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Impact of developmental stage and harvest time of peppermint (*Mentha piperita* L.) on growth and volatile oil yield

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Peppermint, Mentha piperita L., Developmental stage, Harvest time, Essential oil

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ABSTRACT

To study the effect of three growth stages of peppermint (*Mentha piperita* L.) (pre-budding, budding and 50% flowering) and three harvest times during the day (7:00 am, 12:00 pm and 5:00 pm) in the growth and yield of volatile oil, the experiment was conducted in the fields of the agricultural research station (Al-Bandar area) affiliated to the College of Agriculture, Al-Muthanna University, under the environmental conditions of Al-Muthanna Governorate located in southern Iraq, at latitude 31.32139° N and 45.30407° E, for the period from 15/2/2024 to 16/6/2024, and was implemented according to a randomized complete block design with three replications. The results of the study showed the significant effect of the developmental stages of the plant on all the studied traits. Peppermint plants harvested at 50% flowering stage were significantly superior and gave the highest values in plant height (61.33 cm), number of branches (27.38 branch. plant^{-1}), shoot fresh weight (19.07 gm. plant $^{-1}$), shoot dry weight (4.74 gm. plant^{-1}), fresh weight of leaves (12.80 gm. plant^{-1}), dry weight of leaves (3.10 gm. plant^{-1}), percentage of volatile oil (2.40 %) and volatile oil yield per plant (74.06 µL. plant^{-1}). While harvest times had a significant effect on the traits of fresh weight of the vegetative mass, fresh leaf weight, volatile oil content, and volatile oil yield per plant only, which was excelled by the peppermint plants that harvested at 7.00 am and gave the highest values (17.00 gm. plant^{-1}, 11.50 gm. plant^{-1}, 2.29 % and 65.20 µL. plant^{-1}) respectively.

تأثير المرحلة التطورية وموعد الحصاد لنبات النعناع الفلفلي في النمو وحاصل الزيت الطيار

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الخلاصة

لدراسة تأثير ثلاث مراحل نمو لنبات النعناع الفلفلي *Mentha piperita* L. (ما قبل التبرعم ، التبرعم و 50% تزهير) وثلاثة مواعيد حصاد خلال اليوم الواحد (7:00 صباحاً، 12:00 ظهراً و5:00 مساءً) في نمو وحاصل الزيت الطيار، أجريت التجربة في حقول محطة البحوث الزراعية (منطقة البندر) التابعة لكلية الزراعة جامعة المثنى، تحت الظروف البيئية لمحافظة المثنى الواقعة جنوب العراق عند خط عرض 31.32139° شمالاً و45.30407° شرقاً، للفترة من 2024/2/15 إلى 2024/6/16، ونفذت وفق تصميم القطاعات العشوائية الكاملة وبثلاث مكررات، أظهرت نتائج الدراسة التأثير المعنوي للمراحل التطورية للنبات في جميع الصفات المدروسة. أذ تفوقت نباتات النعناع المحصودة عند مرحلة 50% تزهير بشكل معنوي وأعطت أعلى القيم في ارتفاع النبات (61.33 سم)، عدد الأفرع (27.38 فرع. نبات$^{-1}$)، الوزن الطازج للكتلة الخضرية (19.07 غم. نبات$^{-1}$)، الوزن الجاف للكتلة الخضرية (4.74 غم. نبات$^{-1}$)، الوزن الطازج للأوراق (12.80 غم. نبات$^{-1}$)، الوزن الجاف للأوراق (3.10 غم. نبات$^{-1}$)، النسبة المئوية للزيت الطيار (2.40٪) وحاصل الزيت الطيار لكل نبات (74.06 ميكرولتر. نبات$^{-1}$). بينما كان لأوقات الحصاد تأثير معنوي في صفات الوزن الطري للمجموع الخضري ووزن الأوراق الطري ومحتوى الزيت الطيار وحاصل الزيت الطيار للنبات الواحد فقط، والتي تفوقت بها نباتات النعناع التي تم حصادها عند الساعة 7.00 صباحًا وأعطت أعلى القيم (17.00 غم نبات$^{-1}$، 11.50 غم نبات$^{-1}$، 2.29٪ و 65.20 ميكرولتر نبات$^{-1}$) على التوالي، ولم يكن لأوقات الحصاد اي تأثير معنوي على صفات النمو الخضري الأخرى.

الكلمات المفتاحية: النعناع الفلفلي , Mentha piperita L ، المرحلة التطورية ، وقت الحصاد ، الزيت الطيار.

INTRODUCTION

Species of the genus *Mentha* belong to the family Lamiaceae (Beetmnet, 2011) and are found in many different environments, growing widely in Asia, Africa, Europe, North America and Australia(. Mint plants usually grow in moist places and in moist soil, so they often grow near rivers, lakes and in damp places. They grow well in partially shaded places but can also tolerate sunny place.(Silva *et al.* 2009). Peppermint (*Mentha piperita* L.), which belongs to the genus *Mentha*, is a hybrid resulting from the hybridization process between the species *Mentha spicata* and the species *Mentha aquatic* (Singh et al. 2015). It is distributed in many regions of the world. It is an important perennial herb that has been used since ancient times as a natural source for the treatment of many medical conditions, due to the fact that its volatile oil contains several main active compounds, namely menthol (29%), menthone (20-30%), and methyl acetate (1-3%), in addition to containing more than 40 other compounds.

Peppermint leaves contain approximately 1.2-3.9% volatile oil, which consists of approximately 52% monoterpenes, 9% sesquiterpenes, in addition to small amounts of aromatic hydrocarbons (9%), aldehydes 9%, lactones 7%, and alcohol 6% (Saharkhiz *et al.* 2012). (Afzal, 2010). Lorenzi and Matos (2002) indicated that peppermint is widely used as an anthelmintic, antispasmodic, antiseptic, stomach stimulant, carminative, and others. Shkurupii *et al.* (2006) indicated that inhaling peppermint oil vapor treats respiratory congestion and that drinking peppermint tea is beneficial in treating mouth and throat inflammation and cough. Peppermint oil is used externally in the form of ointments or creams to treat muscle and nerve pain, headaches, migraines, and chickenpox (Datta *et al.* 2011). Ground peppermint leaves have been used since ancient times to polish teeth, they are also used in the manufacture of toothpastes to clean and polish natural teeth, Mint is beneficial for children's gums as it reduces pain and gives them germ-free teeth, Menthol, which is the main component of peppermint volatile oil, is used in the manufacture of medicines, oral cleaning and sterilization solutions, toothpastes, cosmetics, and food products (Singh and Pandey, 2018).

Many scientific studies have been conducted to demonstrate the significant impact of environmental factors such as temperature, humidity and light, as well as the impact of

agricultural operations such as planting dates, fertilization, irrigation and harvest dates on the growth and content of medicinal and aromatic plants, including mint. A number of researchers have confirmed the impact of these factors on its quality and type, including: Kızıl and Tonçer (2006) on (*Mentha spicata* L, Llorens-Molina *et al*. (2014) on *Mentha longifolia* L. and Bufalo *et al*. (2015) on *Mentha spicata* L. Alsafar and Al-Hassan (2009) pointed out that the yield and composition of the volatile oil of mint species are affected by the interaction between the genotype, environmental factors, crop age, harvest time, season, distillation method, and storage type.

The developmental stage of plant and the time of harvest are among the most important factors affecting the quantity and quality of the volatile oil (Clar and Menary, 2006). Alsafar and Al-Hassan(2009) pointed out that the quantity and quality of the volatile oils of the mint plant are affected by several factors, including the environment, agricultural season, crop age, harvest time, distillation method, and type of storage. Many studies have indicated that the volatile oil content of peppermint varies from one developmental stage to another and from one harvest or collection time to another. Among these studies is the study by Yesile and Özcan (2021) on peppermint and the study by Rohloff *et al.* (2005) on the same plant as well. Due to the medical and nutritional importance of peppermint, and to improve its growth and increase the production of its volatile oil in the southern region of Iraq, this study was conducted, which included identifying the effect of the plant's developmental stage and harvest time on its vegetative growth and the volatile oil content of its leaves.

MATERIAL AND METHODS

Experiment Location and Planting Season

The experiment was conducted in the fields of the Second Agricultural Research Station (Al-Bandar) located on the banks of the Euphrates River in Samawah city (at coordinates 31.32139° N and 45.30407° E) and affiliated to the College of Agriculture, Al-Muthanna University, for the period from 15/2/2024 to 16/6/2024. The physical and chemical properties of the soil of the experimental field were estimated before planting (soil samples were collected randomly at 0-30 cm) in the laboratories of the Soil Department of the College of Agriculture, Al-Muthanna University, as shown in Table (1). The study was conducted during the spring agricultural season of 2024.

Table (1) some physical and chemical characteristics of field soil before planting.

Parameters		Unit	Value
pH			7.6
EC		ds m^{-1}	4.4
O.M		%	0.94
N			16.4
P		mg kg^{-1} soil	12.2
K			180.6
Soil	Clay	%	22.00
	Silt		18.00
	Sand		60.00
	Texture	Sandy loam	

Experimental

Experiment design, cultivation, plant material and treatments

The experiment was designed using a CRBD design with three replications and was implemented as a factorial experiment by randomly distributing treatments to the experimental units. The experimental field area was divided into three replicates (sectors) separated by a distance of 2 meters. Each replicate included 9 experimental units, each with dimensions of (1.5 m x 3 m) and an area of 4.5 m^2, with a distance of 1 meter between each one.

Peppermint stolons cutting were planted inside the experimental units on 15/2 /2024 (Al-Zayadi, 2019), in lines 40 cm. apart from each other, and 30 cm between one plant and another. Equal amounts of triple superphosphate fertilizer (46% P_2O_5) at a rate of 100 kg $P_2O_5.ha^{-1}$ were added to all experimental units at once when planting the stolons cutting, and equal amounts of urea fertilizer (46% N) at a rate of 120 kg $N.ha^{-1}$ were also added after planting in two batches.

Treatments : The first factor included three different developmental stages of the peppermint plant, which are: pre-budding, budding and 50% flowering, while the second factor included three different harvest times during the same day, which are: 7:00 am, 12:00 pm and 5:00 pm, and the overlap between them. and the interaction between them. Thus, the number of experimental units becomes 27.

Harvesting

Peppermint plants were harvested at a rate of ten plants randomly selected from each experimental unit according to the developmental stage of the plant. The plants (pre-budding stage) were harvested on 17/ 5, on one day and at three times. The first batch of plants was harvested at 7:00 am (at a temperature of 29.4 °C and a relative humidity of 31%). The second batch of plants was harvested at 12:00 pm (at a temperature of 38.2 °C and a relative humidity of 10 %). The third batch was harvested at 5:00 pm (at a temperature of 32.6 °C and a relative humidity of 15 %). The plants of the developmental stage (budding stage) were harvested on 31/5, on one day and at three times as well. The first batch of plants was harvested at 7.00 am (at a temperature of 31.5 °C and a relative humidity of 14%), the second batch of plants was harvested at 12.00 pm (at a temperature of 45 °C and a relative humidity of 10%), while the third batch was harvested at 5.00 pm (at a temperature of 39°C and a relative humidity of 10%). While the plants of the developmental stage (50% flowering stage) were harvested on 15/6, on one day and at three times as well, the first batch of plants was harvested at 7.00 am (at a temperature of 30.4°C and a relative humidity of 31%), and the plants of the second batch were harvested at 12.00 pm (at a temperature of 48.5°C and a relative humidity of 10%), while the plants of the third batch were harvested at 5.00 pm at a temperature of 40°C and a relative humidity of 10%).

Extraction of essential oil

The content of peppermint essential oil was estimated as a percentage using the Clevenger apparatus and the hydro-distillation process, using the extraction method described by Aflatuni (2005) with some modifications. 25 g of peppermint leaves were taken, air-dried in a shaded place at 35-40 °C for each treatment, and after grinding, they were placed in the apparatus flask containing 250 ml of water. The boiling process of water with the leaf powder continued for 3 hours. After turning off the apparatus and completing the extraction process, the volume of volatile oil collected in the graduated tube was read and recorded in milliliters. After that, the amount of volatile oil in the sample was calculated as a relative percentage (v/w) using the following equation:

Volatile oil percentage (%) = volume of volatile oil / weight of sample × 100

The volatile oil yield of a single plant was calculated according to the following equation:

Volatile oil yield per plant = dry weight of the leaves of one plant × the percentage of volatile oil

Studied Characteristics

The study included estimating plant height, number of branches, shoot fresh weight, shoot dry weight, fresh leaf weight, dry leaf weight, percentage of volatile oil and volatile oil yield per plant.

Statistical analysis

Statistical analysis of the data was performed using the Genstat, and using the analysis of variance (ANOVA) method. The means of the treatments were compared at the least significant difference (L.S.D.) and at the probability level (0.05) (Al-Rawi and Khalafallah , 1980).

RESULTS AND DISCUSSION

Data shown in Table (2) showed that the developmental stage of the plant had a significant effect in plant height of peppermint, as the plants harvested at the 50% flowering stage were significantly superior and gave the highest values in the trait of plant height (61.33 cm), which did not differ significantly from the budding stage, compared to the pre-budding stage, which gave the lowest values (53.63cm). The results of the same table show that there was no significant effect of harvest times on the plant height trait, and there was no significant effect of the interaction between the two experimental factors.

The results indicated in Table (3) showed that the developmental stage had a significant effect on the number of branches trait, as peppermint plants harvested at the developmental stage (50% flowering) gave the highest values in this trait, reaching 27.38 branch. plant^{-1} which did not differ significantly from the budding stage, which gave highest reaching 24.75 branch. plant^{-1} compared to the pre-budding stage, which gave the lowest value, reaching 16.70 branch. plant^{-1}. The same results also showed that harvest times did not significantly affect the number of branches trait, and the interaction between

the two experimental factors did not have any significant effect on the number of branches of the peppermint plant.

Table (2) Effect of developmental stage and harvest time of peppermint plant on plant height (cm)

Harvest time (Hour)	**Developmental stage**			**Means**
	Pre- budding	**Budding**	**50% Flowering**	
7:00 am	55.30	58.20	60.83	58.11
12:00 pm	52.00	58.30	59.83	56.71
5: 00 pm	53.60	61.50	63.00	59.37
Means	53.63	59.33	61.33	
L.S.D 0.05	Harvest time N.S	Developmental stage 2.73	Developmental stage × Harvest time N.S	

Table (3) Effect of developmental stage and harvest time of peppermint plant on branches number (branch plant^{-1})

Harvest time (Hour)	**Developmental stage**			**Means**
	Pre- budding	**Budding**	**50% Flowering**	
7:00 am	15.20	25.91	28.50	23.20
12:00 pm	16.10	23.83	27.10	22.34
5: 00 pm	18.80	24.50	26.53	23.28
Means	16.70	24.75	27.38	
L.S.D 0.05	Harvest time N.S	Developmental stage 3.59	Developmental stage × Harvest time N.S	

The results indicated in Table (4) showed that the two experimental factors had a significant effect on the shoot fresh weight of peppermint plants. The developmental stage of the plant had a significant effect on this characteristic, represented by the superiority of the plants harvested in the developmental stage (50% flowering) and gave the highest fresh weight of shoot, which reached 19.07gm. plant^{-1}, with a significant difference from the plants harvested in the (budding) stage, which gave a fresh weight of shoot reached 16.33 gm. plant^{-1} and also superior to the plants harvested in the (pre-budding) stage, which gave the lowest fresh weight of shoot reached 12.53 gm. plant^{-1} . The times of harvesting plants during the day had a significant effect on the shoot fresh weight of peppermint plants, as the plants harvested at 7 am excelled and gave the highest fresh weight of shoot reached 17.00 gm. plant^{-1} which did not differ significantly with the weight of the plants harvested at 5 pm (16.00) compared to the plants harvested at 12 pm which gave the lowest weight reached 14.77 gm. plant^{-1}. The interaction between the two experimental factors had no significant effect on this trait.

Table (4) Effect of developmental stage and harvest time of peppermint plant on shoot fresh weight (gm. plant^{-1})

Harvest time (Hour)	**Developmental stage**			**Means**
	Pre- budding	**Budding**	**50% Flowering**	
7:00 am	13.60	17.60	19.80	17.00
12:00 pm	11.40	14.50	18.40	14.77
5: 00 pm	12.60	16.88	19.00	16.00
Means	12.53	16.33	19.07	
L.S.D 0.05	Harvest time 2.19	Developmental stage 2.19	Developmental stage N.S	×Harvest time

The results of Table (5) indicated the significant effect of the developmental stages of peppermint in the dry weight of shoot, as the plants harvested in the developmental stage (50% flowering) excelled and gave the highest weight reached 4.74 gm. plant^{-1}, which did not differ significantly from the plants harvested in the developmental stage (budding), which gave a weight reached 4.65 gm. plant^{-1} , compared to the stage (pre-budding), which gave the lowest dry weight of shoot (3.43 gm. plant^{-1}). Neither the time of plant harvesting during the same day nor the interaction between the two experimental factors had any significant effect on this trait.

Table (5) Effect of developmental stage and harvest time of peppermint plant on shoot dry weight (gm. plant^{-1})

Harvest time (Hour)	**Developmental stage**			**Means**
	Pre- budding	**Budding**	**50% Flowering**	
7:00 am	3.61	4.93	5.15	4.56
12:00 pm	3.27	4.31	4.49	4.02
5: 00 pm	3.41	4.70	4.58	4.23
Means	3.43	4.65	4.74	
L.S.D 0.05	Harvest time N.S	Developmental stage 0.55	Developmental stage N.S	×Harvest time

The data in Table (6) showed the significant effect of the developmental stages of peppermint and harvest times on the fresh weight of leaves. The plants harvested in the developmental stage (50% flowering) were distinguished and gave the highest weight reached 12.80 gm. plant^{-1}, significantly superior to the plants harvested in the (budding) stage, which gave a weight (9.94 gm. plant^{-1}), which in turn significantly superior to the weight of the leaves of the plants harvested in the developmental stage (pre-budding), which gave the lowest weight reached 7.13 gm. plant^{-1}. The significant effect of harvest times on the fresh weight of leaves was clearly demonstrated by the superiority of the harvest time of plants at 7 am, giving the highest weight (11.50 *gm. plant^{-1}*) compared to the lowest weight (8.79 gm. plant^{-1}) in plants that were harvested at 12 pm. The interaction between the two experimental factors did not have a significant effect on this trait.

Table (6) Effect of developmental stage and harvest time of peppermint plant on fresh leaves weight (gm. plant^{-1})

Harvest time (Hour)	Developmental stage			Means
	Pre- budding	Budding	50% Flowering	
7:00 am	8.00	11.16	14.00	11.50
12:00 pm	6.20	8.16	12.00	8.79
5: 00 pm	7.20	10.50	12.40	10.03
Means	7.13	9.94	12.80	
L.S.D	Harvest time	Developmental stage	Developmental stage ×Harvest time	
0.05	1.74	1.74	N.S	

The results in Table (7) show that the developmental stages of peppermint have a significant effect on the dry weight of leaves, as the plants harvested during the developmental stage (50% flowering) outperformed and gave the highest weight (3.10 gm. plant^{-1}) which did not differ significantly with the plants harvested in the (budding) stage, which gave a weight (2.85 gm. plant^{-1}), which in turn also significantly superior to the weight of the leaves of the plants harvested in the developmental stage (pre-budding), which gave the lowest weight reached 1.96 gm. plant^{-1}. Peppermint plants were not significantly affected by harvest times or the interaction between the two experimental factors in the dry weight of leaves.

Table (7) Effect of developmental stage and harvest time of peppermint plant on dry leaves weight (gm. plant^{-1})

Harvest time (Hour)	Developmental stage			Means
	Pre- budding	Budding	50% Flowering	
7:00 am	2.07	3.10	3.15	2.77
12:00 pm	1.85	2.52	3.06	2.49
5: 00 pm	1.97	3.06	3.08	2.66
Means	1.96	2.85	3.10	
L.S.D	Harvest time	Developmental stage	Developmental stage ×Harvest time	
0.05	N.S	0.42	N.S	

The results of the statistical analysis in Table (8) showed the significant effect of the two experimental factors on the percentage of volatile oil in the leaves of peppermint plants, as the plants that were harvested in the two developmental stages (budding) and (50% flowering) excelled with a significant difference in the content of volatile oil in their leaves and gave the highest two percentages with a slight and insignificant difference between them, reaching (2.44% and 2.40%) respectively, compared to the content of the leaves of peppermint plants that were harvested in the developmental stage (pre-budding), which gave the lowest percentage (1.63%).The results of the same table also showed the significant effect of the peppermint harvest times, as the leaves of the plants harvested at 7 am gave the highest volatile oil content, reaching 2.29% , thus significantly superior to the

harvest times at 5 pm and at 12 pm, which gave (2.12% and 2.07%) respectively. The interaction between the two experimental factors had a significant effect on the volatile oil content of peppermint leaves, as the interaction ((budding stage × 7 a.m.) was significantly superior and gave the highest percentage of volatile oil, reaching 2.60%, compared to the lowest percentage resulting from the interaction (pre- budding stage × 12 pm), which was 1.52%.

Table (8) Effect of developmental stage and harvest time of peppermint plant on percentage of volatile oil (%)

Harvest time	**Developmental stage**			**Means**
(Hour)	**Pre- budding**	**Budding**	**50% Flowering**	
7:00 am	1.75	2.60	2.51	2.29
12:00 pm	1.52	2.34	2.34	2.07
5: 00 pm	1.62	2.40	2.35	2.12
Means	1.63	2.44	2.40	
L.S.D	Harvest time	Developmental stage	Developmental stage	×Harvest time
0.05	0.09	0.09	0.16	

The data in Table (9) indicated that the developmental stages of the peppermint plant and its harvest times during the day had a significant effect on the volatile oil yield of a single plant, as the plants that were harvested in the developmental stage (50% flowering) gave the highest amount of volatile oil, which amounted to 74.06 µL. plant^{-1}, compared to the lowest amount given by the plants that were harvested in the developmental stage (pre-budding), which was 31.90 µL. plant^{-1}. The same data also indicated the significant effect of peppermint harvest times during the day, with plants harvested at 7 am significantly superior in volatile oil yield, giving the highest amount of volatile oil, reaching 65.20 µL. plant^{-1}, with a significant difference from plants harvested at 5 pm, which gave an oil quantity of 58.01 µL. plant^{-1}, compared to plants harvested at 12 pm, which gave the lowest oil yield, reaching 52.27 µL. plant^{-1}. The interaction between the two experimental factors had a significant effect on the volatile oil yield, as interaction (budding stage × 7.00 a.m.) was significantly superior in giving the highest amount of volatile oil yield , reaching 80.86 µL. plant^{-1}, compared to the lowest amount of yield produced by interaction (pre- budding stage × 12 pm), which was 27.80 µL. plant^{-1} .

The aim of this experiment was to evaluate the response of peppermint to the effect of different developmental stages of the plant and its harvest times on its vegetative growth and the content and yield of essential oil. The results showed that the developmental stage of peppermint had a significant effect on all studied traits and increased significantly when plants were harvested at the developmental stage (50% flowering), including vegetative growth traits (plant height, number of branches, shoot fresh weight, shoot dry weight, fresh leaves weight and dry leaves weight).

Table (9) Effect of developmental stage and harvest time of peppermint plant on essential oil yield (µL plant^{-1})

Harvest time (Hour)	**Developmental stage**			**Means**
	Pre- budding	**Budding**	**50% Flowering**	
7:00 am	36.00	80.86	78.75	65.20
12:00 pm	27.80	57.70	71.30	52.27
5: 00 pm	31.90	70.00	72.13	58.01
Means	31.90	69.52	74.06	
L.S.D	Harvest time	Developmental stage	Developmental stage ×Harvest time	
0.05	0.46	0.46	0.80	

Piccaglia *et al.* (1993) indicated that the reason for the increase in vegetative characteristics due to the effect of harvesting at the 50% flowering stage may be due to the length of the plant growth period in addition to the more suitable climatic conditions such as temperature or light period.

The results also showed the significant effect of the developmental stages of peppermint on the content and yield of volatile oil in the leaves, as the developmental stage (50% flowering) gave the highest values in these two traits. Many researchers, including (Uyanık and Gürbüz 2015, Katar *et al.* 2018 and Açıkgöz and Kara 2020), have confirmed that the percentage of volatile oils in medicinal and aromatic plants depends on the plant growth period. These results are consistent with what Yesile and Özcan (2021) found in their study on *Menthe piperita* L. and with Çalışkan and Özgüven (2018) on *Mentha arvensi*s. They are also consistent with what Özyazıcı and Kevseroglu (2019) found in their study on *Menthe spicata* L. and *Lavandula angustifolia* Mill.

The results also showed the significant effect of harvest times on some of the studied traits, as peppermint plants harvested at 7.00 a.m. excelled and gave the highest values in the traits of fresh vegetative mass weight, fresh leaves weight, percentage of volatile oil and volatile oil yield per plant, While harvest times had no significant effect on the traits of plant height, number of branches, dry weight of vegetative mass and dry leaves weight. Although there were no significant differences between harvesting hours in most vegetative characteristics, harvesting at 7:00 am was significantly superior in fresh weight of the vegetative group and in fresh weight of leaves. This may be due to the change in temperature and humidity values during the day, which affects the weight of the vegetative group and leaves. Harvest hours had a significant effect on the percentage of volatile oil, as harvesting at 7:00 am gave the highest percentage of oil, decreased at noon, and then increased in the evening, which confirms that the change in the percentage of essential oil is limited every hour. As indicated by Arabacı *et al.* (2015), aromatic plants should not be harvested at random times, but rather in the early morning, especially in the summer, to avoid high temperatures. These results are consistent with the results of Yesile and Özcan (2021) in their study on peppermint, and with Kulan (2013) in his study of basil (*Ocimum basilicum* L.).

CONCLUSION

We conclude from this study that the developmental stage of peppermint plant has a great effect on content and quantity of essential oil, therefore, harvesting is at 50% flowering stage led to obtaining the highest amount of volatile oil. We also concluded that harvesting the plant in the early morning gave the highest yield of volatile oil and the best vegetative qualities of the plant. Therefore, we must avoid random harvesting of plants containing volatile oils and adhere to the plant reaching the appropriate developmental stage as well as the appropriate time for harvesting as well. This is very important from a medical and economic point of view.

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