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Qualitative And Quantitative Properties Of Essential Oil Of *Mentha Pulegium* L. And *Mentha Suaveolens* Ehrh. Affected By Harvest date

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Abstract

The variation in the content of secondary metabolites of medicinal and aromatic plants can be influenced by harvest date and climatic conditions. The objectives of this study were to evaluate the effect of seven harvest dates every two weeks (4th May, 17th May, 31st May, 14th June, 28th June, 12th July and 26th July) on the productivity of the essential oil content and composition of pennyroyal (*Mentha pulegium* L.) and apple mint (*Mentha suaveolens* Ehrh.) grown under environmental conditions of Egypt. Essential oils were extracted and the chemical composition was determined by gas chromatography - mass spectrophotometer (GC-MS).The fresh and dry biomass yields plant^{-1} of the two mint species increased with the delay in harvesting date up to 12th and 26th July. Essential oil percentage ranged from 0.26 to 0.41% in pennyroyal and from 0.07 to 0.22 % in apple mint. The major constituents of pennyroyal oil were found to be pulegone (46.91 to 94.89%) and ρ-menthan-3-one,cis (0.38 to 48.14%). The highest relative concentration of pulegone (94.89%) was obtained at the 2nd harvest date on17th May. The late harvest date on 26th July produced the highest relative concentration of ρ-menthan-3-one,cis (48.14 %.) and the lowest concentration of pulegone (46.91%). In apple mint, the major constituents were l-linalool (58.18 to73.27%) followed by linalyl acetate (6.10 to 24.56%), then α-terpineol (4.57 to7.81%) and 1,8-cineole (1.72 to 4.68%). The highest relative concentrations of l-linalool (73.27%) and1,8-cineole (4.68%) were produced from the 2nd harvest date on 17th May, while the lowest relative percentage (58.18 and 1.72 %) were recorded at the 5th harvest on 28th June. On the other hand, the highest amount of linalyl acetate (24.56%) was recorded in the 5th harvest date. It might be concluded that, the 2nd harvest date on 17th May was found to be suitable for producing the highest relative concentration of pulegone (94.89%) and l- linalool (73.27%) for pennyroyal and apple mint, respectively. While the delay in harvesting date up to 26th July increased the biosynthesis of ρ-menthan-3-one,cis (48.14 %.) and linalyl acetate (24.56%) in pennyroyal and apple mint, respectively. The harvest date affects the rate of transformation of the major constituents of the essential oil of pennyroyal and apple mint plants.

Keywords: Essential oil, harvest date, *Mentha pulegium*, pulegon, *Mentha suaveolen*

INTRODUCTION

Mentha pulegium L. is one of *Mentha* species commonly known as pennyroyal. It is native to Europe, North Africa, Minor Asia and the near East [1]. Plant growth and the essential oil composition can be influenced by the ecological and climatic conditions. Aziz and Craker (2009) [2] stated that pulegone (88.05%) was the main constituent of pennyroyal oil under desert agro-system in Egypt, which is in accordance with some previously studies which revealed that pulegon was the major components in Massachusetts U.S.A (82.61%) [3], Uruguay (73.4%) [4], Bulgarian (45.4%) [5] and Portugal essential oil (35.1%) [6]. *M. pulegium* L. poses antiseptic, antispasmodic, insect repellent, carminative, diaphoretic and anti-inflammatory activities [7], as well as antimicrobial [8], antioxidant [9], anticholinergic, anti-diabetic [10], hepatoprtective [11] and abortifacient [12] properties. Traditionally, herb was used for the treatment of fibrosis and cervical tumors [13]. The essential oil considered as possible candidate for human cancer chemotherapy [14].

Mentha suaveolens Ehrh. (apple mint or woolly mint) is native to Africa, Temperate Asia and Europe. The major constituents of apple mint differ with the chemotypes, menthyl acetate being the major components [15], dihydrocarvone [16] or

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piperitenone oxide and cis-piperitone oxide [17]. On the other hand, Aziz and Craker,2009 [2] stated that the major constituents of apple mint were linalool (35.32%), ρ-menth-1-en-8-ol, (11.08%), and geranyl acetate (10.86%), respectively. *M. suaveolens* essential oil has antimicrobial [18] and antioxidant activities and can be used as a food additive to extend the shelf life of food products [19].

Harvest date affects the content and composition of secondary metabolites of medicinal and aromatic plants. The variation in the content of secondary metabolites is important for the interaction of plants with pathogens and herbivores. The concentration of terpenoids in aromatic plants varies during the day and season [20, 21]. Delaying the harvest date to the end of July promotes conversion of (–)-menthone to (–)-menthol in peppermint plant [22]. Although monoterpenes are predominate in most essential oils, many also contain sesquiterpenes. This study aimed to evaluate the effect of harvest date on the herb fresh and dry weight as well as the productivity of the essential oil content and composition of pennyroyal (*Mentha pulegium* L.), and apple mint (*Mentha suaveolens* Ehrh.) grown under environmental conditions of Egypt.

MATERIALS AND METHODS

Pennyroyal (*Mentha pulegium* L.) and apple mint *(Mentha suaveolens* Ehrh.) plants were established from cuttings originally secured from the Medicinal Plant Program in the Department of Plant Soil *and* Insect Sciences at the University of Massachusetts, Amherst, USA. The rhizomes (8 cm long) were rooted in sandy loam soil in a greenhouse at National Research Centre, Dokki, Cairo Egypt during February and were transplanted on 15th of March into clay pots (30 cm in diameter) filled with 12kg soil. The physical and chemical properties of the investigated soil were determined according to Cotteni *et al*., (1982) [23].

The soil properties of the experimental pots were sandy loam (17% clay, 44.6% silt, and 38.4 % sand) with 1.18% organic matter, pH of 8.2, EC 1.0 dS/m. The available nutrition elements were 170 ppm N, 33 ppm P and 26 ppm K. At the time of transplanting the following fertilizers were added: Sulphur fertilizer as magnesium sulphate (26.6% S) was added at the rate of 4.5 gm pot^{-1}. Nitrogen fertilizer as calcium nitrate (15.5%N) was added at the rate of 8.43 gm pot^{-1}. Phosphorus fertilizer as calcium super *phosphate* (16 % P_2O_5) was added at the rate of 7.2 gm pot^{-1}.

The layout of the experiment was in complete randomized design of three replicates, each replicate included 10 pots. Seven harvest dates were taken during the growing season of pennyroyal and apple mint *plants* on 4th May, 17th May, 31st May, 14thJune, 28th June, 12thJuly and 26thJuly. The metrological data are shown in Table (1).

Fresh and dry herb weights (g plant^{-1}) were recorded and subjected to statistical analysis according to the methods of Sendecor and Cochran (1980) [24] using L.S.D at the level of 5%.Essential oil percentages of the fresh herb (100g) were determined by hydro-distillation for 3 hours using Clevenger-type apparatus, and dried over anhydrous sodium sulfate according to the Egyptian Pharmacopoeia (1984) [25]. Then, essential oil content (percentage and ml plant^{-1}) was calculated.

The essential oils were subsequently analyzed by GC-MS (Chromatography-mass spectrometry) instrument stands at the Laboratory of Medicinal andAromatic Plants, National Research Centre with the following specifications. Instrument: aTRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), *coupled* with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GCMS system was equipped with a TG-WAX MS column (30 mx 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 60°C for 1 min; rising at 3.0 C/min to 240°C and held for 1 min. The injector and detector were held at 240°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 identified using the analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Table 1. Monthly average of metrological data at the experimental area.

Month	Air temperature ºC			Relative humidity %		
	Maximum	Minimum	Average	Maximum	Minimum	Average
May	45	16	25	94	6	47
June	41	18	27	88	11	51
July	41	21	29	100	9	53

RESULTS AND DISCUSSION

Data illustrated in tables (2 and 3) showed that the fresh and dry weight of herb increased with a delay in harvesting date up to 12th and 26th July) for pennyroyal and apple mint, respectively. Pennyroyal essential oil ranged from 0.26 to 0.41% and apple mint oil ranged from 0.07 to 0.22 % of all harvesting times (Tables, 2&3). The maximum essential oil percentage and content for pennyroyal plant was obtained on 28th June, while the minimum ones were recorded on 4th May date as shown in Table (2). The corresponding dates for apple mint plant were the 12th July and 4th May, respectively. Summer environmental conditions (high temperature and long days) favored essential oil biosynthesis by increasing biomass associated to plant modulation of photosynthetic carbon production into the metabolic machinery of monoterpenes biosynthesis [26].

The identified components of pennyroyal and apple mint essential oil are given in Table (4 and 5). The essential oil constituents varied with harvesting dates. Monoterpenes represented the majority (84.26 - 99.44%) of pennyroyal oil in all harvest dates i.e. α-pinene, sabinene, β- pinene, α-myrcene, dl-limonene etc.. The oxygenated compounds were dominated (82.94 - 98.36%) and consisted mainly of 3-octanol, 1,8- cineole, linalool, 3-octanol,acetate, ρ - menthan-3-one,cis, pulegone etc.. and the ρ-menthan-3-one,cis, (0.38- 48.14%). Pulegone (46.91- 94.89%) were the major component of the oxygenated compounds. In contrast, the sesquiterpenes represented a lower percentage (0.36 - 1.63%) in the oil by α -gurjunene and trans-caryophyllene whereas non-oxygenated compounds represented (1.48-2.95%).

Data in Table (4) showed that the major constituents of pennyroyal oil were found to be pulegone and ρ - menthan-3-one,cis, which ranged from 46.91 to 94.89% and from 0.38 to 48.14 %, respectively. Pulegone was found to **be** the major constituent and the highest relative concentration (94.89 %) was obtained at the 2nd harvest date on 17th May. The later harvest date in July 26th produced the highest relative concentration of ρ-menthan-3-one,cis (48.14 %) and the lowest concentration of pulegone (46.91%). It is clear that increasing plant age increased the biosynthesis of ρ-menthan-3-one,cis and decreased the amount of pulegone. It is well known that ρ-menthan-3-one,cis transforms to pulegone and the environmental conditions affect the rate of transformation. These results were in accordance with some previous studies, Aziz *et al*., 2019 [27] found that pulegone (74.43%) was the main component of *M. pulegium* oil in Egypt. Pulegon (82.61%) was the major components of pennykmroyal cultivated in Massachusetts U.S.A [3], Uruguay (73.4%) [4], Bulgarian (45.4%) [5] and Portugal essential oil (35.1%) [6]. Whereas the major essential oil constituent of pennyroyal varies and can be pulegone, piperitenone-piperitone, or isomenthone-neoisomenthol, depends on the specific type of pennyroyal [28]. The major components of Bulgarian pennyroyal oil were identified as pulegone (42.9- 45.4%), piperitenone (21.7-23.1%), isomenthone (11.3-12.8%) [5]. The variations in pulegone levels could be due to the effect of ecological factors during the growing period as **described** before by Vorin *et al*., (1990) [29] who claimed that temperature and photoperiod influenced pulegone level in mint oil.

The results shown in Table (5) clarified that the essential oil composition *of Mentha suaveolens* was characterized by a high percentage of monoterpene**s** (89.79 - 96.01%) with a high percentages of oxygenated compound (90.75 - 96.00 %) and the major constituents were l-linalool (58.18 to73.27%) followed by linalyl acetate (6.10 to 24.56%), then α-terpineol (4.57 to 7.81%) and 1,8-cineole (1.72 to 4.68%). The non-oxygenated compounds ranged from 2.71 to 4.31% and the oil had lower relative concentrations of sesquiterpenes compound (2.67 - 5.69%).These resulted agreed with that reported by Aziz and Craker, 2009 [2] who stated that the major constituents of *Mentha suaveolens* were linalool (35.32%), ρ-menth-1-en-8-ol, (11.08%), and geranyl acetate (10.86%). The highest relative concentration of l-linalool (73.27%) and1,8- cineole (4.68%) were produced from the 2nd harvest time on 17th May, while the lowest one (58.18 and 1.72 %) were recorded at the 5th harvest on 28th June. On the other hand, the highest relative percentage of linalyl acetate (24.56%) was recorded from the 5th harvest date. The variation in the composition of essential oil may be due to plant species and origin, and environmental conditions, especially day length and photon flux density [30].

Table 2. Fresh and dry weight and essential oil of Mentha pulegium L as influenced by harvest time.

Harvest times	Fresh weight g plant^{-1}	Dry weight g plant^{-1}	Essential oil %	Oil yield ml plant^{-1}
4th May	32.24	7.24	0.32	0.10
17thMay	47.60	10.65	0.35	0.17
31st May	54.23	14.23	0.32	0.17
14th June	54.71	13.61	0.26	0.14
28th June	61.48	14.60	0.41	0.25
12th July	75.41	17.61	0.32	0.24
26th July	70.46	16.44	0.31	0.22
LSD	**5.64**	**0.79**	**0.02**	**0.02**

Table 3. Fresh and dry weight and essential oil of Mentha suaveolens (Ehrh.) as influenced by harvest time

Harvest times	Fresh weight g plant^{-1}	Dry weight g plant^{-1}	Essential oil %	Oil yield ml plant^{-1}
4th May	62.59	15.46	0.07	0.04
17thMay	74.05	20.51	0.09	0.07
31st May	93.13	24.54	0.11	0.10
14th June	101.70	26.64	0.12	0.12
28th June	122.60	32.14	0.14	0.17
12th July	125.39	34.30	0.22	0.28
26th July	145.22	41.44	0.20	0.29
LSD	**9.08**	**0.26**	**0.01**	**0.02**

Table 4. The essential oil constituents of *Mentha pulegium* L as influenced by harvest time

R.T	KI	Essential oil constituents	4th May	17th May	31st May	14th June	28th June	12th July	26th July
4.81	913	α –pinene	0.37	0.47	0.47	0.44	0.38	0.46	0.50
5.85	953	Sabinene	0.08	0.07	0.06	0.07	0.07	0.08	0.07
6.01	959	β- pinene	0.29	0.33	0.33	0.32	0.29	0.36	0.37
6.37	971	β–myrecen	trac.	trac.	0.02	0.01	0.06	0.03	0.03
6.83	986	3-octanol	0.43	0.13	0.11	0.12	0.16	0.07	0.03
7.64	1010	dl- limonene	0.58	0.08	trac.	0.1	0.26	0.28	0.11
7.72	1013	1,8- cineole	0.12	0.14	0.19	0.23	0.16	0.21	0.23
10.34	1083	Linalool	trac.	trac.	0.03	0.02	0.03	0.02	0.2
10.88	1095	3- octanol, acetate	trac.	0.29	trac.	0.05	0.29	0.38	0.4
12.59	1138	ρ - menthan-3-one,cis	1.08	0.38	1.45	3.22	30.52	41.97	48.14
12.96	1147	ρ - menthan-3-one,trans	trac.	0.06	0.07	0.11	1.25	1.79	1.72
13.47	1159	Isopulegone	0.95	0.72	0.93	0.84	0.67	0.66	0.57
14.27	1176	linalyl propionate	0.16	0.1	0.12	0.15	0.18	0.12	0.12
16.07	1215	Pulegone	80.20	94.89	94.76	91.64	64.28	52.97	46.91
16.88	1234	cis- isopulegone	trac.	0.11	trac.	trac.	0.11	0.01	0.02
18.09	1261	Dihydroedylan 1	trac.	0.18	0.01	trac.	0.18	0.02	0.02
22.66	1362	α –gurjunene	1.06	0.96	0.64	1.42	0.48	0.24	trac.
23.27	1375	trans-caryophllene	0.57	0.51	0.34	trac.	0.26	0.12	0.26
24.78	1408	Humulen	trac.	0.18	trac.	trac.	trac.	trac.	0.14
		Monoterpenes	84.26	97.95	98.55	97.32	98.89	99.43	99.44
		Sesquiterpenes	1.63	1.65	0.98	1.42	0.74	0.36	0.40
		Oxygenated compounds	82.94	97.00	97.67	96.38	97.83	98.22	98.36
		Non-oxygenated compounds	2.95	2.60	1.86	2.36	1.80	1.57	1.48
		Total	**85.89**	**99.60**	**99.53**	**98.74**	**99.63**	**99.79**	**99.84**

trac. = less than 0.005

Table 5. The essential oil constituents of Mentha suaveolens (Ehrh.) as influenced by harvest time

R.T	KI	Oil constituents	4th May	17th May	31st May	14th June	28th June	12th July	26th July
4.8	912	α-pinene	0.08	0.13	0.13	trac.	trac.	0.1	0.5
5.85	953	Sabinene	0.07	0.13	0.12	trac.	trac.	0.11	0.12
6.01	959	β- pinene	0.18	0.3	0.29	trac.	0.05	0.25	0.27
6.3	969	β -myrcene	0.22	0.28	0.59	0.07	0.06	0.35	0.26
7.59	1009	dl- limonene	0.1	0.19	0.82	trac.	trac.	0.15	0.1
7.71	1013	1,8- cineole	3.97	4.47	4.68	2.49	1.72	4.38	3.89
8.17	1027	α -ocimene y	0.14	0.13	0.31	trac.	trac.	0.2	0.12
9.56	1064	α-terpinolene	0.12	0.16	0.26	0.06	0.06	0.19	0.13
10.2	1080	l- linalool	67.75	73.27	66.86	63.01	58.18	71.44	66.87
10.42	1085	1-octen-3-yl- acetate	trac.	1.74	2.11	1.28	1.26	1.09	0.92
10.86	1095	3-octanol,acetate	2.87	2.64	3.52	2.2	2.03	2	0.92
13.21	1153	ρ- menth-1-en-8-ol,(s)-(-)-	trac.	0.09	0.08	0.1	0.11	0.11	0.11
13.56	1161	Terpinene- 4- ol	0.06	trac.	trac.	0.05	trac.	0.05	0.05
14.21	1175	α-terpineol	6.31	trac.	7.81	4.57	4.64	5.27	4.99
15.89	1211	Geraniol	0.06	trac.	0.08	trac.	trac.	trac.	trac.
16.26	1220	Linalyl acetate	11.01	6.10	6.30	19.62	24.56	9.9	15.65
17.83	1256	Nerayl acetate	0.30	0.16	0.34	0.41	0.72	0.42	0.53
22.69	1363	α-gurjunene	0.11	0.17	0.08	0.15	0.11	0.08	0.08
23.26	1375	Trans- caryophllene	0.09	0.13	trac.	0.13	0.1	0.08	0.07
25.83	1434	Germacerene-D	1.24	2.06	0.88	2.47	2.65	1.49	1.87
26.4	1447	Bicyclogermacrene	0.06	0.11	trac.	0.11	0.1	0.07	0.07
27.54	1473	Dihidro- β -agarofuran	0.42	0.68	0.35	0.6	0.49	0.37	0.36
29.72	1524	Endo-1-bourbonanol	0.06	0.13	trac.	0.09	0.11	trac.	0.03
30.41	1541	Viridiflorol	1.23	1.77	0.91	1.25	1.41	0.77	0.76
30.61	1546	α-eudesmol	0.07	0.09	0.08	0.09	0.1	0.05	0.05
32.38	1588	Guaiol	0.4	trac.	0.46	0.46	0.52	0.28	0.25
34.22	1635	6-epishyobunol	0.07	0.13	trac.	0.11	0.1	0.05	trac.
Monoterpenes			**93.24**	**89.79**	**94.30**	**93.86**	**93.39**	**96.01**	**95.43**
Sesquiterpenes			**3.75**	**5.27**	**2.76**	**5.46**	**5.69**	**3.24**	**3.54**
Oxygenated compounds			**94.28**	**90.75**	**93.49**	**95.79**	**95.52**	**96.00**	**95.15**
Non-oxygenated compounds			**2.71**	**4.31**	**3.57**	**3.53**	**3.56**	**3.25**	**3.82**
Total			**93.88**	**94.9**	**97.06**	**99.32**	**99.08**	**99.25**	**98.97**

trac. = less than 0.005

CONCLUSION

It might be concluded that the 2nd harvest time on 17th May was found to be suitable for producing the highest relative **concentration** of pulegone (94.89%) and l- linalool (73.27%) for pennyroyal and apple mint, respectively. While the delay in harvesting date up to 26th July increased the biosynthesis of ρ-menthan-3-one, cis (48.14 %.) and linalyl acetate (24.56%). The harvest time affects the rate of transformation of the major constituents of the essential oil of pennyroyal and apple mint plants.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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