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Article

Influence of Planting Date on Growth, Yield, and Volatile Oil of Menthol Mint (*M. arvensis*) Under Marginal Land Conditions

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Abstract: *Mentha arvensis*, commonly named menthol mint or Japanese mint, is an important volatile oil-bearing plant having high amounts of natural menthol. *M. arvensis* volatile oil (VO) and menthol have a considerable share in the global trade of medicinal plants for widespread use in the pharmaceutical, food, cosmetic, and fragrance industries. This study was conducted to investigate the response of *M. arvensis* var. piperascens Holmes to planting time (fall and spring) under marginal land conditions. Plants were propagated by three-year-old rhizomes. Obtained results showed that the effect of growing seasons was not significant on the agronomic traits and VO yield. However, the effects of six different impressions during the experiment were significant on the studied parameters. The highest plant height (45 cm), fresh biomass (16692 kg ha^{-1}), dry biomass (4736 kg ha^{-1}), leaf dry yield (2816 kg ha^{-1}), and VO yield (158 L ha^{-1}) were observed in the 1st cuttings. The yield components decreased with the plants aging. The lowest values of the mentioned parameters were in the sixth harvest. Moreover, the VO content varied from 4.6-6.1 %. Menthol (52.19-73.53 %) and menthone (3.42-18.89 %) were identified as the most major VO components of *M. arvensis*. While the menthol percent decreased in the last cuttings, menthone increased correspondingly. Based on the results of this study, it can be suggested that the cultivation of mint in marginal land has a desirable agronomic yield and acceptable chemical properties.

Keywords: Infertile land, *Mentha arvensis*, Menthol, Planting season, Volatile oil.

Introduction

Various *Mentha species* are used from 2000 years ago as spice and medicine [1]. The genus *Mentha* (Lamiaceae) including 25 to 30 species having a vast distribution throughout the temperate and sub-temperate regions [2]. *M. arvensis* (Menthol mint, Corn mint or Japanese mint), as a medicinal, aromatic, and spice plant [3]. *M. arvensis*, as a perennial herbaceous plant, is cultivated in tropical and subtropical countries worldwide, such as China, India, Brazil, Japan, France, and the USA [4,5]. The main reason for *M. arvensis* cultivation is its volatile oil (VO), which mainly accumulates in the leaves [6,7]. Different species of *Mentha*, especially corn mint, are important resources for VO besides citrus peels that are produced prevalently for industrial purposes [8,9]. *M. arvensis* produces high amounts of VO compared with other Mentha species and this oil has the largest share in the global mint market due to its high content of menthol [10,11]. The other constituents of *M. arvensis* VO are neomenthol, menthone, menthyl acetate, 1,8-cineol, phellandrene, ρ-cymene, limonene, piperitone, carvomenthone,

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linalool, and linalyl acetate. The plant leaves and their constituents are used in pharmaceutical, food, flavor, cosmetics, beverages, and allied industries [12,13]. Besides, *M. arvensis* is used as antiseptic, carminative, stomachic, refringent, stimulant, emmenagogue, diuretic, anti-helminthic, sudorific, contraceptive, anodyne, and antispasmodic [14].

Due to the increased global demand for natural menthol and to the management of the trade of menthol-containing VOs in the international markets, off-season production strategies and alternative lands are inevitable for *M. arvensis* [15,16]. Marginal lands with limited accessibility, climate restrictions, high environmental hazards, and fragile ecosystem leading to low soil productivity are classified as infertile lands [17]. When the plants are exposed to biotic and abiotic stresses, the biosynthesis of secondary metabolites is stimulated [18]. Therefore, in the areas where stressors such as the edaphic factor limit the growth of conventional crops, the replacement of medicinal plants will be a reliable priority [19]. To preserve the genetic resources of medicinal plants and provide ever-increasing human demand for natural plant products, the domestication and cultivation of these plants is necessary. However, to avoid the use of productive lands commonly appertain to the cultivation of strategic crops, the use of inefficient or marginal lands to cultivate medicinal and aromatic plants seems like the appropriate alternative way. In this case, while not reducing the production of the main crops, the cheap labor force of these regions will also turn into significant advantages.

The effects of marginal land conditions have not been precisely investigated on the agronomic yield and secondary metabolites production of mint. The present study was carried out to evaluate the agronomic and chemical responses of *M. arvensis* to the different planting seasons under the marginal (inefficient) lands of the Çukurova region affected by the Mediterranean climate of Turkey.

Materials and Methods

Experimental location

A field experiment was conducted for three successive years during 2011-2014 growing seasons at the research field of Field Crops Department of Çukurova University, located in Çukurova region of Turkey (37°01'-N, 35°21'-W and 35 m above sea level). This region reflects a typical Mediterranean climate with hot and dry summers and mild rainy winters. A summary of meteorological data is shown in Table 1. The field soil is considered poor soil, which has caused the field to be classified as marginal land for cultivation. The field has formed from young alluvial soils of the Seyhan River containing pebbles deposits of various sizes in different depths. The lime content of soil profiles was very high, and organic matter was critically low [20]. Sandy clay loam soil (sand: 55.4 %, Silt: 23.8 % and clay: 20.8 %) of the experimental field had the following physicochemical properties: pH 7.68, EC 0.0.51 mmhos cm^{-1}, organic matter 0.64 %, total nitrogen 0.21 %, $CaCO_3$ 32.4 %, P_2O_5 11.2 mg 100 g^{-1}, K_2O 24.6 mg 100 g^{-1}, Zn 3.6 ppm, Fe 6.4 ppm, Mn 2.7 ppm and Cu 0.85 ppm. The sizes of pebbles in the soil were mainly great. The considered field was previously devoted to cereal cultivation, which was abandoned due to negligible productivity.

Plant material, treatments, and experimental design

The plant material was rhizomes of *M. arvensis* var. *piperascens* Holmes. The required amount of rhizomes were obtained from three-year-old mother plants that existed in the research field of Çukurova University. Two different planting seasons (fall and spring) were considered for the experiment. After field preparation, such as plowing, flattening, and fertilizing, the rhizomes were prepared as 15-20 cm pieces and were planted end to end at a depth of 10 cm. The planting dates of fall and spring were 29th November 2011 and 10th May 2012, respectively. Each plot dimensions were 5.1 × 3 m (15.3 m^2) and the distance between rows was 50 cm. The experiment was arranged by using a randomized complete block design with three replications. For fertilizing, 120 kg ha^{-1} of N and 90 kg ha^{-1} of P were employed. Two-thirds of N and all of P were added simultaneously with planting. The rest of the N was applied after the first cuttings. The fertilization regime was unchangingly repeated in the following years. All the recommended agri-

Table 1. Some local meteorological data during experimental years (2011-2014)

	Temperature (°C)											
	Minimum				Maximum				Mean			
	2011	2012	2013	2014	2011	2012	2013	2014	2011	2012	2013	2014
January	6.4	5.2	6.2	7.2	15.7	12.8	15.1	18.1	10.1	9	10.7	12.7
February	6.7	4.3	8.2	7	17.3	15	18.2	19.3	10.9	9.7	13.2	13.2
March	8.3	6.2	9.2	10.4	20.1	17.1	20.8	21.8	13.5	11.7	15	16.1
April	11.6	12.8	13.2	14.1	22.6	25.8	25.4	25.1	16.5	19.3	19.3	19.6
May	15.6	16.6	17.9	16.7	27.1	27	29.9	27.3	21.4	21.8	23.9	22
June	20.5	21.2	20.8	19.8	30.6	32.3	31.5	30.9	25.6	26.8	26.2	25.4
July	24.3	24.2	24	25	33.4	34.1	34	33.2	28.6	29.2	29	29.1
August	24.7	24.6	23.9	25.7	35.0	36	35.4	34.5	29.5	30.3	29.7	30.1
September	21.5	21.9	20.6	21.7	32.5	34.5	32.3	31.8	27.3	28.2	26.5	26.8
October	15.8	17.5	13.8	16.5	28.2	29.6	27.9	27.7	20.8	23.6	20.9	22.1
November	8.0	12.5	13.2	10.5	19.5	23.6	24.4	21.9	12.4	18.1	18.8	16.2
December	5.8	7.8	6.4	9.8	16.8	16.2	15.9	18.7	10.0	12	11.2	14.3
	Rain (mm)				Relative humidity (%)				Insolation (h)			
	2011	2012	2013	2014	2011	2012	2013	2014	2011	2012	2013	2014
January	76.5	262	64.2	35.7	63.7	72.7	64.9	70	4.5	4	4.2	5.3
February	92.4	122.9	55.7	36.5	62.3	58.6	71.8	63.1	5.8	6.1	5.4	6.8
March	107	57.9	54.3	47.7	64.6	56.1	57.8	62.5	6.3	5.5	6.9	7.2
April	78.3	21.4	100.2	22.1	66.6	63.4	63.3	64.9	8.1	8.2	7.1	8.1
May	105.6	79.9	61.5	34.9	64.1	67.3	64.4	66.2	8.5	8.2	9.3	7.8
June	49.4	17.1	0.9	89.8	66.2	60	59.6	66.6	9.9	9.8	10.7	10.4
July	0	14	0	3.5	67.2	52.8	64	70.3	9.6	8.4	10.9	9.6
August	0	0.1	19.8	0.2	62.9	56.9	67.3	70.6	9.5	9.7	11.2	9.1
September	4.1	0	31.9	95.4	60.4	59	59.1	63.3	8.8	10.3	9.2	8.6
October	5.8	63.4	40.1	54.9	47.9	61.2	49.9	64.3	7.98	6.6	8.6	7.7
November	44.1	128.3	6.1	66.5	53.8	66.9	61.4	59.1	6.31	6	6.2	6.3
December	156.4	298.4	21.5	106.4	66.4	76.1	49	72.8	4.03	3.8	4.9	4.3
Total	719.6	1065.4	456.2	593.6								

cultural practices such as weeding and irrigation, etc., were followed as needed. Irrigation was done according to the needs of plants by the sprinkler method.

Harvesting and records

Cuttings were done twice each year at the beginning of the budding stage at ground level (Table 2). For measuring the plant height, ten plants were randomly selected from each plot. To record biomass yield, plants were cut after removing 0.5 m from the plots as the side effect. Also, to determine the leaves and stems dry weight, certain amounts of plants were separated into leaf and stem sections and dried at 37°C for 72 hours.

Volatile oils isolation

To obtain the VO, 50 g of the dried leaves and 500 mL of distilled water were put in a 500 mL round bottom flask equipped with a neo-Clevenger type apparatus. Hydro-distillation was performed for 180 min in three replications. The VO content was calculated based on the dry weight of leaves and expressed as % (w/w). The obtained VOs were dried over anhydrous sodium sulphate and stored at +4°C.

Chemical analysis

A gas chromatography (GC) system (Agilent Technologies, 7890B), which is equipped with a flame ionization detector (FID) and coupled to a

Table 2. The cuttings dates of *M. arvensis* during experimental years

Planting Season	Year	Cutting date	
		1st Cutting	2nd Cutting
Fall	1st year (2012)	23 July	14 November
Spring		06 August	14 November
Fall	2nd year (2013)	10 June	30 September
Spring		10 June	30 September
Fall	3rd year (2014)	2 June	15 September
Spring		2 June	15 September

mass spectrometry detector (MSD) (Agilent Technologies, 5977A), was used for identifying the chemical components of the VOs. The column for separating the compounds was HP-Innowax (Agilent 19091N-116: 60 m × 0.320 mm internal diameter and 0.25 µm film thickness). The carrier gas was Helium (99.999 %) with a 1.3 mL min^{-1} flow rate. The injection volume was set at 1 µl (20 µL VO was dissolved in 1 mL n-hexane). The solvent delay time was 8.20 min. The injection was performed in split mode (40:1). The samples were analyzed with the column held initially at 70°C after injecting with 5 min hold time. Then, the temperature raised to 160°C with 3°C min^{-1} heating ramp and 5 min hold time. Eventually, the temperature reached 250°C with 6°C min^{-1} heating ramp and 5 min hold time. The detector, injector, and ion source temperatures were 270°C, 250°C, and 230°C, respectively. MS scan range was (m z^{-1}): 50-550 atomic mass units (AMU) under electron impact (EI) ionization (70 eV).

The retention indices (RI) were determined by injecting C_7-C_{30} n-alkanes (Sigma-Aldrich) to (GC/FID) system (Agilent Technologies, 7890B) under the same conditions of the analyses of the VOs. The identifications of the VO components were determined by comparing retention indices, mass spectra by the computer library database of the US National Institute of Standards and Technology (NIST), Wiley libraries, other published mass spectra data [20], and our database. Quantification was done by an external standard method using calibration curves generated by running GC analysis of representative compounds.

Statistical analyses

Data were analyzed by using the MSTAT-C computer software program. The means of treatments were compared using the Least Significant Difference (LSD) method at a 0.05 level of probability. The analysis of variance was conducted on the samples to determine variations of parameters between the planting season and cuttings.

Results and discussion

Plant height

Mean comparisons showed that the effect of different cuttings was significant on plant height (Table 3). The 1st cutting had the most plant height (45.4 cm) and results follow a descending trend towards the last cutting (45.4 to 26.2 cm). Based on the experimental years' data, the plant height was higher in the 1st cuttings of each year. Also, the significant results of the interaction effects of planting season and cutting on plant height indicated the comparative advantage of harvest arrangement and fall planting season (Table 3). The cuttings and planting season effect on plant height probably have arisen from the long vegetative growth of the fall planting season compared to the spring planting. Another reason for the more plant height in the 1st cuttings is seemingly the long growing period than the 2nd one [22]. Continuous spring rains and the high relative humidity are also effective in increasing the height of the 1st cutting plants [23]. In another study conducted in the same location in the fertile land on *M. arvensis* var. piperascens (L.) Holmes, the average plant height was recorded at 66.96 and 48.32 cm during the 1st and 2nd cuttings, respectively [24]. Meanwhile, conditions such as inefficient experimental fields and the increasing age of plants have reduced the plant height over

time. Several reports have recorded the relationship between plant height and cutting numbers that are in line with the results of this study [25-27].

Fresh and dry biomass

Although the planting time effect was not significant on fresh and dry biomass, cuttings had considerable differences in the mentioned traits (Table 3). Mean comparisons showed the highest fresh biomass in the 1st cutting (16692 kg ha^{-1}) and the other subsequent cuttings ranked as next, respectively. The 6th cutting had the lowest fresh biomass (5980 kg ha^{-1}). Like the fresh biomass, dry biomass yield was not significantly different on the planting time, but the difference between the cuttings was significant during the years of the experiment. The highest and the lowest dry biomass (4736 and 1733 kg ha^{-1}) were recorded in the 1st and 6th cuttings, respectively. A continuous decreasing trend was observed in plant biomass (Table 3).

Fall-winter production has been recommended as more suitable for optimal yield and quality of this plant [25,28]. Some other records have also shown that the spring planting time was superior [1,29]. However, in this study, no significant difference was found between the two planting times in terms of yield. Rhizomes planted in the fall were in a recession during the colder months and consumed their nutritional sources. They lost a lot of time to return to normal growth as the weather warmed up. Conversely, rhizomes planted in spring were in the development stage and continued to grow and germinate without wasting time. Therefore, plants in both planting seasons began to grow simultaneously with the onset of the spring season. Decreasing soil productivity, scarcity of food resources, and aging the plants lead to a decline in the final yield. These complications are more severe and rapid in marginal lands. The differences between fresh biomass yield can be ascribed by the ecological factors, genotype, and agricultural techniques [23]. Singh *et. al.*[30], and Dastjerdi *et. al.*[31], also noticed similar results considering high fresh biomass with early cuttings. According to Çaliskan [24], the average fresh biomass of *M. arvensis* var. *piperascens* (L.) Holmes was 20447 and 11567 kg ha^{-1} during the 1st and 2nd cutting, respectively. The minimum and maximum recorded values for the fresh biomass by the mentioned researcher were 10370 and 24430 kg ha^{-1}, which is more than the present study. Additionally, Özgüven and Kirici[32], in the same location and Özel and Özgüven[25], in Harranova have reported the high fresh biomass values (27343 and 22966-23067 kg ha^{-1}, respectively). But, in a study on *M. arvensis* L. *f. piperascens* planted in different months, the fresh biomass was much lesser [33]. Whereas the dry biomass is directly related to fresh biomass, these results are coincident with the fresh biomass data. Consequently, the downtrend in dry biomass was repeated according to fresh biomass. As discussed in the fresh biomass, dry biomass is also affected by the genetic makeup [34], climatic conditions [35], and agricultural practices [36]. Similar results have been observed by Santos *et. al.*[37], and Çaliskan and Özgüven [38].

Leaf dry yield

Leaf dry yield was affected by the cutting number and interaction effects of planting season and cutting (Table 3). The leaf dry yield in the 1st cutting (2816 kg ha^{-1}) was the highest. The 2nd and 3rd cuttings (2193 and 2181 kg ha^{-1}) were ranked the next in a similar statistical group. The last three cuttings were grouped with the lowest leaf dry yield. Table 3 indicates that the first cutting of the plants cultivated in the fall had the highest dry leaf production. In both planting seasons, the 1st, 2nd, and 3rd cuttings were outweighed comparing the subsequent cuttings. A decreasing trend in the yield was also observed in leaf dry yield.

Leaf dry yield is affected by numerous endogenous and exogenous factors. In general, plant aging and the long growing seasons impress this trait. Defoliation of the old leaves is more severe. Factors such as the short vegetative period and physiological senescence [35], high temperature [25], and night-day temperature differences [39], may cause leaf dry yield loss as well. These results are in line with the findings of Santos *et. al.*[37]. It has been reported that with increasing duration of insolation and light intensity, the dry yield of plants and dry leaves weight increases by the accumulation of dry matter [40]. Since each year's first cuttings have a long growing season and long sunny days, the plants produce more dry leaf yield.

Table 3. Mean comparison for some agronomic and chemical parameters of *M. arvensis* influenced by planting season and maarginal land conditions

Treatments	Plant height (cm)	Fresh biomass (kg ha^{-1})	Dry biomass (kg ha^{-1})	Leaf Dry yield (kg ha^{-1})	Leaf:Stem ratio	V. Oil content (%)	V. Oil yield (L ha^{-1})
Planting season							
Fall	36.9	11136.4	3115.1	1812.5	1.46	5.26	96.47
Spring	34.7	11043.3	2949.5	1759.2	1.53	5.63	100.97
Cutting							
1st Cut. (1st y.*)	45.4 a	16692.1 a	4736.4 a	2816.8 a	1.46 bc	5.81 ab	158.74 a
2nd Cut. (1st y.)	37.5 b	15560.2 a	4203.7 a	2193.6 b	1.10 c	6.11 a	133.82 b
3rd Cut. (2nd y.)	37.9 b	12628.9 b	3250.5 b	2181.8 b	2.08 a	5.55 b	119.50 b
4th Cut. (2nd y.)	35.9 b	8661.3 c	2339.8 c	1336.3 c	1.36 bc	5.50 b	73.95 c
5th Cut. (3rd y.)	31.9 c	7016.9 cd	1919.3 cd	1176.3 c	1.61 b	5.10 c	59.88 cd
6th Cut. (3rd y.)	26.2 d	5980.1 d	1733.01 d	1010.3 c	1.38 bc	4.60 d	46.44 d
LSD $_{(\%5)}$	3.671	1828.0	552.3	364.6	0.3939	0.3865	22.45
Planting season × cutting							
Fall × 1st Cut. (1st y.)	51.7 a	17100.0	5300.5	3258.5 a	1.52	4.59 fg	150.47
Fall × 2nd Cut. (1st y.)	37.9 bd	15918.8	4207.9	1974.4 b	0.89	6.47 b	128.38
Fall × 3rd Cut. (2nd y.)	41.2 b	12721.7	3191.3	2026.5 b	1.75	6.14 bc	123.79
Fall × 4th Cut. (2nd y.)	34.4 ce	8470.9	2312.2	1366.6 c	1.45	5.39 de	74.72
Fall × 5th Cut. (3rd y.)	30.5 ef	6708.2	1904.9	1215.6 c	1.75	4.78 f	57.87
Fall × 6th Cut. (3rd y.)	25.6 f	5898.9	1773.6	1033.6 c	1.40	4.20 g	43.58
Spring × 1st Cut. (1st y.)	39.1 bc	16284.1	4172.3	2375.0 b	1.39	7.03 a	167.01
Spring × 2nd Cut. (1st y.)	37.1 bd	15201.5	4199.4	2412.9 b	1.31	5.75 cd	139.25
Spring × 3rd Cut. (2nd y.)	34.7 ce	12536.1	3309.6	2337.2 b	2.41	4.96 ef	115.22
Spring × 4th Cut. (2nd y.)	37.4 bd	8851.6	2367.5	1306.0 c	1.27	5.61 cd	73.18
Spring × 5th Cut. (3rd y.)	33.3 de	7325.5	1933.7	1137.1 c	1.46	5.43 de	61.89
Spring × 6th Cut. (3rd y.)	26.8 f	6061.1	1714.4	986.9 c	1.36	4.99 ef	49.29
LSD $_{(\%5)}$	5.191	-	-	515.7		0.5466	-
CV (%)	8.51	13.69	15.12	16.95	21.81	5.88	18.89
Std. Dev.	7.38	4470.72	1239.03	749.67	0.44	0.83	44.93

The means which have no letters are statistically non-significant at 5% probability level
*: Experimental years

Leaf:stem ratio

In this experiment, the highest leaf:stem ratio (2.08) was found in the 3rd cutting (Table 3). The evaluation of the data for leaf:stem ratio showed that the 1st cuttings were superior to the 2nd in all three experimental years. Also, the elongation of the plant growth period in a growing season can significantly reduce leaf:stem ratio at the first cuttings, such as leaf dry weight. The leaf:stem ratio is a criterion of photosynthetic assimilates production. The climatic and genetic factors affect leaf dry yield; these factors also affect the leaf:stem ratio. Solomon and Beemnet [41], and Kassahun *et. al.*[42] reported that with the increasing plant age in *M. arvensis* and *M. piperita*, this ratio was declined. Furthermore, Kumar *et. al.*[43] reported similar results concerning the superiority of leaf:stem ratio in the 1st cuttings.

Content and yield of volatile oil

The VO content presented in Table 3 showed a significant difference between the cuttings and interaction effects of planting season and cutting (Table 3). The VO content of the 2nd cutting was

the highest (6.11 %). Also, 1st cutting was ranked second (5.81 %). The 1st year was superior in terms of VO content. The percentage of VO content reduced in the subsequent cuttings, correspondingly. The interaction effects of planting season and cuttings on the VO content showed that the 1st cutting of spring planting (7.03 %) and the 2nd cutting of fall planting (6.47 %) hold the highest VO content, respectively. On the other hand, further interpretation of the VO content results indicated that the 1st cuttings of both planting seasons had general superiority except the 1st year of fall planting. Even though the average VO yield of spring planting was slightly higher than fall planting, they were not statistically significant. The mean comparisons furthermore showed the significant effect of cuttings on the VO yield (Table 3). The 1st cutting produced the highest VO yield (158.74 L ha^{-1}). The lowest VO yield was also obtained in the last cutting (6th). As known, the yield of VO in mint is a function of leaf dry weight and VO percentage. Accordingly, considering the leaf dry yield, VO yield had a decreasing pattern from the 1st cutting to the end. Except for the 1st year, VO content showed a downward trend in the subsequent years. Continuous decrease in VO content and yield is probably associated with reduced soil fertility in marginal land conditions, just as agronomic yield components have also declined over time. The high VO accumulation in the second harvest of the 1st year can be linked chiefly to the climatic conditions and overwintering plants immediately after planting. Furthermore, the reduced VO content of menthol mint may be associated with inferior marginal soil fertility and increasing plant age over time.

Özel and Özgüven [25] showed that the VO content in the 2nd cuttings of four planting times of *M. arvensis* var. *piperascens* (L.) Holmes was significantly higher than the 1st cutting in the 1st year. While in the 2nd year, in general, the VO content was reduced and the 1st cuttings produced high VOs than the 2nd. There is a similarity between the study mentioned above and the current results. However, in the Çaliskan [24] experiment, the VO content of the 2nd year was higher than the 1st year. Although genetic processes primarily synthesize secondary metabolites, their production is also significantly influenced by environmental factors. The VO biosynthesis and accumulation and their chemical compositions in medicinal plants are strongly influenced by the genetic makeup, agro-techniques (plant density, planting time, harvesting stage, etc.), environmental factors as well as biotic and abiotic stresses [44]. Singh *et. al.*[45] reported that the yield of menthol mint was 94.6 to 200 L ha^{-1}. The VO yield in the study of Çaliskan [24], conducted at the same location in a fertile land was more than the current findings. In another study conducted by Rajeswara Rao [33], the total VO in six cuttings was more than ours. Therefore, it can be concluded that semi-arid climate and land fertility dominate the inefficient or non-fertile land regarding the VO yield.

Chemical properties of volatile oil

GC-MS analysis of *M. arvensis* VO resulted in 39 constituents (Table 4). Menthol was found as the major component (52.19 % - 73.53 %) (Table 4). The maximum amount of menthol was achieved from the 2nd cutting in fall planting. Menthol amount was following a decreasing pattern till the end of the experiment and the last cutting contained the least menthol content (Table 4). Menthone was the second major compound (3.42 to 18.89 %) (Table 4). Menthone was considerably increased in the cuttings of the 3rd year, which there was a decrease in menthol levels in relevant treatments (Table 4). The other major components were α-pinene (0.75-4.12 %), α-thujene (0.2-7.48 %), limonene (2.01-4.06 %), 3-octanol (0.96-2.29 %), bicyclogermacrene (1.42-2.19 %) and piperitone (0.65-2.06 %). Although the amount of menthyl acetate was significant in most spring planting (2.88-5.77 %), it was observed in low amounts in three fall planting treatments (0.11-0.76 %). α-Pinene content was highest in the 2nd, 3rd, and 4th cuttings of both planting seasons. The least data for this compound was observed in the 1st cuttings of both seasons. Although α-thujene was present in high amounts in VO, the percentage of this compound was negligible in the first cuttings of both planting seasons. Even in the 2nd cutting of fall planting,

no trace of this compound was observed. The highest values of α-thujene were identified in the 4th cuttings of both planting seasons. For limonene, the maximum amounts were observed in the 1st cuttings of fall and spring plantings. The maximum amount of this compound also belonged to spring planting. Limonene showed a downward trend in most treatments after the 3rd cuttings. In terms of 3-octanol biosynthesis, the 1st cuttings of both planting seasons were superior to the other treatments (2.1-2.29 %). The maximum of this compound was recorded in fall planting. Menthofuran is known as a quality determinant compound of *M. arvensis*. This compound was rare (0.04-0.11 %) or absented in most cuttings in both planting seasons. Camphene and neoisomenthyl acetate were only observed in one treatment. Pinocarvone was also absent in all fall planting.

Seemingly, the reduction in menthol levels in the last cuttings is probably related to plant aging. A corresponding decrease in limonene levels in the last cuttings has led to a decrease in menthol content. Limonene is a precursor for menthol biosynthesis [46]. The amount of menthol production follows a sigmoid chart so that the amounts enhance from the 10th day to the 18th day at a constant rate. Then, menthol increases from the 18th day to the 40th day with a higher growth rate and finally remains constant. More menthol biosynthesis occurs in 30 days after the maximum menthone accumulation [47,48]. Therefore, harvest time has a significant effect on the amounts of chemical compounds. Based on European Pharmacopoeia [49], the acceptable amounts of main compounds in dementholized *M. arvensis* oil are; menthol (30-50 %), menthone (17-35 %), isomenthone (5-13 %), menthyl acetate (1.5-7 %), 1,8-cineole (max. 1.5 %), limonene (1.5-7 %), isopulegol (1-3 %), pulegone (max. 2 %), and carvone (max. 2 %). Verma *et. al.*[12] reported that the main component of *M. arvensis* were menthol (62-82 %), menthone (3.4-19 %), isomenthone (2.3-6 %), menthyl acetate (0.5-4.4 %), limonene (0.27-4.74 %), isopulegol (0.4-1.5 %), pulegone (tr-0.7 %), neomenthol (1.3-2.4 %) and carvone (tr-0.1 %) for 4 cultivars of *M. arvensis* VO at 5 different stages of growing period. The menthol amount in the current study was higher than Özel and Özgüven [50] records (22.58-33.25 %) during six cuttings. But, compared with Özgüven and Kirici [32] (69.06-72.19 %) and Çaliskan[24] (65.52-76.19 %) in the same location, the menthol amount of the present study was much lesser.

Oxygenated monoterpenes formed the predominant constituents of *M. arvensis* VO (76.1-86.98 %). On the whole, the amounts of this chemical group had a relatively superior fall planting. The highest values of oxygenated monoterpenes were recorded in the 2nd cutting of fall planting and the lowest was recorded in the 1st cutting of spring planting (Table 5).

Hydrocarbon monoterpenes were in the second place of chemical grouping (6.7-15.89 %). The highest amount of these compounds was obtained in the 2nd year cuttings of both planting seasons. Other chemical groups were identified in minor concentrations. Oxygenated sesquiterpenes (0.3-1.91 %) were at their maximum level in the 3rd year of spring planting. Although this chemical group also had high values in the 3rd year of fall planting, the highest amount among all treatments was related to the 1st cutting of this season. Except for the 4th cuttings of both planting seasons, no significant changes were observed in the amounts of sesquiterpene hydrocarbons of other treatments (0.67-3.72 %). Thus, the 4th cuttings had the lowest level of sesquiterpene hydrocarbons. The synthesis of ester (0-5.77 %) compounds in spring planting cuttings was significantly higher than in fall planting. Also, unidentifiable compounds (other) were observed only in the 1st cutting of fall planting (Table 5). Oxygenated monoterpenes such as menthol, menthone and their isomers, menthyl esters, and piperitone are the main chemical constituent groups of *M. arvensis* that determine the specific flavour of the plant[51].

Conclusions

All in all, the fall and spring planting date were not an influential factor on the yield and volatile oil production of *M. arvensis* cultivated in marginal land. This situation is probably due to the moderate winter temperate, early spring, and relatively warm summer of the Çukurova region. The highest biomass yield was obtained in the two cuttings of

Table 4. Volatile oil composition of *M. arvensis* influenced by planting season and marginal land conditions (%)

					Fall Planting Season						Spring Planting Season					
					1st Year		2nd Year		3rd year		1st Year		2nd Year		3rd year	
No.	RT	RI	RI_L	Compounds	1st Cut.	2nd Cut.	3rd Cut.	4th Cut.	5th Cut.	6th Cut.	1st Cut.	2nd Cut.	3rd Cut.	4th Cut.	5th Cut.	6th Cut.
1	8.722	1028	1031	α-Pinene	0.75	2.41	4.12	2.78	1.79	2.07	0.99	3.09	2.56	2.63	1.54	1.87
2	9.688	1072	1035	α-Thujene	0.20	-	6.68	7.09	5.90	6.49	0.21	4.80	6.76	7.48	6.43	5.80
3	9.778	1076	1078	Camphene	-	-	-	-	-	-	-	0.95	-	-	-	-
4	10.113	1091	1097	2-Hexanone	-	-	0.19	0.13	0.15	0.13	0.07	0.12	0.16	0.19	0.12	0.15
5	10.822	1117	1120	β-Pinene	1.00	0.86	0.80	0.84	0.68	0.46	1.16	0.86	0.80	0.81	0.74	0.68
6	11.142	1127	1130	Sabinene	0.61	0.51	0.52	0.51	0.52	0.47	0.75	0.61	0.43	0.46	0.54	0.54
7	12.286	1164	1168	β-Myrcene	0.99	0.85	0.79	0.77	1.02	0.76	1.25	0.93	0.76	0.74	0.88	0.74
8	13.643	1206	1209	Limonene	3.15	2.51	2.98	2.57	2.25	2.51	4.06	2.71	2.78	2.45	2.42	2.01
9	14.014	1216	1219	1,8-Cineole	0.31	0.19	0.22	0.21	0.20	0.19	0.29	0.24	0.29	0.17	0.21	0.21
10	14.486	1228	1228	*trans*-2-Hexenal	-	0.03	0.04	0.03	0.04	0.04	0.12	0.02	-	0.03	0.02	0.04
11	20.915	1391	1393	3-Octanol	2.29	1.27	1.30	1.39	1.12	1.17	2.10	1.68	1.30	1.16	0.96	1.13
12	24.245	1472	1495	Isomenthone	-	-	5.05	3.78	-	2.46	6.29	-	2.32	-	4.86	-
13	25.132	1494	1503	Menthofuran	0.06	-	0.04	-	0.08	0.07	-	0.02	0.11	-	0.07	0.09
14	25.367	1500	1503	Menthone	9.88	10.85	3.42	4.49	18.89	16.48	4.05	10.09	6.91	7.32	15.05	18.63
15	26.889	1538	1556	Neoisomenthyl acetate	0.94	-	-	-	-	-	-	-	-	-	-	-
16	27.364	1550	1558	Linalool	0.80	1.05	0.78	0.91	0.83	0.77	0.96	1.85	0.84	0.68	0.83	1.02
17	28.634	1582	1559	Pinocarvone	-	-	-	-	-	-	0.43	0.55	-	-	0.57	0.61
18	28.745	1585	1585	Menthyl acetate	0.19	-	0.11	-	-	0.76	5.77	-	-	4.45	3.12	2.88
19	28.975	1591	1642	Levomenthol	1.00	-	0.82	5.76	0.14	0.42	-	0.21	0.94	0.88	0.22	0.22
20	31.003	1643	1652	Menthol	66.58	73.53	64.99	62.61	57.12	55.83	61.60	64.56	65.20	64.17	53.44	52.19
21	32.268	1677	1681	Lavandulol	1.43	0.75	0.63	1.23	1.03	0.84	1.37	1.00	0.78	1.45	0.79	0.75
22	32.434	1681	1697	α-Humulene	0.32	0.18	0.15	0.13	0.14	0.16	0.26	0.21	0.16	0.12	0.15	0.14
23	33.092	1698	1706	α-Terpineol	1.02	0.61	0.70	0.75	0.75	0.77	0.78	0.76	0.75	0.75	0.76	0.71
24	33.75	1716	1719	Germacrene D	0.68	0.58	0.55	0.35	0.66	0.55	1.15	0.97	0.93	0.27	0.73	0.76
25	34.316	1731	1731	Neryl acetate	0.42	-	-	0.22	0.41	0.35	-	-	-	-	-	-
26	34.351	1732	1737	α-Muurolene	-	0.17	0.19	-	-	0.14	-	-	0.17	0.19	0.13	0.20
27	34.419	1734	1738	Piperitone	-	-	0.65	1.77	1.85	1.79	-	-	1.84	1.68	2.06	1.86
28	35.235	1755	1755	Bicyclogermacrene	2.12	2.01	1.78	-	1.48	1.53	2.03	2.19	-	-	1.42	1.44
29	35.581	1765	1777	δ-Cadinene	0.33	0.13	0.17	0.19	0.15	0.25	0.28	0.24	0.25	0.11	0.23	0.15
30	38.98	1847	1847	Geraniol	-	-	-	-	-	-	0.28	-	-	-	-	-

table 4. (continued)

No.	RT	RI	RI_L	Compounds	Fall Planting Season						Spring Planting Season					
					1st Year		2nd Year		3rd year		1st Year		2nd Year		3rd year	
					1st Cut.	2nd Cut.	3rd Cut.	4th Cut.	5th Cut.	6th Cut.	1st Cut.	2nd Cut.	3rd Cut.	4th Cut.	5th Cut.	6th Cut.
31	44.723	1995	1995	Caryophyllene oxide	0.37	0.11	0.19	0.02	0.12	0.15	-	0.03	0.15	0.20	0.18	0.16
32	45.265	2010	2010	Humulene epoxide	-	-	-	0.11	0.11	0.11	-	0.09		0.10	0.12	0.11
33	45.489	2020	-	Unknown	-	-	-	-	-	-	0.11	-	-	-	-	-
34	45.863	2033	-	Unknown	-	-	-	-	-	-	0.22	-	-	-	-	-
35	46.056	2040	2042	Nerolidol	0.09	0.04	0.05	0.03	0.48	0.39		0.04	0.04	0.03	0.50	0.42
36	46.519	2057	2057	Ledol	0.36	-	0.13	-	-	0.31	0.14	-	0.21	-	-	0.24
37	48.524	2133	2146	(-)-Spathulenol	0.32	0.06	0.13	0.04	0.14	0.21	0.08	0.05	0.11	0.04	0.08	0.17
38	49.995	2195	2195	α-Cadinol	0.77	0.09	0.04	0.02	0.22	0.31	0.36	0.24	0.43	0.03	0.35	0.25
39	50.767	2232	2223	α-Bisabolol	-	-	0.30	0.21	0.10	0.10	-	0.02	-	0.02	0.10	0.09

RT: Retention time; RI: Retention indices calculated against n-alkanes (C7-C30) on HP-Innowax column
RI_L: Retention indices that were calculated in different literatures

Table 5. Grouped compounds of *M. arvensis* influenced by planting season and marginal land conditions (%)

Grouped compounds (%)	Fall planting season						Spring planting season					
	1st Year		2nd Year		3rd year		1st Year		2nd Year		3rd year	
	1st Cut.	2nd Cut.	3rd Cut.	4th Cut.	5th Cut.	6th Cut.	1st Cut.	2nd Cut.	3rd Cut.	4th Cut.	5th Cut.	6th Cut.
Oxygenated monoterpenes: 9, 12, 13, 14, 16, 17, 19, 20, 21, 23, 27, 30	81.08	86.98	77.3	81.51	80.89	79.62	76.1	79.28	80	77.1	78.86	76.29
Monoterpene hydrocarbons: 1, 2, 3, 5, 6, 7, 8	6.7	7.14	15.89	14.56	12.16	12.76	8.42	13.95	14.1	14.57	12.55	11.64
Oxygenated sesquiterpenes: 31, 32, 35, 36, 37, 38, 39	1.91	0.3	0.84	0.43	1.17	1.58	0.58	0.47	0.94	0.42	1.33	1.44
Sesquiterpene hydrocarbons: 22, 24, 26, 28, 29	3.45	3.07	2.84	0.67	2.43	2.63	3.72	3.61	1.51	0.69	2.66	2.69
Alcohol: 11	2.29	1.27	1.3	1.39	1.12	1.17	2.1	1.68	1.3	1.16	0.96	1.13
Aldehyde: 10	-	0.03	0.04	0.03	0.04	0.04	0.12	0.02	-	0.03	0.02	0.04
Ester: 15, 18, 25	1.55	0	0.11	0.22	0.41	1.11	5.77	0	0	4.45	3.12	2.88
Ketone: 4	-	-	0.19	0.13	0.15	0.13	0.07	0.12	0.16	0.19	0.12	0.15
Other: 33, 34	-	-	-	-	-	-	0.33	-	-	-	-	-
Total (%)	96.98	98.79	98.51	98.94	98.37	99.04	97.21	99.13	98.01	98.61	99.62	96.26

the 1st year. Also, the dry leaf yield was higher in the first cutting of the 1st year. The highest percentage and yield of volatile oil were related to the 1st year of the experiment. Oxygenated monoterpenes, monoterpene hydrocarbons, and sesquiterpene hydrocarbons formed the most extensive chemical group compounds in *M. arvensis* volatile oil, respectively. Menthol and menthone as oxygenated monoterpenes were identified as the major constituents of *M. arvensis*. Menthol levels increased only in the 1st cuttings of the 1st year of both growing seasons. In the 2nd and 3rd years of both growing seasons, menthol was constantly reduced in the 2nd cuttings. Menthone content decreased with the plants aging. Menthol and menthone content followed a reverse pattern so that, with more menthol content, the menthone accumulation was repressed. The agronomic yield, volatile oil production, and menthol content of *M. arvensis* were severely reduced by the aging of plants and soil fertility in marginal land conditions. Given the socio-economic conditions prevailing in areas with marginal lands, the cultivation of some medicinal plants in these lands can be recommended economically.

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