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Effects of Harvesting Stages, Harvesting Hours and Drying Methods on Essential Oil Content of Lemon Balm Grown in Eastern Mediterranean

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Abstract: This study was conducted to determine the best harvesting stage, harvesting hours and drying method of lemon balm grown at two sites with different altitudes to obtain the highest essential oil yield. The experimental design was an Randomized Complete Block Design in a split-split plot arrangement with four replications comparing three harvesting stages (before flowering, at flowering and after flowering), three harvesting hours (6 am, 12 and 7 pm) and three drying methods (sun drying, shadow drying and fresh leaves). Essential oil content was between 0.064 and 0.073% in the lemon balm grown in Sinanli and it varied from 0.08-0.14% the lemon balm grown in Batiayaz. Essential oil content was affected from harvesting stages, harvesting hours and drying methods in both locations. When the harvesting stages were in consideration, the highest oil content was obtained before flowering in Sinanli, whereas the highest oil content was obtained after flowering in Batiayaz. The most suitable harvesting hours were in the morning (6 am) in Sinanli and in the evening (7 pm) in Batiayaz. The highest oil content was obtained from shadow drying in both locations. β-caryophllene and germacrene-d were the major volatiles, whereas, neral and geranial, the important essential oil components of lemon balm, were found in low quantities, while the other important component citronellal was not detected in the lemon balm grown in both locations.

Key words: Lemon balm, *Melissa officinalis*, harvesting stage, harvesting hour, drying method, oil yield, oil components

INTRODUCTION

Lemon balm or sweet balm *Melissa officinalis* (L.) is native to the countries bordering the Mediterranean sea, however, it grows from the Alps to Pyrenees. In the natural forms, leaves are ovate and serrate growing in opposite pairs. Yellow-white to purple colored flowers grow in clusters at the joints and flowering occurs from July to September. The leaves have fine hairs on both sides and emit a lemony scent when crushed. Lemon balm grows widely in the roadsides, bushy areas and wine yards in all parts of all the Mediterranean region of Turkey. There is no report on the commercial production of the plant in Turkey. Almost all of the domestically consumed and exported products have been gathered from the nature.

Traditionally, the leaves of the plant are used as herbal tea for colds, gastrointestinal disorders, headache, insomnia and calming nerves in Turkey. The leaves and young flowering shoots of lemon balm are antibacterial, antiviral, antispasmodic, carminative, sedative, digestive, emmenagogue and tonic[1-4]. The plant is widely used in alternative medicine due to its rich content of aldehydes and terpenic alcohol. Most of its pharmacological properties have been attributed to the principle constituents. Dry leaves of lemon balm contain 0.02-0.30% essential oil. As a result of low essential oil content, lemon balm oil has a very high price level[5]. The chemical composition of its essential oil has been extensively studied. The main compounds of the essential oil of lemon balm leaves are citral (geranial and neral), citronellal, geraniol, linalool, β-caryophyllene, β-caryophyllene oxide, germacrene D and ocimene[6-15]. Many studies have been conducted to determine essential oil content and composition of lemon the balm. However, information about harvesting stages, harvesting hours and drying methods on the essential oil content of lemon balm is limited. The objectives of this study were to determine the best suitable harvesting stage, harvesting hours and drying method of lemon the balm to obtain the highest essential oil yield.

MATERIALS AND METHODS

Experimental plant materials were collected from two sites having different altitudes in Sinanli (36°06 N, 36°04 E, altitude: 100 m) and in Batiayaz (36°08′ N, 35°59′ E, altitude: 450 m) villages located west of city of Antakya,

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Turkey. Plants were harvested before flowering (30 June in Sinanli and 7 July 1999 in Batiayaz), at flowering (20 July in Sinanli and 30 July 999 in Batiayaz) and after flowering stages (15 August in Sinanli and 25 August 1999 in Batiayaz). In Sinanli, the soil was a clay silt loam with a pH of 7.5, an organic matter content of 0.8% and water holding capacity of 0.34 cm^3. In Batiayaz, the soil was clay silt loam with a pH of 8.3, organic matter content of 1.4 % and water holding capacity of 0.52 cm^3. The maximum and minimum temperatures and relative humidity during the months of the study were given in Table 1. Phenotypic appearance of the plants grown in both locations was similar. In each location and harvesting stage, plants were harvested in different hours of a day, in the morning at 6 am, at noon at 12 pm and in the evening at 7 pm. Harvested plants were separated into three drying groups, freshly distilled, sun dried and shadow dried for each location. Fresh leaves were separated from the stem and only leaves were used for the distillation of the oil. Essential oil was separately distilled from fresh leaves, sun dried leaves and shadow dried leaves with four replications. The volumetric essential oil content was determined using Neo-Clevenger apparatus with 500 mL flask, 20 g drug, 250 mL water, 2 h distillation time by applying hydro-distillation method. The distillation unit consisted of a retort (boiling flask), a condenser and a decanter (receptive flask). Plant leaves were immersed in double their volume of distilled water and then boiled. The condensate was collected in the receptive flask and the oil was removed with a pipette and stored in glass vials. The extracted oils were stored at a temperature of 4°C in well-filled, tightly closed glass vials wrapped in aluminum foil to avoid exposure to light and oxygen. The oil was analyzed by HP (Hewlett Packard) 6890 series GC/MS, equipped with a fused silica capillary column supelco (50 m, 0.25 mm of internal diameter, film thickness of 0.2 μm). The injector temperature was 250°C. The column temperature was initially 120°C (5 min) and then was gradually increased at a rate of 5°C/min up to 200°C. After staying 5 min at 200°C, the temperature was gradually increased at a rate of 10°C/min up to 240°C. The carrier gas was helium at a working rate of 1.5 mL/min with 2 μL injection volume.

Harvesting stages, harvesting hours and drying methods data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure in the Statistical Analysis System software (SAS Institute). The experimental design was a Randomized Complete Block (RCBD) in a split-split plot arrangement with four replications. Harvesting stages (before flowering, at flowering and after flowering) were employed as main plots, harvesting hours (6 am, 12 and 7 pm) as split plots and drying methods (sun drying, shadow drying and fresh leaves) as split-split plots. Mean differences were ascertained using Duncan's Multiple Comparison procedure.

RESULTS AND DISCUSSION

ANOVA summary for the effects of harvesting stages, harvesting hours and drying methods on the essential oil content of *Melissa officinalis* were represented in Table 2.

The essential oil content varied between 0.064% and 0.077 % in Sinanli, however, the variation was not significant (Table 3). The highest essential oil content (0.077%) was obtained at flowering stage and the lowest (0.064%) was obtained at after flowering stage in Sinanli. When Batiayaz was in consideration, the effect of harvesting stages on the essential oil content was significant (Table 3). The highest essential oil content (0.135%) was obtained before flowering and the lowest (0.079%) was obtained at after-flowering stage. Harvesting after flowering stage caused a significant decrease in the essential oil content in both locations. Present results showed that the beginning of flowering is the best suitable harvesting stage for lemon balm. Further delay of harvest decreased the essential oil content of lemon balm. Present results are in good agreement with the findings of Kordana *et al.*[16]. The essential oil contents found in our study were lower than those previously reported for lemon balm[13,17]. However, the essential oil content reported in this study was within the limit of the studies of Gasic *et al.*[18], Ceylan *et al.*[19] and Klimek *et al.*[20].

Effect of harvesting hours on the essential oil content was significant in both locations (Table 3). As reported by previous researchers, essential oil content of lemon balm showed diurnal variation[13]. The highest essential oil content (0.081%) was obtained at 6 am when the plants were covered in dew in Sinanli. Whereas, the highest essential oil content was obtained at 7 pm in Batiayaz. Adzet *et al.*[13] reported similar pattern of diurnal essential oil variability as observed in Sinanli. The diurnal differences in the essential oil content between the two locations most probably resulted from available moisture and disturbed forest canopy in Batiayaz compared with in Sinanli.

Essential oil content was also significantly affected by drying methods at either location (Table 3). When drying methods were in consideration, similar result was obtained from Sinanli and Batiayaz. The highest oil content (0.084% in Sinanli and 0.122% in Batiayaz) was obtained from shadow drying and the lowest (0.099% in Sinanli and 0.060% in Batiayaz) was obtained from fresh

Table 1: Climatic data during the study in Sinanli and Batiayaz

	Atmospheric temperature (°C)						Relative humidity (%)	
	Min		Max		Mean		Mean	
Month	Sinanli	Batiayaz	Sinanli	Batiayaz	Sinanli	Batiayaz	Sinanli	Batiayaz
May	13.6	11.4	38.3	34.5	21.2	19.1	50.0	37.3
June	18.1	15.8	37.9	33.2	26.7	23.7	39.0	32.4
July	16.0	13.2	42.0	35.4	29.2	24.5	51.1	38.7
August	14.4	10.8	40.2	37.7	28.1	26.3	52.0	41.5
September	13.8	9.1	37.8	32.6	25.3	22.6	54.3	44.6

Table 2: ANOVA summary for the effects of harvesting stages, harvesting hours and drying methods on the essential oil content of *Melissa officinalis*, in the two experiment sites

Source	df	Sinanli	Batiayaz
Harvesting stage	2	0.001	0.029**
Replication	3	0.002	0.001
Error a	6		
Harvesting hour	2	0.003**	0.004**
Harvesting stage x harvesting hour	4	0.005**	0.005**
Error b	18		
Drying method	2	0.006**	0.058**
Harvesting stage x drying method	4	0.001	0.007**
Harvesting hour x drying method	4	0.001*	0.003**
Harvesting stage x harvesting hour x drying method	8	0.001*	0.001
Error c	54		

*,** Indicate significance at p = 0.05 and 0.01 levels, respectively

Table 3: Effects of harvesting stage, harvesting hour and drying method on the essential oil content of *Melissa officinalis* in the two experiment sites

	Essential oil content (%)	
Treatments	Sinanli	Batiayaz
Harvesting stage	0.073	0.135a
Before flowering	0.077	0.103b
At flowering	0.064	0.079c
After flowering		
Harvesting hour	0.081a	0.102b
Morning (6 am)	0.069b	0.098c
Noon (12 pm)	0.064c	0.117a
Evening (7 pm)		
Drying method	0.059c	0.060b
Fresh plant	0.072b	0.106a
Sun dried	0.084a	0.122a
Shadow dried		

Numbers within columns followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 level

extraction for both locations. The temperature above 35°C causes essential oil loss; therefore, shadow drying was recommended since the temperature under sunlight exceeds 35°C that accelerate vaporization of volatiles in the summer months.

Harvesting stage and harvesting hour interaction was significant in both locations. (Table 4). In Sinanli, the highest essential oil content (0.097%) was obtained with before flowering stage 6 am harvest time while the lowest (0.058%) was obtained with after flowering stage 7 pm harvest time. In Batiayaz, the highest oil content (0.150%) was obtained with before flowering 12 pm harvest time, as

Table 4: Interaction effect of harvesting stages and harvesting hours on the essential oil content of *Melissa officinalis* in the two experiment sites

	Harvesting hour					
	Sinanli			Batiayaz		
Harvesting stage	6 am	12 pm	7 pm	6 am	12 pm	7 pm
Before flowering	0.097a	0.061c	0.062c	0.127b	0.150a	0.129b
At flowering	0.058c	0.087b	0.084b	0.112d	0.077f	0.119c
After flowering	0.087b	0.060c	0.046d	0.067g	0.067g	0.102e

Numbers followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 level

Table 5: Interaction effect of harvesting stages and drying methods on the essential oil content of *Melissa officinalis* in the two experiment sites

	Drying method					
	Sinanli			Batiayaz		
Harvesting stage	Fresh	Sun	Shadow	Fresh	Sun	Shadow
Before flowering	0.067	0.066	0.087	0.060cd	0.163a	0.183a
At flowering	0.058	0.082	0.090	0.062cd	0.120b	0.126b
After flowering	0.051	0.067	0.074	0.057d	0.083cd	0.097bc

Numbers followed by the same letters are not significantly different according to Duncan's Multiple Range Test at the 0.05 level

the lowest (0.067%) was obtained with after flowering stage 6 am and 12 pm harvest times.

Harvesting stage and drying method interaction was significant only in Batiayaz (Table 5). The highest essential oil (0.183%) content was obtained with before flowering stage and shadow drying while the lowest (0.057%) was obtained with after flowering stage and fresh extraction.

Interaction effect of harvesting hours and drying methods on the essential oil content was significant in both locations (Table 6). The highest essential oil content was obtained with shadow drying with 6 am and 7 pm harvest times in Sinanli (0.091%) and Batiayaz (0.168%), respectively.

It was observed that the essential oil content of the plants grown in Sinanli was generally lower than the plants grown in Batiayaz. This may due to the microclimate, soil, moisture and altitude differences between these two locations. The plants in Sinanli grew under full sunlight; however, the plants in Batiayaz grew

Table 6: Interaction effect of harvesting hours and drying methods on the essential oil content of *Melissa officinalis* in the two experiment sites

Harvesting hour	Drying method: Sinanli Fresh	Sinanli Sun	Sinanli Shadow	Batiayaz Fresh	Batiayaz Sun	Batiayaz Shadow
6 am	0.076c	0.075c	0.091a	0.057c	0.125b	0.122b
12 pm	0.061e	0.073c	0.074c	0.066c	0.113b	0.116b
7 pm	0.039f	0.067d	0.086b	0.056c	0.127b	0.168a

Numbers followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 level

Table 7: Essential oil components of *Melissa officinalis* grown in the two experiment sites

Compound (%)	Sinanli	Batiayaz
β-Caryophllene	26.80	28.38
Germacrene-d	27.42	27.24
Neral (Citral a)	0.24	0.21
Geranial (Citral b)	0.41	0.39
Cyclohexanol	0.95	1.27
β-Sesquiphellandrene	1.87	2.19
Ethanopentaleno	1.36	1.24
Germacrene-d-4-ol	2.04	1.89
Caryophyllene oxide	8.65	9.05
α-muurolene	2.81	2.94
Bicyclogermacrene	6.94	7.69
Total	79.49	82.49

under partially disturbed forest canopy. Therefore, plants in Sinanli flowered and matured earlier than the plants in Batiayaz.

Volatile oil of lemon balm plants grown in two different sites had similar pattern of constituents. This may probably be due to the same genotypic background of the plants grown in two locations. The major volatiles found were β-caryophllene, germacrene-d, β-sesquiphellandrene, ethanopentaleno, germacrene-d-4-ol, caryophyllene oxide, α-muurolene and bicyclogermacrene. These represent over 79.49 and 82.49% of total volatile content for Sinanli and Batiayaz, respectively. The remaining compounds were each in very small amounts (less than 1%) in the plants in both locations. The important essential oil components of lemon balm, neral and geranial, were found in low quantities. The other important component citronellal was not detected in either location. The composition of the essential oil disagreed with the findings of the previous authors[7,9,10,13-15,21-24]. The major essential oil compounds citral (neral and geranial) content was within the range of 10-40% in their studies. In the current study, however, citral represented 0.65 and 0.60% in Sinanli and Batiayaz, respectively. This may be resulted from the genetic make up of the plant grown in both locations.

CONCLUSIONS

In the current study, we found that the essential oil content of lemon balm was significantly affected by harvesting stages, harvesting hours and drying methods in both experiment sites. Harvesting before flower initiation and at flowering stages were found to be the best stages to harvest the plant to obtain the highest essential oil yield. Essential oil content of lemon balm showed diurnal variation in both locations. Morning (6 am) and evening (7 pm) harvests had yield advantage over noon (12 pm) harvest in both locations. Among drying methods, the highest essential oil content was obtained from shadow drying for both locations. Major volatiles in the samples obtained from both locations were β-caryophllene, germacrene-d, caryophyllene oxide, bicyclogermacrene, α-muurolene, β-sesquiphellandrene and germacrene-d-4-ol that represent over 79.49 % and 82.49 % of total volatile content for Sinanli and Batiayaz, respectively.

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