ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Journal of Agronomy 4 (2): 103-108, 2005
ISSN 1812-5379

Determination of Agronomic and Essential Oil Properties of Peppermint (*Mentha piperita* L.) in Various Ages of Plantation

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Abstract: Peppermint (*Mentha piperita* L.) is one of the high demand aromatic medicinal plants and essential oil extracted from the crop is extensively used in the fragrance, flavor and pharmaceutical industries. A field experiment was carried out during the periods of 1998-2001 in the Central Black Sea region climatic condition to investigate variation of yields, essential oil content and components during the years and harvesting periods in two local cultivated peppermint (*M. piperita*) varieties (from Gaziantep and from Adana) from Turkey and to determine economical yield levels. Since one harvest was made in both varieties in the 1st year, yield was low. Each variety was harvested two times in the 2nd and 3rd years and maximum total yields were obtained in the 2nd year in both varieties. While variety 1 was harvested one time in the 4th year, variety 2 did not grow well enough for harvest. Essential oil content of both varieties was higher in the 2nd harvest in 1999 and 2000. While higher menthol contents (56.7 and 56.4%) of the variety 1 were obtained from the 2nd harvests of 1999 and 2000, menthol contents were lower in the variety 2 in the 2nd harvest due to delayed harvests. Good quality of essential oil in the variety 1 was only obtained from 2nd harvests of 2nd and 3rd years, 1999 and 2000. But essential oil of variety 2 was good quality all years, except those of 2nd harvest of 2nd and 3rd years. While economic yields levels of variety 1 were obtained in 2nd (fresh herbage 25.96 t ha^{-1}) and 3rd years (fresh herbage 20.40 t ha^{-1}), economic yield level of variety 2 was obtained only in 2nd year with 26.25 t ha^{-1} fresh herbage yield and was lower than other years.

Key words: *Mentha piperita*, peppermint, yields, essential oil, menthol

INTRODUCTION

Peppermint (*Mentha piperita* L.), which is a hybrid between *M. spicata* and *M. aquatica* belonging to Labiatae, is one of the most important essential oil plants. Essential oil extracted from the crop contents mainly menthol and menthone and it is used extensively in pharmaceutical, flavor and perfumery industries and in aromatherapy. Peppermint is, therefore, cultivated commercially in many countries. But, mint oil is not produced commercially in Turkey and the consumption of peppermint oil was satisfied by importation. Ozel and Ozguven[1] estimated that 25.2 tones of peppermint essential oil and 14 tones of menthol were imported from different countries costing about $ 631.114.

Recent studies related to growth of peppermint in Turkey showed that this crops could be grown successfully in the different climatic conditions of Turkey and varying climatic conditions could affect both yields and quality properties of the plant[2,3]. It was also concluded from previous studies that peppermint could be harvested two times in the temperature climate of Cukurova, South Turkey, while it could be harvested ones in the cooler climatic conditions of high altitude regions[3].

The quality of mint oil is dependent upon both climatic factors effecting biosynthesis, such as photoperiod[4-6], temperature[7], drought[8] and biological factors, such as ontogeny[9] and ageing[10,11]. Agronomic applications such as planting time[1,12], harvest time[13,14], fertilization and irrigation[15] can, also, affect essential oil quality. According to the pharmacopoeia and standards of peppermint oil, menthol content of peppermint oils should not be less than 50% and menthon and methyl acetate contents should be between 10-30 and 5-20%, respectively[16]. According to the previous studies, reduction of pulegone to menthone, the precursors of menthols, results in high quality oil in the peppermint oil. Clark and Menary[17] explained that inter-conversion of essential oil components was affected by coenzymes such as $NADPH_2$ and the accumulation of the coenzymes varied due to light intensity, temperature, photoperiods, high and low day temperatures.

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Table 1: Weather parameters during the period of the study

	Monthly rainfall (mm)				Mean daily temperature (°C)				Sunny time per day (h)			
Months	1998	1999	2000	2001	1998	1999	2000	2001	1998	1999	2000	2001
March	37	34	36	19	3.4	7.9	5.7	11.3	2.6	5.0	5.9	5.9
April	25	67	91	39	10.3	12.8	15.0	13.5	5.1	7.8	5.2	5.4
May	117	47	88	92	17.2	16.1	14.9	14.4	9.7	8.2	7.0	7.8
June	7	34	15	6	19.4	20.7	18.7	20.2	9.4	7.5	8.8	9.4
July	6	2	0	1	22.3	23.7	24.6	23.5	9.2	9.0	10.8	9.8
August	16	22	61	1.2	21.1	23.2	22.5	23.3	11.0	8.4	9.0	8.9
September	16	26	95	20	18.0	19.1	19.3	19.6	7.2	9.3	8.4	8.7

Because of broad agricultural potential, efforts have been made on cultivation and breeding studies of essential oil plants recently. After cultivation and improvement studies and suitable agricultural management, Turkey will be able to produce more than the amounts of essential oil needed in Turkey. This study evaluates the result of field experiment conducted in order to determine agronomic adaptability, economical periods and oil quality of *Mentha piperita* L. in climatic conditions having elevations up to 610 m above the sea level. The variations of yields, essential oil content and component were studied as long as harvest periods in the study.

MATERIALS AND METHODS

Experimental design and agronomic practices: The cuttings of running rhizomes in two local varieties of *Mentha piperita* L. obtained from Gaziantep (variety 1) and Adana (variety 2) growers in Turkey were rooted into a medium with mixture of washed sand, horse mature and field soil (1:1:1). Then the rooted crops were transferred to the field prepared and fertilised (50 kg ha^{-1} N and 100 kg ha^{-1} P_2O_5) from Agricultural Faculty of Gaziosmanpasa University in Tokat on April 20, 1998. The study area is located between 35.3° and 37.9° N. latitudes, 39.5° and 40.5° E. longitudes with an altitude of 650 m. The weather parameters for the study area were given in Table 1. The soil was clay-loam soil with pH 7.8, organic carbon 1.70% and 11.6 and 279.0 kg ha^{-1} available P (P_2O_5) and K (K_2O), respectively. The crops were examined for four years between 1998 and 2001. During the vegetation periods, agronomic operations were applied. The crops were harvested ones in 1998 and twice 1999 and 2000. Although one of the varieties was harvested in 2001, the other did not grow well enough for harvest (Table 2). Fresh herbage (biomass) yields (t ha^{-1}), dry herbage yields (t ha^{-1}), dry leaves yields (kg ha^{-1}) and essential oil yields (L ha^{-1}) were measured for each harvest.

Determination of essential oil content and composition: The leaves separated and dried at 35°C were subjected to distillation in a modified Clevenger system for 2 h (1:15 w/v). The essential oils were stored in glass bottles at 4°C until GC analysis.

Table 2: The details of harvesting schedule for both varieties

Years and harvests	Variety 1	Variety 2
1998-1. harvest	25.07.1998	28.07.1998
1999-1. harvest	05.07.1999	15.07.1999
1999-2. harvests	26.08.1999	18.09.1999
2000-1. harvest	07.07.2000	17.07.2000
2000-2. harvests	03.09.2000	18.09.0000
2001-1. harvest	20.07.2001	-

Essential oil components were determined by Carlo Erba Gas Chromatography equipped with Flame Ionization Detector (FID) and electronic integrator, using bonded phase fused silica capillary column coated with methyl silicone. The operating conditions were as follows: Injection temperature 250°C, hydrogen carrier gas flow 1.5 mL min^{-1}, temperature programming 60°C (3 min) -180 at 3°C min^{-1}. Helium carrier gas flow rate was 1.5 min^{-1}. The mass spectra were obtained at 70 eV. Injector temperature was 230°C and ion source temperature was 250°C. The identification of components was based on comparison of their relative retention times with those of authentic standards.

Statistical analyses: The data obtained from each harvests with 3 replications were subjected to statistical variance analyses using F test according to Snedecor[18]. Variables in the each variety were subjected separately to a variance analysis. The means of the variables were grouped by using Least Significant Difference (LSD) values at 5% level of probability.

RESULTS AND DISCUSSION

Plant height and yield properties: The plant height (cm), fresh herbage (t ha^{-1}), dry herbage (t ha^{-1}), dry leaves (kg ha^{-1}) and essential oil yields (L ha^{-1}) for both varieties were significantly influenced by harvests (Table 3). The growing performances of varieties were partially different for each one. Growing performance of variety 2 was decreased more rapidly than that of the variety 1 and the variety 2 did not grow well enough to harvest in 2001 (Table 2).

Table 3: The variation of plant height, yields and essential oil content according to harvest periods and years

Variables	Plant height (cm)	Fresh herbage yield (t ha^{-1})	Dry herbage yields (t ha^{-1})	Dry leaves yields (kg ha^{-1})	Essential oil yields (kg ha^{-1})	Essential oil contents (%)
Variety 1						
1998-1. harvest	54.00	6.67	1.86	1220.00	25.00	2.10
1999-1. harvest	60.80	15.30	4.59	2800.00	55.80	2.00
1999-2. harvest	51.00	10.66	2.68	1540.00	39.90	2.80
2000-1. harvest	56.30	13.50	3.78	2300.00	38.90	1.70
2000-2. harvest	37.10	6.90	1.79	1090.00	30.30	2.80
2001-1. harvest	31.60	4.20	1.13	700.00	12.00	2.20
LSD	4.14	1.33	0.38	225.00	3.40	0.26
CV (%)	4.69	7.68	8.07	7.69	5.68	6.49
Variety 2						
1998-1. harvest	65.60	7.83	2.03	1340.00	24.20	1.80
1999-1. harvest	59.80	14.90	4.17	2620.00	66.70	2.50
1999-2. harvest	42.50	11.35	2.98	1720.00	48.20	2.80
2000-1. harvest	42.40	7.64	2.28	1410.00	22.00	1.60
2000-2. harvest	28.50	4.10	1.23	730.00	14.70	2.00
2001-1. harvest	-	-	-	-	-	-
LSD	3.24	0.65	0.20	117.40	3.10	0.05
CV (%)	3.50	3.78	4.12	3.98	4.77	1.30

LSD: Least Significant Difference ($p<0.05$), CV: Coefficient of Variation

Table 4: Total yield values of varieties according to years

	Fresh herbage (t ha^{-1})		Dry herbage (t ha^{-1})		Dry leaves (kg ha^{-1})		Essential oil (L ha^{-1})	
Years	Var 1	Var 2	Var 1	Var 2	Var 1	Var 2	Var 1	Var 2
1998	6.67	7.83	1.86	2.03	1220	1340	25.0	24.2
1999	25.96	26.25	7.27	7.15	4340	4340	95.7	114.9
2000	20.40	11.74	5.57	3.51	3390	2140	69.2	36.7
2001	4.20	-	1.13	-	700	-	12.0	-

Table 5: The variation of essential oil components according harvest periods and years (%)

Variables	Menthol	Menthone	Menthafuran	Methyl acetate	Pulegon	1.8 cineol
Variety 1						
1998-1. harvest	41.30	36.55	4.97	2.10	2.96	9.65
1999-1. harvest	43.20	31.75	4.23	2.58	2.68	13.93
1999-2. harvests	56.40	16.90	2.50	5.14	2.12	13.89
2000-1. harvest	36.60	40.26	3.43	1.98	3.07	12.83
2000-2. harvests	56.70	13.92	1.88	5.10	2.83	11.79
2001-1. harvest	40.80	33.50	3.02	4.75	2.85	11.80
LSD	0.64	4.67	2.05	1.54	ns	ns
CV (%)	3.03	5.87	23.72	16.66	12.51	8.88
Variety 2						
1998-1. harvest	50.42	15.90	4.07	3.42	2.42	13.58
1999-1. harvest	53.43	15.27	3.30	3.73	2.55	14.03
1999-2. harvests	44.34	25.07	2.46	3.08	3.10	13.80
2000-1. harvest	51.91	17.01	2.61	4.07	2.55	13.81
2000-2. harvests	41.11	25.00	3.75	3.15	3.31	12.56
2001-1. harvest	-	-	-	-	-	
LSD	5.15	3.78	0.72	0.75	0.34	ns
CV (%)	3.85	6.87	8.05	7.80	4.42	7.25

LSD: Least Significant Difference ($p<0.05$), CV: Coefficient of Variation

The variation of plant height between two varieties was significantly different. The highest value for plant height was obtained for the variety 1 at the 1st harvest of the 2nd year (60.8 cm) while for the variety 2 at the 1st harvest in the 1st year (65.6 cm). The plant heights for the 1st harvests in 1999 and 2000 (60.8 and 56.3 cm for variety 1 and 59.8 and 42.4 cm for variety 2, respectively) were significantly higher than those of the 2nd harvests for both varieties (51.0 and 37.1 cm for variety 1 and 42.5 and 28.5 cm for variety 2 in 1999 and 2000, respectively). The suitable climatic conditions such as photoperiods[4,6] and temperature[19] during the 1st harvest periods in each year increased the plant height for the 1st harvests. As far as the years were concern, plants heights increased in the 1st and 2nd years and decreased rapidly in the 3rd and 4th years as a result of increasing plant density based on enhancement shoot with continuously cutting and physiological ageing[20].

The fresh and dry herbage yields, dry leaves yield and oil yields were measured after each harvest for both varieties during the study. The yields as well as plant height were varied significantly in each harvest. The yields of both varieties for the 1st years (6.67 and 7.83 t ha^{-1} for variety 1 and variety 2, respectively) were significantly lower than those of the 2nd and 3rd years (Table 3 and 4) due to poor growth of crops as a consequence of less carbohydrate in the plants[19]. The maximum yields (fresh and dry herbage yields) for both varieties were recorded at 1st harvest of the 2nd years with 15.30 (fresh) and 4.59 t ha^{-1} (dry) in variety 1 and 14.90 (fresh) and 4.17 t ha^{-1} (dry) in the variety 2. The maximum leaves yields were recorded as 2800 and 2620 kg ha^{-1} for variety 1 and variety 2, respectively at the 1st harvests of the 2nd years. The reason for this is due to availability of optimum plant density at the 2nd year and long and suitable climatic conditions during the 1st harvest periods.

The 2nd harvest yields of both varieties were, also, higher in the 2nd years than that of other years. Maximum total herbage yields for both varieties were, therefore, obtained in the 2nd year (Table 4). After the 2nd year, the yields decreased gradually for both varieties due to weak growth plants. As a result of continuously propagating plants from running stolone and encouraging of lateral bloom by cutting, the increasing plant density per area caused a weak growth for plants in 2000 and 2001 vegetation periods. The increased plant densities also caused fall down lower leaves due to insufficient light density and poor aeration[21]. The yields in variety 2 decreased more rapidly than those of variety 1 and plants did not grow well enough for harvest in 2001 vegetation periods.

Essential oil percentage and oil yield: Results obtained showed that the highest essential oil content was recorded for the 2nd harvest of the 2nd and 3rd year (2.8%) for variety 1 and for the 2nd harvest of the 2nd year (2.8%) for variety 2. Weather parameters such as atmospheric temperature and rainfall may influence essential oil contents in the peppermint[7]. It is also claimed that the increase of the oil content in the 2nd cutting might be attributed to the higher temperature[22] and light density[7]. It can be considered that the density of peltate and capitate trizomes synthesizing and storing essential oil in the plants could be more per leaves area in the 2nd cutting. On the other hand, the lowest essential oils contents in variety 1 (1.7%) and 2 (1.6%) were observed for the 1st harvest at the 3rd year. The lower essential oil content was probably due to low temperature with 14.9°C in May (Table 1) prevailing during active growth periods.

The essential oils yield is a product of biomass yield and essential oil concentration in the plants. Although the higher oil contents in the 2nd cuttings was obtained for the 2nd harvests of 1999 and 2000, the maximum oil yields with 55.8 and 66.7 L ha^{-1} in variety 1 and 2 were measured in the 1st cuttings of 1999 due to the height leaves yield of the 1st harvests. On the contrary to drug leaves yields, maximum oil yields in the 1st harvest of 1999 were obtained for the variety 2 (66.7 L ha^{-1}) due to the higher oil contents (2.0 and 2.5% for variety 1 and for variety 2, respectively) (Table 3). The total maximum oil yields of both varieties (95.7 and 114.9 L ha^{-1} for variety 1 and 2, respectively) were recorded in the 2nd year (Table 4). Similar result in oil yields of the some *Mentha* species was observed by Ozguven and Kirici[3]. The records are also higher than that of Rajeswara Rao[23].

Essential oil contents components: It can be concluded from Table 5 that menthol, menthon, menthofuran and methyl acetate contents varied significantly for the variety 1, while variations of pulegone and 1.8 cineol contents were not significantly important. However, all essential oil components, except for 1.8 cineol, were significantly important for variety 2. Menthol and menthon are the main components in the peppermint oil and they confer characteristic aroma. Cineol and methyl acetate are also considered desirable components while menthofuran and pulegon are undesirable[17]. Differences in menthol contents were statically significant for each harvest. Menthol contents for variety 1 were higher for the 2nd harvest in 1999 (56.4%) and in 2000 (56.7%), while higher menthol contents for variety 2 were obtained for the 1st harvests in 1998 (50.42%), 1999 (53.43%) and 2000 (51.91%). The differences between the two varieties might be attributed to differentiation in the growing and harvest periods and genetic statuses of the varieties. Since variety 1 initiated growing in early spring prevailing lower temperature and rainy phases, the 1st harvest of this variety was made in the beginning of summer and transformation of menthol to menthone decreased because of lower temperatures and light density[7,15]. Although the harvesting of 1st year, 1998, was made in hot summer days, menthol contents in the variety 1 decreased (41.3%) because of high branching causing immature leaves in the harvest[10,24].

Variety 2 was grown more slowly than variety 1 and grew in later spring periods. The 1st harvest at the variety 2 was, there fore, harvested in the hot summer days and maturity in the 2nd harvest was delayed (September). Although temperatures, photoperiods and light densities increased menthol contents of variety 2 in 1st cutting, shortening photoperiods, decreasing temperature and light density in mid September slowed transformation menthone to menthole and increased menthon and menthofurane contents[6,7,24].

Because of the fact that herbage yields and oil quality varied according to climatic and biological factors, yield potential and oil quality were studied during growth periods of the two local varieties originated from Turkey. Therefore, the relationship between oil quality and harvest periods for years was analyzed statically. It was concluded that the yields for both varieties at the 2nd and 3rd years were significantly higher than those of the 1st year. While total yields of 2nd year were nearly same for both varieties, the yields of the 3rd year were lower in the variety 2 than 1. Economic yields levels of variety 1 were, therefore, obtained in 2nd year with 25.96 t ha^{-1} fresh herbage yields and 3rd years with 20.40 t ha^{-1} fresh herbage yields (Table 4). But, economic yield level of variety 2 was obtained only in 2nd year with 26.25 t ha^{-1} fresh herbage yield and was lower than other years (Table 4).

The varieties had different growing periods and the differentiation effected oil quality. The oils with high quality were obtained from the crops growing and harvested in hot temperature periods. Variety 1 had started growing in early spring and oil quality in the 1st cutting was lower than that of the 2nd harvest. However, the variety 2 started growing in the later spring periods and oil quality for the 1st harvest was higher than those of the 2nd cutting.

As a result, variety 1 may be advisable for long-term plantations (3 year). But plantations of variety 2 should be deteriorated at the end of two year due to decreased economical yields. The variety 2 may also be advisable for the climatic conditions with high temperature because of delayed growing in the spring.

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