Effect of Time of Harvesting on Yield and Quality of *Melissa officinalis* L. in Doon Valley, India

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Singh, *et al.*: Effect of Time of Harvesting on Yield and Quality of *Melissa officinalis*

A field experiment on the effect of time of harvesting on yield and quality of *Melissa officinalis* L. was conducted under the agroclimatic conditions of Doon valley, Uttarakhand in order to assess the performance of four harvesting times (H_1-120 days, H_2-140 days, H_3-160 days and H_4-180 days after planting). The fresh and dry herbage and oil yield of the aerial parts showed greater response in H_3 i.e. harvesting at 160 days after planting, followed by H_2 harvesting time. The quality of essential oil was evaluated using GC and GC-MS analysis. Geranial (24.53 %) and neral (18.80 %) were the major constituents found in the essential oil followed by trans-caryophyllene (7.70 %).

Key words: *Melissa officinalis* L., harvesting times, fresh and dry herbage, essential oil, geranial

Melissa officinalis L. (family Lamiaceae), commonly known as 'Lemon balm', is a perennial herbaceous plant, distributed in the Mediterranean region and Asia[1]. It is used to give fragrance to different food and beverage products. Traditionally, the leaves of the plant are used as herbal tea for colds, gastrointestinal disorders, fever, headache, rheumatism,

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insomnia and calming nerves. The leaves and young flowering shoots are antibacterial, antiviral, antiseptic, antispasmodic, carminative, sedative, digestive, emmenagogue and tonic[2-7]. The essential oil and extract can be used as antioxidant[8-10] and also as antibacterial and antifungal[8,11].

Many studies have been conducted for determining the essential oil content and composition of the plant[4,11-15], but the studies on agronomic practices are limited. Effect of spacing and nutrient management on biomass and oil yield of *M. officinalis* was studied in India[12]. Studies on effect of planting practice (at sun and in shade) and harvesting time on the oil yield and composition is also carried out[15]. In another study this is reported that the more suitable time for harvesting of *M. officinalis* is before flowering stage[2]. Studies revealed that harvesting time, distillation and drying methods influenced the quantity and chemical composition of this plant[13,14]. The main constituents in *M. officinalis* oil were citral (neral, geranial), citronellal, citronellol, linalool, geranyl acetate[11-15], although, there were reports for quantitative variation in the major compounds of oil.

Keeping in view the economic potential of lemon balm crop, the aim of this study was to determine the effect of different harvesting times on herbage, oil yield and quality of essential oil as well as to determine the best harvesting time of *M. officinalis* cultivated under the agroclimatic condition of Doon valley of Uttarakhand (India).

The experiment was conducted at the research field of Centre for Aromatic Plants (CAP), Selaqui, located at Dehradun, Uttarakhand (India) at an altitude of 680 m above sea level during year 2008-09. Live germplasm of *M. officinalis* was collected from Dhanolti (altitude 2400 m) and cultivated for experiment under the climatic conditions of Doon valley. The specimen was duly identified in Centre for Aromatic Plants (CAP) and deposited here (Voucher specimens no. CAP-87). The soil was sandy loam (68.8 % sand, 25.6 % silt, 5.6 % clay), with pH 7.4, EC 0.159 dsm^{-1}. 66 kg/ha total nitrogen, 230.72 kg/ha available phosphorus, 58.91 kg/ha available potassium and 0.47% organic carbon. Four harvesting times i.e. H_1–120 days after planting, H_2–140 d after planting, H_3–160 d after planting and H_4–180 d after planting were considered as treatments. Before planting, farmyard manure (20 ton/ha) was thoroughly mixed and a basal dose of nitrogen (80 kg/ha), phosphorus (60 kg/ha) and potassium (40 kg/ha) was applied. The rooted plants were transplanted in the month of December, 2008 at 60×45 cm population density. The design of experiment was in randomized block design (RBD) with three replications. In each replication, 5 plants were randomly selected for recording of different parameters. Growth and yield parameters namely, plant height (cm), fresh and dry herb yield and essential oil yield (kg/ha) were recorded in all the treatments.

Fresh and shade dried leaves with small twigs (aerial parts) of each sample (300 g) of different harvesting stage were separately hydro distilled for 3 h using a Clevenger-type apparatus. The collected oil sample was dried over anhydrous sodium sulphate and stored in sealed vials at 4º until analyzed.

GC analysis was carried out using Agilent Technology 6890 N gas chromatograph data handling system fitted with FID using N_2 as the carrier gas. The column was HP-5 capillary column (30 m×0.32 mm, 0.25 μm film thickness) and temperature program was used as follows: initial temperature of 60º (hold: 2 min) programmed at a rate of 3º/min to a final temperature of 220º (hold: 5 min). Temperatures of the injector and FID were maintained at 210º and 250º, respectively.

The GC-MS analysis was carried out on a Perkin Elmer Clarus 500 gas chromatograph equipped with a data handling system. The sample was injected directly in split less mode. The column was an Rtx®-5 capillary column (60 m×0.32 mm, 0.25 μm film thickness). Helium (He) was the carrier gas at a flow rate 1.0 ml/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI+ mode. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current

TABLE 1: EFFECT OF DIFFERENT HARVESTING TIMES ON GROWTH AND YIELD OF LEMON BALM *(MELISSA OFFICINALIS)*

Treatments	Plant height (cm)	Herb yield (kg/ha)		Oil yield (kg/ha)	
		Fresh	Dry	Fresh	Dry
H_1	43.64	10451	2299	3.72	2.45
H_2	43.80	19219	4228	6.83	4.51
H_3	58.00	21664	4766	7.70	5.08
H_4	54.67	14904	3279	5.30	3.50
*CD at 5%	7.14	67.37	14.81	2.39	1.58

All values are given in average; **P*=0.05; H_1-120 days; H_2-140 d; H_3-160 d and H_4-180 d after planting

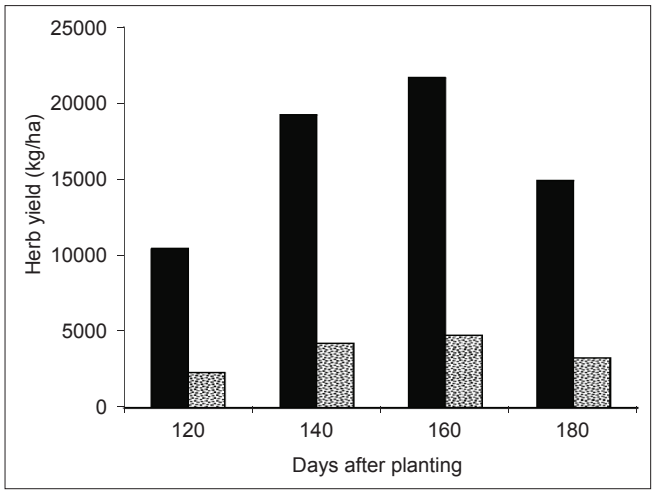

Fig. 1: Herb yield as influenced by harvesting times in *Melissa officinalis*.
The effect of harvesting times on fresh and dry herb yield (■ Fresh ▩ Dry) has shown.

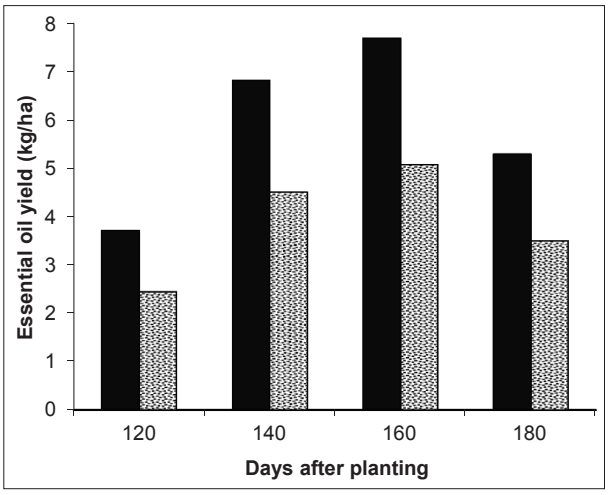

Fig. 2: Essential oil yield as influenced by harvesting times in *Melissa officinalis*.
The effect of harvesting times on fresh and dry essential oil yield (■ Fresh ▩ Dry) has shown.

TABLE 2: IDENTIFIED COMPOUNDS (% IN ESSENTIAL OIL) IN *MELISSA OFFICINALIS*

Compounds	RI	% in oil
Monoterpene hydrocarbon		
cis-ocimene	1043	0.58
Oxygenated monoterpenes		
3-hexen-1-ol	851	0.27
1-octen-3-ol	982	0.52
6-methyl-5-hepten-2-one	985	1.19
Linalool	1100	4.79
p-menth-1-en-8-ol	1145	1.19
Citronellal	1159	4.43
Isomenthol	1199	1.00
Neral	1247	18.80
Trans-geraniol	1249	4.20
Linalyl acetate	1261	3.32
Geranial	1277	24.53
Thymol	1290	0.31
Geranyl acetate	1382	4.62
Sesquiterpene hydrocarbons		
Trans-caryophyllene	1417	7.70
α-humulene	1467	0.56
Germacrene-D	1487	1.02
Oxygenated sesquiterpene		
Caryophyllene oxide	1573	4.74

(TIC) chromatograms. Temperature program was used as follows: initial temperature of 60º (hold: 2 min) programmed at a rate of 3º/min to a final temperature of 220º (hold: 5 min). The temperatures of the injector, transfer line and ion source were maintained at 210º, 210º and 200º, respectively.

Identification of the individual components was made by matching their recorded mass spectra with the library (NIST/Pfleger/Wiley) provided by the instrument software, and by comparing their calculated retention indices with literature value[16].

The recorded data on the effect of different harvesting times on growth, herb and oil yield of *M. officinalis* is given in Table 1. Average oil content (%) in fresh and dry herb was recorded as 0.05% and 0.14%, respectively. The data of plant height was recorded at different harvesting treatments, and it was recorded maximum (58.00 cm) in harvesting time H_3 followed by H_4 (54.67 cm). The effect of harvesting times on herb and essential oil yield (both fresh and dry herb) has shown in figs. 1 and 2. The fresh and dry herb yield and essential oil yield (kg/ha) were consecutively increased in H_1 to H_3 harvesting and decreased in H_4 harvesting. The maximum fresh and dry herb yield (21664 and 4766 kg/ha, respectively) was recorded in H_3 harvesting followed by H_2 (19219 and 4228 kg/ha, respectively). Similarly, maximum fresh and dry oil yield (7.70 and 5.08 kg/ha, respectively) was recorded in H_3 harvesting followed by H_2 (6.83 and 4.51 kg/ha, respectively), H_4 (5.30 and 3.50 kg/ha, respectively) and H_1 (3.72 and 2.45 kg/ha, respectively). In a previous study, it is reported that 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[2].

The percentage composition of the essential oil, obtained by the aerial parts of *M. officinalis* is given in Table 2. The GC and GC-MS analysis of the oil

revealed the occurrence 18 constituents of which geranial and neral were found in the major portions, which constituted 24.53 and 18.80 %, respectively in the oil. The other compounds, which were also detected in appreciable amounts in the essential oil, found to be trans-caryophyllene (7.70 %), linalool (4.79 %), caryophyllene oxide (4.74 %), geranyl acetate (4.62 %) and citronellal (4.43 %). Comparison of the present analytical data with those of a previous study[15] was found to be in good agreement with the quality of Lemon balm oil. No great differences in the contents of neral, geranial, geranyl acetate, caryophyllene oxide and other notable constituents were found.

It is concluded from this study, that harvesting time influenced the herbage and essential oil yield as well as quality of *Melissa officinalis*. The highest fresh and dry herbage and oil yield was obtained at harvesting after 160 days of planting, and lowest in harvesting at 120 d after planting, so H_3 harvesting was recommended to obtain highest yield.

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