity and plant density. Industrial Crops and Products 25(3), 282– 287. https://doi.org/10.1016/j.indcrop.2006.12.007.

Chandini B, Randeep K, Ravendra K, Pantnagar OP. 2019. The impact of chemical fertilizers on our environment and ecosystem. Research Trends in Environmental Sciences, Second Edition, Chapter 5, 69-86.

Chaudhry MA, Rehman A, Naeem MA, Mushtaq N. 1999. Effect of organic and inorganic fertilizers on nutrient contents and some properties of eroded loess soils. Pakistan Journal of Soil Science 16, 63-68.

Ciampitti IA, Vyn TJ. 2012. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: a review. Field Crops Research 133, 48–67. https://doi:10.1016/j.fcr.2012.03.008.

Davis P. 1982. Flora of Turkey (*S. spicigera* (C. Koch) Boiss.). Edinburgh University Press 7, 320-321.

Dhaliwal SS, Naresh RK, Mandal A, Singh R, Dhaliwal MK. 2019. Environmental and sustainability indicators dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: a review. Environmental and Sustainability Indicators 1(2), 1-14. https://doi.org/10.1016/j.indic.2019.100007.

El-Zaeddi H, Martínez-Tomé J, Calín-Sánchez A, Burló F, Carbonell-Barrachina A. 2017. Irrigation dose and plant density affect the volatile composition and sensory quality of dill (*Anethum graveolens* L.). Journal of the Science of Food and Agriculture 97(2), 427-433. https://doi.org/10.1002/jsfa.7890.

Esmaielpour B, Rahmanian M, Khorramdel S, Gharavi H. 2018. Effect of organic fertilizers on nutrient content and essential oil composition of savory (*Satureja hortensis* L.). AgriTECH 38(4), 433-441. https://doi.org/10.22146/agritech.28324.

Faker Baher Z, Rezaei MB, Abbasizadeh B. 2001. Quantitative and qualitative evaluation of the essential oil of savory (*S. hortensis*). Iranian Journal of Medicinal and Aromatic Plants 11, 37-51.

Fallaha S, Rostaei M, Lorigooini, Abbasi Surkia A. 2018. Chemical compositions of essential oil and antioxidant activity of dragonhead (*Dracocephalum moldavica*) in sole crop and dragonhead-soybean (Glycine max) intercropping system under organic manure and chemical fertilizers. Industrial Crops and Products 115, 158-165. https://doi.org/10.1016/j.indcrop.2018.02.003.

Farzaneh M, Kiani H, Sharifi R, Reisi M, Hadian J. 2015. Chemical composition and antifungal effects of three species of Satureja (*S. hortensis*, *S. spicigera*, and *S. khuzistanica*) essential oils on the main pathogens of strawberry fruit. Postharvest Biology and Technology 109, 145-151. https://doi.org/10.1016/j.postharvbio.2015.06.014.

Fernandez R, Scull R, Gonzales J.L, Crespo M, Sanchez E, Carballo C. 1993. Effect of fertilization on yield and quality of chamomile (*Matricaria reculita* L.): aspects of mineral nutrition of the crop. Memorias 11th Congreso Latinoamericano de la Ciencia del Suelo, Second Edition, Congresso Cubcno de la Ciencia del Suelo, Berlin, Germany, 891-894.

Gholami Sharafkhanah A, Jahan M, Banaian Aval M, Kochaki A, Rezvani-Moghaddam P. 2015. Effect of organic, biologic and chemical fertilizer on agroecology and essential oil yield in *Saturja hortensis* L. in Mashhad conditions. Journal of Agroecology 7(2), 179-189. https://agry.um.ac.ir/article_34725.html?lang=en#:~:text=10.22067/JAG.V7I2.50231.

Giovannini Costa G, Kelly Vilela S, Bertolucci SKW, Chagas JH, Oliveira Ferraz E. 2013. Biomass production, yield and chemical composition of peppermint essential oil using different organic fertilizer sources. Agricultural Sciences 37(3), 202-210.

https://doi.org/10.1590/S1413-70542013000300002.

Gokturk T. 2021. Chemical composition of *Satureja spicigera* essential oil and its insecticidal effectiveness on *Halyomorpha halys* nymphs and adults. Zeitschrift Fur Naturforschung. C, Journal of Biosciences 25(76), 451-457. https://doi.org/10.1515/znc-2021-0041.

Hashemi P, Yarahmadi A, Azizi KH, Sabouri B. 2008. Study of the effects of N fertilization and plant density on the essential oil composition and yield of *Cuminum cyminum* L. seeds by HS–SME. Chromatographia 67, 253–257. https://doi.org/10.1365/s10337-009-1269-7.

Karimi A, Gholamalipour Alamdar, E, Avarseji Z, Nakhzari Moghaddam A. 2021. Effect of planting density and number of hand weeding times on weeds control and relationship between morphological characteristics with content and yield of essential oil of *Satureja hortensis* L. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 69, 417-426. https://doi:10.11118/actaun.2021.039.

Keshavarz H, Modarres-Sanavy SAM, Mahdipour-Afra M. 2019. Organic and chemical fertilizers affected yield and essential oil of two mint species. Journal of Essential Oil Bearing Plants 21, 1674-681. https://doi.org/10.1080/0972060X.2018.1497545.

Khademi Doozakhdarreh SF, Khorshidi J, Morshedloo MR. 2022. Essential oil content and components, antioxidant activity and total phenol content of rosemary (*Rosmarinus officinalis* L.) as affected by harvesting time and drying method. Bulletin of the National Research Centre 46, 199. https://doi.org/10.1186/s42269-022-00902-0

Khorshidi J, Fakhr Tabatabaei M, Omidbaigi R, Sefidkon F. 2009. Effect of densities of planting on yield and essential oil components of fennel (*Foeniculum vulgare Mill* var. Soroksary). Journal of Agricultural Science 1(1), 152-157. https://DOI:10.5539/jas.v1n1p152.

Kizil S, Toner O. 2005. Effect of row spacing on seed yield, yield components, fatty oil and essential oil of *Nigella sativa* L. Crop Research (Hisar) 30, 107-112.

Mallanagouda B. 1995. Effects of NPK and FYM on growth parameters of onion, garlic and coriander. Journal of Medicinal and Aromatic Plant Science 4, 916-918.

Morteza E, Akbari G.A, Modares Sanavi S.A.M, Aliabadi Frahani H. 2009. Effects of sowing date and planting density on quality features in valerian (*Valeriana officinalis* L.). Journal of Ecology and the Natural Environment 1(9), 201-205.

Nasir Khan M., Mobin M, Zahid A. 2017. Fertilizers and their contaminants in soils, surface and groundwater. Earth Systems and Environmental Sciences https://doi.org/10.1016/B978-0-12-409548-9.09888-2.

Nejatzadeh F. 2015. Effect of biological and chemical nitrogen fertilizers on growth, yield and essential oil composition of dill (*Anethum graveolens* L.). New Cellular and Molecular Biotechnology Journal 5(19), 77–84. http://dorl.net/dor/20.1001.1.22285458.1394.5.19.7.9.

Nigussie A, Lule B, Gebre A. 2015. Effect of plant population density on growth and yield of artemisia (*Artemisia annua* L.). International Journal of Advanced Biological and Biomedical Research 3(4), 384–390. http://dx.doi.org/10.18869/IJABBR.2015.384.

Okoh OO, Saelimenko AP, Asekun OT, Afolayam A.J. 2008. The effects of drying on the chemical components of essential oils of *Calendula officinalis* L. African Journal of Biotechnology, 7(10), 1500-1502.

Omidbigi R. 2008. Production and processing of medicinal plants. Volume 3, Fifth Edition, Astan Quds Razavi Publications. Mashhad, Iran, 397. (In Persian).

Osakabe Y, Osakabe K, Shinozaki K, Tran LS. 2014. Response of plants to water stress. Frontiers in Plant Science 5 (86), 1-8. https://doi.org/10.3389/fpls.2014.00086.

Poshtdar A, Abdali Mashhadi A, Moradi F, Siadat SA, Bakhshandeh A. 2016. Effects of different sources of nitrogen fertilizer and applied rates on essential oil content and composition of peppermints. Journal of Herbal Drugs 7(1), 51-57.

Ram M, Ram D, Roy SK. 2003. Influence of organic mulching on fertilizer nitrogen use efficiency and herb and essential oil yields in geranium (*Pelargonium graveolens*). Bioresource Technology 87(3), 273-278.

https://doi.org/10.1016/S0960-8524(02)00232-8.

Rossini MA, Maddonni GA, Otegui ME. 2011. Inter-plant competition for resources in maize crops grown under contrasting nitrogen supply and density: variability in plant and ear growth. Field Crops Research 121, 373–380.

https://doi:10.1016/j.fcr.2011.01.003.

Rostaei M, Fallah S, Lorigooini Z, Abbasi Surkia A. 2018. The effect of organic manure and chemical fertilizer on essential oil, chemical compositions and antioxidant activity of dill (*Anethum graveolens*) in sole and intercropped with soybean (Glycine max). Journal of Cleaner Production 199, 18-26. https://doi.org/10.1016/j.jclepro.2018.07.141.

Roussos P.A, Gasparatos D, Kechrologou K, Katsenos P, Bouchagier P. 2017. Impact of organic fertilization on soil properties, plant physiology and yield in two newly planted olive (*Olea europaea* L.) cultivars under Mediterranean conditions. Scientia Horticulturae 220, 11–19. https://doi:10.1016/j.scienta.2017.03.019.

Said-Al Ah HAH, Hussein MS, Abd El-Kader AA. 2010. Effect of nitrogen fertilizer and/or some foliar application on growth, herb yield, essential oil and chemical composition of dragonhead. Journal of Medical Food 2(1), 12-28.

Saki A, Mozafari H, Karimzadeh-Asl K, Sani B. 2019.

Plant yield, antioxidant capacity and essential oil quality of *Satureja mutica* supplied with cattle manure and wheat straw in different plant densities. Communications in Soil Science and Plant Analysis 50 (1), 1-11. https://DOI:10.1080/00103624.2019.1670835.

Sefidkon F, Jamzad Z. 2004. Essential oil composition of *Satureja spicigera* (C. Koch) Boiss. from Iran. Flavour and Fragrance Journal 19, 571–573. https://doi.org/10.1002/ffj.1357.

Seif Sahandi M, Naghdi Badi H, Mehrafarin A, Khalighi-Sigaroodi F, Sharif M. 2019. Changes in essential oil content and composition of peppermint (*Mentha piperita* L.) in response to nitrogen application. Journal of Medicinal Plants 18(72), 81-97.

http://jmp.ir/article-1-2704-en.html

Skubij N, Dzida K. 2019. Essential oil composition of summer savory (*Satureja hortensis* L.) (cv. Saturn) depending on nitrogen nutrition and plant development phases in raw materials cultivated for industrial use. Industrial Crops and Products 135, 260-270. https://DOI:10.1016/j.indcrop.2019.04.057.

Tawfeeq AM. 2017. Factors affecting essential oil production in rosemary (*Rosmarinus officinalis* L.). Ph.D. thesis, School of Biological Sciences, University of Reading, 171.

Tehrani Sharif H, Sharifi Ashoorabadi E, Tajali AA, Makizadeh Tafti M. 2015. Effect of plant nutrition systems on qualitative and quantitative yield of purple basil (*Ocimum basilicum* L.). Iranian Journal of Medicinal and Aromatic Plant Research 31(2), 283-306.
https://dx.doi.org/10.22092/ijmapr.2015.101470.

Tuttolomondo T, La Bella S, Leto C. 2016. Effects of plant density on the number of glandular trichomes and on yield and quality of essential oils from oregano. Natural Product Communications 11(6), 849-852.

https://doi.org/10.1177/1934578X1601100638.

Yang L, Wen KS., Ruan X, Zhao YX, Wei F, Wang Q. 2018. Response of plant secondary metabolites to environmental factors. Molecules 23(4), 762.

https://dx.doi.org/10.3390%2Fmolecules23040762.

Yousefi B, Sefidkon F, Lebaschy MH, Mirza M. 2021. Effects of different densities and feeding with organic fertilizers on percent, yield and chemical composition of essential oil in *Satureja mutica* under rain-fed conditions. Iranian Journal of Medicinal and Aromatic Plant Research 38(1), 102-118.

https://doi://10.22092/IJMAPR.2021.354198.2976.

Zakerian F, Sefidkon F, Abbaszadeh B, Kalate Jari S. 2020. Effects of water stress and mycorrhizal fungi on essential oil content and composition of *Satureja sahendica* Bornm. Journal of Agricultural Sciences and Technology 22(3), 789-799.

Zoratti L, Karppinen K, Escobar AL, Häggman H, Jaakola L. 2014. Light-controlled flavonoid biosynthesis in fruits. Frontiers in Plant Science 5, 1-16. https://doi:10.3389/fpls.2014.00534