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The effect of different harvest dates on the yield and quality properties of rosemary (*Rosmarinus officinalis* L.) plant

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Abstract

The purpose of this research carried out in 2018 under Eskişehir ecological conditions was to determine the effect of six different harvest dates (T_1: 16 August, T_2: 29 August, T_3: 14 September, T_4: 28 September, T_5: 15 October and T_6: 31 October) on the yield and quality properties rosemary (*Rosmarinus officinalis* L.). The experiment was established according to a randomized complete-block design with four replications. The composition of the essential oil obtained by the hydro-distillation from the dry leaves of the rosemary plant was analyzed by GC-MS. In the experiment carried out, the mean values of plant height (cm), fresh herb yield (t ha^{-1}), dry herb yield (t ha^{-1}), dry leaf yield (t ha^{-1}), essential oil ratio (%), essential oil yield were determined as 61.87 cm, 15.78 t ha^{-1}, 5.80 t ha-1, 3.55 t ha^{-1}, 0.69% and 23.92 l ha^{-1}, respectively. In the essential oil samples obtained from the harvests made at different dates, 35 different components were identified. The values of the camphor, 1-verbenone, α-pinene, borneol and 1,8-cineol components determined as the main component in the analyzed essential oil samples ranged from 11.67 to 14.78%, from 8.47 to 11.77%, from 8.74 to 11.82%, from 8.61 to 11.12% and from 7.01 to 9.07%, respectively.

Key words: Rosemary, *Rosmarinus officinalis*, different harvest dates, essential oil

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Farklı hasat zamanlarının biberiye (*Rosmarinus officinalis* L.) bitkisinin verim ve kalite özellikleri üzerine etkisi

Özet

Bu araştırma, farklı hasat zamanlarının (Z_1: 16 Ağustos, Z_2: 29 Ağustos, Z_3: 14 Eylül, Z_4: 28 Eylül, Z_5: 15 Ekim ve Z_6: 31 Ekim) biberiye (*Rosmarinus officinalis* L.) bitkisinin verim ve kalite özellikleri üzerine etkisini belirlemek amacıyla Eskişehir ekolojik koşullarında 2018 yılında yürütülmüştür. Deneme, tesadüf blokları deneme desenine göre 4 tekerrürlü olarak kurulmuştur. Biberiye bitkisinin drog yapraklarından su distilasyonu yöntemiyle elde edilen uçucu yağın kompozisyonu GC-MS ile analiz edilmiştir. Yürütülen çalışmada, ortalama bitki boyu (cm), yeşil herba verimi (t ha^{-1}), kuru herba verimi (t ha^{-1}), kuru yaprak verimi (t ha^{-1}), uçucu yağ oranı (%) ve uçucu yağ verimi değerleri sırasıyla 61.87 cm, 15,78 t ha^{-1}, 5.80 t ha^{-1}, 3.55 t ha^{-1}, % 0,69 ve 23.92 l ha^{-1} olarak tespit edilmiştir. Farklı zamanlarda yapılan hasatlardan elde edilen uçucu yağ örneklerinde 35 farklı komponent olduğu tespit edilmiştir. Analiz edilen uçucu yağ örneklerinde ana bileşen olarak tespit edilen camphor, 1-verbenone, α-pinene, borneol ve 1,8-cineol bileşenlerin değerleri sırasıyla %11.67-14.78, %8.47-11.77, %8.74-11.82, %8.61-11.12 ve 7.01-9.07 arasında değişmiştir.

Anahtar kelimeler: Biberiye, *Rosmarinus officinalis*, farklı hasat zamanları, uçucu yağ

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1. Introduction

Rosemary (*Rosmarinus officinalis* L.) plant, an important species of the Lamiaceae/Labiatae family, is an important medicinal and aromatic plant naturally distributed in the Mediterranean region [1, 2, 3]. The genus Rosmarinus has *R. officinalis*, *R. laxiflorus*, *R. eriocalyx* and *R. lavandulaceus*, which are distributed in different parts of the world. *R. officinalis* has varieties of erectus, humilis and albiflorus [4, 5]. Rosemary having a strong taproot and a plant height of 50-100 cm, needle-shaped leaves and pale blue flowers is an evergreen, perennial, in bush form and cross-fertilization plant [1, 2, 6].

The leaves of the rosemary plant are used as spice and herbal tea mixtures. Otherwise, the essential oil obtained from rosemary plants is used in perfume, food and pharmaceutical industry [1, 7]. Also, rosemary is widely used in aromatherapy and folk medicine, as well as being used as an ornamental plant in different regions of the world [8, 9, 10]. Rosemary is well known a medicinal plant and has been used as anti-oxidant, anti-cancer, antidiabetic, anti-inflammatory, antimicrobial, antifungal, antimutagenic, antispasmodic, wound healing, strengthening capillaries of hair and protecting liver [2, 7, 11, 12, 13, 14, 15].

In some countries such as France, Spain, Portugal and Greece, rosemary is cultivated to meet the demand for the use of essential oils in the food, perfume, cosmetic and pharmaceutical industries [7]. Many other countries meet the needs of rosemary by harvesting from natural plants. In Turkey, rosemary collected from the natural flora of Mersin and Adana are presented to domestic and foreign markets [16]. Rosemary has attracted attention as an important medicinal and aromatic plant in recent years due to the industrial demand for the essential oil obtained from the plant. In order to meet this demand for both the essential oil and the drug leaves of the rosemary, the cultivation of the plant in field conditions is becoming increasingly important.

Studies on the volatile oil components of the rosemary plant have shown that the plant has different chemotypes such as eucalyptol/1,8-cineole (Italy, Morocco and Tunisia), camphor-borneol (Spain) and α-pinene-verbanon (France and Corsica) in different parts of the world [5, 17, 18, 19]. Many studies conducted on rosemary's essential oil ratio and its components have shown that many factors are effective on essential oil content and its composition [1, 5, 7, 21, 30]. One of the most important factors affecting the rate and composition of essential oil is the different harvest dates. The studies conducted to determine the optimum harvest date in order to obtain the desired yield and quality product in the rosemary plant revealed different findings [5, 7].

The aim of the experiment was to determine the effect of harvest at different dates on the yield and quality of the rosemary in Eskişehir ecological conditions.

2. Materials and methods

The field experiment of the study was conducted in the province of Eskişehir. The province located in Aegean, Marmara and Central Anatolia Region with 788 meters altitude has semi-arid and cool continental climate.

The average annual precipitation of the long years is 349,8 mm. In the region, the summer months are hot, dry (but summer nights are cold) while the winter months are snowy, frosty and cold. The differences between day and night temperatures are high. In addition to the low annual precipitation in the region, its distribution throughout the year is not ideal for the cultivation of some plants. In 2018, total annual precipitation of 371.8 mm, average temperature of 13.5 °C and an average humidity of 69.93% were recorded in Eskişehir (Table 1).

Table 1. Meteorological data of the experimental site* (2018)

Months	Total precipitation (mm)	Mean temperature (ºC)	Relative humidity (%)
January*	30,00	1,40	82,60
February	28,80	5,60	78,60
March	49,80	8,90	69,80
April	16,80	13,60	58,70
May	72,00	16,40	72,40
June	60,60	19,30	68,20
July	42,00	21,90	63,60
August	19,30	22,70	60,70
September	3,80	18,30	62,70
October	30,10	13,00	74,30
November	18,60	7,40	77,60
Total/Mean	**371,80**	**13,50**	**69,93**

***Data were taken from Eskisehir Regional Meteorological Service*

Soil at a depth of 60 cm was sampled before the experiment and subjected to a physicochemical analysis. Data for the years and locations are demonstrated in Table 2. The results showed that the soil was Clay loam with pH 7.6, salt (22.25%), clay (35.91%), organic matter (1.73%) and amount of macro nutrients were, total N (0.13 %), total P (36 ppm).

Table 2. Analysis of the soil sample taken from the area where the experiment was conducted

Physical analysis				
Depth (cm)	**Soil texture**	**Sand (%)**	**Salt (%)**	**Clay (%)**
0-60	Clay loam	41,83	22,25	35,91
Chemical analysis				
pH	**Lime (%)**	**Organic matter (%)**	**Total nitrogen (%)**	**P_2O_5 (ppm)**
7,60	8,46	1,73	0,13	36,00

** Analysis was conducted in the laboratory of Forestry, Soil and Ecology Research Institute.*

In this research, the seedlings obtained from Afyonkarahisar Medicinal and Aromatic Plants Center were used as plant material. Seedlings used in the field experiment were propagated by clonal method. The field experiment was established in 2017 with 4 replications according to randomized complete block design. This study was carried out in experimental area of Eskişehir Forest Nursery Directorate. Each plot has six rows with 5.0 m in length. Seedlings were transplanted at a distance of 0.35 m between plants and 0.75 m between rows on 10.05.2017 [18, 21]. The dry materials used in this study were harvested the specified dates in the second year of the plantation. The plots were kept weed free. The plots were irrigated by system of drip irrigation. The frequency of irrigation was determined by taking into account the monthly average air temperatures. The plots were irrigated when necessary. The plants were irrigated 6 times in the second year of the plantation. No fertilizer application has been made to experiment. Plants were harvested at six different dates T_1: 16 August, T_2: 29 Auğust, T_3: 14 September, T_4: 28 September, T_5: 15 October and T_6: 31 October) at intervals of 15 days each. Plants in plots were harvested by cutting 20-25 cm above soil surface. The fresh plants harvested was dried at 35 °C for 48 hours in the drying-oven. The essential oil contents of the samples taken from the dried leaves were determined by the water distillation method in Clevenger apparatus for 3.5 hours. Essential oil samples were stored in refrigerator at 4 °C until the composition analysis.

The essential oil composition of the oil samples was analyzed by gas chromatography (Agilent 5975C) coupled to mass spectrometry (Agilent 5975C) using capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 μm) [31].

The experiment findings were subjected to variance analyses according to a Randomized Complete Block Design. The differences between the mean values were determined by Least Significant Difference Method (LSD) via TARIST statistical package program [22].

3. Results

The harvest of the rosemary plant in the ecological conditions of Eskişehir was made 6 dates in 2-week periods, the first harvest of which was made on 16 August 2018. There was a time difference of about 75 days between the first harvest and the last harvest. The climatic changes due to seasonal differences in harvesting dates affected the growth and development of the rosemary plant and caused differences in the yield and quality of the product. In addition, the variation in vegetation period of the plants due to different harvest dates caused a change in the yield of dry herb and dry leaf [23; 24, 30].

The results of the study showed that the different harvest dates had a significant effect on the dry herb yield, dry leaf yield and essential oil content, but different harvest dates did not have significant effect on plant height, fresh herb yield and essential oil yield (Table 3).The lowest plant height (57.75 cm) was obtained from the first harvest, while the highest plant height (65.24 cm) was found from the last harvest. However, all values of plant height were found in the same group. The mean plant height value obtained from the experiment was recorded as 61.87 cm (Table 3). This situation showed that the varying climatic conditions and vegetation period due to different harvest dates are not significantly effective on plant height.

When the fresh herb yields obtained from the study were examined, it was observed that the lowest fresh herb yield (13,92 t ha^{-1}) was obtained from the first harvest and the highest value (16,44 t ha^{-1}) was obtained from the last harvest. The mean value of fresh herb yield was determined as 15,78 t ha^{-1} for different harvest dates (Table 3). Due to the late harvest dates, increased vegetation periods and the climate changes did not cause a significant increase in fresh herb yield as in plant height.

Different harvest dates had significant effect on dry herb yield and dry herb values formed two different groups. While the value of the first harvest date was in the second group, all other values were in the first group. The highest dry herb value (6.32 t ha) was obtained from the last harvest date in the same group, except for the first harvest date. The lowest dry herb yield value (4.62 t ha^{-1}) was determined at the first harvest date. The average value was 5.8 t ha^{-1} (Table 3). This situation showed us that the vegetative growth of the rosemary plant in the ecological conditions of our region generally ends at the end of August. It is observed that the assimilate substances produced by the photosynthesis of the rosemary plant in September and October were used in dry matter accumulation rather than plant growth.

The part of the rosemary plant, which is used both as a spice and as extracting of the essential oil, is the dry leaves. Therefore, dry leaf yield in the unit area is considered to be an important factor in rosemary cultivation. Experimental results revealed that the different harvest dates significantly affected the dry leaf yield (at $p<0.01$ level).

Different harvest dates occurred statistically two different groups for the dry leaf yields. The values of dry leaf yields obtained from the harvests on October 15 and 31 were in the first group while others were in the second group. While the highest dry leaf yield (4,31 t ha^{-1}) was recorded from the last harvest date it was observed that the lowest yield value (2,99 t ha^{-1}) was obtained from the first harvest date. At the same time, the average dry leaf yield value was 3.55 t ha^{-1} (Table 3). As it was understood from the values given above, the late harvest dates (in September and October) did not significantly increase the values of the plant height and the fresh herb yield but caused a significant increase in the dry herb and dry leaf yield values. This situation can be explained by the fact that the rosemary plant used the assimilate substances produced by photosynthesis in September and October and not in plant growth but in dry matter accumulation under the ecological condition of our region [25, 26, 30]. In Eskişehir ecological conditions, decreases in mean monthly temperatures in September and October and decreases in day length had a negative effect on plant growth (Table 1).

Table 3. Average values of rosemary plants harvested at different dates, different groups formed and analysis of variance

Harvest Dates	**Plant Height (cm)**	**Fresh Herb Yield (t ha^{-1})**	**Dry Herb Yield (t ha^{-1})**
16 August 2018	57,75	13,92	4,62 **B**
29 August 2018	58,75	15,85	5,91 **A**
14 September 2018	62,75	16,25	5,98 **A**
28 September 2018	62,50	16,04	5,69 **A**
15 October2018	64,25	16,21	6,31 **A**
31 October 2018	65,24	16,44	6,32 **A**
Mean	**61,87**	**15,78**	**5,80**
L.S. D. (%):	-	-	0,72
F_{values}:	2,825**ns**	2,653**ns**	13,26****
C.V. (%):	6,84	8,61	11,52
Harvest Dates	**Dry Leaf Yield (t ha^{-1})**	**Essential Oil Ratio (%)**	**Essential Oil Yield (l ha^{-1})**
16 August 2018	2,99 **B**	0,79 **A**	23,51
29 August 2018	3,21 **B**	0,75 **A**	24,16
14 September 2018	3,28 **B**	0,77 **A**	25,19
28 September 2018	3,28 **B**	0,71 **A**	25,06
15 October 2018	4,20 **A**	0,57 **B**	24,07
31 October 2018	4,31 **A**	0,50 **B**	21,50
Mean	**3,55**	**0,69**	**23,92**
L.S.D. (%):	0,47	0,08	-
F_{values}:	24,99****	45,42****	1,70**ns**
C.V. (%):	15,88	17,05	9,21

ns: not significant; **: p<0,01; L.S.D.(%): Least significant difference; C.V.: Coefficient of variance.

The changing harvest dates were significantly influential on the essential oil content of the rosemary plant. The highest essential oil content (0,79%) was obtained from the first harvest in August when the average monthly temperature was highest, and the lowest value (0,50%) was obtained from the harvest at the end of October when the average temperature was the lowest. The contents of essential oil extracted from harvested plants in August and September were higher than the amount of volatile oil obtained from the harvests in October (Table 3). This change in the essential oil content values can be explained by the higher average monthly temperatures in August and September compared to October under Eskişehir ecological conditions, respectively (Table 1). As is known, increasing average monthly temperatures create an effect of temperature stress on aromatic plants and cause an increase in essential oil contents [23, 27, 30].

In the study carried out, different harvest dates did not significantly affect the yield of essential oil. The lowest yield of essential oil (21,5 l ha^{-1}) was obtained from the sixth harvest date (on 31 October), while the highest value (25,19 l ha^{-1}) was obtained from the third harvest date (on 14 September) (Table 3). The essential oil yield values in the unit area appear as a function of the volatile oil content and the dry leaf yield values. In the study, there was an increase in the yield of dry leaves in parallel with the delayed harvest dates, while the decrease in the essential oil content was observed. This can explain the fact that different harvest dates were not significantly effective on the yield of essential oil.

Thirty-five components were identified accounting for 94.1-96.1 % in the oil. Camphor, 1-verbenone, α-pinene, borneol and 1,8-cineole/eucalyptol were the main components of the oil from rosemary. While α-pinene, one of these

main components, was monoterpene hydrocarbon, the other four compound (camphor, 1-verbenone, borneol and 1,8-cineole) were oxygenated monoterpenes. Depending on the different harvest dates, the camphor ratio (11.67-14,78%) changed. The highest camphor rate was taken from the fifth harvest date (on 15 September), while the lowest value was obtained from the first harvest date (on 16 August). While the camphor rate increased from the first harvest date to the fifth harvest date, on the other hand, in the sixth harvest date, the camphor value decreased again. In the other main components of the essential oil, no regular changes were determined depending on the harvest dates (Table 4). The change in essential oil components due to harvest dates can be explained by varying average temperatures and day length [26, 28, 29].

Table 4. Essential oil composition of rosemary (*Rosmarinus officinalis* L.) plant harvested at different dates

		Different Harvest Dates						
RT	**Compounds**	**16 Aug.**	**29 Aug.**	**14 Sept.**	**28 Sept.**	**15 Oct.**	**31 Oct.**	**Mean**
23,794	Camphor	11,67	12,08	12,10	12,94	14,78	13,85	**12,90**
30,435	l-Verbenone	8,47	11,44	11,46	13,61	11,50	11,77	**11,38**
7,521	α-Pinene	10,26	8,74	8,75	10,87	11,25	11,82	**10,28**
30,155	Borneol	9,90	8,61	8,62	9,78	11,12	10,95	**9,83**
12,868	1,8-Cineole	7,01	7,01	7,01	7,06	9,07	8,47	**7,61**
24,642	Linalool	4,06	3,87	3,88	3,94	4,03	4,18	**3,99**
26,955	Caryophyllene	5,54	4,48	4,49	3,47	2,27	2,37	**3,77**
12,570	DL-Limonene	3,58	3,07	3,07	3,38	3,40	3,45	**3,32**
26,266	(-)-bornyl acetate	3,68	4,37	4,38	2,39	1,54	1,46	**2,97**
8,605	Camphene	2,76	2,22	2,22	2,50	2,78	2,91	**2,56**
38,464	15-Crown-5	1,36	3,44	3,45	1,78	0,60	1,74	**2,06**
33,286	trans-Carveol	3,05	2,10	2,10	1,10	2,20	1,05	**1,93**
30,001	α-Terpineol	1,62	1,79	1,79	1,77	2,03	1,94	**1,82**
32,324	Nopol	1,58	1,71	1,71	1,66	1,82	1,74	**1,70**
24,943	Pinocamphone	1,53	1,69	1,69	1,55	1,98	1,72	**1,69**
10,955	δ-3-Carene	1,77	1,57	1,57	1,75	1,69	1,61	**1,66**
33,292	Phenylurea	-	1,03	1,03	1,92	1,08	2,15	**1,44**
29,488	cis-Verbenol	1,37	-	-	-	-	-	**1,37**
29,494	trans-Verbenol	-	1,42	1,42	1,15	1,27	1,15	**1,28**
37,946	18-Crown-6 ether	1,32	1,92	1,93	0,46	0,21	1,10	**1,15**
15,453	α-Terpinolen	1,36	1,30	1,30	1,15	0,91	0,81	**1,14**
15,061	ρ-Cymene	1,01	0,83	0,83	1,06	1,24	1,36	**1,05**
23,417	chrysanthenone	1,14	1,02	1,02	0,95	0,96	0,98	**1,01**
10,253	verbenene	0,96	0,82	0,82	1,26	0,97	1,06	**0,98**
11,403	β-Myrcene	1,03	0,92	0,92	0,97	0,99	1,03	**0,98**
31,579	δ-Cadinene	0,96	0,92	0,92	1,07	0,54	0,74	**0,86**
29,338	α-Homulene	1,08	0,93	0,93	0,74	0,58	0,61	**0,81**
27,056	Terpinen-4-ol	0,67	0,71	0,71	0,74	0,96	0,86	**0,78**
30,767	trans-α-Bisabolene	0,84	0,88	0,88	0,66	0,60	0,60	**0,74**
29,831	α-Amorphene	1,21	0,92	0,92	0,39	0,52	0,26	**0,70**
28,273	Menthol	0,35	-	-	1,01	-	-	**0,68**
36,082	(-)-Caryophyllene oxide	0,92	0,71	0,71	0,49	0,60	0,64	**0,68**
9,733	β-Pinene	0,69	0,63	0,63	0,52	0,74	0,63	**0,64**
14,157	γ-Terpinene	0,69	0,59	0,59	0,69	0,59	0,58	**0,62**
28,804	(S)-cis-Verbenol	0,63	0,59	0,59	0,50	0,64	0,56	**0,58**
	< 0,50	5,95	5,69	5,56	4,73	4,60	3,88	**5,07**
	Total	**100,00**	**100,00**	**100,00**	**100,00**	**100,00**	**100,00**	

4. Conclusions and discussion

In Eskişehir ecological conditions, the fifth harvest date (on 15 October) may be recommended to obtain the highest dry leaf yield in rosemary cultivation, while the third harvest date (on 14 September) can be recommended for the highest yield of essential oil.

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