

EFFECTS OF INTRA-ROW SPACING ON HERBAGE YIELD, ESSENTIAL OIL CONTENT AND COMPOSITION OF *MICROMERIA FRUTICOSA*

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Abstract

Micromeria fruticosa is a perennial medicinal plant grown in the rocky areas of the Mediterranean region. A study was conducted to determine the effects of five different intra-row spacings on essential oil content, essential oil composition, herbage and essential oil yield of *M. fruticosa*. The essential oil was extracted by hydrodistillation and analyzed by GC-MS. Essential oil content was not affected significantly by intra-row spacings. More than 20 compounds were identified in each intra-row spacing. Monoterpene ketones constitute most of the essential oil of *M. fruticosa*. Pulegone (>57%) was the major component followed by *iso*-menthone (>15%) and piperitenone (>7%). Herbage and essential oil yield/ha were increased with the decreasing of intra row spacing. It was concluded that *M. fruticosa* could be planted at 60 x 15 cm spacing distance to have maximum herbage and essential oil yield.

Rezumat

Micromeria fruticosa este o plantă medicinală perenă ce crește în zonele stâncoase din regiunea mediteraneană. Studiul a fost efectuat pentru a determina efectul cultivării *M. fruticosa* la cinci distanțe diferite între rânduri, asupra conținutului de ulei esențial, compoziției în ulei esențial și randamentului privind producția de plante și ulei esențial de *M. fruticosa*. Uleiul esențial a fost extras prin hidrodistilare și analizat prin GC-MS. Conținutul în ulei esențial nu a fost afectat în mod semnificativ de distanțele dintre rânduri. Mai mult de 20 de compuși diferiți au fost identificați pentru fiecare distanță dintre rânduri. Cetonele monoterpene constituie cea mai mare parte din uleiul esențial de *M. fruticosa*. Pulegona (> 57%) a fost componentul major, urmată de *iso*-mentonă (> 15%) și piperitenonă (> 7%). Randamentul dezvoltării plantelor și compoziția în ulei esențial / hectar au crescut odată cu scăderea spațierii dintre rânduri. S-a ajuns la concluzia că *M. fruticosa* ar putea fi plantată la 60 x 15 cm distanță de spațiere între rânduri, pentru a avea randament maxim în ceea ce privește dezvoltarea plantelor și compoziția uleiului esențial.

Keywords: Essential oil composition, essential oil content, herbage yield, *Micromeria fruticosa*, pulegone

Introduction

Micromeria (Lamiaceae), a perennial aromatic herb naturally grown in the rocky areas of the Mediterranean parts of Turkey, is represented by 14 species and 22 taxa, 12 of them being endemic. Traditionally, the plant is used in alternative medicine as herbal tea for the treatment of colds, abdominal pains, eye infections, high blood pressure, and heart disorders due to the principal constituents of its essential oil [1, 3, 10, 11]. Dried leaves of the plant are also used as a spice to give flavor to soups and foods in the Eastern Anatolia [6]. Dry leaves of *M. fruticosa* contain 1.2 and 4 % essential oil [6, 10]. The chemical composition of *M. fruticosa* essential oil was extensively studied. The main compounds of *M. fruticosa* essential oil are pulegone, piperitenone, linalool, 1,8-cineole, and isomenthone [1, 2, 3, 5, 7, 10]. Essential oils from different plant species have been shown to possess antimicrobial activity [9]. The major essential oil constituent of *Micromeria*, pulegone, was reported to have antifungal [2], antimicrobial [5], and antioxidant [10] activities.

The essential oil content and composition of *Micromeria* species were studied extensively by many researchers [1, 2, 3, 4, 5, 8, 10] but few studies have been performed on cultivation of *Micromeria* [8]. *M. fruticosa* is a row crop therefore, determining suitable row spacing is one of the leading approaches to maximize of essential oil content. The objective of the current study was to determine the optimal intra-row spacing that will maximize essential oil content, essential oil yield and herbage yield of *M. fruticosa*.

Materials and Methods

Micromeria fruticosa plants were collected from Amanos mountains in the Eastern Mediterranean part of Turkey. The collected plants were grown in a nursery to screen the superior high yielding genotypes. A high yielding selected genotype was vegetatively propagated by stem cuttings. The rooted cuttings were transferred in 4-row plots, 5 m long with 5 intra-row spacing at the experimental field of the Mustafa Kemal University. The planting densities were 60 x 35 cm (47.580 plants/ha), 60 x 30 cm (55.500 plants/ha), 60 x 25 cm (66.666 plants/ha), 60 x 20 cm (83.333 plants/ha) and 60 x 15 cm (110.833 plants/ha). The experimental design was a randomized complete block with three replications. The crop was fertilized with 75 kg/ha of N and 75 kg/ha of P₂O₅. Drip irrigation was applied during the growing period. The soil of experimental plots was a clay silt loam with pH of 7.4, having 1.1% organic matter, 0.11% total nitrogen content, and water

holding capacity of 0.36 cm³. The daily climatic data were recorded by using HOBO weather station, (Onset Computer Corporation, USA). The long-term monthly mean temperatures from January to December were 8.2, 9.6, 13.2, 17.2, 21.2, 24.8, 27.2, 27.7, 25.6, 20.9, 14.0 and 9.4°C, respectively. The long-term monthly mean precipitations from January to December were 172.7, 156.8, 141.3, 101.5, 90.4, 21.9, 21.9, 8.0, 39.8, 74.0, 114.2 and 172.1 mm, respectively.

The above ground parts of the plants were harvested at the onset of the flowering in the first week of June 2008 and 2009. At harvest, a sample of 10 plants was randomly selected from each plot to determine plant height, dry leaf weight/plant and dry stem weight/plant. Essential oil content, herbage and essential oil yield were determined by harvesting the central two rows of the 4-row plots. Harvested plant materials were dried under shaded, airy place for a week. The dried leaf samples (25 g) were subjected to steam distillation for 3 h using a Clevenger-type apparatus. The essential oil percentage was expressed as v/w with respect to the dry matter of the initial material.

Essential oils were analyzed by gas chromatography using a Hewlett-Packard 6890 gas chromatograph interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (30 m×0.25 mm coated with 5% phenyl methyl silicone, 0.25 µm film thickness, Hewlett-Packard, Palo Alto, CA, USA) was used. The carrier gas was helium with a flow rate of 1.3 mL/min. The amount of the samples injected was 0.1 µL in split mode (50:1). The oven temperature was initially 50°C, increased at a rate of 2°C/min to 90°C, 5°C/min to 210°C and finally isothermal for 5 min. The injector and detector temperatures were maintained at 250 and 280 °C, respectively. The quadrupole mass spectrometer was scanned over the range 50-550 amu at 1.53 scan/s, with an ionizing voltage of 70 eV. The essential oil components were identified by comparison of their mass spectra with those of a computer library or with authentic compounds.

Data were analyzed separately for each year using the GLM (General Linear Model) procedure of SAS (Statistical Analysis System) to evaluate the effects of plant population on herbage yield and essential oil content of *M. fruticosa*.

Results and Discussion

The relative amounts of each component were determined by the GC-MS analysis (Table I).

Table I
Composition of the essential oil from *Micromeria fruticosa* grown in 15, 20, 25, 30 and 35 cm intra-row spacing in 2008 and 2009

Compound	RT	Percentage of essential oil component in five intra row spacings									
		15 cm		20 cm		25 cm		30 cm		35 cm	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
<i>β</i> -Pinene	5.30	1.61 $\pm$ 0.08	1.21 $\pm$ 0.01	1.34 $\pm$ 0.03	1.08 $\pm$ 0.05	2.12 $\pm$ 0.14	3.62 $\pm$ 0.32	1.42 $\pm$ 0.07	2.18 $\pm$ 0.15	1.84 $\pm$ 0.08	2.25 $\pm$ 0.10
Sabinene	5.74	0.73 $\pm$ 0.01	0.26 $\pm$ 0.01	0.78 $\pm$ 0.01	0.15 $\pm$ 0.01	0.86 $\pm$ 0.02	0.37 $\pm$ 0.01	0.73 $\pm$ 0.04	0.21 $\pm$ 0.01	0.81 $\pm$ 0.01	0.87 $\pm$ 0.01
Myrcene	7.40	0.82 $\pm$ 0.01	0.95 $\pm$ 0.01	0.61 $\pm$ 0.01	0.09 $\pm$ 0.01	0.55 $\pm$ 0.01	0.43 $\pm$ 0.01	0.57 $\pm$ 0.02	0.46 $\pm$ 0.01	0.59 $\pm$ 0.01	0.35 $\pm$ 0.01
Limonene	8.59	2.08 $\pm$ 0.02	1.17 $\pm$ 0.15	2.40 $\pm$ 0.06	3.02 $\pm$ 0.02	2.37 $\pm$ 0.02	2.98 $\pm$ 0.26	2.41 $\pm$ 0.01	2.86 $\pm$ 0.09	2.10 $\pm$ 0.10	1.98 $\pm$ 0.08
1,8-Cineole	8.80	0.15 $\pm$ 0.01	0.40 $\pm$ 0.01	0.10 $\pm$ 0.01	0.25 $\pm$ 0.01	0.24 $\pm$ 0.01	0.37 $\pm$ 0.01	-	0.94 $\pm$ 0.01	0.34 $\pm$ 0.01	0.66 $\pm$ 0.01
Iso-Menthone	22.99	17.66$\pm$0.25	19.34$\pm$0.16	15.2$\pm$0.24	18.27$\pm$0.60	17.97$\pm$1.05	18.06$\pm$1.04	18.09$\pm$0.50	17.25$\pm$0.45	16.21$\pm$0.82	17.13$\pm$0.35
Cis-isopulegone	27.76	0.76 $\pm$ 0.01	0.28 $\pm$ 0.01	0.71 $\pm$ 0.01	0.88 $\pm$ 0.01	0.78 $\pm$ 0.01	0.5 $\pm$ 0.01	0.82 $\pm$ 0.32	0.46 $\pm$ 0.01	0.57 $\pm$ 0.01	0.68 $\pm$ 0.01
Trans-isopulegone	28.34	0.85 $\pm$ 0.01	0.63 $\pm$ 0.05	0.93 $\pm$ 0.01	1.09 $\pm$ 0.03	1.07 $\pm$ 0.04	1.14 $\pm$ 0.14	1.13 $\pm$ 0.85	2.15 $\pm$ 0.03	1.06 $\pm$ 0.01	1.36 $\pm$ 0.04
<i>β</i> -Caryophyllene	29.15	1.16 $\pm$ 0.01	1.90 $\pm$ 0.01	1.40 $\pm$ 0.01	2.16 $\pm$ 0.06	1.02 $\pm$ 0.02	2.03 $\pm$ 0.28	1.12 $\pm$ 0.29	2.08 $\pm$ 0.01	1.34 $\pm$ 0.03	1.20 $\pm$ 0.02
Pulegone	31.02	60.34$\pm$1.32	57.52$\pm$0.85	62.92$\pm$1.53	56.63$\pm$1.37	60.04$\pm$1.75	57.16$\pm$1.04	61.2$\pm$1.86	57.06$\pm$2.04	60.75$\pm$1.86	58.06$\pm$1.05
Germaene-D	32.51	0.18 $\pm$ 0.01	0.20 $\pm$ 0.01	-	0.16 $\pm$ 0.01	0.21 $\pm$ 0.01	0.30 $\pm$ 0.01	0.22 $\pm$ 0.01	0.35 $\pm$ 0.01	0.21 $\pm$ 0.01	-
2-Cyclohexen-1-ol	32.86	0.80 $\pm$ 0.01	1.70 $\pm$ 0.20	0.64 $\pm$ 0.01	0.71 $\pm$ 0.01	0.74 $\pm$ 0.01	0.41 $\pm$ 0.01	0.73 $\pm$ 0.01	1.12 $\pm$ 0.01	0.67 $\pm$ 0.01	0.49 $\pm$ 0.01
Piperitone	32.96	1.20 $\pm$ 0.23	1.14 $\pm$ 0.08	1.80 $\pm$ 0.04	1.24 $\pm$ 0.06	1.41 $\pm$ 0.31	1.90 $\pm$ 0.23	1.44 $\pm$ 0.07	1.98 $\pm$ 0.03	1.05 $\pm$ 0.01	0.95 $\pm$ 0.02
Bicyclogermacrene	33.21	0.19 $\pm$ 0.01	0.31 $\pm$ 0.01	0.23 $\pm$ 0.01	0.29 $\pm$ 0.01	0.18 $\pm$ 0.01	0.12 $\pm$ 0.01	0.30 $\pm$ 0.01	0.45 $\pm$ 0.01	0.37 $\pm$ 0.01	0.32 $\pm$ 0.01
5-Hexen-2-one	35.71	0.30 $\pm$ 0.02	0.28 $\pm$ 0.01	0.55 $\pm$ 0.01	0.43 $\pm$ 0.01	0.46 $\pm$ 0.03	0.37 $\pm$ 0.22	0.33 $\pm$ 0.01	0.37 $\pm$ 0.01	0.34 $\pm$ 0.01	0.28 $\pm$ 0.01
2-Cyclohexen -one	35.90	-	0.22 $\pm$ 0.01	0.27 $\pm$ 0.01	0.87 $\pm$ 0.01	0.22 $\pm$ 0.01	-	0.27 $\pm$ 0.01	0.41 $\pm$ 0.01	0.41 $\pm$ 0.01	0.26 $\pm$ 0.01
4-Nitro-o-cresol	37.57	0.23 $\pm$ 0.01	0.18 $\pm$ 0.01	0.21 $\pm$ 0.01	0.42 $\pm$ 0.01	0.19 $\pm$ 0.01	0.3 $\pm$ 0.01	0.25 $\pm$ 0.01	0.3 $\pm$ 0.01	0.82 $\pm$ 0.01	0.74 $\pm$ 0.01
Piperitenone	37.90	9.19$\pm$0.25	10.3$\pm$0.30	8.81$\pm$0.56	9.74$\pm$1.02	8.28$\pm$0.43	7.65$\pm$0.82	7.32$\pm$0.72	7.07$\pm$0.70	9.14$\pm$0.27	8.80$\pm$0.20
Caryophyllene oxide	39.07	0.35 $\pm$ 0.01	0.32 $\pm$ 0.01	0.45 $\pm$ 0.01	0.63 $\pm$ 0.01	0.37 $\pm$ 0.01	0.34 $\pm$ 0.01	0.31 $\pm$ 0.01	0.24 $\pm$ 0.01	-	0.39 $\pm$ 0.01
2-Butanone	39.71	0.23 $\pm$ 0.01	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01	-	0.13 $\pm$ 0.01	0.16 $\pm$ 0.01	0.11 $\pm$ 0.01	0.2 $\pm$ 0.01	0.21 $\pm$ 0.01	0.47 $\pm$ 0.01
Patchulane	42.68	0.43 $\pm$ 0.01	-	0.30 $\pm$ 0.01	0.46 $\pm$ 0.01	0.34 $\pm$ 0.01	0.32 $\pm$ 0.03	0.37 $\pm$ 0.01	0.4 $\pm$ 0.01	0.32 $\pm$ 0.01	0.65 $\pm$ 0.01
Total		99.26	98.45	99.79	98.57	99.55	98.53	99.14	98.54	99.15	97.89

The values ($\pm$ SEM) are expressed as means of three different determinations and were obtained from electronic integration measurements using selective mass detector.

More than twenty essential oil components were identified in the essential oils obtained from 15, 20, 25, 30 and 35 cm intra-row spacings in 2008 and 2009. Three components identified in the essential oil of *M. fruticosa* in concentrations greater than 7% were pulegone, *iso*-menthone and piperitenone which did not vary significantly among intra-row spacings in both years. More than seventeen components were present in amounts less than 3%. Among the essential oil components, pulegone was the major component (>56%) while *iso*-menthone was the second major component (>15%) of the essential oil of *M. fruticosa* grown in different intra-row spacings. The high content of monoterpenic ketones is responsible for the main odor and flavor characteristics of the essential oil. Pulegone was the most encountered component in *M. fruticosa* [1,2, 3, 5, 7, 10]. Contrary to the earlier reports, menthalactone, guaiacol, menthol, and menthone were not detected in the current study.

Significant differences among intra-row spacings were detected for studied plant parameters, except that for plant height and essential oil content (Table II, III). Plant height increased with the decreasing intra-row spacing in both years. However, increases in plant height were not significant in both years. Environmental factors such as the quantity and quality of light affected plant height. Light density, as well as the red and far red ratio, plays an important role on plant height.

Table II

Effects of intra-row spacing on *Micromeria* plant height, dry leaf weight, dry stem weight, leaf/stem ratio in 2008 and 2009

Intra-row spacing	No. Plants (ha)	Plant height (cm)		Dry leaf weight (g/plant)		Dry stem weight (g/plant)	
		2008	2009	2008	2009	2008	2009
15 cm	47,580	109.73	120.12	24.38	24.33	30.58	31.00
20 cm	55,500	109.53	118.93	25.77	27.00	33.28	34.00
25 cm	66,666	108.03	115.81	28.27	29.67	34.44	36.33
30 cm	83,333	98.33	115.43	30.02	35.33	35.70	38.00
35 cm	110,833	96.57	113.94	30.94	39.67	35.71	41.33
LSD 0.05		N.S.	N.S.	2.76	2.44	3.92	1.79

N.S. = Not significant

Dry leaf weight/plant significantly reduced as intra-row spacing reduced in 2008 and 2009 (Table II). The lowest and the highest dry leaf weight/plant values were obtained from 15 and 35 cm intra-row spacings, respectively in both years. Decreased dry leaf weight/plant in narrower intra-rows was resulted from the increase in competition among the plants and decrease of the space allocated for every plant.

Similar to dry leaf weight/plant, the value of dry stem weight/plant significantly increased with the increasing the intra-row spacing (Table II). The highest dry stem weight/plant was obtained from 35 cm intra-row spacing while the lowest was obtained from 15 cm in both years of the study.

Table III
Effects of intra-row spacing on *Micromeria* herbage yield, essential oil content, essential oil yield in 2008 and 2009

Intra-row spacing	No. Plants (ha)	Herbage yield (kg/ha)		Oil content (%)		Oil yield (l/ha)	
		2008	2009	2008	2009	2008	2009
15 cm	47,580	2702.5	2696.8	4.80	4.63	129.66	124.86
20 cm	55,500	2147.2	2316.6	4.75	4.66	102.01	107.84
25 cm	66,666	1884.4	1977.7	4.88	4.47	92.01	88.08
30 cm	83,333	1665.9	1961.0	4.83	4.58	80.53	89.59
35 cm	110,833	1472.3	1887.3	4.85	4.71	71.10	88.73
LSD 0.05		131.62	165.42	N.S.	N.S.	6.09	8.47

N.S. = Not significant.

Effects of intra-row spacing on herbage yield/ha were highly significant (Table III). The highest and the lowest herbage yield pertained to the intra-row spacing of 15 and 35 cm, respectively in both years. As intra-row spacing decreased from 35 cm to 15 cm, herbage yield increased from 1472.3 to 2702.5 kg/ha in 2008 and from 1887.3 to 2696.8 kg/ha in 2009. The number of plant per hectare increased with the decrease of intra-row spacing. Hence, the closest intra row spacing had the highest herbage yield per hectare in both years.

Intra-row spacing had no significant effect on essential oil content in both years (Table III). Essential oil contents varied between 4.75 and 4.88% in 2008 and between 4.47 and 4.71% in 2009. Essential oil content observed in the current study are higher than the previously published studies, which reported oil content between 2.6 and 3.5 % depending on environmental conditions and management [3, 7, 10].

When essential oil yield/ha was taken into consideration, the highest essential oil yield was obtained from 15 cm intra-row spacing while the lowest oil yield was obtained from 35 cm intra-row spacing in both years (Table III). Oil yield increase in lower intra-row spacing was due to the increased herbage yield per hectare. Available solar radiation, soil moisture and soil fertility are not utilized in maximum rate by the plants grown in wider intra-row spacings. Choosing the proper plant density eliminates the limitations for utilization of temperature, solar radiation, soil moisture and soil fertility to produce maximum yield.

Conclusions

The results showed that the effects of intra-row spacing on essential oil content of *M. fruticosa* were not insignificant at the studied range of intra-row spacing. More than twenty components were determined by the GC-MS analysis of the essential oil obtained from different intra-row spacings. Most of the essential oil components consist of monoterpenoids. Pulegone was the major component followed by *iso*-menthone and piperitenone. Dry leaf and stem weight/plant significantly increased through the use of wider intra-rows; however herbage and essential oil yield/ha reduced through the use of wider intra-rows.

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