

## Effect of Different Nitrogen Doses on Agricultural and Quality Characteristics of *Mentha x piperita* L. and *Mentha spicata* L. Species

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### ABSTRACT

The effect of various amounts of nitrogen (N) fertilizer (0, 50, 100, 150 and 200 kg ha<sup>-1</sup>) on the growth and quality characteristics of *Mentha x piperita* and *Mentha spicata* species was studied under Turkey's Uşak ecological conditions in 2017-2019. In the study, harvests in the vegetation period of 2018 and 2019 were combined and the average plant height and essential oil content, total fresh and dry herbage, dry leaf and essential oil yields, and the composition of the essential oil was examined. The plant height, total yields and essential oil content of both mint species increased significantly with N fertilizer applications. The highest total yields and essential oil contents in both mint species were obtained from 200 kg N ha<sup>-1</sup>. The main components of the essential oil were identified as menthol and menthon in *Mentha x piperita* and carvone and limonene in *Mentha spicata*. The highest menthol content (44.66%) was obtained from 100 kg N ha<sup>-1</sup>, while the highest carvone content (59.90%) was obtained from 200 kg N ha<sup>-1</sup>. The total yields of *Mentha x piperita* increased significantly with increase in N fertilization up to 200 kg ha<sup>-1</sup>, while the total yields of *Mentha spicata* increased with increase in N fertilization up to 150 kg ha<sup>-1</sup>.

**Keywords:** Carvone, Essential oil, Menthol, Mint fertilization.

### INTRODUCTION

Mint is a perennial, valuable spice and essential oil plant belonging to the Lamiaceae family (Baydar, 2016). *Mentha x piperita*, *Mentha spicata*, and *Mentha arvensis* are the three most important cultivated mint species in the world. Because the essential oils contain menthol, *Mentha arvensis* and *Mentha x piperita* are widely produced in countries such as India, China and the USA. While *Mentha x piperita* cultivation is suitable in temperate climates, *Mentha arvensis* is more suitable for tropical and subtropical climates (Telci *et al.*, 2011). *Mentha spicata* is mostly used as a spice, and its essential oil is rich in carvones (40-80%). In Turkey, the cultivation

of this species is dominant (Baydar, 2016). *Mentha spicata* (spearmint) is a herbaceous perennial herb with strongly spreading rhizomes under the ground. The fresh and dried plants and their essential oils are widely used in food, cosmetic, confectionery, chewing gum, toothpaste, and pharmaceutical industries (Bensabah *et al.*, 2013). *Mentha x piperita* (peppermint), a hybrid between *Mentha spicata* and *Mentha aquatica*, is one of the most important essential oil plants. Peppermint essential oil containing mainly menthol and menthone is used extensively in pharmaceutical, food, cosmetic, flavor, and perfumery industries and in aromatherapy (Telci and Şahbaz, 2005a). The yield and quality of cultivated plants are affected by the genetic potential of

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the plant, the climate and soil factors as well as by many other agronomic practices (Kaleem *et al.*, 2010; Aghaye Noroozlo *et al.*, 2019; Souri *et al.*, 2019a). Fertilization and application of chemical fertilizers, as an important agronomic practice, has significant effect on yield and quality of crops and, in this regard, nitrogen is a unique mineral. Nitrogen fertilization more than any other mineral nutrients can influence plant growth and productivity (Souri and Hatamian, 2019; Souri *et al.*, 2019b). In medicinal and aromatic plants, nitrogen can influence the content and chemical composition of essential oil depending on many factors such as environmental and agrotechnical conditions (Aboukhalid *et al.*, 2017). For this reason, care should be taken to apply the fertilizer in sufficient quantities, at the appropriate time and form. As it is known, excessive or insufficient nitrogen fertilizer applications cause losses in the yield and quality of the product, and, especially, excessive nitrogen application causes environmental problems over time (Moniruzzaman *et al.*, 2014). The nitrogenous fertilizer needs of the plants vary greatly depending on the plant species, climate and soil physiochemical conditions (Souri, 2016; Hatamian *et al.*, 2018). Therefore, it is necessary to determine the most appropriate plant N requirement by field experiments when plants cultivation is to be introduced in new and different regions.

The aim of this study was to evaluate the effect of different nitrogen doses on the agricultural and quality characteristics of *Mentha×piperita* and *Mentha spicata* under Turkey's Uşak ecological conditions.

## MATERIALS AND METHODS

The study was carried out in a farmer's field (38° 35' 36.89" N and 29° 26' 53.77" E) located in Turkey's Uşak Province, in 2017-2019. Meteorological data of the experimental site are given in Table 1. Soil texture of the site was clay-loam with 7.81

pH (as determined in KCl), 9.44 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 1305 kg ha<sup>-1</sup> K<sub>2</sub>O, 0.615 dS m<sup>-1</sup> EC, and 9.9% lime. The available potassium content of the soil was high, but the available phosphorus content was very low. Cuttings of 8-10 cm length were taken (on 24.05.2017) from the plantation of the *Mentha×piperita* and *Mentha spicata* in the field of the Eskişehir Osmangazi University, Faculty of Agriculture. The cuttings were planted in the rooting mixture (1/2 sand+1/2 soil) on 25.05.2017 and after about 1.5 months it was ready for transplanting in the experimental site. Rooted cuttings were planted in plots (6.5 m<sup>2</sup>) on 24.07.2017. Plants were planted 30 cm apart, in 45 cm rows. The experiment was established in a split plots design in randomized blocks with three replications. Fertilizer was applied at each site before planting at the rate of 50 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. Experimental plots were treated with five different nitrogen doses (0, 50, 100, 150 and 200 kg ha<sup>-1</sup>). Ammonium sulfate (20.5%, N) and triple super phosphate (42%, P<sub>2</sub>O<sub>5</sub>) were used as the sources of nitrogen and phosphorus, respectively. Half of the nitrogen doses were given in the spring when the plants were 10-15 cm, and the other half was given after the first cutting. Plots were irrigated frequently (intervals of 7-10 days) with a drip irrigation system. Weed control was done by hand. In the study, the first and last rows of each plot and 0.3 m from both ends of the rows were excluded as border effect. Harvests were done by hand at the beginning of flowering of mint species by cutting the plants approximately 5-10 cm high from the soil surface.

The plants were not harvested in 2017, as they did not reach harvest maturity. It was harvested twice a year in both 2018 and 2019. Harvest times are given in Table 2. In this article, harvests in the vegetation period of 2018 and 2019 were combined and total yields were given.

The harvested plants were dried in the oven at 35°C for 48 hours. In order to determine the essential oil content, 1.0 liter of water was added to 100 g of dry leaf

**Table 1.** Meteorological data of the experiment site.

Months	Total Precipitation (mm)			Mean Temperature (°C)		Mean Relative Humidity (%)	
	LYA <sup>a</sup>	2018	2019	2018	2019	2018	2019
January	58.4	71.8	87.9	3.6	1.6	73.2	80.9
February	58.5	63.8	17.3	6.7	5.4	73.9	65.6
March	51.1	76.8	20.8	9.3	7.8	67.6	58.4
April	57.7	6.4	40.1	15.4	10.5	47.5	60.4
May	43.1	102.4	36.9	17.2	16.7	61.5	55.5
June	24.1	54.6	37.3	20.2	20.9	59.4	58.6
July	15.5	58.2	8.9	23.7	22.8	49.3	47.0
August	9.5	37.8	0.4	24.3	24.4	48.0	42.8
September	17.2	0.1	22.1	20.4	19.4	46.8	52.2
October	44.9	74.1	6.4	14.8	16.6	59.1	55.3
November	56.3	68.7	47.9	9.5	11.6	67.7	63.1
December	74.3	110.3	88.3	3.6	5.5	79.1	71.3
Total	510.6	725.0	414.3	-	-	-	-
Mean	-	-	-	14.1	13.6	61.1	59.3

<sup>a</sup> Long Year Average of between 1986 and 2016 years (Mean of long-term).

**Table 2.** Harvest times of *Mentha spicata* and *Mentha*×*piperita*.

Species	2018		2019	
	First cutting	Second cutting	First cutting	Second cutting
<i>Mentha spicata</i>	28.06.2018	08.09.2018	04.07.2019	14.09.2019
<i>Mentha</i> × <i>piperita</i>	10.07.2018	14.09.2018	15.07.2019	21.09.2019

sample from each application and extracted by hydro distillation for 3 hours using the Clevenger apparatus. Samples of essential oil were obtained by water distillation and were stored in a refrigerator at 4°C until the composition analysis. The compositions of the essential oil samples were analyzed by gas chromatography (Agilent 5975C) coupled to mass spectrometry (Agilent 5975C) using capillary column (HP Innovax Capillary; 60.0 m×0.25 mm×0.25 µm). Helium was used as the carrier gas at 0.8 mL/min flow rate. Essential oils were diluted 1:100 ratios with hexane to analyze its composition. GC-MS analysis was carried out at split mode (40:1). The samples injection volume was adjusted as 1 µL and injection temperature as 250 °C. The oven temperature was programmed as 60°C for 10 minutes, increased at 4°C min<sup>-1</sup>, to 220°C, and held at 220°C for 10 minutes. MS spectra were monitored between 35-450 amu and the ionization mode used was electronic

impact at 70eV. The relative percentage of the components was calculated from GC-MS peak areas. Wiley and Adams libraries data were used for the identification of essential oil components. The percent compositions of the volatiles were obtained using the FID detector. The identification of the components were made by comparing the spectra taken from the MS detector with the spectra in the libraries given above. When difficulties were encountered in identification, standard substances were given to the device and the identification process was carried out. In the study, the values obtained by combining the cuttings during the year were subjected to analysis of variance using split plots design in randomized blocks. The analysis of the data was done in the SPSS statistical software program and the significant differences were determined by the Tukey test at a significance level P≤ 0.01.



## RESULTS AND DISCUSSION

Plant height increased significantly in application of nitrogen in both mint species compared to the control plants. The highest plant height of both mint species was obtained in the nitrogen application of 200 kg ha<sup>-1</sup>. While plant height in *Mentha spicata* increased significantly with increase in N application up to 150 kg ha<sup>-1</sup>, plant height in *Mentha×piperita* increased significantly up to 200 kg ha<sup>-1</sup> nitrogen dose (Table 3). Plant height values of both mint species were higher in 2018. This was probably due to the higher temperatures in 2018 compared to 2019 (Tugay *et al.*, 2000). Similar to these findings, it has been reported that plant height increases with increasing nitrogen doses in mint species (Singh and Singh, 1989; Kothari and Singh, 1995; Yeşil, 2012; Büyükbayraktar, 2014; Sheykholeslami *et al.*, 2015).

The total fresh and dry herbage yields of both mint species were higher in 2018 than in 2019 and difference between years was significant ( $P < 0.01$ ) (Table 3). These differences were due to the annual change of climatic factors such as temperature (Tugay *et al.*, 2000; Telci and Şahbaz, 2005b). The highest total fresh and dry herbage yields were obtained from 200 kg N ha<sup>-1</sup> in both mint species. However, there was no significant difference between 150 and 200 kg N ha<sup>-1</sup> in *Mentha spicata* (Table 3). Kothari and Singh (1995) reported that the highest total fresh herb yield in *Mentha gracilis* was obtained from 300 kg N ha<sup>-1</sup>, but there was no significant difference between 200 and 300 kg N ha<sup>-1</sup>. The finding that fresh herb yield increased with increasing nitrogen was consistent with the results of Zheljzkov *et al.* (2010), Yeşil (2012) and Büyükbayraktar (2014). In addition, this finding was in agreement with the results of Yeşil (2012) and Büyükbayraktar (2014), who noted that dry herbage yield increased with increasing nitrogen. Similar results of higher growth traits and fresh yield of leafy vegetables due

to application of nitrogen fertilizers have been also reported in other crops (Mohammadipour and Souri, 2019; Souri *et al.*, 2019b).

In the study, total dry leaf yield was taken between 2.66-5.55 t ha<sup>-1</sup> in *Mentha spicata* and 2.30-4.97 t ha<sup>-1</sup> in *Mentha×piperita*. Similar to other yields, dry leaf yields were higher in 2018 than 2019. In the average of years, the highest dry leaf yields were recorded for 200 kg N ha<sup>-1</sup> in both mint species. *Mentha spicata* showed significant increases in dry leaf yield up to 150 kg N ha<sup>-1</sup>, while *Mentha×piperita* showed significant increases up to 200 kg N ha<sup>-1</sup> (Table 3). Yeşil (2012) determined that dry leaf yield in *Mentha spicata* increased up to 50 kg N ha<sup>-1</sup>. The dry leaf yield of mint varies according to the genetic structure of the plant (Tugay *et al.*, 2000) as well as the cultivation practices (Singh *et al.*, 1995; Telci and Şahbaz, 2005b).

The effects of the years and nitrogen on essential oil content in both mint species were found to be significant. The essential oil content in *Mentha spicata* was higher in 2019 compared to 2018 (Table 3). This is probably due to the average temperature values of June, which was the first cut time and higher in 2019 than in 2018. On the other hand, the content of essential oil in *Mentha×piperita* was lower in 2019 due to the lower temperature in September, which was the second cut time (Table 1). There was no significant difference between nitrogen doses in *Mentha spicata*. Essential oil content increased significantly in *Mentha×piperita* up to 100 kg N ha<sup>-1</sup>. The highest content of essential oil in both mint species was obtained from the application of 200 kg N ha<sup>-1</sup> (Table 3). The results of the study are in agreement with the observations by Arabaci and Bayram (2004) and Büyükbayraktar (2014) who reported that essential oil content increases using nitrogen fertilizer.

The effect of nitrogen applications was significant on total essential oil yield in both mint species. Total essential oil yields in both mint species were higher in 2018 than

**Table 3.** The effects of years and different nitrogen doses on average yield components of *Mentha spicata* and *Mentha x piperita*.

	Species	Years	Nitrogen doses (kg ha <sup>-1</sup> )					Mean	Years (Y)	F values		
			0	50	100	150	200			Nitrogen (N)	Y×N	CV (%)
Plant height (cm)	<i>M. spicata</i>	2018	46.23 <sub>c</sub>	58.00 <sub>b</sub>	66.98 <sub>a</sub>	67.45 <sub>a</sub>	68.43 <sub>a</sub>	61.42 <sub>A</sub>	27.483 <sub>*</sub>	141.925 <sub>**</sub>	4.827 <sub>**</sub>	22.31
		2019	31.18 <sub>d</sub>	43.03 <sub>c</sub>	53.62 <sub>b</sub>	59.40 <sub>ab</sub>	62.07 <sub>a</sub>	49.86 <sub>B</sub>				
		Mean	38.71 <sub>d</sub>	50.52 <sub>c</sub>	60.30 <sub>b</sub>	63.43 <sub>ab</sub>	65.25 <sub>a</sub>	55.64				
	<i>M. x piperita</i>	2018	33.27	39.92	47.47	52.08	59.05	46.36 <sub>A</sub>	55.369 <sub>*</sub>	148.347 <sub>**</sub>	0.989 <sub>ns</sub>	29.60
		2019	18.62	27.88	33.92	42.02	46.27	33.74 <sub>B</sub>				
		Mean	25.95 <sub>e</sub>	33.90 <sub>d</sub>	40.70 <sub>c</sub>	47.05 <sub>b</sub>	52.66 <sub>a</sub>	40.05				
Fresh herbage yield (t ha <sup>-1</sup> )	<i>M. spicata</i>	2018	19.52	30.11	38.92	42.99	45.32	35.37	9.052 <sub>ns</sub>	242.633 <sub>**</sub>	1.288 <sub>ns</sub>	30.93
		2019	13.98	28.00	36.26	40.93	44.03	32.64				
		Mean	16.75 <sub>d</sub>	29.06 <sub>c</sub>	37.59 <sub>b</sub>	41.96 <sub>a</sub>	44.67 <sub>a</sub>	34.00				
	<i>M. x piperita</i>	2018	17.17	28.64	32.09	37.16	42.51	31.51 <sub>A</sub>	119.542 <sub>**</sub>	38.213 <sub>**</sub>	1.308 <sub>ns</sub>	36.79
		2019	9.07	19.44	22.28	30.19	33.12	22.82 <sub>B</sub>				
		Mean	13.12 <sub>e</sub>	24.04 <sub>d</sub>	27.19 <sub>c</sub>	33.68 <sub>b</sub>	37.81 <sub>a</sub>	27.17				
Dry Herbage Yield (t ha <sup>-1</sup> )	<i>M. spicata</i>	2018	6.24	9.65	11.66	13.47	13.73	10.95	11.428 <sub>ns</sub>	268.501 <sub>**</sub>	0.854 <sub>ns</sub>	30.70
		2019	4.04	8.05	10.02	11.31	12.34	9.15				
		Mean	5.14 <sub>d</sub>	8.85 <sub>c</sub>	10.84 <sub>b</sub>	12.39 <sub>a</sub>	13.03 <sub>a</sub>	10.05				
	<i>M. x piperita</i>	2018	5.43	8.80	9.46	10.68	12.20	9.32 <sub>A</sub>	119.542 <sub>**</sub>	38.213 <sub>**</sub>	1.308 <sub>ns</sub>	34.79
		2019	2.63	5.51	6.89	8.33	8.98	6.47 <sub>B</sub>				
		Mean	4.03 <sub>e</sub>	7.16 <sub>d</sub>	8.17 <sub>c</sub>	9.51 <sub>b</sub>	10.59 <sub>a</sub>	7.89				
Dry leaf yield (t ha <sup>-1</sup> )	<i>M. spicata</i>	2018	2.99 <sub>d</sub>	4.33 <sub>c</sub>	4.94 <sub>b</sub>	5.79 <sub>a</sub>	5.71 <sub>a</sub>	4.75	6.565 <sub>ns</sub>	167.189 <sub>**</sub>	4.156 <sub>*</sub>	24.21
		2019	2.33 <sub>c</sub>	4.51 <sub>b</sub>	4.71 <sub>b</sub>	5.04 <sub>ab</sub>	5.39 <sub>a</sub>	4.40				
		Mean	2.66 <sub>d</sub>	4.42 <sub>c</sub>	4.83 <sub>b</sub>	5.41 <sub>a</sub>	5.55 <sub>a</sub>	4.57				
	<i>M. x piperita</i>	2018	2.90	4.50	5.05	5.15	6.08	4.74 <sub>A</sub>	119.542 <sub>**</sub>	38.213 <sub>**</sub>	1.308 <sub>ns</sub>	31.90
		2019	1.69	3.06	3.41	3.81	3.86	3.17 <sub>B</sub>				
		Mean	2.30 <sub>d</sub>	3.78 <sub>c</sub>	4.23 <sub>b</sub>	4.48 <sub>b</sub>	4.97 <sub>a</sub>	3.95				
Essential oil content (%)	<i>M. spicata</i>	2018	1.63	1.98	1.98	2.02	2.02	1.93 <sub>B</sub>	1896.000 <sub>**</sub>	11.781 <sub>**</sub>	1.411 <sub>ns</sub>	9.43
		2019	1.96	2.04	2.18	2.24	2.28	2.14 <sub>A</sub>				
		Mean	1.79 <sub>b</sub>	2.01 <sub>a</sub>	2.08 <sub>a</sub>	2.13 <sub>a</sub>	2.15 <sub>a</sub>	2.03				
Essential oil content (%)	<i>M. x piperita</i>	2018	2.38	2.55	2.49	2.64	2.60	2.53 <sub>A</sub>	119.542 <sub>**</sub>	38.213 <sub>**</sub>	1.308 <sub>ns</sub>	9.03
		2019	2.00	2.32	2.47	2.51	2.68	2.40 <sub>B</sub>				
		Mean	2.19 <sub>c</sub>	2.44 <sub>b</sub>	2.48 <sub>ab</sub>	2.58 <sub>ab</sub>	2.64 <sub>a</sub>	2.46				

\* Significant at the P≤ 0.05 probability level, \*\* Significant at the P≤ 0.01 probability level, ns: Not significant.

Table 3 continued....

**Continue of Table 3.** The effects of years and different nitrogen doses on average yield components of *Mentha spicata* and *Mentha×piperita*.

Species	Years	Nitrogen doses (kg ha <sup>-1</sup> )					Mean	F values				
		0	50	100	150	200		Years (Y)	Nitrogen (N)	Y×N	CV (%)	
Essential oil yield (l ha <sup>-1</sup> )	2018	48.70	86.00	98.20	116.90	115.90	93.10	0.452 ns	205.529 **	1.391 ns	28.24	
	2019	46.60	90.90	102.30	112.50	122.30	94.90					
	Mean	47.70 d	88.50 c	100.20 b	114.60 a	119.10 a	94.00					
	2018	69.30	114.40	125.30	136.20	157.20	120.50 A	119.542 **	38.213 **	1.308 ns	35.64	
	2019	34.20	71.30	84.20	96.00	103.70	77.90 B					
	Mean	51.80 e	92.80 d	104.80 c	116.10 b	130.40 a	99.20					
The main components of essential oil (%)	<i>M. Spicata (Carvone)</i>	2018	57.25 b	59.32 a	60.26 a	57.70 b	57.89 b	58.48 B	180.420 **	15.207 **	22.981 **	2.91
		2019	57.84 b	59.97 ab	58.80 b	61.65 a	61.91 a	60.03 A				
		Mean	57.54 b	59.64 a	59.53 a	59.67 a	59.90 a	59.25				
	<i>M. ×piperita (menthol)</i>	2018	42.88 a	43.31 a	43.65 a	42.16 a	39.01 b	42.20	5.903 ns	32.514 **	3.140 *	4.70
		2019	44.48 a	44.21 a	45.68 a	42.44 a	41.93 a	43.74				
		Mean	43.68 a	43.76 a	44.66 a	42.30 b	40.47 c	42.97				

\* Significant at the P≤ 0.05 probability level, \*\* Significant at the P≤ 0.01 probability level, ns: Not significant.

in 2019. The effect of years in *Mentha×piperita* was significant (Table 3). This is due to the fact that the dry leaf yields of *Mentha×piperita* in 2018 were higher than in 2019. The highest total essential oil yield was obtained from the application of 200 kg N ha<sup>-1</sup> in both mint species. However, total essential oil yield in *Mentha spicata* increased significantly up to 150 kg N ha<sup>-1</sup> (Table 3).

Alsafar and Al-Hassan (2009) reported that essential oil yield increased as the growth rate increased with increasing fertilization. Court *et al.* (1993) reported that there was a significant increase in essential oil yield in *Mentha×piperita* up to 180 kg N ha<sup>-1</sup>. Similarly, many researchers such as Mitchell and Farris (1996) and Zheljzkov *et al.* (2010) found that more essential oil yields were produced with the increased nitrogen application.

The total number of components compounds of *M. spicata* and *M.×piperita* depended on the nitrogen application and were 39 and 35, respectively (Table 4 and 5). Carvone and limonene were determined as the main components in *Mentha spicata*, while menthol and menthone were determined as the main components in *Mentha×piperita* (Tables 4 and 5). Carvone which has the highest content among the components of *Mentha spicata* essential oil, was detected at the highest rate (59.90%) in the application of 200 kg N ha<sup>-1</sup>, while the highest limonene content (18.65%) was in the control plants (Table 4). *Mentha spicata* essential oil containing 55-65% carvone has been reported to be suitable for industrial use (Telci *et al.*, 2004). The values of carvone content obtained in the study were between these values (Table 4).

Considering the average of years, it was determined that the content of carvone

**Table 4.** The effect of different nitrogen doses on essential oil composition of *M. spicata*.

	Retention	Compounds	Nitrogen doses (kg ha <sup>-1</sup> )				
			0	50	100	150	200
1	11.31	$\alpha$ -Pinene	0.97	0.83	0.82	0.79	0.87
2	14.71	$\beta$ -pinene	1.36	1.19	1.18	1.09	1.19
3	15.30	Sabinene	0.65	0.60	0.44	0.56	0.62
4	17.11	Myrcene	3.21	3.07	3.19	3.05	3.26
5	18.77	Limonene	18.6	16.97	17.32	16.41	17.77
6	19.25	1.8-Cineole	3.99	3.68	3.58	3.01	3.27
7	26.36	3-Octanol	0.11	0.12	0.06	0.09	0.06
8	29.08	<i>Trans</i> -sabinene hydrate	0.94	1.13	1.43	1.40	1.56
9	29.28	$\gamma$ -Terpinene	0.59	0.61	0.62	0.62	0.60
10	29.57	L-Menthone	-	0.25	0.18	0.13	0.00
11	29.84	<i>cis</i> -3-hexenyl isovalerate	0.15	0.07	0.05	0.11	0.06
12	31.29	$\beta$ -Bourbonene	2.19	2.06	2.01	2.19	2.01
13	32.24	<i>cis</i> -Sabinene hydrate	-	0.00	0.00	0.03	0.00
14	32.42	Menthyl acetate	0.05	0.00	0.00	0.06	0.00
15	32.88	$\beta$ -Cubebene	0.07	0.06	0.06	0.08	0.00
16	33.51	$\beta$ -Elemene	0.72	0.82	0.90	0.88	0.82
17	33.85	beta-Caryophyllene	1.62	1.65	1.54	1.59	1.56
18	34.14	<i>cis</i> -Dihydrocarvone	0.42	0.19	0.23	0.17	0.13
19	34.33	<i>cis</i> -Isodihydrocarvone	0.19	0.11	0.11	0.11	0.00
20	34.34	<i>trans</i> -Dihydrocarvone	0.43	0.38	0.48	0.34	0.48
21	34.72	L-Menthol	-	0.47	0.81	0.51	0.29
22	35.30	Pulegone	0.22	0.23	0.14	0.22	0.22
23	35.73	Dihydrocarvyl acetate	0.41	0.29	0.19	0.29	0.21
24	35.94	bicyclosesquiphellandrene	0.21	0.24	0.26	0.37	0.16
25	36.13	(+)-Epi-bicyclosesquiphellandrene	-	0.08	0.16	0.08	0.08
26	36.65	$\alpha$ -Terpineol	0.24	1.59	1.54	2.02	1.51
27	37.25	Germacrene D	2.07	1.40	1.53	1.48	1.50
28	37.40	Neodihydrocarveol	0.77	0.33	0.17	0.23	0.28
29	37.54	<i>cis</i> -Carvyl Acetate	0.05	0.00	0.00	0.06	0.00
30	37.87	<i>Trans</i> -Carvyl acetate	0.26	0.30	0.32	0.46	0.30
31	37.94	Bicyclogermacrene	0.44	0.53	0.34	0.59	0.57
32	38.11	Carvone	57.5	59.64	59.53	59.67	59.90
33	40.21	<i>Trans</i> -Carveol	0.33	0.38	0.29	0.29	0.24
34	41.02	<i>cis</i> -Carveol	0.33	0.29	0.19	0.31	0.27
35	43.37	<i>cis</i> -Jasmone	-	0.05	0.00	0.09	0.00
36	44.99	Caryophyllene oxide	-	0.03	0.00	0.02	0.00
37	46.09	1.10-di-epi-Cubenol	0.04	0.04	0.00	0.09	0.00
38	47.93	spathulenol	0.19	0.12	0.13	0.19	0.13
39	50.06	$\alpha$ -Cadinol	0.04	0.04	0.04	0.09	0.04
Total			99.4	99.80	99.79	99.73	99.90

increased slightly with increasing nitrogen doses. Similar to the findings of the study, it was reported that the content of carvon increased with increasing nitrogen application (Singh and Singh, 1986; Büyükbayraktar, 2014).

Menthol, which has the highest content among the components of *Mentha*×*piperita*

essential oil, was detected at the highest rate (44.66%) in the application of 100 kg N ha<sup>-1</sup>, while the highest menthone content (18.65%) was in the application of 200 kg N ha<sup>-1</sup> (Table 5). Mitchell and Farris (1996) reported that high nitrogen in *Mentha*×*piperita* could delay oil maturation, because it possibly reduces stress. Singh and

**Table 5.** The effect of different nitrogen doses on essential oil composition of *M. x piperita*.

	Retention time	Compounds (%)	Nitrogen doses (kg ha <sup>-1</sup> )				
			0	50	100	150	200
1	11.31	$\alpha$ -Pinene	0.77	0.78	0.76	0.69	0.72
2	14.72	$\beta$ -pinene	1.18	1.18	1.16	1.07	1.10
3	15.30	Sabinene	0.66	0.66	0.64	0.60	0.62
4	16.91	$\beta$ -myrcene	0.14	0.22	0.16	0.29	0.22
5	18.75	Limonene	2.00	1.97	1.98	2.02	1.76
6	19.25	1.8-Cineole	6.51	6.27	6.28	5.89	5.81
7	20.03	$\beta$ -Ocimene	-	0.16	0.00	0.08	0.08
8	20.33	cis-Ocimene	0.14	0.00	0.18	0.07	0.09
9	21.96	p-Cymene	0.10	0.00	0.14	0.00	0.00
10	26.36	3-Octanol	0.20	0.20	0.18	0.13	0.12
11	29.09	Trans-sabinene hydrate	1.33	1.78	1.55	2.55	1.84
12	29.28	$\gamma$ -Terpinene	1.06	1.04	1.17	1.01	1.80
13	29.59	Menthone	17.6	19.9	18.1	21.29	23.55
14	30.06	Menthofurane	1.66	1.49	1.18	1.28	1.29
15	30.52	Isomenthone	2.77	3.16	3.09	3.31	3.50
16	31.29	$\beta$ -Bourbonene	0.44	0.44	0.34	0.44	0.50
17	31.36	Neomenthyl acetate	0.19	0.06	0.11	0.56	0.07
18	31.94	Linalool	0.14	0.14	0.15	0.14	0.14
19	32.22	cis-Sabinene hydrate	0.17	0.08	0.14	0.00	0.00
20	32.45	Menthyl acetate	4.88	3.18	4.39	2.71	3.49
21	33.04	Neoisomenthyl acetate	0.17	0.06	0.10	0.07	0.07
22	33.39	Neomenthol	3.33	2.97	3.33	3.20	2.97
23	33.58	b-Elemene	1.21	1.26	1.25	0.95	1.23
24	33.97	$\beta$ -caryophyllene	3.42	2.28	2.37	2.57	2.56
25	34.35	Neoisomenthol	0.78	0.65	0.69	0.54	0.41
26	34.96	Menthol	43.6	43.7	44.6	42.30	40.47
27	35.38	(Z)- $\beta$ -Farnesene	0.40	0.38	0.37	0.39	0.39
28	35.52	Isomenthol	0.22	0.17	0.23	0.10	0.09
29	35.6	Pulegone	0.13	0.13	0.17	0.17	0.09
30	36.75	$\alpha$ -Terpineol	0.16	0.15	0.17	0.14	0.13
31	37.25	Germacrene D	2.31	2.41	2.15	2.42	2.48
32	37.76	Piperitone	0.65	0.69	0.65	0.68	0.73
33	37.94	Bicyclogermacrene	0.28	0.29	0.27	0.31	0.31
34	38.07	Carvone	0.21	0.25	0.00	1.15	0.47
35	47.04	Viridiflorol	0.97	0.97	0.95	0.94	0.94
Total			99.8	99.8	99.1	99.08	100.00

Singh (1989) found the highest menthol content (47.55%) at a dose of 100 kg N ha<sup>-1</sup> in India conditions. Court *et al.* (1993) obtained the highest menthol content (44.95%) at dose of 60 kg N ha<sup>-1</sup> under Canadian conditions. Components of secondary (bioactive) metabolites, which are effective substances of medicinal and aromatic plants, vary depending on environmental factors, cultivation practices, and the genotypes (Moradkhani *et al.*, 2010).

There were positive correlations between plant height, fresh and dry herbage yields,

dry leaf yield, essential oil yield in *M. spicata*. Positive correlations were observed between the carvone content and fresh herbage yield, dry leaf yield, essential oil content and essential oil yield in *M. spicata* (Table 6).

On the other hand, there were positive correlations between plant height, fresh and dry herbage yields, dry leaf yield, essential oil content, and yield in *M. x piperita*. Negative correlations were observed between the menthol content and all parameters examined in *M. x piperita* (Table 7).



**Table 6.** Coefficient of correlation between the examined parameters of *Mentha spicata* in relation to nitrogen application.

	Plant height	Fresh herbage yield	Dry herbage yield	Dry leaf yield	Essential oil content	Essential oil yield
Fresh Herbage Yield	0.894**					
Dry Herbage Yield	0.946**	0.975**				
Dry Leaf Yield	0.862**	0.963**	0.964**			
Essential Oil Content	0.330ns	0.589**	0.462*	0.508**		
Essential Oil Yield	0.807**	0.967**	0.926**	0.964**	0.714**	
Carvone	0.235ns	0.391*	0.285ns	0.376*	0.575**	0.481**

\* Significant correlation at a level of  $P \leq 0.05$ , \*\* Significant correlation at a level of  $P \leq 0.01$ , ns: Not significant.

**Table 7.** Coefficient of correlation between the examined parameters of *Mentha x piperita* in relation to nitrogen application.

	Plant height	Fresh herbage yield	Dry herbage yield	Dry leaf yield	Essential oil content	Essential oil yield
Fresh Herbage Yield	0.975**					
Dry Herbage Yield	0.961**	0.972**				
Dry Leaf Yield	0.923**	0.925**	0.971**			
Essential Oil Content	0.756**	0.774**	0.737**	0.624**		
Essential Oil Yield	0.950**	0.956**	0.983**	0.984**	0.740**	
Menthol	-0.732**	-0.703**	-0.680**	-0.646**	-0.382*	-0.649**

\* Significant correlation at a level of  $P \leq 0.05$ , \*\* Significant correlation at a level of  $P \leq 0.01$ , ns: Not significant.

significantly decreased at doses of 150 and 200 kg N ha<sup>-1</sup>.

## CONCLUSIONS

All nitrogen doses applied in the study caused a significant increase in the plant height, total fresh and dry herb yield, dry leaf yield, essential oil content, and yield in both mint species compared to the control plants. The total yields of *Mentha x piperita* increased significantly with increase in nitrogen fertilization up to 200 kg N ha<sup>-1</sup>. However, total yields of *Mentha spicata* increased significantly with increase in nitrogen fertilization up to 150 kg N ha<sup>-1</sup>. Nitrogen fertilization significantly increased the content of carvone in *M.spicata*, but no significant difference was detected between the applied nitrogen doses. The highest menthol content in *M x piperita* was obtained from 100 kg N ha<sup>-1</sup> and the menthol content

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### اثر مقادیر مختلف نیتروژن روی ویژگی های زراعی و کیفیت گیاهان گونه *Mentha spicata* L. و *x piperita* L.

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#### چکیده

اثر مقادیر مختلف نیتروژن (N) شامل (0، 50، 100، 150، و 200 کیلوگرم در هکتار) روی رشد و کیفیت گونه *Mentha x piperita* L. و *Mentha spicata* L. در شرایط زیست بومی منطقه Uşak در ترکیه در سال های 2017-19 بررسی شد. در این پژوهش، برداشت های محصول در دوره رشد 2018 و 2019 با هم ترکیب شد و میانگین ارتفاع گیاه و محتوای اسانس، وزن کل سبزینه های تازه و خشک، وزن برگ خشک و عملکرد اسانس، و ترکیب شیمیایی اسانس بررسی شد. ارتفاع گیاه، عملکرد کل، و محتوای اسانس هر دو گونه نعا با افزایش مقدار نیتروژن داده شده به طور معناداری افزایش یافت. بیشینه عملکرد کل و محتوای اسانس در هر دو گونه نعا از تیمار 200 کیلوگرم نیتروژن در هکتار به دست آمد. اجزای اصلی اسانس در *Mentha x piperita* L. به صورت منتول و منتون و در *Mentha spicata* L. به صورت carvone و limonene شناسایی شد. بیشترین مقدار



محتوای منتول (44/66٪) از تیمار 100 کیلو گرم در هکتار نیتروژن به دست آمد، در حالیکه بیشترین محتوای carvone برابر (59/90٪) در تیمار 200 کیلو گرم در هکتار نیتروژن بود. عملکرد کل *Mentha x piperita* L. با افزایش مقدار نیتروژن تا حد 200 کیلوگرم در هکتار افزایش نشان داد، ولی عملکرد کل *Mentha spicata* L. با افزایش نیتروژن تا حد 150 کیلوگرم در هکتار افزایش یافت.