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Effects of harvest stage and diurnal variability on yield and essential oil content in *Mentha* × *piperita* L.

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Abstract: This study aimed at investigating the effect of ontogenetic and diurnal variability on yield and quality criteria of *Mentha piperita*. The research was conducted in the Ordu province of Turkey for two years. The trial was established in a randomised complete block design with 3 replications according to factorial regulations. Four different harvesting periods (budding, first flowering, 50% flowering, 100% flowering) and three harvesting hours (9:00 a.m., 1:00 p.m., 5:00 p.m.) were evaluated. A significant difference was determined between the trial years in all characters examined: plant height 55.9–69.0 cm, fresh herb yield 3 236–16 438 kg/ha, drug-herb yield 1 748–2 862 kg/ha, fresh leaf yield 3 477–4 506 kg/ha, drug leaf yield 968–1 253 kg/ha, essential oil ratio 2.0–2.5% and essential oil yield that varied between 19–28 L/ha. The highest yields were obtained at the beginning of flowering in drug leaf yield and 100% flowering period for all other characteristics. Diurnal variability was statistically significant only in the rate of essential oil, and the highest rate occurred in the 9:00 a.m. harvest.

Keywords: peppermint; quality features; aromatic plant; environmental factor; medicine

Mentha species are cultivated commercially in many countries for their valuable essential oil. Mint, a plant grown by people in their gardens, in front of their houses, or in the fields in Turkey since time immemorial, has become an export product worth $1 030 523 today (Yaldız and Çamlıca 2018). *Mentha* × *piperita* L., which is the best known natural source of menthol, is also known as peppermint, and it is a hybrid of *Mentha spicata* and *Mentha aquatica* and grown in many parts of the world (Singh et al. 2015). The oil obtained from its leaves is known as *Mentha* × *piperita* oil, *Mentha* oil, and peppermint oil (Herro and Jacob 2010), and it is used as raw material in the food, medicine, and cosmetics industries (Özgüven and Kırıcı 1999, Moghaddam et al. 2013, Baydar 2019). Furthermore, it is consumed extensively in the form of tea in traditional medicine (McKay and Blumberg 2006).

Medicinal and aromatic plants are greatly affected by environmental conditions (temperature, light, moisture) and cultural practices (planting dates, irrigation, fertilisation, spraying, harvest time). These factors have much more effect on medicinal plants than other cultivated plants. Because in medicinal plants, quality is as important as yield, and it is not desired to be below the quality criteria specified in pharmacopoeias. Therefore, it is necessary to determine the most appropriate harvest time, depending on the ecological conditions in which medicinal plants to be cultivated in different regions are grown. There are many studies examining the effects of environmental factors on agricultural and quality characteristics of the *Mentha* genus (Kızıl and Tonçer 2006, Llorens-Molina et al. 2014, Bufalo et al. 2015, Özyazıcı and Kevseroğlu 2019).

The aim of this study was to examine the effects of different harvest times and harvest hours on yield and quality characteristics of *Mentha* × *piperita* L., which is used as an essential oil source in the pharmaceutical, food and cosmetic industries.

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MATERIAL AND METHODS

Climatic characteristics of the research area. The research was conducted in the trial area of the Ordu University, Faculty of Agriculture in 2017 and 2018. In the first trial year, the total amount of precipitation was 62.9 mm, the average temperature value was 15.1 °C, relative humidity was 68.6%, and in the second trial year, the total amount of precipitation was 87.2 mm, the average temperature was 16.3 °C, relative humidity was determined to be 75.7%. When the vegetation period (April–September) meteorological data of both trial years are compared, the total precipitation (61.2 mm), relative humidity (75.3%), and temperature (20.9 °C) in the 2018 trial year values were higher than the 2017 trial year (41.5 mm, 73%, 19.7 °C, respectively).

Soil characteristics of the research area. The soil in the research area has a clayey-loamy, with soil pH close to neutral and with low contents of lime content, organic matter, nitrogen, phosphorus and potassium.

The material used in the research. *Mentha* × *piperita* L., which was used as material, was obtained from the Ordu University, Faculty of Agriculture Field Crops Medicinal and Aromatic Plants Collection Garden. The rhizomes taken from the soil on March 8, 2017, were developed in a perlite-peat (1:1), and the seedlings were planted in the field on May 17, 2017. Hoeing was performed for weed control throughout the vegetation period, and irrigation was carried out considering rainfall, soil and plant factors.

Method. The trial was established in random blocks in 3 repetitions according to factorial regulations; the parcel width is 1.2 m, the parcel length is 3.6 m, the parcel area is 4.32 m^2. Planting of mint was done with 40 cm between lines, 30 cm row spacing, and 12 plants per row. In the experiment, a distance of 1 m was left between the parcels and 2 m between the blocks. Diammonium phosphate (18%) and triple superphosphate (42%) fertilisers were applied at the dose of 50 kg per hectare with the calculation of pure substance, and after each harvest, 50 kg/ha diammonium phosphate (18%) fertiliser was applied to the plots again as pure nitrogen.

To determine the ontogenetic variability, it was harvested during budding, beginning of flowering, 50% blooming, 100% blooming, and for diurnal variability at the hours of 9:00 a.m., 1:00 p.m., 5:00 p.m. The study examined plant height, the yield of fresh herbage, the yield of drug herbage, the yield of drug leaves, essential oil ratio and yield of essential oil. The essential oil was obtained with the help of the Clevenger device. For each treatment, 30 g of grounded mint leaves that were dried at 35 °C were boiled for 3 h in a distillation flask. At the end of boiling, the amount of essential oil accumulated in the graduated pipe was read and recorded in mL (Yeşil 2012).

In the analysis of the data, three-way ANOVA was used in the trial design of completely randomised block, and the difference between the means was determined by the Duncan's multiple range test. 5% significance level was used in calculations and interpretations; all calculations were made in Minitab 19 (Minitab Inc., State College, USA) statistics program.

Harvest dates. Table 1 shows the harvest dates.

Table 1. Harvest dates

Budding	First flowering	%50 flowering	%100 flowering
31. 07. 2017	08. 08. 2017	15. 08. 2017	31. 08. 2017
20. 07. 2018	27. 07. 2018	05. 08. 2018	14. 08. 2018

RESULTS AND DISCUSSION

Out of the examined characteristics, a three-way analysis of variance (year × harvest time × hour triple interaction) was not found significant ($P < 0.05$), only the main effects of year and harvest time factors were statistically significant.

Plant height. It is known that plant height varies according to climate and soil factors, cultivation techniques, genotype and year. In this study, plant

Table 2. Main effects of the year and harvest time factors for plant height (cm)

Factor		Mean	Standard error
Year (n = 36)	2017	49.0	1.116
	2018	79.7	1.781
Harvest date (n = 18)	budding	55.9^b	4.086
	first flowering	66.2^a	4.058
	%50 flowering	66.4^a	3.732
	%100 flowering	69.0^a	4.523
Hour (n = 24)	9:00 a.m.	64.8	3.842
	1:00 p.m.	64.4	3.777
	5:00 p.m.	63.9	3.423

There is a difference between the harvest time means without a common letter ($^*P < 0.05$)

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height values between years were significant at the level of $P < 0.001$, and plant height of 49.0 cm and 79.7 cm was determined in the first and second trial year, respectively. A significant change in values over the years is due to the fact that the plants that spend the winter in the soil benefit from the spring precipitation in the second year and start to develop earlier. Independent of the year and hour factors, it is seen that there is a difference at the level of $P < 0.001$ between the plant height averages of the harvest periods (Table 2). The harvests at the beginning of flowering (66.239 cm), 50% flowering (66.489 cm) and 100% flowering (69.050 cm) were in the same group. The plant height value (55.9 cm) determined during the budding period was significantly lower ($P < 0.001$) than the plant heights determined in other harvest periods, but was consistent with the findings of Özyazıcı and Kevseroğlu (2019). In addition, the findings obtained in both trial years are higher than the values reported by Can and Katar (2020), which are suitable for the development periods of the species. The increase in plant height during the harvest period is due to the progress of the plant's development period as well as the more suitable climatic conditions such as temperature, precipitation or photoperiod (Piccaglia et al. 1993).

When harvesting hours are examined, it is seen that plant height is not affected by changing temperature and humidity during the day ($P < 0.05$), regardless of year and harvest time, plant height is sequenced at 9:00 a.m. (64.8 cm), 1:00 p.m. (64.4 cm) and 5:00 p.m. (63.9 cm). Similarly, Kulan (2013) also reports that the plant height of *Ocimum basillicum* L. is not affected by harvest times.

Fresh herb yield. When Table 3 is examined, the fresh herb yields determined in the first trial year were statistically included in the same group ($P < 0.05$), but the highest yield was determined in 100% flowering period (4 714 kg/ha), the beginning of flowering (4 136 kg/ha), budding (3 236 kg/ha) and 50% flowering period (2 992 kg/ha). In the second trial year, the highest fresh herb yield was observed in the 100% flowering period (16 438 kg/ha) and the lowest fresh herb yield in the budding period (10 399 kg/ha), which was found statistically significant ($P < 0.05$). At the beginning of flowering (12 812 kg/ha) and the harvest of 50% flowering (12 773 kg/ha) period, the sequencing continued as above-mentioned. These findings are similar to the results of Arslan et al. (2010), Özyazıcı and Kevseroğlu (2019). Researchers emphasised that ontogenetic variability has an effect on fresh herbage yield in their research on species *M. arvensis* and *M. spicata*. Also, Kızıl and Tonçer (2005) reported that they obtained the highest fresh herb yield, drug-herb yield and drug leaf yield from the harvest at the 100% flowering period during the study they conducted on *Thymus kotschyanus* species. The effect of diurnal variability on fresh herb yield was not statistically significant ($P < 0.05$). Fresh herb yield, which was determined as 9 135 kg/ha 9:00 a.m. harvest, decreased at 1:00 p.m. (8 059 kg/ha) and increased again at 5:00 p.m. harvest (8 118 kg/ha). It is thought that this difference in efficiency, which occurs according to hours, is due to the ambient humidity.

Drug herb yield. It is seen that the drug-herb yield (962 kg/ha) in the first trial year is significantly lower ($P < 0.001$) than the drug-herb yield determined in the second trial year (3 584 kg/ha) (Table 4). This difference is caused by *Mentha × piperita* L. plant's spreading to the area by forming a rhizome and creating more aboveground parts in the second year

Table 3. Main effects of the year and harvest time factors for fresh herb yield (kg/ha)

Factor	Factor level	2017 (n = 9)		2018 (n = 9)	
		mean	standard error	mean	standard error
Harvest date	budding	3.236^{Ab}	429.61	10.399^{Ca}	1.004
	first flowering	4.136^{Ab}	347.29	12.812^{Ba}	860
	%50 flowering	2.992^{Ab}	335.07	12.773^{Ba}	700
	%100 flowering	4.714^{Ab}	506.06	16.438^{Aa}	848
Hour (n = 24)	9:00 a.m.	9.135	1 196.82		
	1:00 p.m.	8.059	1 087.51		
	5:00 p.m.	8.118	1 002.12		

There is a difference between the harvest time averages without a common capital letter in the same year (*$P < 0.05$). There is a difference between the year averages without common lowercase letters at the same harvest time (*$P < 0.05$)

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Table 4. Main effects of the year and harvest time factors for drug-herb yield (kg/ha)

Factor		Mean	Standard error
Year (n = 36)	2017	962	64.41
	2018	3.584	155.43
Harvest date (n = 18)	budding	1.748^{c}	306.89
	first flowering	2.330^{b}	329.01
	%50 flowering	2.153^{b}	330.88
	%100 flowering	2.862^{a}	414.12
Hour (n = 24)	9:00 a.m.	2.482	352.74
	1:00 p.m.	2.146	294.71
	5:00 p.m.	2.192	273.28

There is a difference between the harvest time means without a common letter (*P < 0.05)

(Özgüven and Kırıcı 1999, Telci 2001). Regardless of the trial years and harvest hours, it is stated that there is a significant difference between harvest time averages (P < 0.05). The highest drug herb yield was at 100% flowering period (2 862 kg/ha), followed by the beginning of flowering (2 330 kg/ha), 50% flowering (2 153 kg/ha) and budding (1 748 kg/ha) periods. Consistent with this study, researchers who conducted studies on various types of the subject found the highest drug herb yield at 100% flowering period (Karık et al. 2007, Kızıl 2009, Karaca Öner and Sonkaya 2020).

Regarding the harvesting hours, no statistically significant difference (P < 0.05) was found in the herb yield, the yield ranking was 2 482 kg/ha at 9:00 a.m., 2 192 kg/ha at 5:00 p.m. and 2 146 kg/ha at 1:00 p.m. harvest. Arabacı et al. (2015) stated that in their study examining the effect of diurnal variability on yield characteristics in *Coridothymus capitatus* L. genotypes, the time between sunrise and sunset did not have an effect on drug-herb yield, and similar findings were obtained in this study.

Fresh leaf yield. The fresh leaf yield of 5 735 kg/ha in the second trial year is significantly higher (P < 0.001) than the 2 194 kg/ha fresh leaf yield obtained in the first trial year (Table 5). This yield difference between years can be explained by the number of plants per unit area is low in the first year, while in the second trial year, *Mentha* × *piperita* L. increases the number of plants per unit area by forming rhizomes. Considering the harvest times, the highest fresh leaf yield was detected in 100% flowering period (4 506 kg/ha), and it was followed by the beginning of flowering (4 149 kg/ha), 50% flowering (3 727 kg/ha), and budding (3 477 kg/ha), and this difference between the harvesting periods at the P < 0.05 level was significant. In parallel with this study, Karaca Öner and Sonkaya (2020) reported that they determined the highest fresh leaf yield in *Origanum onites* L. at 100% flowering and the least fresh leaf yield during the budding period. Although there was no statistical difference (P < 0.05) between the harvesting hours, 9:00 a.m. harvests (4 174 kg/ha) took the first place, followed by 5:00 p.m. (3 862 kg/ha) and 1:00 a.m. (3 859 kg/ha) harvests. Changing temperature and humidity values during the day are thought to affect fresh leaf yield mathematically, though not statistically.

Drug leaf yield. The difference in drug leaf yield between the trial years was significant at the level of P < 0.001, 588 kg/ha in the first trial year and 1 681 kg/ha drug leaf was obtained in the second trial year. This yield difference between the years is due to the fresh leaf yield. In the second year, the herbaceous yield of the plant that spread through the field through its rhizomes, increased and this has affected the leaf yield positively. The drug leaf yield determined as 1 253 kg/ha at the beginning of flowering was 1 202 kg/ha during the 100% flowering period, 1 115 kg/ha during the 50% flowering period and 968 kg/ha during the budding period, but this difference between yields was not statistically significant (P < 0.05). When the harvesting hours were examined regardless of the trial years, and harvest periods, the 9:00 a.m. harvest (1 199 kg/ha) took the first place, followed by the 1:00 p.m. (1 114 kg/ha) and 5:00 p.m. (1 089 kg/ha) harvest. Drug herb yield difference between harvest hours was not statisti-

Table 5. Main effects of the year and harvest time factors for fresh leaf yield (kg/ha)

Factor		Mean	Standard error
Year (n = 36)	2017	2.194	117.90
	2018	5.735	209.28
Harvest date (n = 18)	budding	3.477^{c}	405.95
	first flowering	4.149^{ab}	440.18
	%50 flowering	3.727^{bc}	484.10
	%100 flowering	4.506^{a}	587.86
Hour (n = 24)	9:00 a.m.	4.174	461.59
	1:00 p.m.	3.859	412.27
	5:00 p.m.	3.862	393.21

There is a difference between the harvest time means without a common letter (*P < 0.05)

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Table 6. Main effects of the year and harvest time factors for drug leaf yield (kg/ha)

Factor		Mean	Standard error
Year	2017	588	33.17
(*n* = 36)	2018	1.681	68.09
	budding	968	137.22
Harvesting date	first flowering	1.253	141.21
(*n* = 18)	%50 flowering	1.115	159.80
	%100 flowering	1.202	163.57
Hour	9:00 a.m.	1.199	149.77
(*n* = 24)	1:00 p.m.	1.114	125.30
	5:00 p.m.	1.089	116.55

cally significant ($P < 0.05$). This situation shows that the drug leaf yield is not affected by the changing ecological factors during the day (Table 6).

Essential oil rate. In Table 7, it is shown that the main effects of all factors are statistically significant. The difference in the essential oil ratio between the trial years was significant at the level of $P < 0.001$, and 2.6% and 1.8% essential oil ratio were obtained in the first and second trial year. The difference in yield between years can be explained by the high rainfall and high relative humidity in the second trial year. It is known that ecological factors such as temperature, day length, exposure time and vegetation period have an effect on yield and yield-related properties (Hadiana et al. 2008, Ramakrishna and Ravishankar 2011). The ratio of essential oil between the harvest times was significant ($P < 0.01$). It is seen that the highest amount of essential oil occurs in the harvests at 100% flowering (2.5%), followed by budding (2.2%), 50% flowering (2.1%) and the beginning of flowering (2.0%). The result obtained from the study corresponds to those published by Aflatuni et al. (2006), who studied four mint species and reported that the highest essential oil ratio was obtained at the beginning of flowering, which differed from the findings of Topalov et al. (1991), Çalışkan and Özgüven (2018), who reported that it occurred at 50% flowering period. Duriyaprapan and Britten (1982) reported that the essential oil rate increased at 100% flowering period. Consistent with these findings, Arabacı et al. (2015) stated that the rate of an essential oil varies according to the climate, environment, topographic conditions, genetic structure and morphogenetic, ontogenetic and diurnal variability, which are called individual variability.

In addition, it has been emphasised by various researchers that the essential oil ratio of medicinal and aromatic plants depends on the development period of the plant (Kızıl and Tonçer 2006, Lakušič et al. 2013, Uyanık and Gürbüz 2015, Katar et al. 2018, Wahba et al. 2018, Açıkgöz and Kara 2020). Considering the harvest hours, the difference in essential oil ratio between hours was significant at the level of $P < 0.05$, in terms of the ratio; the 9:00 a.m. harvest was in the first place with 2.3%, the essential oil rate decreased at 1:00 p.m. (2.1%) and rose again at 5:00 p.m. (2.2%). This hourly change in the essential oil ratio shows as Arabacı et al. (2015) stated, that harvesting of medicinal and aromatic plants should not be done at a random time and the time when the essential oil rate reaches the highest point should be determined. In addition, the researchers reached similar results in their studies and stated that the highest amount of essential oil was obtained in the morning harvest, then the rate of essential oil fell over the noon and increased again in the afternoon. As stated in the same study, the hourly change in the rate of essential oil may depend on the ambient temperature and humidity values at the time of harvest. In parallel with these results, there are studies reporting that the rate of essential oil in various medicinal and aromatic plants is affected by diurnal variability (Khalid et al. 2009, Yıldırım et al. 2019).

Essential oil yield. In terms of essential oil yield, there was a significant difference at the level of $P < 0.001$ between the trial years, the essential oil yield,

Table 7. Main effects of the year and harvest time factors for essential oil rate (%)

Factor		Mean	Standard error
Year	2017	2.6	0.065
(*n* = 36)	2018	1.8	0.046
	budding	2.2^{B}	0.131
Harvest date	first flowering	2.0^{C}	0.125
(*n* = 18)	%50 flowering	2.1^{BC}	0.114
	%100 flowering	2.5^{A}	0.126
Hour	9:00 a.m.	2.3^{a}	0.119
(*n* = 24)	1:00 p.m.	2.1^{b}	0.100
	5:00 p.m.	2.2^{ab}	0.110

There is a difference between the harvest time averages without a common capital letter ($^*P < 0.05$). There is a difference between the hour averages without common lowercase letters ($^*P < 0.05$)

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which was 15 L/ha in the first trial year, increased to 30 L/ha in the second trial year. The high yield of essential oil in the second year of the study can be explained by the higher yield of drug leaves in the second year compared to the first year. As it is known, essential oil yield depends on the essential oil ratio and drug leaf yield. Although the essential oil ratio was low in the second trial year, the drug leaf yield was higher than the first trial year.

When the harvest periods were compared among themselves, the difference in essential oil yield was significant (P < 0.01), the highest yield occurred in the 100% flowering period (28 L/ha), the beginning of flowering (24 L/ha), 50% flowering (21 L/ha) and the budding period (19 L/ha) harvests. There are studies revealing the effect of ontogenetic variability on essential oil yield. Özyazıcı and Kevseroglu (2019) detected the highest essential oil yield in *Mentha spicata* L. and *Lavandula angustifolia* in 50% flowering period; Kızıl and Tonçer (2006) and Karaca Öner and Sonkaya (2020) detected 100% flowering period in *Mentha spicata* L. var. *spicata* and *Origanum onites* L.

Essential oil yield, which depends on drug leaf yield and essential oil ratio, was not statistically affected by harvest hours (P < 0.05), but mathematically, 25 L/ha in 9:00 a.m. harvest, 22 L/ha in 1:00 p.m. harvest, 22 L/ha in 5:00 p.m. harvest oil yield was obtained (Table 8). The emergence of the highest yield of essential oil at the morning hours was due to the increase in temperature and decrease in ambient humidity later in the day, resulting in a decrease in the yield of fresh leaf and essential oil ratio. Researchers working on the subject also reached similar conclusions (Kumar et al. 2013).

Table 8. Main effects of the year and harvest time factors for essential oil yield (L/ha)

Factor		Mean	Standard error
Year	2017	15	1.04
(n = 36)	2018	30	1.44
Harvest date (n = 18)	budding	19^{a}	2.04
	first flowering	24^{ab}	2.01
	%50 flowering	21^{a}	2.45
	%100 flowering	28^{a}	3.12
Hour (n = 24)	9:00 a.m.	25	2.41
	1:00 p.m.	22	2.14
	5:00 p.m.	22	2.01

There is a difference between the harvest time means without a common letter (*P < 0.05)

In conclusion, according to the study results, a significant increase occurred in the second trial year in other properties examined, except for the essential oil ratio. The highest drug leaf yield was obtained for all other factors at harvest in the 100% flowering period. In terms of diurnal variability, a statistical difference was detected only in the essential oil ratio, and the highest essential oil ratio was found in the harvest at 9 a.m. In the light of the data obtained from the study, in *Mentha* × *piperita* L. species, 100% flowering period and 9 a.m. harvest can be recommended.

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