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Effect of Different Planting Times on Essential Oil Components of Different Mint (*Mentha* spp.) Varieties*

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Abstract: This study was carried out to determine the effect of different planting times on the essential oil components of different mint varieties (*Mentha arvensis* var. *piperascens*, *M. piperita* Mitcham, *M. piperita* Eskişehir and *M. piperita* Prilubskaja). The field trials were conducted at the GAP Agricultural Research Station under Harran Plain conditions in 1993 and 1994. The mint oil components, α-pinene (0.49-1.00%), β-pinene (1.38-2.12%), 1,8-cineole (2.64-10.85%), menthone, menthofuran (28.09-49.52%), menthole (22.55-38.89%), pulegone (0.00-1.32%), menthyl acetate (0.46-6.78%) and β-caryophyllene (0.54-2.84%), were determined. The results indicated that the essential oil components were affected by planting times, mint varieties and cutting numbers. The highest menthole ratio was obtained from *M. arvensis* var. *piperascens* (33.50-38.89%), from second cutting and autumn transplantation. Therefore, autumn transplantations are more suitable under Harran Plain conditions.

Key Words: Mint varieties (*Mentha piperita* L., *M. arvensis* var. *piparescens* Holmes), Planting times, Essential oil components

Değişik Dikim Zamanlarının Farklı Nane (*Mentha* spp.) Tiplerinin Uçucu Yağ Bileşenlerine Etkisi

Özet: Harran Ovası koşullarında farklı dikim zamanlarının farklı nane tür ve tiplerinin (*Mentha arvensis* var. *piparescens*, *M. piperita* Mitcham, *M. piperita* Eskişehir Nanesi ve *M. piperita* Prilubskaja) uçucu yağ bileşenlerine etkisinin saptandığı bu araştırma, 1993 ve 1994 yıllarında Şanlıurfa ilinde bulunan GAP Tarımsal Araştırma ve Geliştirme İstasyonu'nda yürütülmüştür. Nane uçucu yağlarında α-pinen (% 0.49-1.00), β-pinen (% 1.38-2.12), 1,8-cineol (% 2.64-10.85), menthon, menthofuran (% 28.09-49.52), menthol (% 22.55-38.89), pulegon (% 0.00-1.32), menthylacetat (% 0.46-6.78) ve β-caryophyllen (% 0.54-2.84) bileşenleri belirlenmiştir. Çalışmada, uçucu yağ bileşenlerinin dikim zamanlarından, nane tiplerinden ve biçim zamanlarından etkilendiği gözlenmiş ve en yüksek menthol oranı (% 33.50-38.89) *M. arvensis* var. *piperascens* nane tipinde, sonbahar dikimlerinde ve genel olarak 2. biçimde saptanmıştır. Harran Ovası koşulları için Sonbahar dikimlerinin daha uygun olduğu söylenebilir.

Anahtar Sözcükler: Nane türleri (*M. piperita* L., *M. arvensis* var. *piparescens* Holmes), Dikim zamanları, Uçucu Yağ Bileşenleri

Introduction

Peppermint oil is one of the most important essential oils. It is used in pharmaceuticals, cosmetics and flavourings all over the world. Therefore, mint is produced commercially in many countries. Mint and its essential oils are yet to be commercially produced in Turkey. Therefore, mint oil and menthole requirements are largely met by imports. For example, in 1995, approximately 25.2 tonnes of mint oil and 14 tonnes of menthole were imported from different countries costing $631,114,00 (Anonymous, 1995). In this sense, these crops, which grow naturally in Turkey, can easily be cultivated and provide income to small farmers and also diversify the crop system.

These crops are being cultivated on small farms around the city of Şanlıurfa, Turkey, and consumed fresh and/or in dried form. Therefore, under Şanlıurfa conditions, mint cultivation can become widespread, yielding the highest quality and quantity (Özel and Özgüven, 1999).

Some researchers have shown that the differences in the quality of mint oils were dependent upon

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environmental factors that affected the biosynthesis (Franz *et al.*, 1984; Muray *et al.*, 1986; Brun *et al.*, 1990; Vorin *et al.*, 1990). The menthole ratio was dependent on the direct or indirect effects of these factors, which led to changes in the components of oil, from pulegon to menthone, from menthone to menthole and from menthole to menthyl acetate (Muray *et al.*, 1986). Mint grown under short day and high temperature conditions had a lower menthole ratio (Franz *et al.*, 1984). Short day conditions caused a reduction in the menthofuran contents at the end of the growing season and the menthone content was high in younger leaves (Clark *et al.*, 1984). The major component of mint oil is menthone, which is present in younger leaves located at the top of the plant (Brun *et al.*, 1990). The oil composition of young leaves grown under long day conditions changed from menthofuran to menthone and menthole, and menthone was a major component of the oil of the leaves grown under short day conditions (Vorin *et al.*, 1990). Menthone and menthofuran contents were greatly affected by changes in the growing seasons (Court *et al.*, 1993).

This study was carried out to determine the effect of different planting times on essential oil components of various mint cultivars under Harran Plain conditions.

Materials and Methods

Plant Materials

Four mint varieties, *Mentha arvensis* var. *piperascens* (L.) Holmes, *M. piperita* L. cv. Mitcham, *M. piperita* L. cv. Prilubskaja and *M. piperita* L. cv. Eskişehir, were used in this study. The rhizomes were procured from the Field Crops Department, Agriculture Faculty, Çukurova University in Adana.

Field Studies

This study was carried out at the Southeastern Anatolia Project (GAP) Agricultural Research Station near Koruklu village in Şanlıurfa, during the 1992-1993 and 1993-1994 seasons.

Four mint varieties and four planting times (15th October and 15th and November as well as 1st April and 1st May) were used. Experiments were arranged in split plots with four replications. The results were analysed with the split-split plot as mint varieties in the main plots and dates of planting in subplots, and cutting numbers in minor plots. Plant rhizomes, which had been cut 8-10 cm, were replanted into the experimental area with 40 cm x 15 cm spacing. Applications of 5 kg N/da and 5 kg P_2O_5/da and 6 kg K_2O/da were made uniformly in all plots. A little water was applied immediately after each planting to ensure adequate moisture for sprouting. Subsequently, irrigation, weeding and hoeing were performed when needed. In addition, 5 kg N/da was applied uniformly on all plots after each harvest. In this procedure, three harvests were taken from *M. piperita* Prilubskaja and two from the other varieties at the onset of the flowering.

Steam Distillation

Essential oil was obtained by steam distillation of 10 g of dried leaves for 2h according to the method proposed by Tansı (1991).

Gas Chromatography

Quantitative analyses were performed on a Tracor 560 gas chromatograph equipped with a flame ionisation detector. Oil samples were taken by GC under the following conditions:

Column	Stainless steel (% OV-1 Cromosorb W.H.P. 80/100 mesh, b'X1/4"SS GC 8932)
Temperature program	85-220 ºC increased by 3 ºC/min.
Injection temperature	85 ºC
Detector temperature	230 ºC
Carrier gas	N2 with 30 ml/min.
Sample size	5 ml
Recorder	Hewlett Packard 3396A
Chart speed	0.2 mm/min

The quantitative identification of different constituents was achieved by comparing their retention time as well as the GC investigation of authentic samples of the different components. Relative content (%) of individual constituents in the oil was proportionally calculated on the basis of the peak area corresponding to each component. Mean values of the replications are presented in Tables 1 and 2.

Table 1. The Mean of Essential Oil Components as Affected by Planting Times, Mint Varieties and Cutting Numbers, in 1993.

Treatments	α-Pinene	β-Pinene	1,8-cineole	Menthone + Menthafuran	Menthole	Pulegone	Menthyl acetate	β-Caryophyllene
				PLANTING TIMES				
15th October	0.53 a	1.64	6.85 b	37.32 b	31.15 b	0.70 c	3.21 a	1.13 a
15th November	0.49 c	1.65	6.97 a	36.36 c	31.55 a	0.64 d	3.00 b	1.13 a
1st April	0.52 ab	1.63	5.51 d	39.8 a	28.65 c	0.91 a	2.84 b	1.06 b
1st May	0.50 bc	1.63	6.57 c	39.76 a	28.55 c	0.82 b	3.31 a	0.86 c
LSD (5%)	0.026	NI	0.106	0.620	0.341	0.041	0.162	0.056
				MINT VARIETIES				
M. arvensis	0.46 d	1.51 c	3.69 c	28.39 d	33.52 a	0.39 c	0.46 d	0.73 c
Mitcham	0.58 a	1.95 a	8.79 b	43.8 a	28.48 c	0.76 b	2.70 c	1.70 a
Eskişehir	0.52 b	1.38 d	2.64 d	35.77 c	28.75 c	0.33 c	5.14 a	1.08 b
Prilubskaja	0.49 c	1.68 b	9.34 a	42.95 b	29.42 b	1.32 a	3.75 a	0.78 c
LSD (5%)	0.025	0.057	0.161	0.701	0.438	0.062	0.211	0.080
				CUTTING NUMBERS				
1st Cut	0.50 b	1.63	6.15 b	36.48 c	31.09 a	0.84 a	2.54 a	1.27 a
2nd Cut	0.52 a	1.64	6.18 b	37.33 b	30.72 b	0.68 b	2.89 b	0.94 b
3rd Cut	0.50 b	1.65	8.93 a	49.52 a	22.55 c	0.83 a	6.13 a	0.54 c
LSD (5%)	0.019	NI	0.120	0.493	0.350	0.035	0.110	0.058

Table 2. The Mean of Essential Oil Components as Affected by Planting Times, Mint Varieties and Cutting Numbers, in 1994.

Treatments	α-Pinene	β-Pinene	1,8-cineole	Menthone + Menthafuran	Menthole	Pulegone	Menthyl acetate	β-Caryophyllene
				PLANTING TIMES				
15th October	0.78 b	1.69 b	7.06 b	31.21 c	33.38 a	0.79 b	4.36 a	2.59 a
15th November	0.84 a	1.71 b	7.56 a	31.08 c	33.95 a	0.87 a	3.51 c	1.64 c
1st April	0.84 a	1.89 a	7.37 a	34.30 a	29.06 b	0.69 c	4.10 b	1.81 b
1st May	0.79 ab	1.75 b	7.11 b	33.18 b	29.33 b	0.78 b	4.42 a	1.66 c
LSD (5%)	0.056	0.072	0.224	0.557	0.572	0.031	0.195	0.097
				MINT VARIETIES				
M. arvensis	0.51 d	1.31 b	3.45 c	28.09 d	38.89 a	0.00 d	0.93 d	2.48 b
Mitcham	1.00 a	2.02 a	10.28 b	31.63 c	30.98 b	0.85 b	6.31 a	2.84 a
Eskişehir	0.76 b	1.41 b	2.73 d	32.49 b	30.19 b	0.64 c	5.14 b	1.76 c
Prilubskaja	0.59 c	2.12 a	10.85 a	35.86 a	27.54 c	1.35 a	4.03 c	1.06 d
LSD (5%)	0.063	0.155	0.409	0.701	0.860	0.037	0.381	0.098
				CUTTING NUMBERS				
1st Cut	0.78 b	1.68 c	6.77 c	33.45 c	31.35 b	0.98 b	2.80 c	2.51 a
2nd Cut	0.84 a	1.78 b	7.03 b	29.42 c	33.25 a	0.49 c	4.72 b	1.67 b
3rd Cut	0.84 a	1.97 a	10.24 a	40.52 a	24.45 c	1.15 a	6.78 a	0.61 c
LSD (5%)	0.043	0.069	0.184	0.620	0.518	0.032	0.184	0,073

Results and Discussion

In total, nine essential oil components (α-pinene, β-pinene, 1,8-cineole, menthone + menthofuran, menthole, pulegone, menthyl acetate and β-caryophyllene) were identified, but it was not possible to differentiate menthone from menthofuran and therefore these compounds are shown together.

The main effects of different applications (planting times, mint varieties and cutting numbers) on the essential oils of mint were grouped according to LSD (5%) and are illustrated in Tables 1 and 2.

α-pinene

The percentage of α-pinene was significantly affected by mint varieties, planting times and cutting numbers. The highest level of α-pinene was obtained from the Mitcham variety and from the second cutting in both years; however, according to the planting times, there was a difference between 1993 and 1994. The highest α-pinene level was attained from 15^{th} October planting for the first year, and 15^{th} November and 1^{st} April plantings for the second year (Tables 1 and 2). The variations in α-pinene according to different applications may be due to the different reactions of mints with various genotypes to ecological factors. In general, the reason why the second cuttings gave the highest α-pinene level could be the rise in temperature during vegetation. These results are in good agreement with the findings of Duriyaprapan *et al.* (1986), who postulated that α-pinene levels increased with increases in daily temperature.

β-pinene

In 1993, the β-pinene levels of experimental materials were affected only by the varieties. On the other hand, in 1994, it was found that the mint varieties, planting times and cutting numbers had significant effects on the β-pinene levels. The highest β-pinene levels were recorded on the 15^{th} November planting time and from the third cutting in the first year. In addition, the Mitcham variety was found to be the highest β-pinene producing variety in the first year. In the second year, the Prilubskaja variety was evaluated as the variety which had the highest β-pinene level. In addition, the 1^{st} April planting and the third cutting produced the highest β-pinene level in the second year (Tables 1 and 2). It is thought that the differences between the β-pinene levels stemmed from the differences in genotypes and the differences in the reactions of plants against ecological variations during the growing period. Our findings are in harmony with Muray *et al.* (1986), who found that β-pinene was affected by the ecological factors and cutting times disorderly.

1,8-Cineole

The 1,8-cineole levels of the mint varieties examined were significantly affected by the applications in both years of the study. The highest 1,8-cineole levels were obtained from the 15^{th} November planting, from the Prilubskaja variety and from the third cutting (Tables 1 and 2). These findings are supported by Muray *et al.* (1986), Virmani and Datta, (1970) and Ceylan (1978), who claimed that the 1,8-cineole level was influenced by the genotype and growth conditions. Clark and Menary (1979) found that the increase in 1,8-cineole level depending upon cutting numbers resulted from the fact that the photoperiod affected the essential oils and the 1,8-cineole levels increased with the increase in light intensity.

Menthone + Menthofuran

The levels of menthone + menthofuran were significantly affected by the applications in both years. The highest menthone + menthofuran levels, excluding the mint varieties, were obtained from the 1^{st} April planting and from the third cutting. While in 1993 the Mitcham variety gave the highest menthone + menthofuran levels, in 1994 the Prilubskaja variety produced the highest level of menthone + menthofuran (Tables 1 and 2). The highest menthone + menthofuran levels in the spring plantings (1^{st} April and 1^{st} May) and the late cuttings may have stemmed from the fast growth of plants in these periods under the influence of ecological factors and from being rich in young shoots and young leaves. These results are supported by Clark and Menary (1984), who stated that the young leaves were rich in menthone, and by Brun *et al.* (1990), who postulated that the prime compound of a young leaf was menthone.

Menthole

Menthole levels were affected by the planting dates, mint varieties and cutting numbers in both experimental years. The highest menthole levels were recorded in the 15^{th} November plantings and in the *M. arvensis* variety. The effects of cutting numbers varied according to the year of the experiment. In the first year the first cutting, and in the second year the second cutting produced the highest menthole levels (Tables 1 and 2). Overall, the autumn plantings (15^{th} October and 15^{th} November) had higher menthole levels. This may have been due to the effects of environmental factors such as temperature and photoperiod throughout the growing period. Duriyaprapan *et al.* (1986) and Franz *et al.* (1984) found that high temperature and the combination of low day and night temperature caused a decrease in the menthole level. Muray *et al.* (1986) claimed that the factors affecting plant biosynthesis such as temperature, light intensity and photoperiod affected the level of menthole as well.

Pulegone, Menthyl acetate and β-caryophyllene

The levels of pulegone, menthyl acetate and β-caryophyllene were affected by planting times, mint varieties and cutting numbers in both experimental years. The highest pulegone levels were attained from the 1st April planting, from the Prilubskaja variety and from the first cutting in 1993. On the other hand, in 1994, the highest pulegone levels were recorded in the 15th November planting, in the Prilubskaja variety and in the third cutting (Tables 1 and 2). The variations in pulegone levels depending upon applications and experimental years were probably due to the effects of ecological factors during the growing period. Some researchers (Clark and Menary, 1979; Muray *et al.*, 1986; Vorin *et al.*, 1990) claimed that environmental factors (i.e. temperature and photoperiod) influenced pulegone levels in mint essential oils.

The highest menthyl acetate levels were recorded in the 1st May planting and the third cutting in both years. In 1993, the Eskişehir variety and, in 1994, the Mitcham variety had the highest menthyl acetate levels (Tables 1 and 2). It is thought that the dependence of menthyl acetate level upon the year resulted from variations in ecological factors. In addition, the reason for high menthyl acetate in the first and last plantings and late cuttings may have resulted from the environmental conditions. Our findings are in good agreement with those of Vorin *et al.* (1990), who found that menthyl acetate levels varied throughout the growing season and increased with the late cuttings.

β-caryophyllene levels of the experimental materials were found to be highest in the 15th October planting, the first cutting and in the Mitcham variety, in both years (Tables 1 and 2). This could be due to the fact that different genotypes planted at different times reached cutting maturity at different times and, eventually, the ecological differences during the cutting period affected the β-caryophyllene level.

To conclude, the essential oil components of different mint varieties were significantly affected by the planting times, cutting times and years. In addition to the existence of menthole and menthone + menthofuran, which are the prime compounds of essential oils at high concentrations, α-pinene, β-pinene, 1,8-cineole, pulegone, menthyl acetate and β-caryophyllene were also detected at lower levels.

The highest menthole level was recorded in the *M. arvensis* variety followed by the Prilubskaja variety. The highest menthole concentration was obtained from the autumn plantings. Since the level of menthole varied depending on the cutting and years, further studies should concentrate on the determination of optimum cutting times and diurnal variability that are suitable for the region.

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