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Evaluation of Essential Oil, Morphological Traits and Crop Yield in *Satureja mutica* Fisch. & C. A. Mey. in Rainfed Cultivation under Different Row Spacing

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ABSTRACT

Rainfed cultivation of drought-tolerant medicinal plants is a way to decrease of using water in agricultural systems. *Satureja mutica* (white savory) needs little water requirement and it is tolerant to drought. To investigate the change in essential oil (EO) quality and quantity, plant yield and yield components of white savory under different planting densities in rainfed cultivation, an experiment was conducted by completely randomized factorial design with three replications during 2017- 2018 and 2018- 2019 crop years. The row spacing treatments were 25×50 cm, 50×50 cm and 75× 50 cm. Results showed that the highest percentage of plant survival (63.37%) was observed in the lowest density (75×50 cm). The highest plant height (35.40 cm) was observed in the second year× low density. The highest average crown diameter (34.35 cm), the highest fresh weight of the plant (49.01 g), and the maximum plant dry weight (23.54 g) were obtained in the treatments of medium density planting× second year. The highest wet yield (2940.46 $kgha^{-1}$), dry yield (1319.00 $kgha^{-1}$) and EO yield (26.62 $kgha^{-1}$) were obtained in the treatment of high planting density× year2. The highest EO percentage (2.04%) was found in the treatment of low planting density (75×50 cm). the highest percentage of thymol (57.7%) was obtained in 50 × 50 cm row spacing and the highest percentage of carvacrol (7.59%) was obtained from 75 × 50 cm row spacing. In rainfed conditions, the suitable distance between the cultivation lines is 25 × 50 cm.

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1. Introduction

The cultivation of economic plants with low water requirements that can be grown in dry land conditions is a way to protect water resources. *Satureja mutica* Fisch. & C. A. is an Iranian native plant used in medicinal and health industries (Gohari *et al.*, 2011), and grows in the north of Iran (Gohari *et al.*, 2011), Caucasus and Turkmenistan (Jamzad, 2012). The white savory has little Water requirement and as a drought-resistance valuable medicinal plant can be cultivated in dry farming conditions (Karimi *et al.*, 2022).

Plant density is a management cultivation factor influencing plant growth. Plant yield is influenced by inter-specific competition for environmental growth factors and the maximum yield is achieved when this competition is minimized (Villalobos *et al.*, 2017). The plant spacing is an important factor in determining the microenvironments in the field. Planting density affects the absorption of radiation, water and nutrients in plants by changing the crown and root system structure (Wu *et al.*, 2016). Increasing planting density increases competition among plants for light, water, and nutrients (Ciampitti and Vyn, 2012; Rossini *et al.*, 2011) and subsequently cause abiotic stress in plants and decrease the 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 sunlight. The ability of the plant to collect environmental resources, including solar radiation energy, water, organic and inorganic nutrients depend

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on plant spacing (De-Yang *et al.*, 2016). High-density planting increases the competition between plants for resources, which results in limited resources being discharged (Jiang *et al.*, 2013). The improved canopy construction can lead to an optimal leaf area index that can boost photosynthetic ability through efficient solar radiation interception (Wang *et al.*, 2015). Although lower plant densities, in general, leads to the growth of larger single plants, optimal density encourages better yield per unit area (Yousefi *et al.*, 2021; Qiu *et al.*, 2013).

Based on a model presented by Deng et al. (2012b) when planting density is less than optimal, biomass yield per area increases linearly with density, and when planting density is greater than optimal plant density, the yield of the plant is reduced by the ratio of -1/3 second exponent of plant density. Therefore, it is important to find the optimal density for the cultivation of plant species under specific ecological and environmental conditions. In rainfed cultivation, due to the limitation of nutrient resources and soil moisture, the presence of optimal density to better absorption of nutrients, and better use of soil water is more important to achieve the max yield production (Naghdi Badi *et al.*, 2004).

Little information is available about the effects of row spacing on yield traits of *Satureja mutica* plants. To fill this information gap, this field experiment was conducted.

2. Materials and methods

2.1. Experimental designs and treatments

This experiment was performed as a completely randomized factorial design with three planting densities and three replications. The experiment was carried out in the two crop years (2017-2018 and 2018-2019). The row spacing treatments included three densities: LD = 50 × 75 cm row spacing, MD = 50 × 50 cm row spacing and HD = 50 × 25 cm row spacing. The area of each plot was 3 × 4 = 12 m^2.

2.2. Experimental conditions and plant material

The seeds of white savory, provided by the Forests and Rangelands Research Institute of Iran, 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 a temperature of 18-24 C° and a humidity of 35%.

In the early spring, the seedlings were transferred to the research field in Mehregan Research Station of Kermanshah Agricultural and Natural Resources Research and Education Center at 34˚, 9ˊ latitude, 47˚, 9ˊ longitude, and 1270 m altitude.

The results of farm soil analyses are presented in Table 1. The meteorological statistics for two experimental crop years, (Table 2) and monthly and annual rainfall (Table 3) were obtained from the nearest weather station (Kermanshah weather station). Mechanical methods were used for controlling weeds and no fertilizers, herbicides or pesticides were used during the project.

Table 1. Physical and chemical characteristics of fertilizers treatment and control

Fertilizer treatment	Soil Texture	EC (ds/m)	pH	Absorbable phosphor (ppm)	Absorbable potassium (ppm)	Organic carbon (%)
Farm soil (without fertilizer)	Silty-Clay	0.70	7.03	12.20	520	1.13

2.3. Measurement of morphological traits and calculation of yield traits

Various morphologic traits, including plant height, crown diameter, average wet weight and average dry weight of the plant were measured, and then wet yield ($kgha^{-1}$) and dry yield ($kgha^{-1}$) were calculated.

2.4. Essential oil extraction

After the plants reach the 50% flowering stage, in late August, savory plants were cut, 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 the net weight of the essential oil was calculated and kept in a refrigerator (4 °C).

2.5. EO percentage and EO yield calculation

The EO percentage was calculated by the W/W method using the following formula:

EO% = EO weight (g) / plant dry weight (g) ×100 (Khademi Doozakhdarreh *et al.*, 2022). The EO yield was obtained by multiplying the EO percent by the dry plant yield per hectare (Yousefi *et al.*, 2021).

2.6. GC and GC/MS analysis

After diluting 1 μL of essential oil in 2 μL of dichloromethane, samples were analyzed by a gas chromatograph with specifications (Ultra Fast Model) Thermo-UFM and with Chrom-Card A/D data processor, cap-column Ph-5 (non-polar) made by Thermo Company with a length of 10m and an inner diameter of 0.1 and 0.4 µm thickness, the inner surface of the device was coated with a stationary phase of 5% dimethyl siloxane phenyl made by Thermo Company, Italy. Column temperature program: Initial temperature 60 °C, start to final temperature 285 °C, which added every minute 80 °C to them, then stopped at this temperature for 3 minutes, detector type was FID with 290 °C. The temperature of the injection chamber was 280°C, the carrier gas was helium and the inlet pressure to the column was set at 0.5 kg cm^{-2} (Zakerian *et al.*, 2020).

GC/MS device was Varian 3400 connected to the mass spectrometer (Saturn II), with an ion telephoto system and ionization energy of 70 electron volts. It has a semi-polar DB-5 column (length 30 m, inner diameter 0.25 mm and thickness of static phase layer equal to 0.25 microns). Column head gas pressure was set at 35 pounds per square inch, temperature 40 °C to 250 °C with increasing speed of 4 °C per minute, injection chamber temperature 260 °C and line transfer temperature 270 °C. The retention indexes were calculated by injection of normal hydrocarbons (C_7-C_{25}) under the same conditions as essential oil injection, by a computer program and in BASIC language. Chemical compounds in EO were identified by comparison of spectra with different sources (Adams, 2017).

2.7. Data analysis

The analyses of variance and Pearson's correlation (p= %5) were done by SPSS (ver.16) software's. Mean values were compared using multiple ranges of Duncan's test. The principal component analysis was performed by Minitab (ver.16) software's.

Table 2. Metrological statistics in Kermanshah weather station

Crop years	Absolute maximum temperature (˚C)	Absolute minimum temperature (˚C)	Average of maximum temperature (˚C)	Average of minimum temperature (˚C)	Average annual temperature (˚C)	Average humidity (%)	Average annual evaporation (mm)	Annual precipitation (mm)	Average of long time annual precipitation
2016-2017	40.8	8.2	24.8	7.7	16.6	37	1931	394.5	470.7
2017-2018	43.5	10.6	23.6	8.3	16.74	45	162.6	434	470.7

Table 3. Monthly and annual distribution rainfall statistics (mm) in Kermanshah weather station

Crop years	October	November	December	January	February	March	April	May	June	July	August	September
2016- 2017	0	1	14.7	75.4	80.8	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

3. Results and discussion

3.1. Analysis of variance

The results of the analysis of variance (Table 4) showed that there are significant differences between years for the traits of plant height, canopy diameter, plant fresh weight, plant dry weight, wet yield, dry yield and essential oil yield per hectare at the level of 1%. The plant survival percentage showed a significant difference at the level of 5% between the years. The EO percentage was not significantly different between the years.

Significant differences were shown among planting row spacing treatments for the traits of plant survival, canopy diameter, plant fresh weight, plant dry weight, wet yield, dry yield and EO yield at the level of 1%, but the traits of plant height and EO percentage had no significant differences among the planting row spacing.

The interaction effect of year × row spacing was significant for plant height, plant dry weight, wet yield, dry yield, and EO yield ($P<0.01$), as well as the plant wet weight and EO ($P<0.05$) (Table 4).

3.2. Comparison of means

The comparison of means by Duncan's test (Table 5) showed that the highest plant height (35.40 cm) was observed in second year× low density. The highest average of crown diameter (34.35 cm), the highest fresh weight of the plant (49.01 g), and the maximum plant dry weight (23.54 g), belonged to treatments of medium density planting× second year. The highest wet yield (2940.46 kgha^{-1}), dry yield (1319.00 kgha^{-1}) and EO yield (26.62 kgha^{-1}) were obtained in the treatment of high planting density× year2

Table 4. Results of the variance analysis for yield and yield components in *S. mutica* plants cultivated under rainfed conditions and different row spacing during two crop years.

Source of variations	df	Plant survival	Plant height	Plant crown	Plant wet weight	Plant dry weight	Wet yield	Dry yield	EO percent	EO yield
Year (Y.)	1	3855.0*	1531.0**	2152.0**	5036.0**	1674**	10900000**	3474000**	0.07^{ns}	1501.0**
Rep. (R.)	4	327.91	29.00	29.87	12.66	8.36	31650	23410	0.04	5.09
Row spacing (R. S.)	2	1568.0**	0.86^{ns}	84.12**	1056.0**	241.6**	9977000**	2498000**	0.05^{ns}	904.6**
Y.× R. S.	2	117.97^{ns}	112.9**	14.35^{ns}	32.52*	26.76**	709600**	187200**	0.15*	53.03**
Error	8	1.42	13.48	8.84	5.40	3.74	1.73	9.23	0.04	6.59
CV %		2.15	11.87	10.22	5.34	9.04	0.07	0.31	10.07	13.22

* and ** = significant difference at the level of 5% and 1% respectively, and ns= no significant difference. EO = essential oil

Table 5. Mean comparison for interaction effect of year and row spacing for yield and yield components in *S. mutica* plants cultivated under rainfed conditions.

Treatments	Means Squares ± SD				
	Plant survival (%)	Plant height (cm)	Plant crown (cm)	Plant wet weight (g)	Plant dry weight (g)
Year1 (Y1)×HD	34.03±9.10^{a}	25.76±0.88^{b}	18.58±2.69^{c}	15.51±2.49^{d}	7.02±0.8^{d}
Y1×MD	51.39±8.67^{a}	32.75±0.7^{ab}	23.83±3.08^{bc}	21.15±3.17^{c}	12.01±2.07^{c}
Y1×LD	47.92±9.55^{a}	28.19±6.45^{ab}	24.12±3.08^{bc}	25.14±3.31^{c}	12.18±2.677^{c}
Year2 (L2)×HD	51.39±5.24^{a}	32.61±3.12^{ab}	32.58±4.5^{a}	36.76±2.19^{b}	16.49±0.687^{b}
Y2×MD	52.78±12.70^{a}	33.81±5.49^{a}	34.35±2.13^{a}	49.01±3.93^{a}	23.54±0.76^{a}
Y2×LD	72.92±23.66^{a}	35.4±2.25^{a}	29.75±4.62^{ab}	48.53±2.56^{a}	23.03±1.28^{a}
Treatments	Means Squares ± SD				
	Wet yield (kg×ha^{-1})	Dry yield(kg×ha^{-1})	EO percent (%)	EO yield(kg×ha^{-1})	
Year1 (Y1)×HD	1240.8±199.39^{c}	561.66±63.64^{cd}	2.00±0.19^{a}	11.14±0.51^{cd}	
Y1×MD	846±126.76^{d}	480.40±82.71^{d}	2.15±0.62^{a}	10.15±2.52^{d}	
Y1×LD	670.29±88.26^{d}	324.70±71.22^{e}	1.98±0.03^{a}	6.43±1.37^{e}	
Year2 (L2)×HD	2940.46±175.25^{a}	1319.00±54.70^{a}	2.02±0.09^{a}	26.66±1.21^{a}	
Y2×MD	1960.5±157.19^{b}	941.38±30.18^{b}	1.84±0.08^{a}	17.30±1.23^{b}	
Y2×LD	1294.12±68.32^{c}	614.11±34.15^{c}	2.15±0.04^{a}	13.19±0.69^{c}	

The common letters show no significant difference between traits (α= 0.05); HD = high density; MD = medium density; LD = low density; EO = essential oil.

3.3. Pearson's correlation estimation

Pearson's correlation estimation (Table 6) showed that plant survival had a significant positive correlation with plant wet weight (r= 0.62**), plant dry weight (r= 0.64**) and plant crown (r= 0.56**). Plant height showed a significant positive correlation with the traits of plant crown (r= 0.72**), plant dry weight (r= 0.47**) and plant wet weight (r= 0.43**) (r= 0.56**). The trait of plant crown showed a highly significant positive correlation with the plant dry weight (r= 0.76**), plant wet weight (r= 0.74**), dry yield (r= 0.37**) and wet yield (r= 0.34**). Plant wet weight had a highly significant positive correlation with the plant dry weight (r= 0.96**). Plant dry weight was positively correlated with the wet yield (r= 0.32*), dry yield (r= 0.39**), and EO yield (r= 0.42**). Plant wet yield showed a highly significant positive correlation (r= 0.98**) with the dry yield and EO yield dry yield (r= 0.94**). The trait of dry yield showed a highly significant positive correlation (r= 0.97**) with the EO yield.

3.4. Principal components analysis for yield traits

Results of PC analysis showed that the PC1 and PC2 had Eigenvalues of more than 1 and in total represented 100% of cumulative variances (Table 7). The traits of Plant survival (0.41), plant wet weight (0.33) and plant dry weight (0.29) had the highest positive contributions; and the traits of plant highet (-0.53), wet yield (-0.47), dry yield (-0.46) and EO yield (-0.44) had the highest negative share in the PC1. The traits of Plant height (0.41), Plant crown (0.51), Plant wet weight (0.43) and Plant dry weight (0.45) had the highest positive share, and EO percent (-0.16) had the highest negative share in the PC2.

The PCA diagram (Fig. 1) showed that the highest EO percentage was obtained in 50×75 cm row spacing (first quarter) The highest EO yield, dry yield and wet yield were obtained from the 50 × 25 cm row spacing treatment (second quarter). The highest value of plant survival, plant wet weight, plant dry weight and plant crown were obtained from 50 × 50 cm row spacing (Fig. 1).

Table 6. Pearson's correlation estimation between morphologic and yield traits in *S. mutica* plants cultivated under rainfed conditions and different row spacing.

Traits	Plant survival	Plant height	Plant crown	Plant wet weight	Plant dry weight	Wet yield	Dry yield	EO percent	EO yield
Plant survival	1								
Plant height	0.30*	1							
Plant crown	0.56**	0.72**	1						
Plant wet weight	0.62**	0.43**	0.74**	1					
Plant dry weight	0.64**	0.47**	0.76**	0.96**	1				
Wet yield	-0.04	0.33*	0.34*	0.29*	0.32*	1			
Dry yield	0.01	0.35*	0.37**	0.31*	0.39**	0.98**	1		
EO percent	0.16	0.18	0.15	0.04	0.15	-0.08	-0.03	1	
EO yield	0.07	0.37**	0.39**	0.33*	0.42**	0.94**	0.97**	0.20	1

*, **. Correlation is significant at the 0.05 level and 0.01 level respectively

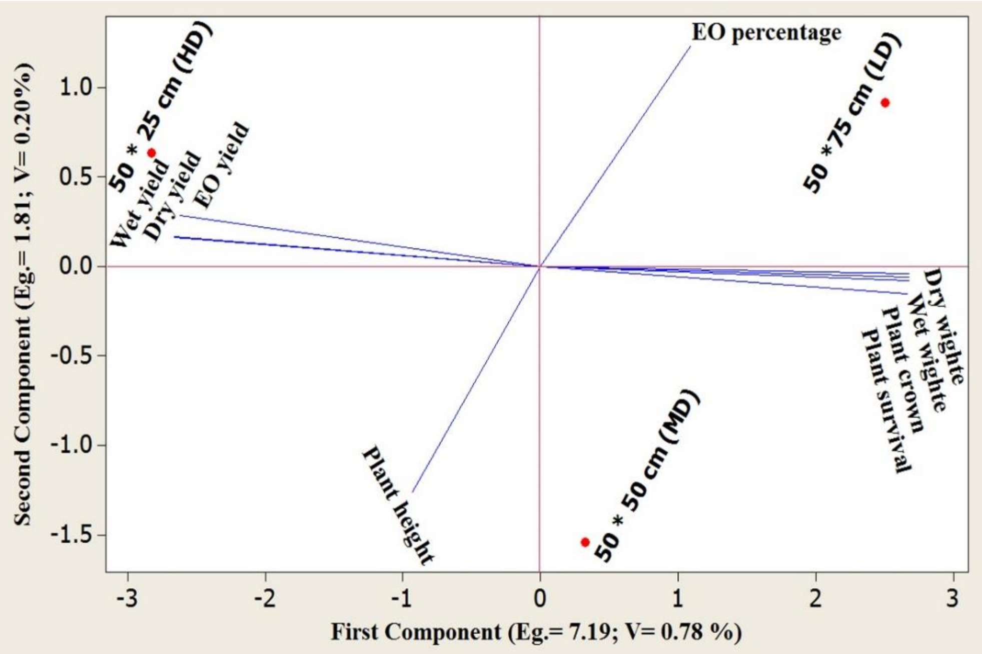


Figure 1. The biplot of morphological and yield traits in *satureja mutica* plants cultivated under rainfed conditions and different planting densities. (LD = low density; MD = medium density; HD = high-density Eg. = Eigenvalue; EO = essential oil; V = variance).

Table 7. The proportion of yield traits in PC1 to PC3, Eigenvalue, variance proportion and cumulative variance of components in *satureja mutica* plants cultivated under rainfed conditions and different row spacing treatments.

Variables	PC1	PC2	PC3
Plant survival	0.37	-0.06	0.04
Plant height	-0.16	-0.67	0.17
Plant crown	0.36	-0.18	-0.43
Plant wet weight	0.36	-0.12	-0.27
Plant dry weight	0.36	-0.11	0.10
Wet yield	-0.37	0.01	-0.22
Dry yield	-0.37	0.01	-0.73
EO percent	0.14	0.69	0.01
EO yield	-0.37	0.07	0.35
Eigenvalue	7.19	1.81	0.0
Proportion of Variance (%)	0.78	0.20	0.0
Cumulative Variance (%)	0.78	0.98	1.0

3.5. Chemical compounds of essential oil

Using GC, nine chemical compounds were identified in the EO samples (Table 8). The main compounds of EO were Carvacrol, thymol, γ- terpinene and ρ-cymene. According to Fig. 2a and 2b the highest percentage of ρ- cymene (13.27%) was observed in 75 × 50 cm row spacing. The highest percent of carvacrol (7.59%) in 75 × 50 cm and γ- terpinene (22.10%) were obtained in 25 × 50 cm row spacing. The highest amount of thymol (50.66%) was obtained in 50 × 50 cm row spacing. The highest total percentage of thymol + carvacrol (57.17%) was obtained in 50 ×50 cm row spacing (Fig. 2a and 2b).

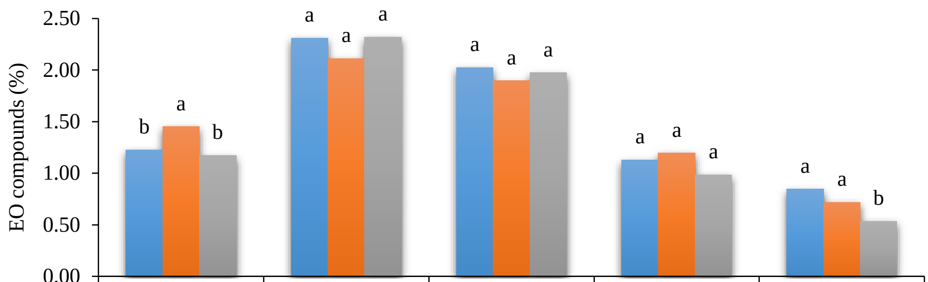
3.6. Principal components analysis for EO chemical compounds

The results of PCA showed that the PC1 and PC2 had Eigenvalues of more than 1 and in total represented 100% of cumulative variances (Fig. 3). The compounds of ρ- cymene (0.41%), camphene (0.38), α- Terpenine (0.38), carvacrol (0.32), and β- pinene (0.27) had the highest positive contributions; and the compounds of Trans- caryophyllene (-0.40), thymol (-0.39) α- pinene (-0.39) had the highest negative share in the PC1. The compounds of **γ-** Terpinene (0.56), camphene (0.49) and β- pinene (0.42 had the highest positive share, and carvacrol (-0.37) had the highest negative share in the PC2.

The PCA diagram (Fig. 3) showed that the compounds of Trans – caryophyllene, and thymol (third quarter) had the same trends. These compounds were correlated to the medium-density treatment and the highest percentages of these compounds were obtained from the 50 × 50 cm row spacing treatment. The compounds of a- terpinene, γ- terpinene and ρ-cymene had the same trend (first quarter) and were correlated to high-density treatment. The highest percentages of a- terpinene, γ- terpinene and β- pinene were obtained from 25 × 50 cm row spacing. The compounds of ρ- cymene and carvacrol showed the same trend and they were correlated to low density treatment. The highest amounts of ρ- cymene and carvacrol were obtained from 75 × 50 cm row spacing (Fig. 3).

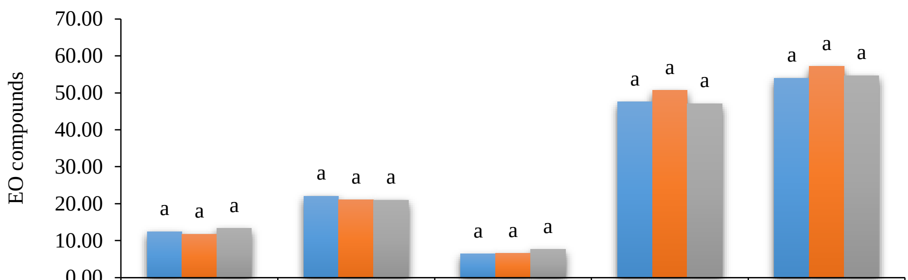
Table 8. Specification of EO chemical compounds identified in *S. spicigera* under rainfed conditions and different O. Fs. treatments

Classification	Chemical name	RT	RI	Formula
Phenol monoterpenoids	Carvacrol	4.07	1289.96	$C_{10}H_{14}O$
	Thymol	3.96	1281.22	$C_{10}H_{14}O$
Bicyclic monoterpenes	α-pinene	0.87	931.13	$C_{10}H_{16}$
	β- pinene	0.87	931.13	$C_{10}H_{16}$
	Trans-caryophyllene	5.33	1376.02	$C_{15}H_{24}$
	Camphene	1.16	953.1	$C_{10}H_{16}$
Isometric monoterpenes	γ-terpinene	1.63	1051.59	$C_{10}H_{16}$
	α- terpinene	1.63	1051.21	$C_{10}H_{16}$
Benzene alkyl	ρ-cymene	1.36	1014.15	$C_{10}H_{14}$



	Trans Caryophyllene	α- Terpenine	β-Pinene	α-Pinene	Camphene
25*50	1.23	2.31	2.03	1.13	0.85
50*50	1.45	2.11	1.90	1.19	0.72
75*50	1.17	2.32	1.97	0.99	0.53

Figure 2(a). Mean comparison of EO chemical compounds in *S.mutica* planted under rainfed conditions and different row spacing treatments



	p-Cymene	γ-Terpinene	Carvacrol	Thymol	Thymol + Carvacrol
25 * 50 cm	12.40	22.10	6.41	47.56	53.96
50 * 50 cm	11.76	21.03	6.51	50.66	57.17
75 * 50 cm	13.27	20.97	7.59	47.06	54.65

Figure 2(b). Mean comparison of EO chemical compounds in *S.mutica* planted under rainfed conditions and different row spacing treatments

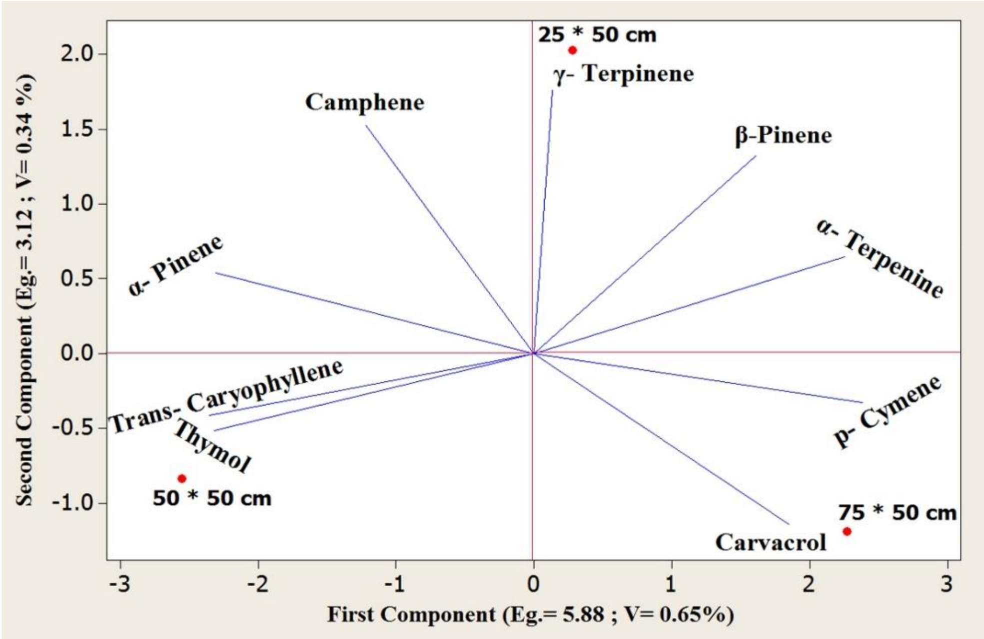


Figure 3. The Biplot of EO chemical compounds in *S. mutica* plants cultivated under rainfed conditions and different row spacing treatments

The plant spacing affected the morphological and yield traits of *S. mutica* plants. The highest fresh weight of the plant (49.01 g), and the maximum plant dry weight (23.54 g), were obtained in medium density planting× second year and the highest plant height (35.40 cm) was observed in the second year × low density. Haque and Sakimin (2022) have reported that reducing the plant density leads to increases in the biomass of a single plant.

In summer savory (*Satureja hortensis* L.) plants, under irrigation, the highest plant height (53.03 cm) was obtained at 35 × 35-row spacing (Mohammadpour *et al.*, 2012). The maximum plant weight in *Satureja bachtiarica* (under irrigation conditions) was observed in 25 × 50-row spacing (Mirjalili *et al.*, 2022). According to Xiao et al. (2006), in sunflower plants, by the increase in plant density, the competition for receiving light between plants increases and the plant height decreases but some authors believed that in some crop plants, increasing in planting density leads to increasing of plant height (Matsuo *et al.*, 2018). In *S. sahandica* plants, under irrigation cultivation, the highest single plant shoot yield (22.98 g) was obtained in the 80 × 80 cm row spacing (Abbaszadeh *et al.*, 2014).

In our study, the highest wet yield (2940.46 $kgha^{-1}$), dry yield (1319.00 $kgha^{-1}$) and EO yield (26.62 $kgha^{-1}$) were obtained in the treatment of high planting density (25 × 50 cm row spacing) × year2.

In a recently study, in rainfed cultivation of *S. spicigera* plants, the highest wet yield, dry yield and essential oil yield were obtained at the 25 × 50 cm row spacing (Yoosefi *et al.*, 2022). A study under irrigation conditions has showed that, In *S. mutica* plants, the highest fresh-weight yield and dry-weight yield have been obtained in 25 × 50 cm row spacing (Saki *et al.*, 2019). In *S. sahandica* plants, the highest flowering shoot yield (1587.5 $kgha^{-1}$) and the highest essential oil yield (14.53 $kgha^{-1}$) have been achieved in the 20 × 20 cm row spacing (Abbaszadeh *et al.*, 2014), and in *Satureja hortensis* plants the highest wet yield, dry yield and essential oil yield has been obtained at the 30 × 50 cm row spacing (EL-Leithy *et al.*, 2017).

In some industrial and crop plants, under irrigation conditions, row spacing has affected on the yield and morphological traits (Haarhoff and Swanepoel, 2021; Bernhard and Below, 2020; Haarhoff and Swanepoel, 2020). In soybean cultivation, the highest seed yield has been obtained from a 30 cm row spacing (Caliskan *et al.*, 2007). The highest yield of cotton has been obtained in three plants per square meter (Yang *et al.*, 2014). In tomato, by increasing plant density, shoot biomass has significantly reduced (Ravneet *et al.*, 2021). In *Carthamus tinctorious*, morphological and yield traits significantly were affect by row distances, and 30 cm row distance had the highest yield (Eryiğit *et al.*, 2014). In soybean plants, by increasing Planting density, the yield has been increased by 24.5% more than the standard row planting pattern (Gulluoglu *et al.*, 2016). The highest yield of sesame has been obtained at the 37.5 ×10 cm row spacing (Bakhshandeh, 2009).

The Plant density significantly affected the EO yield, but it had no significant effect on EO percentage. The highest EO percentage (2.04%) was obtained in 50 × 75 cm row spacing (low density) but the highest EO yield (27.28 kgha^{-1}) was obtained in 25 × 50 cm row spacing (high density).

Based on Callan et al. (2007) the planting density affects the yield and quality of aromatic herbs. Under irrigation conditions, Saki et al. (2019) reported that the highest EO content and EO yield, in *S. mutica* plants, have been observed in 25 × 50 cm row spacing (high plant density). In *Satureja hortensis* plants (under irrigation conditions), the EO content and EO yield have significantly increased by increasing planting density (Karimi *et al.*, 2021). Under irrigation cultivation, the highest essential oil percentage (2.01%), in *S. sahandica* plants, has been achieved in the 80×80 cm row spacing (Abbaszadeh *et al.*, 2014). In *Satureja bachtiarica*, the highest EO percent has been obtained in low-density planting (50 × 75 cm row spacing) and the highest EO yield was observed in 50 ×25 cm row spacing (Mirjalili *et al.*, 2022).

Khazaie et al. (2008) reported that in *Thymus vulgaris* plants, oil production was decreased by increasing planting density, but *Hyssopus officinalis* plants showed no response to planting density. In fennel plants (Khorshidi *et al.*, 2009) and in mint plants (Kizil and Toner, 2005), the EO content has been significantly affected by different planting densities. However, the EO percentages were not influenced by density in Peppermint and Spearmint plants (Nigussie *et al.*, 2015) and *Artimisia annua* plants (Aflatuni *et al.*, 2005). Saki et al. (2019) reported that in *S. mutica* plants, under irrigation conditions, high plant densities (25 × 50 cm row spacing) lead to higher plant production. Based on Bahreininejad et al. (2022), under dry farming, the highest wet yield (2844 kgha^{-1}) and the highest dry yield (1433 kgha^{-1}), in *S. spicigera* plants, have been obtained in row spacing 25× 50 cm. In *Satureja bachtiarica* plants, under irrigation conditions, the highest EO yield has been observed in 25 × 50 cm row spacing (Mirjalili *et al.*, 2022).

Competition among plants of the same species often results in power-law relations between measures of crowding, such as plant density, and average size, such as individual biomass. These dictate that following a given power-law exponent and a constant of proportionality, how plant biomass increases with decreasing plant density (Assaad *et al.*, 2020). These power-law relations are significant to crop production (Friedman, 2016). Depending on the plant species, plant genetics (Pant and Sah, 2020; Deng *et al.*, 2012a; Guo *et al.*, 2011), environmental conditions (Ilkaee et al., 2017) and nutritional conditions (Mook and van Der Toorn, 2022; Pant and Sah, 2020; Zhang and Tielbörger, 2020), the response of plants to planting density differ, so different and sometimes contradictory reports have been given about the effect of planting density on plant yield.

4. Conclusion

We concluded that the rainfed cultivation of *S. mutica* plants is possible in the mountainous areas with an annual rainfall of about 450 mm and more, in the north, west and northwest of Iran. We recommended the 50 × 25 cm row spacing for *S. mutica* cultivation in rainfed conditions.

Abbreviation

EO= essential oil, ES= enriched straw, FS= farm soil, HD= high density, LD= low density, MD= medium density, O. C. = organic carbon, O. F. = organic fertilizer, RMC= rotten cow manure

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

The first author [B. Y.]: implementation of the research project and writing the article

The second author [H. S.]: cooperation in the implementation of the research project and statistical analysis of the data

Informed consent

The authors declare not to use any patients in this research.

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