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**Original Article**

# Cattle Manure Influences Plant Yield, Antioxidant Capacity and Essential Oil Quality of Sahandi Savory (*Satureja sahendica* Bornm.) under Different Plant Densities

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## Abstract

Sahandi savory (*Satureja sahendica* Bornm.) is a medicinally endemic plant, which may be affected by organic fertilizers and cultivated methods. To investigate the effect of cattle manure (30 ton/ha) as organic fertilizers and plant density (high plant density: 80000, medium plant density: 40000, and low plant density: 26666 plan $ha^{-1}$), an experiment was carried out as split plot with three replications during 2017 and 2018 on plant yield, total phenol content (TPC), total flavonoid content (TFC), essential oil (EO) yield and composition of Sahandi savory in the rainfed condition. Fresh weigh yield (FWY) and dry weight yield (DWY) increased under manure application at high/medium plant density in second year. The highest TPC and TFC were observed with manure application at high plant density in 2018. The highest DPPH scavenging activity was observed at high plant density under manure application in second year. In second year under manure application, medium and high plant densities increased EO yield by 44% and 35% compared to low plant density. The GC and GC/MS analysis showed that the main constitutes of Sahandi savory EO were p-cymene (36.03-56.37%), thymol (21.59-32.26%) and γ –terpinene (6.21-22.01%), representing different concentrations by using the organic fertilizers and plant densities. To sum up, the use of cattle manure and high plant density can result in higher plant production.

**Keywords:** Essential oil composition, Thymol, Plant density, Total phenol content

## Introduction

Savory (*Satureja* L.) is included in the major taxa of Lamiaceae family, which originates from Mediterranean region [1]. This genus is represented in the flora of Iran with 14 species, eight of them (*S. edmondii, S. intermedia, S. isophylla, S. kallarica, S. bakhtiarica, S. khusstanica, and S. sahendica*) being endemic of Iran [2]. Sahandi savory (*S. sahendica*) is distributed in northwestern and western Iran. It is a late flowering species (late summer and fall), growing on rock walls, and rocky slopes [3]. Sahandi savory is a perennial, branched and bushy aromatic plant with 10 to 30 cm high [4]. The leaves are long, elliptical complete covered with fine hairs [4]. The volatile oils of savory isolated from their aerial parts represent aromatic and medicinal features [5]. The fresh and dried aerial parts have been consumed in many culinary purposes such as stuffings, stews, dishes containing meat and poultry, sausages, and greengroceries [6]. In folk medicine, the leaf and stem of savory have been used for curing diarrhea, inflammation of the stomach and intestines, urinary infections, wounds, asthma, and as antimicrobial, tonic, antiseptic, flatulent, digestive, diuretic, carminative, and aphrodisiac drugs. In addition, we can mention the biological activities including antioxidant, antifungal, antibacterial, antidiabetic and anti-inflammatory properties of savory [7].

The management of plant nutrition with fertilizer is important to optimize the agricultural products [8]. In arid and semi-arid regions like most parts of Iran the soil nutrients are low. Thus, the soil reinforcement with fertilizer allows producing and developing the

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sustainability of crop production [9]. Currently, there is a challenge in Iran to improve the essential oil (EO) productivity and antioxidant capacity of sahandi savory in terms of human health and environmental concerns [10]. Generally, organic fertilizer such as manure can improve the plant productivity with rarely environmental concerns [11,12]. Today, it is recognized that application of cattle manure as sail amendment in plant production is useful with no environmental concerns [12,13].

Organic fertilizers are widely used to improve the agricultural production [12,13]. Although the improvement of EO quality and quantity, and antioxidant capacity with different organic and chemical fertilizers in medicinal plants has been well documented [14, 15, 16, 17, 18], there is little information regarding the cattle manure on plant yield and EO production in medicinal and aromatic plants (MAPs). Recently, MAPs have widely applied for their high capacity in neutralizing the toxic free radicals [19]. Hence, attempts in investigation of natural antioxidants in MAPs have been increased [20].

The cultivation management is a major factor in plant development and yield. Plant density is a management cultivation factor influencing the plant growth with adjusting soil water and nutrients [21]. Plant density induces especial situation for plant with changing the plant environmental and edaphic factors such soil moisture and canopy temperature. Regarding the environmental, geographical and edaphic factors, the plants adopt their appropriate plant density to use the optimum nutrients and water [22].

In the recent years, the excessive use of chemical fertilizers in agricultural systems boosts environmental challenges and concerns globally. The use of organic fertilizers is the strategy to subside the undesirable effects of plant production. There is no published work on the effect of cattle manure, as the main alternatives of chemical fertilizers in Iran, on sahandi savory. Therefore, we investigated the effects of organic fertilizers (cattle manure) and plant density (i) on plant yield, EO yield and composition, and (ii) on antioxidant activity of sahandi savory in rainfed condition.

## Material and Methods

### Plant Material and Growth Condition

Sahandi savory seeds were obtained from Research Institutes of Forests and Rangelands (RIFR), Iran. They were sown in the foam trays filled with a mixture of peat moss and vermiculite (1:1 volume) at a greenhouse condition in RIFR. Two-month seedlings were transplanted in the open field in Absard (52º 20″ E and 35º 42″ N), a city in the central district of Damavand county, Tehran province, in April 2017. The mean annual temperature was 11 °C with the minimum and maximum as -24 °C in January–February and 37 °C in July–August, respectively. Mean annual rainfall was 333 mm (Table 1). PH and EC of the soil (0-20 cm) was respectively 8.3 and 0.8 ds $m^{-1}$.

**Table 1** Meteorological information of the planting area

| Year | Average Temperature (Centigrade) | Annual rainfall (mm) |
|---|---|---|
| 2017 | 12.38 | 321.5 |
| 2018 | 11.95 | 487 |

### Treatment Details

The present study was conducted as split plot design in three replications during 2017 and 2018. The main plot was fertilized application in two levels (control: no manure application, cattle manure: 30 t/ha) and sub plot was plant density in three levels (high density: 80000 plant/ha, medium density: 40000 plant/ha, and low density: 26666 plant/ha). Cattle manure was added the soil before transplanting. During the experiment, no pesticide and chemical fertilizers were used and we manually controlled the weeds. In both years, plants were harvested at flowering stage above the soil surface at September.

### Fresh Weight Yield (FWY) and Dry Weight Yield (DWY)

To measure fresh weigh, plants were cut from the bottom of stem, and to measure dry weight they immediately were maintain in the envelopes and dried at 40º C in the dark condition till got a constant weight [12].

### Determination of Total Phenolic Content (TPC)

Folin–Ciocalteu reagent was selected to measure TPC spectrophotometrically [23]. 100 µl of the MeOH solution of the precisely measured weight of investigated plant 1–10 (2.54, 2.58, 2.25, 4.03, 4.80, 2.13, 4.62, 1.47, 1.58, 15.05 mg/mL respectively) were mixed with 0.75 mL of Folin–Ciocalteu reagent and allowed to stay at 22º C for 5 min. The mixture was supplied with 0.75 ml of $NaHCO_3$. Absorbance was measured at 725 nm by UV–VIS spectrophotometer (Varian Cary 50) after 90 min at 22 ºC. Standard curve was calibrated by Gallic acid (0–100 mg/; r > 0.99). The results were represented as mg Gallic acid (GA) /g Dry weight. (Fig. 1)

### Determination of Total Flavonoid Content (TFC)

The method with aluminum chloride was applied to measure the total flavonoid content [24]. Briefly, the mixture containing 0.5 mL of sample and 300 µL of $NaNO_2$ (1:20 w/v) was vortexed for 10 s and left to stand at 24 ºC for 5 min. After that, the reaction mixture was changed by 300 µL of $AlCl_3$ (1:10 w/v), 2 mL of NaOH (1 M) and 1.9 mL of distilled water, and then vortexed for 10 s.

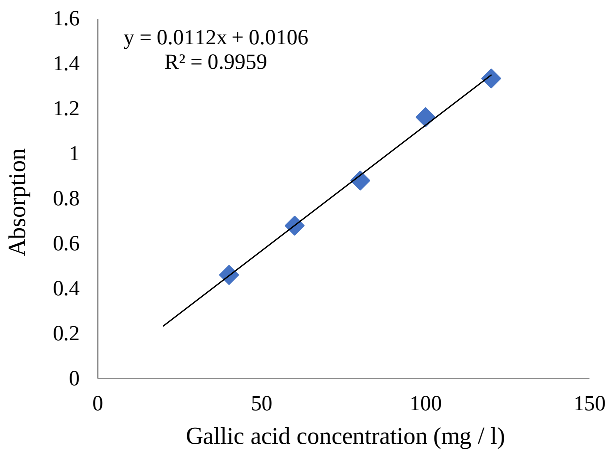


**Fig. 1** Standard Gallic acid curve

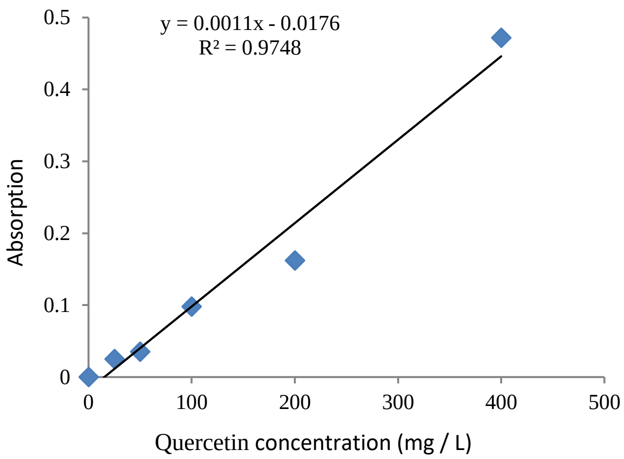


**Fig. 2** The standard curve and standard equation of total flavonoids in quercetin

The absorbance was determined at 510 nm. Quercetin concentrations ranging from 0 to 1200 µg/mL were prepared and linear fit was used for calibration of the standard curve (Fig. 2).

Radical Scavenging Activity

Free radical scavenging activities of the extracts was measured using a DPPH radical described by Brand-Williams *et al*. (1999) 0.1 ml of the extract solution was mixed with 1.0 ml of DPPH solution and 4 ml of methanol. The absorbance wavelength was 517 nm performed by UV–vis spectrophotometer (Varian Cary 50). The scavenging effect was determined as follow:
(1) DPPH scavenging% = [1/ (A 517 nm, sample – A 517 nm, control)] × 100.

Essential oil (EO) Content and Yield

Lemongrass EO content was quantified based using the method described by the European Pharmacopoeia for oil production [25]. Briefly, 100 g of dried aboveground plant parts were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The oil samples were dehydrated by placing them in dark glass bottles containing anhydrous sodium sulfate. The samples were maintained at 4 ºC until they were analyzed by gas chromatography and/or mass spectroscopy. EO yield was determined as the amoun of EO extracted from total dry weight per hectare.

Essential Oil Extraction

In order to identify the EO content, during peak flowering season, 100 g of dried aerial parts from each treatment were hydrodistilled in the Clevenger type apparatus for 3 hr. The EO content was measured and reported as v/w percentage. The EO yield (kg ha−1) was measured with multiplying the EO content with the plant yield of the experimental treatments. Anhydrous sodium sulfate was used to dry EO samples, and finally the samples were stored at 4 °C to further analysis of GC and GC-MS.

Gas Chromatography (GC) Analysis

Thermo-UFM ultrafast gas chromatograph equipment with a ph-5 fused silica column (10m length × 0.1 mm id., film thickness 0.4 µm) was used to analyze EOs. Oven temperature was maintained at 60 ºC for 5 min and then programmed to 285 ºC at a rate of 5 ºC min-1; flame ionization detector (FID) and injector temperature were 290 ºC and 280 ºC, respectively; helium was applied as carrier gas with an inlet pressure of 0.5 kg cm-2.

Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS analyses were accomplished by Varian 3400 GC-MS system equipment with AOC-5000 auto injector and DB-5 fused silica capillary column (30 m × 0.25 mm i.d.; film thicknesses 0.25 µm). Temperature was programmed from 60 °C to 250 °C with 3 °C min-1; Injector and interface temperature were 260 °C and 270 °C, respectively; acquisition mass range of 40–340 amu; ionization voltage of 70 eV; the carrier gas was helium at a velocity of 45 cm sec-1.

Component Identification

Homologous series of n-alkanes (C7–C25) determined the retention index for all volatile constituents. According to Adams, the components of oil were identified by matching their retention indices (RI) and mass spectra. EO components were identified by GC/MS spectroscopy.

Statistical Analysis

The data (n = 3) were subjected to one-way analysis of variance (ANOVA) and using the SAS software package for Windows (SAS, version 9.3, SAS Institute, Cary, NC). Duncan's multiple range tests showed the comparison of mean values. The data were statistically investigated at 5% probability level.

## Results

### The Yield of Fresh and Dry Weight

Fresh weight yield (FWY) was influenced by year, fertilizer, plant density, the mutual interactions of year and fertilizer, fertilizer and density, and tripe interaction of year, fertilizer, and plant density (P≤0.05, Table 3). FWY in second year was higher compared to first year. In plants treated with manure application and low plant density, 2.54 fold of FWY was observed in second year relative to first year. Manure increased the FWY particularly in second year. In second year and high plant density, we found a 58% increase of FWY under manure application relative to no manure application. In second year under manure application, medium plant density increased FWY by 12% in comparison to low plant density (Table 2). Dry weight yield (DWY) was affected by year, fertilizer, plant density, and the interaction of year and fertilizer (P≤0.05, Table 3). In second under manure application, medium and high plant density increased DWY by 20% and 16% compared to no manure application (Table 2).

**Table 2** Fresh weight yield (FWY), dry weight yield (DWY), and branch numbers of sahandi savory under organic fertilizer and plant density

| Year | Organic Fertilizer | Plant density | FWY | DWY | The number of branch |
|---|---|---|---|---|---|
| 2017 | Control | Low | 353.3±24.9 ef | 96.0±5.3 g | 78.7±2.4 f |
| | | Medium | 326.3±26.7 ef | 109.0±2.9 fg | 80.0±2.1 f |
| | | High | 263.3±12.5 f | 128.7±6.5 d-f | 79.0±2.3 f |
| | Manure | Low | 421.3±41.8 e | 113.3±6.7 e-g | 79.7±1.6 f |
| | | Medium | 363.3±9.4 ef | 139.3±2.4 de | 79.7±2.6 f |
| | | High | 306.7±9.8 f | 149.3±3.2 d | 85.3±3.1 f |
| 2018 | Control | Low | 900.0±16.3 c | 208.0±9.6 c | 99.7±2.4 e |
| | | Medium | 683.3±32.9 d | 219.7±6.1 c | 101.7±2.1 de |
| | | High | 650.0±57.1 d | 234.0±4.3 c | 107.0±3.1 cd |
| | Manure | Low | 1066.0±56.3 b | 268.3±16.5 b | 110.1±2.b c |
| | | Medium | 1203.3±77.6 a | 324.7±2.6 a | 115.0±2.1 b |
| | | High | 1031.7±95.1 b | 311.3±7.3 a | 122.7±3.1 a |

**Table 3** Fresh weight yield (FWY), dry weight yield (DWY), and branch numbers of sahandi savory under organic fertilizer and plant density

| Significance | Organic Fertilizer | FWY | DWY | The number of branch |
|---|---|---|---|---|
| | Year (Y) | ** | ** | ** |
| | Fertilizer (F) | ** | ** | ** |
| | Y*F | ** | ** | ** |
| | Density (D) | ** | ** | ** |
| | Y*D | ns | ns | ns |
| | F*D | ** | ns | ns |
| | Y*F*D | ** | ns | ns |

**Table 4** Total phenolic content (TPC), Total flavonoid content (TFC), and DPPH scavenging activity of sahandi savory under organic fertilizer and plant density

| Year | Organic Fertilizer | Plant density | TPC (mg GA/g DW) | TFC (mg QE/g DW) | DPPH scavenging activity (%) |
|---|---|---|---|---|---|
| 2017 | Control | Low | 25.5±1.5 e | 6.2±0.48 f | 73.7±1.2 f |
| | | Medium | 27.6±1.5 e | 7.0±0.35 ef | 80.3±6.3 c-e |
| | | High | 27.5±1.5 e | 6.4±0.20 f | 76.0±1.4 ef |
| | Manure | Low | 30.8±1.2 d | 6.8±0.08 ef | 78.3±1.6 d-f |
| | | Medium | 32.4±1.1 cd | 7.5±0.08 cd | 77.3±1.6 d-f |
| | | High | 33.6±0.7 bc | 7.4±0. 31 de | 77.7±1.6 d-f |
| 2018 | Control | Low | 25.6±1.5 e | 8.0±0.16 cd | 78.7±1.2 d-f |
| | | Medium | 27.2±1.2 e | 8.2±0.21 cd | 82.3±1.2 b-d |
| | | High | 26.2±1.6 e | 8.4±0.12 c | 80.0±1.6 de |
| | Manure | Low | 34.0±1.2 bc | 9.5±0.49 b | 85.0±0.8 a-c |
| | | Medium | 35.7±1.3ab | 10.3±0.61ab | 86.7±1.2ab |
| | | High | 36.7±0.8a | 11.0±1.14a | 87.3±1.7a |

**Table 5** Total phenolic content (TPC), Total flavonoid content (TFC), and DPPH scavenging activity of sahandi savory under organic fertilizer and plant density

| Significance | Organic Fertilizer | TPC (mg GA/g DW) | TFC (mg QE/g DW) | DPPH scavenging activity (%) |
|---|---|---|---|---|
| | Year (Y) | * | ** | ** |
| | Fertilizer (F) | ** | ** | ** |
| | Y*F | ns | ** | * |
| | Density (D) | ** | ** | * |
| | Y*D | ns | ns | ns |
| | F*D | ns | ns | ns |
| | Y*F*D | ns | ns | ns |

Essential Oil (EO) Content and Yield

EO content in second year was higher compared to first year. Increased EO content was found with manure application. In second year under manure application, high plant density increased EO content by 16% compared to low plant density. EO yield in second year was significantly greater than first year (Fig. 3).

The Number of Branches

The number of branches was affected by year, fertilizer, plant density, and the interaction of year and fertilizer (P≤0.05, Table 3). We observed greater branch number in second year in comparison with first year. Manure application increased the number of branches. In second year under low plant density, manure application enhanced the branch number by 12% relative to no manure application. The number of branches increased with enhanced plant density. In plants supplied with manure in second year, high plant density reached the maximum branch number to be 122.7 branches (Table 2).

Total Phenolic Compound (TPC), Total Flavonoid Contend (TFC), and DPPH-Scavenging Activity

TPC was affected by year, organic fertilizer, and plant density (P≤0.05, Table 5). Under low plant density and manure application, TPC in first year plants increased by 13% in comparison to second year plants. We observed the improved TPC under manure application especially in second year. TPC increased by 38% with manure application at high plant density in 2018 (Table 4). Year, organic fertilizer, plant density, and the interaction of year and organic fertilizer significantly changed TFC (P≤0.05, Table 5). TFC in second year was greater than first year. Under manure application and low plant density, 29% augmentation of TFC was observed in second year relative to first year. Boosted TFC was observed with manure application. In second year under high plant density, manure application increased TFC by 30% in comparison to no manure application (Table 4).
DPPH scavenging activity was significantly affected by Year, organic fertilizer, plant density, and the interaction of year and organic (P≤0.05, Table 5). The highest DPPH scavenging activity was observed at low plant density under manure application in second year to be 87.3% (Table 4). We observed the significant increase of EO yield in medium and high plant density.
In second year under manure application, medium and high plant densities increased EO yield by 44% and 35% compared to low plant density (Fig. 4).

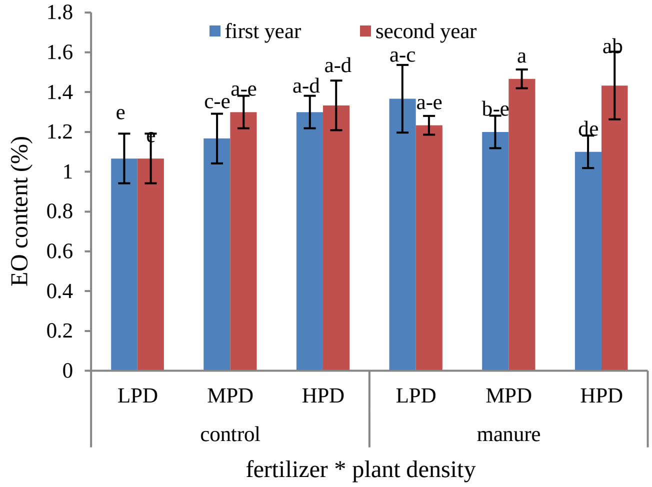


**Fig. 3** Essential oil (EO) content under organic fertilizer and plant density during 2017 and 2018

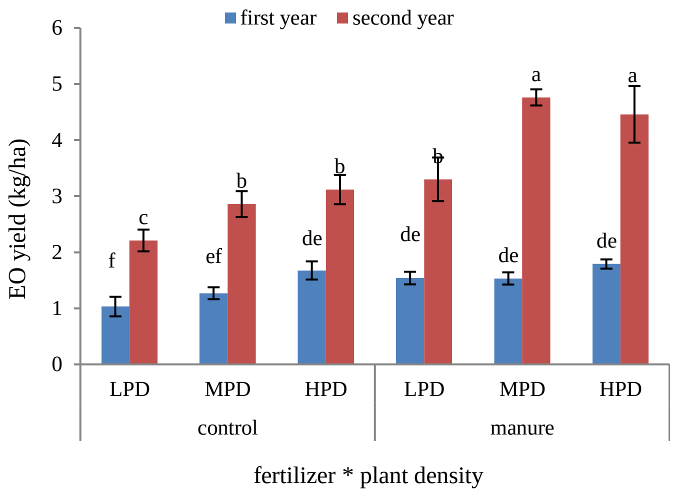


**Fig. 4** Essential oil (EO) yield under organic fertilizer and plant density during 2017 and 2018

Chemical Profile of EO

The GC and GC/MS analysis of *S. sahandica* EO identified 25 compounds (Tables 6 and 7), and their majority belonged to oxygenated monoterpenes. p-cymene (36.03-56.37%), thymol (21.59-32.26%) and γ –terpinene (6.21-22.01%) were the main constitutes of *S. sahandica* EO. p-cymene in first year (48.1%) was higher than that in second year (44.2%). In contrast, γ–terpinene in second year (10.4%) was greater compared to first year (17.2%). Manure application increased p-cymene, but decreased γ–terpinene and thymol. The highest p-cymene was observed at medium plant density in first year (Table 6). γ –terpinene at medium plant density in second year was the maximum to be 22.01% (Table 7). The greatest thymol was found at low plant density as 31.70% (Table 6). β-Pinene, (E-B)-Ocimene, linalool, thymol acetate are the compounds that were appeared at second year. In contrast, camphene, 1,8-cineol, carvacrol methyl ether, β – bisabolene, and trans-sabinene hydrate were the compound that were vanished at second year.

## Discussion

The optimum FWY and DWY were found in plants supplied with cattle manure in second year. Manure due to its essential elements for plant growth such as nitrogen, phosphorus, potassium, and zinc plays a significant role on plant growth. It also enriches the soil by increasing the organic matter, which can improve the physicochemical properties of soil such as structure, aeration, soil moisture-holding capacity, and nutrients [26, 27].

Rehman *et al*. (2016) indicated the increased plant growth and biomass with manure application [26]. The FWY and DWY in second year (2018) and high plant density was higher than first year (2017) in a similar plant density. In low plant density, we observed the reduction of FWY and DWY because of lower leaf area and later canopy development. In rainfed condition, the open canopy cannot control sunlight rate, and it makes adverse effects on soil properties such as texture, moisture, and nutrients.

Antioxidant capacity increased over time, with manure application and increased plant density.

**Table 6** Essential oil compassion of sahandi savory under manure fertilizer and plant density (HPD: high plant density, MPD: medium plant density, LPD: low plant density) in 2017.

| Compound | RI (Retention index) | Control | | | Manure application | | |
|---|---|---|---|---|---|---|---|
| | | HPD | MPD | LPD | HPD | MPD | LPD |
| α-Thujene | 934.61 | 1.11 | 1.05 | 0.54 | 0.88 | 0.68 | 0.54 |
| α-Pinene | 946.09 | 0.75 | 0.78 | 0.57 | 0.65 | 0.57 | 0.53 |
| Camphene | 962.66 | 1.78 | 0.17 | - | 0.12 | 0.08 | - |
| Myrcene | 976.79 | 1.84 | 1.65 | 1.15 | 1.43 | 1.56 | 1.25 |
| α -Phellandrene | 1007.94 | 0.18 | 0.21 | 0.28 | 0.18 | 0.22 | 0.20 |
| α -Terpinene | 1043.62 | 1.38 | 0.88 | 0.50 | 1.34 | 1.16 | 0.68 |
| p-Cymene | 1053.41 | 47.57 | 56.37 | 48.68 | 43.13 | 44.36 | 48.55 |
| Limonene | 1055.84 | 0.26 | 0.16 | 0.18 | 0.30 | 0.26 | 0.25 |
| γ-Terpinene | 1084.13 | 13.22 | 8.46 | 6.21 | 14.75 | 12.71 | 7.41 |
| Cis-Sabinene hydrate | 1100.00 | 0.18 | 1.81 | 0.35 | 0.19 | 0.24 | 0.23 |
| *Trans*-Sabinene hydrate | 1130.48 | - | 0.13 | 0.23 | - | - | 0.19 |
| Borneol | 1219.85 | 0.33 | 0.41 | 0.44 | 0.37 | 0.28 | 0.35 |
| Terpinen-4-01 | 1225.44 | - | 0.27 | 0.30 | 0.20 | - | 0.28 |
| Thymol | 1320.15 | 26.53 | 23.48 | 31.70 | 27.13 | 30.95 | 32.26 |
| Carvacorol | 1330.04 | 1.12 | 1.02 | 1.52 | 3.72 | 1.11 | 1.80 |
| Thymol-acetate | 1363.79 | 0.39 | 0.16 | 0.46 | 0.40 | 0.44 | 0.49 |
| (E) - Caryophyllene | 1476.54 | 0.85 | 0.78 | 0.70 | 0.92 | 0.78 | 0.39 |
| β - bisabolene | 1534.32 | 0.21 | 0.13 | 0.16 | 0.19 | 0.15 | - |
| Spathulenol | 1649.09 | 0.15 | 0.17 | 0.36 | 0.31 | 0.44 | 0.37 |
| Caryophyllene Oxide | 1659.41 | 0.55 | 0.86 | 1.55 | 1.08 | 1.17 | 1.07 |
| Total | - | 98.53 | 98.95 | 95.88 | 97.62 | 97.33 | 96.84 |

**Table 7** Essential oil compassion of *S. sahandica* under manure fertilizer and plant density (HPD: high plant density, MPD: medium plant density, LPD: low plant density) in 2018.

| Compound | RI (Retention index | Control | | | Manure application | | |
|---|---|---|---|---|---|---|---|
| | | HPD | MPD | LPD | HPD | MPD | LPD |
| α-Thujene | 1.36 | 1.08 | 1.00 | 1.08 | 0.99 | 0.90 | 937.56 |
| α-Pinene | 0.98 | 0.71 | 0.67 | 0.76 | 0.76 | 0.69 | 946.29 |
| β-Pinene | 2.21 | 1.86 | 1.73 | 1.75 | 1.77 | 1.59 | 982.42 |
| Myrcene | 0.30 | 0.21 | 0.20 | 0.26 | 0.27 | 0.25 | 1010.34 |
| α -Phellandrene | - | - | 1.44 | - | - | - | - |
| α -Terpinene | 1.79 | 1.68 | - | 1.45 | 1.97 | 1.22 | 1045.08 |
| p-Cymene | 47.49 | 43.49 | 47.50 | 48.74 | 36.03 | 42.09 | 1054.62 |
| (E-B)-Ocimene | - | 0.24 | 0.16 | 0.16 | 0.21 | 0.14 | 1063.99 |
| Limonene | 0.30 | - | - | - | - | - | 1056.98 |
| γ-Terpinene | 18.84 | 18.23 | 15.18 | 15.32 | 22.01 | 14.03 | 1084.53 |
| *cis*-Sabinene hydrate | - | 0.16 | 0.22 | 0.16 | 0.15 | 0.22 | 1100.00 |
| Linalool | 0.39 | 0.39 | 0.46 | 0.48 | 0.37 | 0.45 | 1112.92 |
| Borneol | - | 0.37 | 0.41 | 0.50 | 0.37 | 0.36 | 1224.81 |
| Terpinen-4-01 | 0.54 | 0.79 | 0.81 | 0.75 | 0.61 | 0.94 | 1309.85 |
| Thymol | 21.59 | 26.05 | 25.74 | 24.04 | 29.86 | 31.14 | 1322.00 |
| Carvacorol | 1.11 | 1.06 | 1.09 | 0.97 | 0.82 | 1.29 | 1326.82 |
| Thymol-acetate | 0.88 | 0.98 | 0.67 | 0.62 | 1.07 | 0.88 | 1364.32 |
| (*E*) - Caryophyllene | 0.69 | 0.75 | 0.89 | 0.77 | 0.85 | 0.84 | 1480.12 |
| Spathulenol | 0.51 | 0.28 | 0.25 | 0.34 | 0.37 | 0.31 | 1654.17 |
| Caryophyllene Oxide | 0.47 | 0.69 | 0.71 | 0.80 | 0.62 | 0.89 | 1664.11 |
| Total | 99.44 | 99.03 | 99.13 | 98.97 | 99.08 | 98.24 | 22740.93 |

Flavonoids and phenolics contents increased with manure application. It is proved that the greater amounts of phenolic in sahandi savory can be characterized by the role of organic fertilizers in the secondary metabolism of plants which induces the acetate shikimate pathway, producing more flavonoids and phenolics [30]. Additionally, because of the higher photo–pathogenic stress in organic farming, this in turn may induce abiotic stress, and increase phenolics [31]. Organic fertilizers increased antioxidant potential of broccoli [31], *Satureja hortensis* L. [32] and fennel [33]. Antioxidant activity of second year was higher in respect to first year. Antioxidant activity of plants can increase by time due to the change in physiological responses of plants to the duration that plant is exposed the environmental parameters such as temperature and light [31]. Antioxidant activity Increased with boosted plant density. The increased competition of plants to obtain sufficient light, nutrients, and water is associated with high plant density, which results in raising the antioxidant activities such as TPC. The increased TPC due to high plant density was observed by Taleie *et al*. (2012) in Stevia rebaudiana Bertoni [34], Lombardo *et al*. (2009) in globe artichokes [35], and by Danesi *et al*. (2014) in palm tree kale [36].

Increased EO yield was observed in plants supplied with the interaction of manure application and second year relative to non-application of manure and first year. Previous studies have revealed improved EO quantity and quality of some medicinal plants [12,37-40]. Manure upgrades the soil characteristics along with increasing the benefit microorganism in rhizosphere. It improves the plant growth, nutrients uptake, and stimulates physiological and biochemical pathways of plants such as EO production [41]. In the present study, we observed an increased EO yield at high plant density. EO production is influenced by the interaction of various factors like plant ontogeny, site, photosynthesis rate, photoperiodic modulation, moisture, salinity, temperature etc. In general, the factors affecting photosynthesis can promote the EO production. Plant density by changing the canopy influences the sunlight rate, soil moisture, canopy temperature, soil nutrients, which finally changes the photosynthesis and EO production [42].

The main EO components of sahandica savory were thymol, p-cymene, γ-terpinene. Sefidkon *et al*. (2004) indicated that the main constituents of sahandica savory

EO were thymol (19.6–41.7%), p-cymene (32.5–54.9%) and γ-terpinene (1.0–12.8%), which is similar to our work [3]. In present study, we observed different amounts of main constituents under year, manure application, and plant density. Askary *et al*. (2018) showed that 30 t/ha manure increased the thymol of Thymus daenensis and Thymus vulgari EOs. The change in EO composition under different treatments is associated with the organic fertilizer of the given compound and plant condition [12]. The difference in chemical composition of EO is dependent on the physiology of the whole plant [16]. Cattle manure influences the major compound, due to the fact this organic fertilizer release minerals and conserve soil moisture that affect the secondary metabolism of plants [43, 44]. In addition, the cellular metabolism and biomass production will be improved by these advantages. Consequently, an enhanced vegetative growth with an increase of glandular trichomes is observed [15, 43, 44, 45]. There was a different responses of plant over the time. Unlike our study, Saki *et al*. (2019) found that thymol identified of *Satureja mutica* EO in second year was higher than first year due to the enhanced oil glands and improved terpene biosynthesis. Askary *et al*. (2018) found the highest carvacrol in no application of manure and first year [12]. It is reported that use of fertilizers significantly changes the aroma profile of the Eos [43, 44, 46].

## Summary

The present study attempts to find the best plant density on growth and secondary products of sahandi savory under manure application. Plant density is an eminent factor in determining the quality and quantity of plants. In addition, organic fertilizers due to its environmental-friendly effects are strongly capable to be used as an alternative of chemical fertilizers. Therefore, we evaluated the effect of plant density and cattle manure on growth and EO of sahandi savory. We observed that high plant density and manure application caused the highest FWY and DWY. To obtain optimum antioxidant capacity and EO quality and quantity, we can use medium/high plant density with manure application and harvest time in second year.

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