Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(5):919-933

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effect of drying period and harvesting times on herb, essential oil content and its constituent's from different parts of *Melissa officinalis*

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ABSTRACT

This research aimed to investigate the effect of drying period and harvesting dates at different plant parts on vegetative herb, volatile oil content and its composition of Melissa officinalis harvested 8 cuts. Increasing of the drying times led to decreased of herb, leaves and stem weights in all cuttings and 4th cut yielded the highest weights of herb, leaves and stem in all drying periods (0, 5, 10 and 20 days). However, the highest weights were obtained from fresh herb (207.1-190.0g/plant; 16.6-15.3 ton/fed.), fresh leaves (117.1-110.6g/plant; 9.3-8.05 ton/fed.) and fresh stem (90.1-90.4 g/plant; 7.2-7.2 ton/fed.) in 4th cut in the first and second seasons, respectively. Essential oil percentage of Melissa herb and leaves increased by the drying process, where it reached the highest % in plants dried for a period of 5 days followed by drying period of 10 days and then drying for 20 days. Stems contain neglectable amount of essential oil compared to herb and leaves and failed in many cuttings and completely disappeared by drying particularly at 20 days in all cuttings. In general, plants harvested in 1st cut and dried for 5 days gave the highest essential oil % (0.307-0.32 % in herb and 0.39-0.40% in leaves) and oil yield in herb (10.2-10.8 l/fed.) as well as fresh leaves in 1st cut gave the highest oil yield (9.63-10.08 l/fed.) followed by 7th cut compared to the rest of cuttings in the first and second seasons, respectively. Plant parts, drying process and cut dates have an impact on the geranial and neral contents. The highest contents of neral and geranial were obtained from leaves, herb and stem. Increasing drying period decreased geranial and neral contents. Plants harvested in 1st cut gave the highest neral and geraniol contents.

Keywords: *Melissa officinalis*, harvesting date, drying periods, Essential oil, GC-MS.

INTRODUCTION

Awareness on harvesting time, drying type, and period of drying are essential to obtain higher yield, essential oil content and better quality [1, 2]. Natural drying (drying in the shade) is most widely used methods because of its lower cost [3]. Drying is the common and basic method for post-harvest preservation of medicinal and aromatic plants and their quality [4]. It is necessary for decreasing the large volumes of freshly medicinal plants and to become easier for transporting and storage, to extend product shelf life, minimize packaging requirements and reduce shipping weights without a decrease of photochemical [5, 6]. Drying process increases the shelf life by slowing microorganism's growth and preventing certain biochemical reactions that might alter the organoleptic characteristics [6, 7], increase of oil yields, accelerates distillation by improving the heat transfer [8-11]. However, may be lost essential oil due to volatilization and mechanical damage of secretory structures (oil glands) during harvesting and drying [11]. The biosynthesis of secondary metabolites controlled genetically, and also strongly affected by the environmental influences of a particular growing region, agronomic conditions, harvesting time and

type of processing [12, 13]. Harvesting time is very important to obtain higher essential oil content and better quality [14]. In addition, for maximum oil production, long days and high light intensities are required during the maturation period for maximum oil production [15]. However, Murray et al. [16] and Court et al. [17] noticed that harvesting time of the peppermint is a key factor influencing the essential oil composition. Thus optimizing the harvesting time is of fundamental importance for maximizing the quality of essential oil.

Lemon balm (*Melissa officinalis*, Lamiaceae) is a perennial herb native to South Europe and used in traditional medicine because plant secondary metabolites have been shown to benefit a broad spectrum of health conditions. It used for their sedative and antispasmodic effects, antioxidant, antimicrobial and antiviral activities and inhibit division of tumor cells as well as for treating gastrointestinal disorders [18-22], and it is very well known for its ability to reduce stress and anxiety, promote sleep, improve appetite, and ease pain and discomfort associated with digestion. Moreover, several studies suggest that lemon balm is beneficial for a wide variety of human disorders such as cancer, HIV-1, Alzheimer's disease, attention deficit hyperactivity disorder, indigestion, gas, insomnia, and hyperthyroidism [21,23-27].

Although the composition of essential oil from lemon balm (*Melissa officinalis* L.) has been much studied [21,28,29], the interference of certain factors such as drying period and harvesting times (cutting numbers) that influenced the yield and composition of essential oil, remains to be explored so far. With respect to lack of information about drying period and harvesting times effects on essential oil yield, this research was conducted to determine and optimize the harvesting times (cutting numbers) for distillation of essential oil from different parts of *M. officinalis*, and to determine the richest parts of the plant and to identify the best chemical profile of the essential oil related to the optimization of post-harvesting drying period.

EXPERIMENTAL SECTION

Plant material and optimization of growing conditions

Seeds were sown in the nursery on 15th November throughout 2010 and 2011. Four months after seed sowing, the seedlings were subsequently transplanted into the field on15th March of both seasons into plots 3x3.5m on rows, with 60cm a part and 20 cm between the seedlings. This study was carried out during 2011/2012 and 2012/2013 growing seasons at the experimental farm of the Faculty of Pharmacy, Cairo University, Giza governorate, Egypt. Before sowing, physical and chemical properties of the soil of the experiment were determined by standard methods [30]. The soil texture was sandy loam, having a physical composition as follows: 51.1% sand, 25.0% silt, 23.9% clay and 0.5% organic matter. The results of soil chemical analysis were as follows: pH= 8.05; E.C (m.mohs/cm) = 4.90; and available N, P and K =0.07, 0.53 and 2.80 mg kg-1, respectively.

Sample preparation

During each growing season, the plants were harvested 8 times on1st August (four and a half months), 1st November (seven and a half months), 1st February (ten and a half months), 15th March (nearly a year), 1st May (year and two months), 1st July (year and 4our months), 1st September (year and six months) and 1st December (year and nine months) respectively, after transplantation. The plants were harvested at 5-10 cm above the soil. *M. officinalis* plants were harvested when the plants just before flowering stage when the content and quality of essential oil is the highest [31, 32]. The fresh non flowering plant materials from each harvest date were divided into two halves. The first half as a whole aerial parts; the second half was separated to leaves and stems. All of them (whole aerial parts, leaves and stems) were divided into four groups: the first group of samples was given by fresh aerial parts, leaves and stems; the second group of samples was dried at room temperature in a shaded and well ventilated place for 5 days; the third group of samples was dried at room temperature, in a shady and well ventilated place for 10 days; the fourth group of samples was dried at room temperature, in a shady and well ventilated place for 20 days until reaching a constant weight. Fresh and dried material of different samples (leaves, stems, and whole aerial parts) was weighted (g plant^{-1}). The fresh and dry weights/plant or /fed and essential oil content of the fresh and dry samples of each collection was determined. Meteorological data at Giza, during the growing seasons are shown in (Table A).

Isolation of essential oils

Representative plant samples, differing each other for drying period and harvest times, were submitted hydro distillation using a Clevenger-type apparatus according to the method described in the British Pharmacopoeia [33]. Essential oil yield was expressed as ml 100 g^{-1} fresh or dry material), while essential oil yield per plant was

expressed as ml plant^{-1}. The essential oils were collected and dehydrated over anhydrous sodium sulphate and kept in refrigerator until GC-MS analyses.

Table A: Meteorological data during the two growing seasons

Month		2010/2011		Month		2011/2012		Month		2012/2013	
	T(°C) Max.	T(°C) Min.	RH %		T(°C) Max.	T(°C) Min.	RH %		T(°C) Max.	T(°C) Min.	RH %
October	39.1	17.9	56	October	39.2	14.7	55	October	34.1	17.1	60
November	32.8	13.6	56	November	26.9	10.3	65	November	32.0	9.9	66
December	29.4	8.0	56	December	23.6	7.8	69	December	30.6	1.1	61
January	24.1	7.3	65	January	23.1	4.2	59	January	28.4	5.4	61
February	26.4	7.2	56	February	24.8	4.5	57	February	28.8	6.6	56
March	30.3	8.2	55	March	33.0	7.6	54	March	37.6	8.8	48
April	38.5	10.9	49	April	38.4	13.0	44	April	38.3	11.5	47
May	38.8	14.3	47	May	39.4	15.5	45	May	43.8	15.7	44
June	40.5	18.9	52	June	42.0	20.1	49	June	46.2	20.5	48
July	39.3	21.8	56	July	40.5	23.8	55	July	38.9	21.6	57
August	38.7	22.4	57	August	40.1	22.9	54	August	40.1	22.2	55
September	36.4	20.8	58	September	37.6	18.3	57	September	42.5	17.8	54

Source: Meteorological data of Giza (CLAC, Egypt), average values; T (°C) Max. and Min. are monthly average, maximum and minimum temperatures; RH is monthly average relative humidity

Gas chromatography/mass spectrometry (GC-MS) analysis

Chromatography-mass spectrometry (GC-MS) instrument stands with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was helium at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 40 ^{o}C for 1 min; rising at 4.0 ^{o}C/min to 160 ^{o}C and held for 6 min; rising at 6 ^{o}C/min to 210 ^{o}C and held for 1 min. The injector and detector temperatures were held at 210 ^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Statistical analysis

Except for the constituents of the essential oil, the data in this study were analyzed with the analysis of variance (ANOVA) using JMP 10 program (SAS Institute, NC, USA). The mean values of treatments were compared using Tukey's HSD test. Values accompanied by different letters are significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Biomass Productivity

From Tables (1-3), it can be concluded that weights (g/plant or ton/fed) of the all aerial fresh herb, leaves and stem decreased by drying process; increasing of the drying times led to decreased of herb, leaves and stem weights in all cuttings in both seasons. It is also found that the 4th cut yielded the highest weights of herb, leaves and stem in all drying periods (0, 5, 10 and 20 days) in both seasons. For fresh weight of herb, the 4th cut gave the highest values followed by the 5th and 1st cuts, while the 2nd and 8th cuts gave the lowest herb fresh weights. Regarding the effect of drying periods, the maximum herb weight was obtained from fresh plants without drying, while drying the herb for prolonged periods 10 and 20 days significantly reduced the herb weight. When considering the interaction between cut number and dying periods, the highest herb weight (207.1 and 190.0 g/plant) was obtained from fresh Melissa plants cut for the 4th time without any drying treatments in the first and second seasons, respectively.

We found a clear impact of the harvest time on the fresh weight and dry matter of herb, leaves and stems of *Melissa officinalis* (Tables 1-3). The period from February to June is the most appropriate for producing the highest fresh and dry weights. For the leaves weight, plants without drying (fresh plants) had significantly higher leaf weight than the plants that were exposed to dying process for various periods. Plants cut at the 4th cut produced the maximum leaf weight values as compared to other treatments. At the meantime, leaf weights from plants cut at the 2nd and 7th cuts were particularly lower than the other cuts. The interaction between cut number and drying period, where the maximum leaf weight (117.1 and 110.6 g/plant) was obtained from fresh plants at the 4th cut (Table 2) in the first and second seasons, respectively. Similar results were obtained for the stem weight, where the fresh plants without

drying treatment gave the highest stem weight. The 4th cut also was superior to the other cut in terms of the stem weight, while plants harvested at the 1st and 2nd cuts produced the minimum values. Interaction table between drying period and cut number (Table 3) indicates that the maximum stems value (90.1 and 90.4 g/plant) was obtained from fresh plants cut for the 4th time in the first and second seasons, respectively.

Table 1. Effect of drying periods (0, 5, 10, 20 days) and number of cuts on herb weight (g/plant and ton/fed) of *Melissa officinalis* during two successive seasons

Cuts number (harvest date)	1st season							
	herb weight (g)				herb weight (ton/fed)			
	Drying period (days)				Drying period (days)			
	0	5	10	20	0	5	10	20
1st cut (1st August , 2011)	107.3±4.0c*	41.6±2.3fg	32.4±1.8f-j	26.4±1.8f-k	8.5±0.32c	3.3±0.18fg	2.6±0.15f-j	2.1±0.12f-k
2nd cut (1st November, 2011)	71.6±4.6e	22.3±0.53h-k	17.2±0.99jk	15.6±1.6k	5.7±0.37e	1.8±0.04h-k	1.37±0.08jk	1.3±0.12jk
3rd cut (1st February, 2012)	95.0±5.9cd	23.9±2.23h-k	18.9±0.99jk	18.8±0.62jk	7.6±0.47cd	1.91±0.18h-k	1.51±0.08jk	1.49±0.06jk
4th cut (15th March, 2012)	207.1±9.0a	68.5±3.12e	42.5±2.9f	38.0±3.2f-h	16.6±0.72a	5.5±0.25e	3.4±0.23f	3.0±0.26f-h
5th cut (1st May, 2012)	137.5±6.7b	36.8±1.09f-i	23.6±1.5h-k	19.4±1.3jk	11.0±0.53b	2.95±0.09f-i	1.9±0.12h-k	1.55±0.10jk
6th cut (1st July, 2012)	98.4±1.3cd	28.4±0.33f-k	22.6±1.12h-k	20.3±0.4i-k	7.9±0.1cd	2.27±0.03f-k	1.8±0.09h-k	1.6±0.05jk
7th cut (1st September, 2012)	88.6±4.4d	25.2±0.56g-k	17.8±1.12jk	13.6±1.1k	7.1±0.35d	2.02±0.05g-k	1.4±0.09jk	1.07±0.09k
8th cut (1st December, 2012)	92.0±2.15cd	20.3±0.42i-k	14.1±0.29k	12.6±1.07k	7.4±0.17cd	1.6±0.03i-k	1.13±0.02k	1.0±0.09k
2nd season								
1st cut (1st August , 2012)	112.5±1.99c	42.8±1.35g	32.7±1.5g-j	26.4±1.5h-l	8.9±0.16c	3.4±0.11g	2.6±0.12g-j	2.2±0.06h-l
2nd cut (1st November, 2012)	65.5±2.6f	19.4±2.82j-m	15.5±2.4k-m	13.9±2.5lm	5.24±0.20f	1.55±0.23j-m	1.24±0.19k-m	1.1±0.28lm
3rd cut (1st February, 2013)	90.9±1.6d	21.6±1.05i-m	17.9±1.3k-m	17.8±0.48k-m	7.27±0.13d	1.7±0.08i-m	1.44±0.10k-m	1.4±0.06k-m
4th cut (15th March, 2013)	190.9±5.3a	64.4±2.74f	37.4±3.7gh	35.1±2.5g-i	15.3±0.43a	5.2±0.22f	2.9±0.30gh	2.7±0.25g-i
5th cut (1st May, 2013)	142.6±5.8b	35.1±0.98g-i	21.9±2.1i-m	18.7±1.9k-m	11.4±0.47b	2.8±0.08g-i	1.7±0.17i-m	1.6±0.04k-m
6th cut (1st July, 2013)	109.9±3.2c	28.7±0.77h-k	23.2±1.42i-m	19.9±1.25j-m	8.79±0.26c	2.29±0.06h-k	1.8±0.11i-m	1.66±0.09j-m
7th cut (1st September, 2013)	77.2±3.94ef	22.0±3.06i-m	15.1±1.71k-m	11.4±1.3m	6.18±0.31ef	1.76±0.24i-m	1.2±0.14k-m	0.97±0.13m
8th cut (1st December, 2013)	85.1±1.68de	18.9±0.52k-m	12.4±0.73m	11.3±0.9m	6.8±0.13de	1.5±0.04k-m	0.99±0.06m	0.9±0.08m

**Numbers with one or more shared letter within the season are not significantly different at p≤0.05 using Tukey's test*

Table 2. Effect of drying periods (0, 5, 10, 20 days) and number of cuts on the leaves weight (g/plant and ton/fed) of *Melissa officinalis* during two successive seasons

Cuts number (harvest date)	1st season							
	leaves weight (g)				leaves weight (ton/fed)			
	Drying period (days)				Drying period (days)			
	0	5	10	20	0	5	10	20
1st cut (1st August , 2011)	83.9±0.8c	25.9±1.1hi	22.1±0.99h-k	16.4±0.36i-n	6.7±0.07c	2.1±0.09hi	1.77±0.08h-k	1.31±0.03i-n
2nd cut (1st November, 2011)	51.3±2.8f	20.8±0.6h-m	15.8±1.1j-n	10.7±0.8n	4.1±0.22f	1.7±0.05h-m	1.27±0.09j-n	0.85±0.06n
3rd cut (1st February, 2012)	66.3±4.7de	21.6±1.5h-l	11.9±0.98k-n	10.3±0.9n	5.3±0.37de	1.7±0.12h-l	0.96±0.08k-n	0.82±0.07n
4th cut (15th March, 2012)	117.1±4.6a	55.8±2.8f	41.1±1.5g	28.2±1.2h	9.3±0.37a	4.46±0.22f	3.29±0.12g	2.25±0.09h
5th cut (1st May, 2012)	96.1±4.2b	23.9±1.4h-j	17.6±1.1i-n	13.8±0.8j-n	7.69±0.33b	1.91±0.12h-j	1.41±0.09i-n	1.1±0.07j-n
6th cut (1st July, 2012)	71.9±2.5d	20.9±0.9h-m	17.2±0.28i-n	16.0±0.2i-n	5.76±0.20d	1.67±0.07h-m	1.38±0.02i-n	1.28±0.02i-n
7th cut (1st September, 2012)	59.0±1.25ef	19.1±0.17h-n	15.7±0.15j-n	11.2±0.5mn	4.7±0.10ef	1.53±0.01h-n	1.26±0.01j-n	0.89±0.04mn
8th cut (1st December, 2012)	61.4±1.32ef	18.6±0.8h-n	22.4±0.22h-j	11.6±0.45l-n	4.9±0.11ef	1.5±0.06h-n	1.79±0.02h-j	0.93±0.04l-n
2nd season								
1st cut (1st August , 2012)	90.2±2.4b	26.5±0.34hi	22.8±0.8h-k	16.2±0.57j-p	7.22±0.19b	2.1±0.03hi	1.7±0.07h-k	1.29±0.05j-p
2nd cut (1st November, 2012)	55.8±2.3ef	21.2±1.4h-l	14.7±1.6k-p	9.6±0.2p	4.47±0.18ef	1.7±0.11h-l	1.17±0.13k-p	0.76±0.01p
3rd cut (1st February, 2013)	64.6±2.2de	20.2±0.78h-n	12.3±1.2l-p	9.9±0.0.7op	5.2±0.18de	1.6±0.06h-n	0.98±0.09l-p	0.79±0.05op
4th cut (15th March, 2013)	110.6±5.02a	48.5±1.9f	37.3±0.57g	27.7±0.56h	8.05±0.40a	3.88±0.16f	2.98±0.05g	2.21±0.04h
5th cut (1st May, 2013)	99.3±2.8a	24.8±1.1h-j	18.7±0.8i-o	15.0±0.1k-p	7.9±0.22a	1.98±0.09h-j	1.49±0.07i-o	1.2±0.01k-p
6th cut (1st July, 2013)	75.9±3.1c	20.7±0.38h-m	18.3±1.3i-p	16.1±0.4j-p	6.07±0.25c	1.66±0.03h-m	1.46±0.10i-p	1.29±0.03j-p
7th cut (1st September, 2013)	58.4±0.54de	18.5±0.68i-p	15.9±0.29j-p	11.3±0.7n-p	4.7±0.04de	1.48±0.05i-p	1.27±0.02j-p	0.91±0.06n-p
8th cut (1st December, 2013)	65.7±2.5d	25.3±0.39hi	22.3±0.13h-k	11.8±0.38m-p	5.26±0.20d	2.02±0.03hi	1.78±0.01h-k	0.94±0.03m-p

**Numbers with one or more shared letter within the season are not significantly different at p≤0.05 using Tukey's test*

Regarding the yields of herb, leaves and stems (Tables 1, 2, 3), the same behavior was found where the fresh plants without dry treatments produced the largest values, while increasing the drying period decreased the weights to reach their minimum values at the longest drying period tested (20 days). The 4th cut produced significantly higher herb, leaves, and stems yields than the other cuts. The interaction between the drying period and cut number was highly significant (p≤0.0001) in all the parameters, and generally fresh plants at the 4th cut gave the highest values (16.6 and 15.3 ton/fed) for herb, (9.3 and 8.05 ton/fed) for leaves and (7.2 and 7.2 ton/fed) for stems, in the first and second seasons, respectively).

Nurzyńska-Wierdaka et al. [34] noticed that lemon balm, as a perennial plant, provides raw material for 3-4 years and its yield increases from 1.0-1.5 t ha^{-1} (in the first year of cultivation) up to 2.0-4.0 t ha-1 (in the next years) in Poland. The yield of dried lemon balm herb at different locations in New Zealand varies from 870 kg/ha in year of

establishment, up to 13,010 kg/ha (established crop) [35]. Mihajlov et al. [36] concluded that in the first year, yielding 500 kg/ha of above-ground plant dry mass. In the second year, yielding 6,775 kg/ha of above-ground plant dry mass of *Melissa officinalis* in Macedonia. Singh et al. [32] studied the effect of four harvesting times (H1-120 days, H2-140 days, H3-160 days and H4-180 days after planting) on yield and quality of *Melissa officinalis* L. The fresh and dry herbage and oil yield of the aerial parts showed greater response in H3 i.e. harvesting at 160 days after planting, followed by H2 harvesting time.

Table 3. Effect of drying periods (0, 5, 10, 20 days) and number of cuts on the stems weight (g/plant and ton/fed) of *Melissa officinalis* during two successive seasons

Cuts number (harvest date)	1st season							
	stems weight (g/plant)				stems weight (ton/fed)			
	Drying period (days)				Drying period (days)			
	0	5	10	20	0	5	10	20
1st cut (1st August , 2011)	23.3±3.6cd	5.3±1.05fg	3.9±0.52g	1.8±0.33g	1.85±0.28cd	0.43±0.09fg	0.31±0.04g	0.147±0.03g
2nd cut (1st November, 2011)	20.3±4.6c-e	3.8±0.66g	3.1±0.62g	1.1±0.23g	1.59±0.36c-e	0.31±0.05g	0.249±0.05g	0.09±0.02g
3rd cut (1st February, 2012)	28.8±1.5bc	6.25±0.3fg	4.0±0.09g	2.1±0.12g	2.28±0.11bc	0.49±0.02e-g	0.35±0.04g	0.17±0.01g
4th cut (15th March, 2012)	90.1±5.2a	18.4±1.45c-f	12.7±1.0d-g	6.9±0.56e-g	7.2±0.42a	1.47±0.12c-f	1.02±0.08d-g	0.55±0.04e-g
5th cut (1st May, 2012)	41.4±10.3b	8.9±2.1e-g	6.1±1.6fg	3.1±0.74g	3.3±0.81b	0.72±0.17e-g	0.485±0.13fg	0.247±0.06g
6th cut (1st July, 2012)	26.5±1.3cd	6.1±0.5fg	4.0±0.58g	1.8±0.09g	2.09±0.09cd	0.49±0.04fg	0.32±0.05g	0.147±0.01g
7th cut (1st September, 2012)	28.9±3.4bc	6.0±0.57fg	4.3±0.48g	2.1±0.28g	2.36±0.30bc	0.49±0.05fg	0.34±0.04g	0.16±0.02g
8th cut (1st December, 2012)	30.6±1.2bc	6.5±0.02e-g	4.6±0.15fg	2.2±0.14g	2.43±0.08bc	0.52±0.00e-g	0.38±0.0fg	0.179±0.01g
2nd season								
1st cut (1st August , 2012)	22.3±4.4de	5.1±1.5gh	4.0±1.3gh	1.7±0.29h	1.77±0.34de	0.41±0.12hi	0.32±0.10hi	0.13±0.02i
2nd cut (1st November, 2012)	9.6±4.6f-h	2.1±0.94h	1.4±0.64h	0.72±0.33h	0.91±0.23f-i	0.21±0.03i	0.137±0.03i	0.06±0.03i
3rd cut (1st February, 2013)	26.3±2.1cd	5.3±0.4gh	3.77±0.43gh	1.9±0.08h	2.09±0.16cd	0.42±0.03hi	0.30±0.03hi	0.15±0.01i
4th cut (15th March, 2013)	90.4±2.5a	18.4±0.6d-f	13.5±0.39e-g	6.98±0.35gh	7.2±0.20a	1.44±0.07d-g	1.07±0.03e-h	0.55±0.03hi
5th cut (1st May, 2013)	43.3±4.2b	8.5±0.8f-h	6.3±0.7gh	3.18±0.27gh	3.4±0.33b	0.67±0.07g-i	0.50±0.06hi	0.25±0.02hi
6th cut (1st July, 2013)	34.0±5.1bc	6.9±1.1gh	4.9±0.66gh	2.5±0.29gh	2.7±0.43bc	0.55±0.09hi	0.39±0.05hi	0.2±0.02i
7th cut (1st September, 2013)	18.8±4.4d-f	4.2±0.6gh	2.6±0.34gh	1.3±0.30h	1.49±0.35d-g	0.33±0.05hi	0.21±0.03i	0.10±0.02i
8th cut (1st December, 2013)	19.4±1.9d-f	4.2±0.2gh	2.73±0.35gh	1.4±0.18h	1.55±0.15d-f	0.34±0.02hi	0.22±0.03i	0.11±0.01i

**Numbers with one or more shared letter within the season are not significantly different at p≤0.05 using Tukey's test*

Foster [37] mentioned that, plant height below the 60-cm height is well for lemon balm grown and best yield. However, yield of lemon balm established by transplants were substantially greater than yields by direct seeding [38, 39]. Also, Kleitz et al. [39] noticed that, the second season increased lemon balm dry weight yields. Lemon balm yields varied from 0.18-2.35 ton ha^{-1} in the first season and 2.35 to 3.91 ton ha^{-1} in the second season as a result of the year. As well as, Kleitz et al. [39] concluded that lemon balm grew slowly. It generally required the longest period of growth before harvest, whether direct-seeded or transplanted. One harvest of lemon balm was 99-210 days from planting until first harvest and total number of harvests was 1-2 harvest in the first year. In the second year, transplanted Melissa grew faster, allowing more two harvests in the season. Verma et al. [40] found that the aerial parts of plant are harvested after 6 month of transplanting. Best time for harvesting just before the flowers open when the concentration of volatile oil is at its highest. Sari and Ceylan [41] studied the effect of different locations (Menemen and Bozda) on *Melissa officinalis* in Turkey over three years to determine high quality and yield. They found significant variations between locations and years in terms of yield and quality characters. The green herb yield, drug leaves yield and essential oil rate over populations and years were 47.58 cm, 2869 $kg.ha^{-1}$, 496.9 kg ha^{-1} and 0.067% respectively in Menemen while they were 416 kg ha^{-1}, 90.0 kg ha^{-1} and 0.036% respectively in Bozda. The growth of *Melissa officinalis* increased after the first year in both locations; therefore all yields were significantly higher in the second and third years compared to the first year.

Harvesting frequently caused reduction in biomass yield of aromatic plants, thus affecting essential oil yield and can be useful or harmful to oil production, depending on environmental factors. The herbage yield is usually high at first harvest, becomes constant and declines with repeated harvests [42, 43]. Kothari et al. [44] found that biomass yield was greater in the first harvest and gradually declined in the subsequent harvests of *Ocimum tenuiflorum*. Contrary to the decrease in biomass yield, essential oil content in general was lower in the first harvest and increased gradually in subsequent harvests to reach maximum in the fourth harvest.

Rose geranium (*Pelargonium* sp.) has a life span of six to eight years under commercial production and the first harvest is carried out at 6-8 months after planting. Subsequent harvests are then conducted at 3-4 month intervals to avoid losses in oil yield due to leaf senescence [43, 45]. Harvesting of secondary branches of *Ocimum tenuiflorum* led to maximum plant height, plant spread and number of secondary branches during second and subsequent

harvests [35]. Rose-scented geranium plants are normally cut at 15 to 20 cm above ground to allow reestablishment of new leaves for the process of photosythesis [43, 46].

Temperature plays an important role in plant growth and yield and changes in most of their metabolic activities such as photosynthesis, respiration and transpiration [42]. Weiss [43] concluded that geranium maximum leaf growth with high oil content was obtained under warm sunny conditions. These results were in agreement with Kumar et al. [47] who reported that warm environments favored vegetative growth of geranium. Contrary, the low biomass yield during summer in geranium subjected to thermal (atmospheric as well as soil) and moisture stresses was caused by the reduction in levels of photosynthesis and damaging effects of solarization [47, 48].However, the growth in geranium increased under long-day photoperiods and plant dry mass as a consequence of increased chlorophyll content [49]. Similar results reached by Adams and Langton [50]. Contrary, Runkle [51] who suggested that geranium was a day neutral plant. Letchamo and Xu [52] found that variability in shoot yield and essential oil of thyme was associated with photosynthetic activities.

Essential oil content

Essential oil percentage of Melissa herb and leaves increased by the drying process, where it reached the highest % in plants dried for a period of 5 days in both herb and leaves, followed by drying period of 10 days and then drying for 20 days. On the other side, Fresh herb and fresh leaves gave the lowest oil percentage in both seasons and in most cuttings compared to drying periods. The reduced oil% in the fresh material is most likely attributed to the presence of high moisture as compared to the dry material. However, the relationship between oil % and drying period was not linear and the % reached its maximum values in plants dried for only 5 days, then tended to decrease with prolonged drying periods (i.e. 10 and 20 days). Prolonged drying period could adversely affect the shape and structure of oil glands, thus reducing the essential oil %. This particular hypothesis warrants a further investigation using electron microscope in order to have a better idea about the morphological and chemical changes that might occur at various drying period intervals.

Table 4. Effect of drying periods (0, 5, 10, 20 days) and number of cuts on herb essential oil % (v/w) and oil yield (l/fed) of *Melissa officinalis* during two successive seasons

Cuts number (harvest date)	1st season							
	herb essential oil				herb essential oil yield (l/fed)			
	Drying period (days)				Drying period (days)			
	0	5	10	20	0	5	10	20
1st cut (1st August , 2011)	0.1±0.01g-j	0.307±0.01a	0.2±0.01cd	0.153±0.01ef	8.67±0.8a	10.2±0.36a	5.15±0.15b-d	3.25±0.4e-g
2nd cut (1st November, 2011)	0.073±0.0i-k	0.29±0.01a	0.14±0.01e-g	0.113±0.0f-i	4.19±0.27c-f	5.16±0.17b-d	1.92±0.12g-k	1.42±0.19h-l
3rd cut (1st February, 2012)	0.006±0.0l	0.027±0.00l	0.017±0.0l	0.008±0.0l	0.46±0.07j-l	0.51±0.07j-l	0.25±0.05kl	0.12±0.01l
4th cut (15th March, 2012)	0.017±0.0l	0.037±0.0kl	0.023±0.0l	0.013±0.0l	2.75±0.55f-h	1.37±0.2h-l	0.8±0.16i-l	0.21±0.06kl
5th cut (1st May, 2012)	0.023±0.0l	0.16±0.01de	0.107±0.0g-j	0.093±0.01h-j	1.83±0.25g-l	4.68±0.18b-e	2.45±0.26g-i	1.44±0.08h-l
6th cut (1st July, 2012)	0.073±0.01i-k	0.233±0.02bc	0.137±0.02e-g	0.11±0.01g-j	5.76±0.46bc	5.29±0.40bc	2.43±0.25g-i	1.79±0.15g-l
7th cut (1st September, 2012)	0.083±0.01h-j	0.273±0.01ab	0.153±0.00ef	0.12±0.01e-h	5.96±0.86b	5.46±0.14bc	2.18±0.15g-j	1.29±0.06h-l
8th cut (1st December, 2012)	0.07±0.01jk	0.213±0.01c	0.123±0.0e-h	0.107±0.00g-j	5.14±0.38b-d	3.46±0.15d-g	1.39±0.03h-l	1.08±0.12h-l
2nd season								
1st cut (1st August , 2012)	0.11±0.0h-k	0.32±0.0a	0.21±0.01cd	0.16±0.01e-g	9.6±0.15a	10.8±0.4a	5.45±0.36b-d	3.4±0.44e-i
2nd cut (1st November, 2012)	0.077±0.0kl	0.28±0.01ab	0.14±0.01f-i	0.09±0.01i-l	4.0±0.2c-g	4.36±0.7c-f	1.72±0.25h-m	1.23±0.2j-m
3rd cut (1st February, 2013)	0.005±0.0n	0.02±0.01n	0.01±0.0n	0.005±0.0n	0.36±0.01m	0.35±0.1m	0.14±0.01m	0.12±0.05m
4th cut (15th March, 2013)	0.02±0.0n	0.047±0.01l-n	0.02±0.0n	0.008±0.0n	3.06±0.09f-j	2.39±0.3g-l	0.6±0.06lm	0.23±0.04m
5th cut (1st May, 2013)	0.023±0.0mn	0.13±0.01f-j	0.09±0.02i-l	0.08±0.01kl	2.66±0.37f-k	3.66±0.4d-h	1.6±0.32i-m	1.17±0.12j-m
6th cut (1st July, 2013)	0.077±0.01kl	0.247±0.0bc	0.15±0.0f-h	0.113±0.01g-k	6.78±0.93b	5.65±0.2bc	2.75±0.17f-k	1.79±0.13h-m
7th cut (1st September, 2013)	0.09±0.01j-l	0.29±0.01ab	0.16±0.01d-f	0.11±0.01h-k	5.59±0.63b-d	5.11±0.8b-e	1.98±0.27h-m	1.0±0.13k-m
8th cut (1st December, 2013)	0.07±0.00k-n	0.2±0.0c-e	0.11±0.01h-k	0.069±0.03k-m	4.55±0.31c-f	3.03±0.08f-j	1.06±0.13k-m	0.63±0.29lm

**Numbers with one or more shared letter within the season are not significantly different at p≤0.05 using Tukey's test*

Confirmation that the drying in the shade is the best, Agah and Najafian [53] on aerial parts of *Lippa citriodora* concluded that shade drying method is suitable for highest essential oil quantity than (sun-drying and oven-drying). Khorshidi et al. [54] showed that the maximum essential oil percentage (1.8%) was obtained to leaf rosemary followed by mixed leaf and stem and then stem. Also, shade drying was best for essential oil than oven drying (45°C) and sun drying. Argyropoulos and Muller [55] found that drying process decreased the essential oil content in *M. officinalis* herb and the degree of loss was proportional to the drying temperature. For example, 65% of the oil was lost when the herb was dried at 60°C as compared to 16% at 30°C. Agah and Najafian [53] on aerial parts of *Lippa citriodora* concluded that shade drying method is suitable for highest essential oil quantity than (sun-drying and oven-drying). Similarly in some other labiatae members such as *Thymus vulgaris*, drying the herb at 50°C or lyophilization caused a significant loss in the essential oil as compared to natural drying or convective drying at 30

and 40°C [56]. The decrease in oil% at higher temperature was attributed to the loss of oil during water movement from the leaves epidermis as well as the evaporation of essential oil from glands when get ruptured from high temperature. It is also expected that the degree of essential oil loss by high temperature or drying period will differ based on the species, localaization of secretory structures, moisture content and other drying factors.

In the current study, we found that the essential oil content of *M. officinalis* is significantly affected by harvesting times, which is in agreement with some previous reports. For example, it was found that the highest amount essential oil in *Melissa officinalis* plants was in before flowering stage compared to flowering stage and after flowering stage [29]. They concluded that the essential oil yields vary considerably from month-to-month and is also influenced by the micro-environment (sun or shade) in which the plant was growing. *Melissa officinalis* plants cultivated in the sunny plots and collected in April had the highest oil yield, which ranged from 0.3 to 0.5 ml/100 g [57]. Also, Simionatto et al. [58] reported that the oil % varies greatly with a range of 0.12 % to 0.25 % during the first and second cutting. The oil yield was particularly high during the first cut than second cut. The fresh and dry weights as well as oil yield of *Melissa officinalis* plants harvested after 160 days after planting showed superior response than shorter harvesting times (120 and 140 days) [32]. Not only the phonological stage, but also the drying method can have significant influence on the essential oil % in the final product, which was demonstrated in our. In this regard, Verma et al. [40] found that, generally lemon balm dry herb is dried in the shade to preserve the chemical composition of the plant. Too much direct sunlight will cause volatile oils to disappear. Shalaby et al. [59] reported that the oil content of shade-dried leaves of Mellisa officinalis was higher than oven-dried. Also, Fathi and Sefidkon [60] on Eucalyptus sargentii concluded that, the essential oil of shade-dried leaves was higher compared to oven-dried and sun-dried. Agah and Najafian [53] on aerial parts of *Lippa citriodora* concluded that shade drying method is suitable for highest essential oil quantity than (sun-drying and oven-drying). Also, the essential oil content can differ considerably according to the plant part used during drying. For instance, Uyanik and Gurbuz [61] found that, the high essential oil content was determined in leaf (0.13%), while the essential oil amount decreased in herb (0.08%). Abdelmageed et al. [62] studied the effect of post-harvest drying period on the essential oil yield and composition of four different parts from *Etlingera elatior*. They found that the highest yield was obtained from leaves dried for 48 h (0.16% v/w), pseudostems dried for 24 h (0.013% v/w), rhizomes dried for 6 h (0.047% v/w) and inflorescences dried both for 24 and 72 h (0.1% v/w), respectively. While, Jaafar et al. [63] reported that the percentage yield of volatile constituents of the leaves, stems, flowers and rhizomes of *E. elatior* were 0.0735, 0.0029, 0.0334 and 0.0021%, respectively. Faridah et al. [8] studied the essential oils from the leaves and rhizomes of *Alpinia conchigera* dried for different times (0 (fresh), 1, 2, 3 and 7 days of drying, respectively). The highest oil yield was obtained from leaves dried for 7 days (0.300 v/w) and rhizomes dried for 3 days (0.162 v/w) suggesting that post-harvest drying period had a positive effect on the oil yield of both leaf and rhizome.

Table 5. Effect of drying periods (0, 5, 10, 20 days) and number of cuts on leaves essential oil % (v/w) and oil yield (l/fed) of *Melissa officinalis* during two successive seasons

Cuts number (harvest date)	1st season							
	leaves essential oil				leaves essential oil yield (l/fed)			
	Drying period (days)				Drying period (days)			
	0	5	10	20	0	5	10	20
1st cut (1st August , 2011)	0.143±0.01i-k	0.39±0.01a	0.29±0.01cd	0.207±0.0f-h	9.63±0.82a	8.11±0.24ab	5.20±0.26cd	2.69±0.12g-k
2nd cut (1st November, 2011)	0.09±0.01l-n	0.307±0.01bc	0.217±0.0e-g	0.187±0.01g-i	3.68±0.32d-h	5.33±0.46cd	2.75±0.24g-k	1.57±0.07i-m
3rd cut (1st February, 2012)	0.013±0.0n	0.05±0.00n	0.027±0.0n	0.017±0.0n	0.72±0.20lm	0.85±0.07k-m	0.24±0.05m	0.13±0.03m
4th cut (15th March, 2012)	0.02±0.0n	0.057±0.01n	0.03±0.0n	0.02±0.0n	1.89±0.11h-m	2.53±0.35g-l	0.99±0.05j-m	0.45±0.03m
5th cut (1st May, 2012)	0.053±0.0n	0.21±0.01fg	0.177±0.01g-j	0.127±0.0k-m	4.08±0.26d-g	4.19±0.05d-g	2.48±0.08g-l	1.39±0.03i-m
6th cut (1st July, 2012)	0.083±0.0mn	0.287±0.01cd	0.2±0.0f-h	0.16±0.0h-k	4.77±0.15c-f	4.8±0.37c-e	2.77±0.05g-k	2.03±0.03h-m
7th cut (1st September, 2012)	0.133±0.01j-l	0.353±0.01ab	0.247±0.01d-f	0.19±0.02g-i	6.29±0.55bc	5.39±0.28cd	3.09±0.11e-i	1.71±0.14i-m
8th cut (1st December, 2012)	0.083±0.0mn	0.26±0.03c-e	0.16±0.0h-k	0.127±0.0k-m	5.17±1.09cd	5.15±0.74cd	2.85±0.03f-j	1.17±0.07i-m
2nd season								
1st cut (1st August , 2012)	0.14±0.01h-j	0.4±0.0a	0.28±0.01c	0.21±0.01de	10.08±0.15a	8.55±0.12b	4.87±0.09cd	2.75±0.02f-i
2nd cut (1st November, 2012)	0.09±0.0k-m	0.29±0.0bc	0.21±0.01d-f	0.19±0.01e-h	3.88±0.29d-f	5.04±0.35cd	2.48±0.35g-j	1.46±0.11i-m
3rd cut (1st February, 2013)	0.01±0.0n	0.05±0.0mn	0.02±0.01n	0.01±0.0n	0.517±0.02l-n	0.87±0.08k-n	0.19±0.06mn	0.06±0.02n
4th cut (15th March, 2013)	0.02±0.0n	0.06±0.01mn	0.03±0.0n	0.02±0.0n	1.34±0.28j-n	2.31±0.15g-j	0.89±0.01k-n	0.37±0.08l-n
5th cut (1st May, 2013)	0.06±0.01mn	0.21±0.01d-f	0.16±0.02e-j	0.12±0.01j-l	4.78±0.57cd	4.14±0.08de	2.48±0.30g-j	1.40±0.11i-n
6th cut (1st July, 2013)	0.08±0.01l-n	0.28±0.01c	0.2±0.0d-g	0.15±0.0g-j	4.88±0.54cd	4.57±0.16cd	2.93±0.21e-h	2.06±0.12g-k
7th cut (1st September, 2013)	0.13±0.0j-l	0.34±0.02b	0.25±0.01cd	0.18±0.01e-i	5.92±0.20c	5.01±0.30cd	3.13±0.11e-g	1.63±0.14h-l
8th cut (1st December, 2013)	0.08±0.0l-n	0.25±0.03cd	0.16±0.0f-j	0.14±0.01i-k	4.207±0.16de	5.0±0.59cd	2.86±0.02e-h	1.46±0.32i-m

**Numbers with one or more shared letter within the season are not significantly different at p≤0.05 using Tukey's test*

In the current study, the essential oil % in the herb of *M. officinalis* ranged from 0.005 to 0.3%, which agrees with the some previous reports (0.02% to 0.30%) [40]; 0.04 to 0.10% [64]. However higher contents (0.03-0.47%) were

also found from aerial parts [32, 65-69]. Mihajlov et al. [36] mentioned that lemon balm yield of essential oil 0.05% or 0.5 mL oil/kg herb. Nurzyńska-Wierdaka et al. [34] mentioned that oil content in air-dried leaves was 0.3%. Jalal et al. [70] concluded that, the yield of air-dried leaves essential oil was 0.4%. This indicates that various factors including pre-harvest and post harvest factors may contribute to the variability in the content of *M. officinalis* herb products and therefore affect their quality.

In general, first cut gave the highest essential oil % compared to the rest of cuttings followed by seventh cut (tables 7 and 8), which may be a reflection of the optimum growth conditions at the 1st and 7th cuts in August and September during which all plant biochemical reactions are active and presumably the biogenesis of individual volatile molecules reached its peaks as a result of warm temperature. On the other hand, cuts that were done during winter or cold weather (i.e. cut numbers 3 and 4) showed the least content due to the low biochemical activities of the plants during that time. The interaction between cut numbers and drying period was significant where the highest values (0.307 and 0.39%) were obtained from plants at the 1st cut and dried for 5 days in the herb and leaves, respectively.

When comparing the essential oil % in stems (Table 9) with other organs, it can be concluded that stems contain neglectable amount of essential oil compared to herb and leaves and failed in many cuttings and completely disappeared by drying particularly at 20 days in all cuttings.

Table 6. Effect of drying periods (0, 5, 10, 20 days) and number of cuts on stems essential oil % (v/w) and oil yield (l/fed) of *Melissa officinalis* during two successive seasons

Cuts number (harvest date)	1st season							
	stem essential oil				stem essential oil yield (l/fed)			
	Drying period (days)				Drying period (days)			
	0	5	10	20	0	5	10	20
1st cut (1st August , 2011)	0.002±0.001	0.002±0.0	0.001±0.001	nd	0.02±0.01ab	0.01±0.0ab	0.003±0.0b	0.0±0.0b
2nd cut (1st November, 2011)	0.001±0.0	0.001±0.001	0.001±0.001	nd	0.01±0.0ab	0.004±0.0b	0.002±0.0b	0.0±0.0b
3rd cut (1st February, 2012)	nd	nd	nd	nd	0.0±0.0b	0.002±0.0b	0.0±0.0b	0.0±0.0b
4th cut (15th March, 2012)	nd	0.001±0.001	nd	nd	0.0±0.0b	0.013±0.01ab	0.00±0.00b	0.0±0.0b
5th cut (1st May, 2012)	nd	0.001±0.0	nd	nd	0.01±0.0.01ab	0.003±0.0b	0.002±0.0.0b	0.0±0.0b
6th cut (1st July, 2012)	nd	0.001±0.001	nd	nd	0.01±0.0.01ab	0.003±0.0b	0.001±0.0.0b	0.0±0.0b
7th cut (1st September, 2012)	0.001±0.0	0.001±0.001	0.001±0.001	nd	0.03±0.0a	0.01±0.01ab	0.004±0.0b	0.0±0.0b
8th cut (1st December, 2012)	nd	0.001±0.001	nd	nd	0.0±0.0b	0.004±0.0b	0.001±0.0b	0.0±0.0b
2nd season								
1st cut (1st August , 2012)	0.002±0.001	0.002±0.001	0.001±0.001	nd	0.03±0.01a	0.03±0.02ab	0.002±0.0bc	0.0±0.0c
2nd cut (1st November, 2012)	0.001±0.0	0.001±0.0	nd	nd	0.008±0.0a-c	0.001±0.0bc	0.001±0.0c	0.0±0.0c
3rd cut (1st February, 2013)	nd	0.001±0.0	nd	nd	0.0±0.0c	0.003±0.0bc	0.0±0.0c	0.0±0.0c
4th cut (15th March, 2013)	nd	0.001±0.001	nd	nd	0.0±0.00c	0.01±0.01a-c	0.00±0.00c	0.0±0.0c
5th cut (1st May, 2013)	nd	nd	nd	nd	0.00±0.0.0c	0.002±0.0bc	0.00±0.0c	0.0±0.0c
6th cut (1st July, 2013)	nd	0.001±0.0	nd	nd	0.008±0.0.01a-c	0.004±0.0bc	0.001±0.0.0bc	0.0±0.0c
7th cut (1st September, 2013)	0.001±0.001	0.001±0.001	nd	nd	0.008±0.0a-c	0.003±0.01bc	0.001±0.0c	0.0±0.0c
8th cut (1st December, 2013)	nd	0.001±0.001	nd	nd	0.005±0.01bc	0.001±0.0bc	0.00±0.0c	0.0±0.0c

**Numbers with one or more shared letter within the season are not significantly different at p≤0.05 using Tukey's test*

The essential oil production depends on (1) biomass (dry-matter) production, (2) oil content per unit of biomass which they determine the quantity of oil which can be recovered from the plant, whereas (3) oil composition that determine the quality. These aspects can be influenced independently by changes in management which includes harvesting the crop at maximum production or by environmental factors [71].

Peppermint essential oil was remarkably influenced by changes in temperature. Plant dry matter, frequency of oil glands on leaves, morphological development and oil yield responded positively to higher temperatures and the leaf mass ratio showed an increase with increasing day temperature. The combination of high day and low night temperatures produced the greatest leaf mass ratio [72]. As well as, photoperiod has a strong effect on plant growth and yield production [73]. Runkle [41] mentioned that photoperiod may also influence plant height, branching and other plant growth characteristics. It also had a direct impact by adjusting the metabolic pathways relevant, of photosynthetic carbon production and its partitioning to the Rohmer route (non-mevalonate pyruvate-glyceraldehyde-3-phosphate driven isopentenyl pyrophosphate synthesis), which leads to generation of essential oil terpenoids [73]. These metabolic processes have a direct relevance to essential oils obtained from mints. This was evident in the three different Mentha species (*M.arvensis*, *M. citrate*, *M. cardiaca*) were long-day plants, exhibiting substantially higher vegetative proliferation under long day conditions. Shorter-day conditions resulted in slower growth and reduced herbage yield.

The maximum essential oil in geranium was obtained in the leaves, therefore, the greater proportion of leaves in the harvested produce the better oil yield [74]. Murtagh and Smith [75] described oil yield as the mathematical product of leaf yield and oil content.

Determination of the correct harvesting time is extremely important for maximum yield and for highest oil quality. Kumar et al. [47] also found that essential oil yield of geranium was affected by herbage yield and only to a negligible extent by the oil content in the herb. These (herbage yield and oil concentration), according to Murtagh [42], appears to accumulate and fluctuate independently.

The season or month of harvesting was influenced on the oil yield of essential oil plants. Weiss [43] reported that the highest oil content of geranium was observed in July (rainy/monsoon) and lowest in February (spring) in southern India. Doimo et al. [76] reported that not only the seasons and months of harvest affected oil yield, but the geographic area where these plants were grown also influenced yield. Changes of the photoperiod may benefit essential oil yield and composition at the expense of the plant biomass. In three species (*M. arvensis*, *M. citrate*, *M. cardiaca*) experiment, the short-day condition in all three species gave the greatest essential oil content and best composition while the long-day plants produced high oil yield [73]. Contray, the accumulation of *Thymus vulgaris* oil was increased in the leaves under supplemental light as compared to natural light grown plants. It was concluded that the photosynthetic input of CO_2 increased the number or the density of essential oil glands per given leaf area, or it increased both the number and the size at the same time [77]. High or low temperatures may favour the development of oil glands on leaf surfaces. An experiment on Japanese mint revealed that the number of oil glands per unit leaf area on the adaxial leaf surface responded differently to high day temperature treatment than those on the abaxial leaf surface. The oil glands on the adaxial surfaces were greater in number at 35^{o}C day temperature and it was observed that they increased in number with increases in night temperature while those on the abaxial surface remained costant at all temperatures [72].

Table 7. Comparison chemical composition (%) of *Melissa officinalis* essential oils during 1st cut in the second season

Compound	Herb	Leaves	Stem
β-pinene	6.50	4.40	-
limonene	0.29	0.35	0.12
myrcene	0.64	0.68	-
ocimen	0.10	0.10	0.47
limonene oxide	1.83	1.68	0.11
citronellal	0.70	1.28	1.36
menthol	-	-	-
iso-menthol	-	0.87	-
citronellol	3.95	1.70	2.11
nerol	0.40	0.79	0.57
geraniol	0.28	-	0.99
neral	30.25	30.88	18.36
piperitone	-	-	0.44
geranial	38.64	41.23	29.24
eugenol	-	-	-
citronyl acetate	0.53	0.54	-
α-cubebene	0.25	0.32	2.87
geranyl acetate	5.29	3.46	7.06
α-copapene	-	-	0.98
β-cubebene	-	-	0.23
β-caryophyllene	5.38	6.07	5.20
α-humulene	0.42	0.54	0.99
β-selinene	0.16	0.35	0.49
germacrene D	2.09	2.91	1.34
γ-cadinene	0.16	0.43	0.67
farnesene	0.19	0.21	0.07
β-ionene	0.13	-	0.59
verolidol	0.19	-	-
caryophyllene oxide	0.14	0.10	4.84
E-caryophyllene	0.15	0.11	2.25
humulene oxide	0.09	-	0.88
α-cadinol	-	-	0.35

GC/MS analysis

The relative percentage of main constituents of the essential oil extracted from the herb, leaves and stem of *Melissa officinalis* during the first season analyzed with GC-MS are shown in Tables (7-9). The identified compounds were

grouped into three items i.e., major compounds (more than 10%), minor compounds (less than 10% and more than 1%) and trace ones (less than 1%).

Table (7) shows the distribution of essential oil compounds in different parts (herb, leaves and stems). It is clear from Table (7) that each plant part has its distinctive fingerprint from the essential oil compounds. For example, the profile of essential oil from leaves was void of ocimen, β-selinene and farnesene, while in the stem, compounds such as β-pinene, myrcene, menthol, Iso-menthol, eugenol, citronellyl acetate, and verolidol were absent. The essential oil in the fresh herb, leaves and stem was dominated by the geranial and neral, which indicates that the Melissa plant used in this experiment was a geranial and neral chemotype. The highest percentage of the major compounds (geraniol and neral) was found in leaves (41.23%; 30.88%) followed by whole herb (38.64%; 30.25%) and finally the stems (29.24%; 18.36%), respectively. This is in agreement with some previous reports that showed that the major components of *M. officinalis* essential oil are geranial and neral (40, 78-84]. On the other hand, different chemotypes grown at various locations have been found. For example, limonene was the major component in Scotland-grown Melissa, while neral was a minor compound and geranial was absent [85]. Similarly, Basta et al. [86] reported that caryophyllene oxide and β-pinene were the most abundant constituents in the oil of *M. officinalis*, but neral and geranial were not detected in the oil. Citronellal and citral, accompanied by β-caryophyllene, germacrene D, ocimene and citronellol were the main components of essential oil from plants grown in Finland [87]. A study in Turky showed that citronellal, citral, thymol, and β-caryophyllene were recorded as major components in herb [64]. Saeb and Gholamrezaee [29] found that the major components in leaves before flowering stage were decadienal (29.38%), geraniol (25.3%), caryophyllene oxide (8.75%), geranyl acetate (5.41%). This indicates that *Melissa officinalis* has different chemotypes, which may be as a result of the interaction between the environmental conditions in a particular location with the genetic factors to produce this particular chemotype. In this regard, particular environmental conditions might stimulate the expression of certain genes to produce particular functional proteins (i.e. enzymes and transcription factors) that could be involved in the synthesis of certain compounds (chemotype) to help the plant adapt to the surrounding environment. Advances in the metabolomic technique will help identify these compounds that might play a significant role in plant response to the environment. In addition to the geographical location and phonological stage, it is also worth noting that the distribution of compounds in the essential oil is largely affected by the plant part. For example, caryophyllene oxide, citral and β-caryophyllene were the main components in herb oil, while in the leaf, the main components were citral, caryophyllene oxide, and z-citral [88]. In a nother recent study, 27 volatile components in the leaf and 35 components in the stem were identified. β-caryophyllene oxide, geranial, neral, β-caryophyllene and geranyl acetate were the main constituents of the leaf; while n-hexadecanoic acid, (*Z,Z*)-9,12-octadecadienoic acid; dodecanoic acid; β-caryophyllene and geraniol were the main constituents in the stem [89].

Regarding minor compounds, the stems gave the highest % of caryophyllene oxide, E-caryophyllene, citronellal, geranyl acetate geraniol and α-cubebene followed by herb and then leaves, while the highest percentage of citronellol, β-pinene and limonene oxide was obtained from herb followed by stems and then leaves as well as the highest percentage of β-caryophyllene, germacrene D, was obtained from leaves followed by herb and then stems (Table 7). Minor compounds such as geraniol, geranyl acetate, β-carophyllene, and caryophyllene oxide were also reported in the literature [59, 79, 84, 89, 90] reported that nerol, citronellal, citronellol, geranyl acetate, β-caryophyllene, menthol, germacrene D, neryl acetate, linalool, α-pinene, β-pinene, γ-cadinene, caryophyllene oxide and E-caryophyllene compounds were minor compounds (less than 10% and more than 1%). In another study, pentadecanal, geranyl acetate, β-caryophyllene, hexadecanoic acid, caryophyllene oxide and β-caryophyllene were minor compounds constituting less than 5% from the total essential oil [91].

The effects of drying period on the composition of *M. officinalis* essential oil are presented in Tables (8 and 9). It is clear that the main compounds responsible for the odor in Melissa plants differed qualitatively and quantitatively as a result of drying treatments and cut date. In the first cut (1st August), neral and geranial was the dominant compounds under fresh and drying periods except for Melissa herb which dried for 20 days where citronellal was the dominant compound. Drying process led to decrease neral and geranial contents. The highest % of neral and geranial was obtained from fresh herb; but the highest content of citronellal was obtained by herb drying for 20 days. In the fourth cut (15th March), considerable differences in the major compounds (geranial, neral, citronellal, citronellol, geranyl acetate, β-caryophyllene, caryophyllene oxide and E-caryophyllene) were observed due to drying period. The percentage of these compounds varied depending on drying periods. Drying lead to an increase in geranyl acetate, citronellal and citronellol % and decrease in geranial and neral as compared to fresh herb. The highest % of neral and geranial was obtained from fresh herb; citronellal and geranyl acetate from drying for 5 and

10 days, respectively. As well as the highest contents of citronellol, caryophyllene oxide and E-caryophyllene were obtained from drying for 20 days. In the fifth cut (1st May), Neral and geranial was major components in fresh herb. Also, neral, geranial and geranyl acetate was the dominant compound for Melissa herb dried for 10 days. While, neral, geranial and β-caryophyllene was the dominant compound for Melissa herb dried for 5 days. Drying lead to an increase in geranyl acetate and β-caryophyllene and decrease in neral and geranial as compared to fresh herb. The highest % of neral and geranial was obtained from fresh herb; but drying for 5 and 10 days gave the highest contents of β-caryophyllene and geranyl acetate, respectively. In the eighth cut (1st December), neral and geranial compounds were the dominant of fresh herb, and dried herb for 5, 10 and 20 days. Neral, geranial and geranyl acetate was the major of herb drying for 20 days. Neral and geranial contents decreased by drying process and the highest contents were obtained from fresh herb. However, content of geranyl acetate was increased by drying process and dried herb 20 days gave the highest content.

Table 8. Chemical composition (%) of *Melissa officinalis* essential oil during the second season

Compound	Drying Period(days)							
	0	5	10	20	0	5	10	20
	Herb at 1st Cut				Herb at 4th Cut			
β-pinene	6.50	1.26	3.34	1.01	4.66	2.86	1.14	2.22
limonene	0.29	-	-	0.94	-	-	-	-
myrcene	0.64	-	-	0.91	-	-	0.94	-
ocimen	0.10	-	-	1.89	-	-	0.85	-
limonene oxide	1.83	-	0.78	1.38	-	0.32	0.67	-
citronellal	0.70	-	0.69	36.32	-	16.22	5.31	5.10
menthol	-	-	1.43	0.99	-	-	-	-
iso-menthol	-	-	0.39	0.79	-	-	-	-
citronellol	3.95	-	0.96	3.85	2.67	5.00	3.15	10.50
nerol	0.40	-	0.35	4.59	2.21	-	-	-
geraniol	0.28	-	0.42	6.40	1.25	-	-	-
neral	30.25	24.16	25.35	6.44	18.05	17.36	17.05	12.76
piperitone	-	7.48	0.70	1.32	-	-	9.79	1.64
geranial	38.64	37.18	35.15	5.48	19.63	17.88	15.91	13.98
eugenol	-	1.16	1.44	-	4.77	3.89	3.43	2.75
citronyl acetate	0.53	1.34	1.62	-	1.75	-	-	-
α-cubebene	0.25	3.36	1.06	-	2.63	-	-	0.63
geranyl acetate	5.29	4.21	7.40	-	1.87	8.74	12.79	9.96
α-copapene	-	0.88	0.76	-	3.76	-	3.13	-
β-cubebene	-	-	0.93	-	3.27	-	2.30	-
β-caryophyllene	5.38	2.90	0.73	1.50	2.41	2.98	1.23	6.04
α-humulene	0.42	-	0.46	1.10	1.05	2.11	0.93	0.54
β-selinene	0.16	-	0.70	1.01	2.13	1.66	0.65	1.44
germacrene D	2.09	6.91	7.81	3.47	1.58	2.55	1.60	2.60
γ-cadinene	0.16	0.88	0.48	1.17	3.40	3.11	0.47	0.21
farnesene	0.19	0.94	0.53	1.04	1.12	1.13	1.20	1.32
β-ionene	0.13	0.75	-	1.43	1.29	0.44	-	-
verolidol	0.19	-	-	-	0.90	-	-	-
caryophyllene oxide	0.14	1.66	0.50	1.20	0.79	3.10	4.56	12.49
E-caryophyllene	0.15	1.37	1.50	1.27	0.07	6.06	6.33	12.09
humulene oxide	0.09	0.72	1.26	1.39	5.75	2.76	0.66	0.67
α-cadinol	-	-	-	-	0.90	-	-	-

The changes in the essential oil composition as a result of drying time have been studied in other plants. For example, Abdelmageed et al. [92] found that the variation of principal components in the essential oil of *Etlingera elatior* depends on both plant part and drying time. The most prominent compounds identified were 2-cyclohexen-1-one (93.4%) from leaves dried for 6 h, 2-tridecanone (51.6%) from pseudostems dried for 24 h, 1-dodecanol from rhizomes (63.6%) dried for 48 h and from inflorescences (54.5%) dried for 24 h. Faridah et al. [8] found that, the major constituents in the essential oils of fresh, one, two and three- day dried leaves of *Alpinia conchigera* were cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl), while for leaves dried for seven days were 1, 6, 10-dodecatriene, 7 and 11-dimethyl-3-methylene. β-pinene was the major component for fresh and two- day dried rhizomes, while 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) was the major constituent for one, three and seven-day dried rhizomes.

Compared to the harvest times effect on essential oil constituents responsible for the odor in *Melissa officinalis* under study. We find that neral and geranial was the two major and the first harvest (1st August) gave the highest contents of the two compounds followed by fifth harvest (1st May) and eighth harvest (1st December) then fourth harvest (15th March). From this result we can conclude that the temperature and light conditions and other climate

surrounding plant life have a direct and significant impact on both the neral and geranial content. Summer season is a favorite of the plant and increase quality of essential oil followed by spring season then fall season and finally winter season, where temperature and light were low and this was reflected in the lowest in the essential oil quality.

Table 9. Chemical composition (%) of *Melissa officinalis* essential oil during the second season

Compounds	Drying Period(days)							
	0	5	10	20	0	5	10	20
	Herb at 5th Cut				Herb at 8th Cut			
α-thujene	-	-	-	-	-	0.95	0.29	-
α-pinene	-	-	-	-	-	0.70	1.35	-
β-pinene	0.22	0.26	0.40	-	-	0.53	0.49	0.10
limonene	-	0.19	0.66	0.40	-	0.40	0.88	
myrcene	0.11	1.05	-			0.42	0.82	0.06
ocimen	-	-	-	-	-	0.81	0.49	-
linalool	-	-	-	-	-	0.42	0.41	-
limonene oxide	-	0.43	0.37	0.23	-	0.16	0.38	1.82
citronellal	0.23	0.21	3.31	7.44	2.02	3.22	3.29	0.75
borneol	-	-	-	-	-	0.29	0.24	0.33
menthol	-	-	0.57	-	0.98	0.32	0.48	1.16
iso-menthol	-	-	1.26	-	-	0.58	0.41	0.28
citronellol	0.56	0.71	5.83	0.45	8.10	5.33	3.20	6.51
nerol	-	0.21	2.29	-	-	0.58	0.50	0.59
geraniol	-	1.37	2.27	-	-	0.20	0.81	0.64
neral	27.97	21.44	17.63	15.97	20.48	18.87	18.37	10.24
piperitone	5.73	6.29	-	7.84	1.95	1.42	0.93	0.83
geranial	33.86	29.68	17.14	21.84	27.68	20.65	18.00	17.84
eugenol	-	1.67		0.16	1.13	1.50	1.48	0.44
Citronyl acetate	-	-	-	-	1.78	0.66	1.15	1.00
α-cubebene	1.45	-	-	0.64	-	0.60	2.43	0.84
geranyl acetate	3.79	5.33	16.09	3.98	4.90	1.21	8.62	24.17
α-copapene	1.03	-	7.69	1.70	-	1.10	0.88	0.99
β-cubebene	0.33	-	0.89	0.44	-	1.01	1.76	1.87
β-caryophyllene	5.77	13.24	5.78	7.98	9.26	4.99	4.84	7.32
α-humulene	0.46	2.17	0.47	1.76	3.17	3.41	1.97	0.40
β-selinene	-	-	1.03		-	0.21	-	-
germacrene D	4.67	6.57	3.46	4.40	2.30	6.35	2.98	1.39
γ-cadinene	1.54	3.03	1.18	6.64	-	2.51	1.71	0.49
farnesene	-	-	1.33	-	-	-	-	-
β-ionene	-	-	-	-	0.30	0.28	0.26	-
caryophyllene oxide	0.97	2.33	4.01	6.89	1.81	1.92	6.50	8.26
hexadecane	-	-	-	-	2.30	2.48	0.32	
E-caryophyllene	-	0.48	2.02	2.90	4.93	2.76	3.91	5.89
humulene oxide	-			1.30	2.04	1.01	1.60	0.69
α-cadinol	-	-	-	-	0.73	0.93	0.85	0.28

The chemical composition of the essential oil was also observed to be influenced by environmental changes i.e. seasonal changes with different soil water content, temperature and photoperiod. These environmental conditions may increase or decrease different terpenoids in the plant. Studies conducted on two chemotypes of rose-scented geranium showed that weather parameters such as temperature and rainfall influenced the content and chemical constituents of the essential oil. Hot months were observed to favor the accumulation of citronellol while cool months favoured geraniol [45, 93]. Rajeswara Rao et al. [48] studied the response of citronellol concentration in geranium essential oil and observed that it peaked in summer rather than in winter-spring. Other studies on rose-scented geranium indicated that citronellol concentration peaked during the late winter-spring and was minimal in autumn [76].When comparing the citronellol: geraniol ratio, it was found that cold periods (where the minimum dry bulb temperature was reduced to Co_2) resulted in rapid decrease in geraniol as compared to citronellol. The levels of geraniol were reduced in winter but no distinct peak was determined, although it tended to be higher in spring-summer [76]. Increases in day temperatures also increased menthone concentration in Japanese mint but menthanole was not affected much [72].

CONCLUSION

The current study showed that drying period and harvest time and their interaction influence the herb and essential oil accumulation of *Melissa* plants. Different drying period and harvest time not only caused quantitative changes in the essential oil components, but also qualitative changes were found, which put more emphasis on the importance of selecting the proper drying period and harvest time of herbage for essential oil usage. It can be concluded that 4th cut yielded the highest weights of herb, leaves and stem in all drying periods (0, 5, 10 and 20 days). Regarding to

essential oil production, in general, first cut gave the highest essential oil % compared to the rest of cuttings followed by seventh cut. Where the highest values (0.307 and 0.39%) were obtained from plants at the 1st cut and dried for 5 days in the herb and leaves, respectively. The GC/MS analysis revealed the major components of *Melissa officinalis* to be geranial and neral in the fresh herb, leaves and stem. The highest percentage of the major compounds (geraniol and neral) was found in leaves (45.97%; 34.52%) followed by whole herb (37.34%; 29.87%) and finally the stems (29.24%;18.36%). Plants harvested in 1st August (1st cut) gave the highest percentage of neral and geraniol followed by fifth cutting in 1st May, then the eighth cut in 1st December and finally fourth cut in 15th March which gave the lowest content of neral and geraniol. The drying process have an impact on the geraniol and neral, where these compounds increased by increasing the drying period. The highest content of geraniol and neral was obtained from fresh herb followed by herb dried for 5 days, then herb dried for 10 days and finally the lowest content of these compounds was obtained from herb dried for 20 days. These changes could be relevant to the quality of essential oil and its use in certain food and cosmetic applications.

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