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Chemical Profiling of *Mentha spicata* L. var. '*viridis*' and *Mentha citrata* L. Cultivars at Different Stages from the Kumaon Region of Western Himalaya

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ABSTRACT

Two spearmint (*Mentha spicata* L. var. '*viridis*') cultivars viz. 'Neerkalka' and 'Supriya' and one bergamot mint (*Mentha citrata* L.) cultivar 'Kiran' cultivated in the Kumaon region of northern India were investigated for their essential oil content and composition at different stages of crop growth. Essential oil content and composition were both affected by crop age in all cultivars. All the cultivars accumulated maximum essential oil at 150 days after transplanting. The percentage carvone in 'Neerkalka' was higher at 90 days (67.0%) followed by the 150-days-old crop (61.68%), while in 'Supriya', carvone concentration increased at 150 days (72.47%). In 'Kiran', linalool and linalyl acetate were highest in 150- and 180-days-old crops, respectively.

Keywords: composition, essential oil content, Lamiaceae, *Mentha* spp, plant age
Abbreviations: GC, gas chromatography; **GC-MS**, gas chromatography-mass spectrometry

INTRODUCTION

Mints comprise a group of species of the genus *Mentha* (family Lamiaceae). The members of this genus are widely distributed in semi-temperate to tropical agroclimates. The aerial parts of the herb on distillation yields essential oil (EO) containing a large variety of aroma chemicals in varying amounts, such as menthol, menthone, *iso*-menthone, menthofuran, carvone, linalool, linalyl acetate and piperitenone oxide used in pharmaceutical, food, flavour, cosmetics, beverages and allied industries (Spencer *et al*. 1990; Sharma and Tyagi 1991; Kokkini *et al*. 1995; Singh *et al*. 2005; Chowdhury *et al*. 2007; Zheljazkov *et al*. 2010b).

The spearmint and bergamot mint EOs are obtained from *Mentha spicata* L. var. *viridis* and *Mentha citrata* L. plants, respectively and are extensively used in the flavour and cosmetic industries. These mint species are being cultivated in several countries. The annual world production of *M. spicata* and *M. citrata* EOs are 4500 and 4,000 tonnes, respectively, while India produces 2000 t/year of each (Bahl *et al*. 2000; Khanuja 2007). Large efforts to genetically improve mint have resulted in the development of a number of superior varieties such as 'Neerkalka', 'Supriya' and 'Kiran' which are mainly cultivated in the north Indian plains (Virmani *et al*. 1987, 1988; Patra *et al*. 2001; Patra and Kumar 2005; Chauhan *et al*. 2009).

To find a new ecological area for cultivation, prevalent cultivars of spearmint and bergamot mint were introduced into the Kumaon region of Uttarakhand. It is well known that yield and composition of EO is strongly influenced by the development stage of the plant, which further depends on genotype/chemotype and environmental settings (Shah and Gupta 1989; Chalchat *et al.* 1997; Bahl *et al.* 2000; Zheljazkov *et al*. 2010a). Therefore, in this study, the qualitative and quantitative performance of three cultivars belonging to two species viz. *M. spicata*, and *M. citrata* have been examined at different developmental stages from the hilly region of Uttarakhand.

MATERIALS AND METHODS

Plant material and isolation of EO

The origin and special features of the spearmint cultivars ('Neerkalka' and 'Supriya') and bergamot mint cultivar ('Kiran') are summarized in **Table 1**. All the cultivars were planted in January using vegetative propagules at an inter-row spacing of 50 cm. The crops were raised following normal agricultural practices at the experimental farm of the Central Institute of Medicinal and Aromatic Plants, Research Centre, Purara, Bageshwar. The experimental site is located at an elevation of 1250 m with a temperate mild climate. Sampling began at 90 days after planting (DAP) and took place every month at 30-days intervals up to 180 DAP. Freshly harvested samples of all cultivars were hydrodistilled separately in triplicates in a Clevenger-type apparatus for 3 hrs to extract the EO. The EOs were collected, measured, dehydrated by anhydrous sodium sulphate and kept in a cool and dark place prior to analysis.

GC and GC-MS analysis

The GC analysis of the oil samples was carried out on a Nucon 5765 gas chromatograph equipped with an FID using two different stationary phases, BP-20 (30 m × 0.25 mm i.d., 0.25 µm film coating) and DB-5 (30 m × 0.32 mm i.d., 0.25 µm film coating) fused silica capillary columns. Hydrogen was used as the carrier gas at 1.0 ml/min. The temperature programme was: 70-230°C at 4°C/min (for BP-20) and from 60-210°C at 3°C/ min (for DB-5). The injector and detector temperatures were 210 and 230°C, respectively. The injection volume was 0.02 µL neat (syringe: Hamilton 1.0 µL capacity, Alltech USA) and the split ratio was 1:40.

GC-MS analysis of the EOs was carried out on a Perkin-Elmer Turbomass Quadrupole Mass spectrometer fitted with PE-5 fused silica capillary column (50 m × 0.32 mm; 0.25 µm film coating). The column temperature was programmed from 100 to 280°C at 3°C/min using He as the carrier gas at a flow rate of 1 mL/min. The injector temperature was 220°C and MS conditions were: EI mode 70 eV, ion source temperature 250°C. Identification

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Research Note

Table 1 Origin and characteristics of different mint cultivars.

Plant	Cultivar	Origin	Characteristics	Reference
Mentha spicata L. var. '*viridis*'	'Neerkalka'	Interspecific hybridization between *M. arvensis* L. cv. 'Kalka' (2n=96) and *M. spicata* cv. 'Neera'(2n=24)	Essential oil (0.8%), carvone (50-55%) with trace quantity of menthol	Patra *et al*. 2001; Patra and Kumar 2005
Mentha spicata L. var. '*viridis*'	'Supriya'	An northern Himalayan accession	Essential oil (0.35%), carvone (65%)	Virmani *et al*. 1987
Mentha citrata L.	'Kiran'	Induced mutagenesis	Essential oil (0.5%), linalool (48%), linalyl acetate (37%)	Virmani *et al*. 1988

Table 2 Chemical profile of *Mentha spicata* L. var. '*viridis*' cv. 'Neerkalka' at different stages of crop growth.

Compound (%)	RI	Age of plant (DAP)			
		90	120	150	180
α-Pinene	1026	0.50	0.39	0.68	0.77
β-Pinene	1105	0.27	0.26	0.30	0.40
Sabinene	1119	t	t	t	t
β-Myrcene	1158	0.25	0.31	0.61	0.64
Limonene	1194	10.70	14.30	21.01	24.81
1,8 Cineole	1204	0.20	0.24	0.21	0.25
3-Octanol	1394	1.52	1.06	0.88	0.19
Menthone	1460	0.32	0.27	0.20	-
(*E*)-Sabinene hydrate	1463	0.69	0.22	0.20	0.41
iso-Menthone	1488	0.74	0.48	0.47	0.89
β-Bourbonene	1515	0.60	0.54	0.62	0.89
Linalool	1530	t	t	0.23	t
β-Caryophyllene	1589	0.43	t	0.60	0.78
Terpinen-4-ol	1606	-	0.1	t	-
(*Z*)-Dihydrocarvone	1610	0.97	1.87	1.00	1.58
(*E*)-Dihydrocarvone	1624	0.10	0.10	t	t
(*E*)-Dihydrocarvyl acetate	1633	0.15	0.25	0.43	0.39
Menthol	1646	0.65	0.74	2.63	0.80
Germacrene-D	1721	t	t	t	t
Carvone	1751	67.00	59.62	61.68	57.49
(*Z*)-Carvyl acetate	1777	0.55	0.90	0.19	0.12
(*E*)-Carveol	1825	1.27	1.75	1.15	0.85
(*Z*)-Carveol	1866	1.77	2.26	3.12	3.32
(*Z*)-Jasmone	1968	1.03	t	t	t
Piperitenone oxide	2004	2.50	1.21	t	t
Essential oil content (%)*		0.38	0.60	0.75	0.70

RI: retention indices on BP-20 column; t: trace (<0.10%)
DAP: Days after planting; *v/w

Table 3 Chemical profile of *Mentha spicata* L. var. '*viridis*' cultivar 'Supriya' at different stages of crop growth.

Compound	RI	Age of plant (DAP)			
		90	120	150	180
α-Pinene	1026	1.05	0.77	0.92	1.21
β-Pinene	1105	0.84	0.86	0.85	1.02
Sabinene	1119	0.24	0.26	0.28	0.35
β-Myrcene	1158	4.36	2.61	2.21	1.99
α-Terpinene	1177	0.19	0.15	0.19	0.42
Limonene	1194	11.29	12.61	11.72	12.74
1,8 Cineole	1204	1.49	1.58	1.49	2.02
β-Phellandrene	1206	0.29	0.11	0.10	t
(*Z*)-β-Ocimene	1210	0.82	0.44	0.37	0.83
(*E*)-β-Ocimene	1251	0.44	0.22	0.12	t
p-Cymene	1271	0.23	t	0.60	t
Terpinolene	1279	-	t	-	-
3-Octyl acetate	1345	0.84	0.60	-	-
3-Octanol	1394	1.14	1.41	1.67	2.21
(*E*)-Sabinene hydrate	1463	0.36	1.69	2.65	1.78
β-Bourbonene	1515	1.09	0.46	0.47	0.21
Linalool	1530	0.12	0.26	t	t
β-Caryophyllene	1589	1.35	0.93	0.1	t
Terpinen-4-ol	1606	3.21	0.59	0.16	0.31
(*Z*)-Dihydrocarvone	1610	t	2.17	0.16	1.75
(*E*)-Dihydrocarvone	1624	0.12	t	t	t
(*E*)-Dihydrocarvyl acetate	1633	0.21	t	t	0.37
(*E*)-β-Farnesene	1662	t	t	t	t
α-Terpineol	1701	1.07	1.78	0.64	1.41
Germacrene-D	1721	0.39	0.30	t	t
Carvone	1751	62.92	64.77	72.47	62.55
(*Z*)-Carvyl acetate	1777	0.24	0.44	t	0.76
(*E*)-Carveol	1825	1.59	2.00	1.00	0.91
(*Z*)-Carveol	1866	0.31	0.38	0.57	1.08
(*Z*)-Jasmone	1968	2.16	0.61	0.86	t
Piperitenone epoxide	2004	t	0.47	t	-
Viridiflorol	2102	0.72	0.80	0.38	0.42
Essential oil content (%)*		0.46	0.50	0.72	0.70

RI: retention indices on BP-20 column; t: trace (<0.10%)
DAP: Days after planting; *v/w

was done on the basis of retention index (determined with reference to homologous series of *n*-alkanes (C_9-C_{24}) under identical experimental conditions), co-injection with known compounds, an MS Library search (NIST and WILEY), by comparing with the MS literature data (Davies 1990; Adams 1995). The retention times of standards/marker constituents of known EOs were also used to confirm the identities of constituents. The relative amounts of individual components were calculated based on GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

The EO content and composition of spearmint, and bergamot mint cultivars were found to vary with respect to crop age (**Tables 2-4**). The EO content varies from 0.38 to 0.75%, 0.46 to 0.72%, and 0.34 to 0.57% in 'Neerkalka', 'Supriya' and 'Kiran', respectively during different stages. Interestingly, in all the cultivars maximum EO content was obtained when the crop was harvested at 150 DAP; thereafter, it showed a downward trend. A similar trend of EO accumulation was also reported in these cultivars from Indo-gangetic plains (Bahl *et al*. 2000).

The EOs obtained from different cultivars at different crop ages were analyzed by GC and GC-MS. A total of 25, 32 and 25 compounds were identified in the EOs of 'Neerkalka', 'Supriya' and 'Kiran', respectively (**Tables 2-4**). 'Neerkalka' and 'Supriya' were mainly composed of carvone and limonene. In 'Neerkalka', the carvone content ranged between 57.49-67.0% with the highest amount at 90 DAP followed by 150 DAP (61.68%), the lowest value at 180 DAP. Furthermore, the percentage of β-myrcene (0.25-0.64%), limonene (10.7-24.81%) and (*Z*)-carveol (1.77-3.22%) increased while that of 3-octanol (1.52-0.19%) and menthone (0.32-0%) decreases with advancing crop age. The menthol concentration in 'Neerkalka' ranged from 0.65 to 2.63%, which was not reported in the other cultivars of *M. spicata*. The presence of a relatively lower concentration of carvone (compared to other spearmint cultivars), menthone and menthol in trace quantities is due to the hybrid nature (hybrid between *M. arvensis* L. cv. 'Kalka' and *M. spicata* cv. 'Neera') of 'Neerkalka' (Patra *et al*. 2001). On the other hand, in 'Supriya', the percentage of carvone (62.55-72.47%) and (*E*)-sabinene hydrate (0.36-2.65%) increased as crop age progressed, becoming highest at 150 DAP; 3-octanol and (*Z*)-carveol also showed a similar trend but their highest amount was recorded at 180 DAP (2.21 and 1.08%, respectively). A decreasing trend was recorded for β-myrcene (4.36-1.99%), β-bourbonene (1.09-0.21%) and β-caryophyllene (1.35%-trace). Moreover, germacrene-D, 3-octyl acetate, terpinen-4-ol and (*Z*)-jasmone reached the highest levels at 90 DAP, while limonene 1,8 cineole and (*Z*)-carvyl acetate were highest at 180 DAP. The major

Table 4 Chemical profile of *Mentha citrata* L. cultivar 'Kiran' at different stages of crop growth.

Compound	RI	Age of plant (DAP)			
		90	120	150	180
β-Myrcene	1158	1.68	1.08	1.93	2.57
Limonene	1194	0.34	0.26	0.37	0.74
1,8 Cineole	1204	0.35	0.39	t	t
(*Z*)-β-Ocimene	1210	1.09	0.57	1.18	0.98
γ-Terpinene	1239	0.21	t	0.36	0.42
(*E*)-β-Ocimene	1251	0.10	t	t	t
p-Cymene	1271	t	t	0.36	0.42
Terpinolene	1279	-	t	0.24	0.23
Menthone	1460	t	t	t	t
(*E*)-Linalool oxide	1450	t	-	-	-
Linalool	1530	42.04	40.32	46.31	32.86
Linalyl acetate	1546	19.27	29.22	24.51	37.72
β-Caryophyllene	1589	1.53`	1.06	1.14	1.66
Menthol	1646	t	t	0.33	0.45
(*E*)-β-Farnesene	1662	t	t	t	t
α-Humulene	1675	t	t	0.33	0.45
α-Terpineol	1701	4.08	4.61	3.46	2.90
γ-Cadinene	1739	1.00	1.15	1.17	0.89
δ-Cadinene	1742	t	t	-	-
Geranyl acetate	1762	2.27	2.83	2.14	2.26
Citronellol	1782	t	0.13	0.12	-
Nerol	1804	1.09	1.01	1.33	0.76
Geraniol	1858	2.66	3.21	2.36	2.43
Caryophyllene oxide	1980	t	0.12	0.99	0.40
Viridiflorol	2102	1.94	3.46	0.73	0.63
Essential oil content (%)*		0.34	0.50	0.57	0.52

RI: retention indices on BP-20 column; t: trace (<0.10%)
DAP: Days after planting; *v/w

constituents of 'Kiran' EO were linalool (32.86-46.31%), linalyl acetate (19.27-37.72%), α-terpineol (2.90-4.61%), geranyl acetate (2.14-2.83%), geraniol (2.36-3.21%) and β-myrcene (1.08-2.57%). Although there was no regular trend for any component with respect to crop age in 'Kiran', nevertheless linalyl acetate, β-myrcene, β-caryophyllene, limonene, γ-terpinene and α-humulene were highest at 180 DAP, whereas linalool, (*Z*)-β-ocimene and terpinolene were highest at 150 DAP. Furthermore, the amount of α-terpineol, viridiflorol geraniol, geranyl acetate, 1,8 cineole and citronellol were highest at 120 DAP. The EOs obtained from different crop ages thus showed considerable variation in content and composition of all three cultivars. This could be due to expression of different genes at different developmental stages of the plant and further by the environmental factors arising from seasonal variations (Verma *et al*. 2010a, 2010b, 2010c, 2010d).

Carvone-rich spearmint has been investigated in India as well as in other countries. In India, under the climatic conditions of indo-gangetic plains the carvone percentage was varied from 45.9-71.6% in cultivar 'Neerkalka' and 53.3-77.1% in cultivar 'Supriya'. Interestingly, both cultivars were noted to have higher percentage of carvone at early stages of crop growth. Similar, pattern was also noted with cultivar 'Neerkalka' in present study, however, the trend of carvone accumulation in cultivar 'Supriya' was entirely different in present study than that observed in indo gangetic plains (Bahl *et al*. 2000). Further, the carvone percentage was varied from 46.4-53.3% in spearmint grown at different locations in Egypt (El-Wahab and Mohamed 2009). However, the spearmint grown in Iran was found to contain relatively lesser amount of carvone (22.4%) (Hadjiakhoondi *et al*. 2000). The percentages linalool and linalyl acetate in bergamot mint was increased towards crop maturity (Bahl *et al*. 2000). However, no any regular trend was noticed with these constituents in present study. Finally, it was concluded that the yield and chemical composition of spearmint and bergamot mint essential oils was strongly dependent on developmental stage of the plant, and therefore harvesting time is one of the most important factors influencing the oil quality.

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REFERENCES

Adams RP (1995) *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry*, Allured Publishing Corp., Carol Stream, Illinois, USA

Bahl JR, Bansal RP, Garg SN, Naqvi AA, Luthra R, Kukreja AK, Kumar S (2000) Qualitative evaluation of the essential oils of the prevalent cultivars of commercial mint species *Mentha arvensis, spicata, piperita, cardiaca, citrata* and *viridis* cultivated in indo-gangetic plains. *Journal of Medicinal and Aromatic Plants Sciences* **22**, 787-797

Chalchat JC, Garry RP, Michet A (1997) Variation of the chemical composition of essential oil of *Mentha piperita* L. during the growing time. *Journal of Essential Oil Research* **9**, 463-465

Chauhan A, Rahman L, Verma RS, Singh A, Verma RK, Yadav AK, Kukreja AK, Khanuja SPS (2009) Improved varietal development of medicinal and aromatic plants - A review. *Journal of Medicinal and Aromatic Plant Sciences* **31**, 246-255

Chowdhury JU, Nandi NC, Uddin M, Rahman M (2007) Chemical constituents of essential oils from two types of spearmint (*Mentha spicata* L. and *M. cardiaca* L.) introduced in Bangladesh. *Bangladesh Journal of Scientific and Industrial Research* **42**, 79-82

Davies NW (1990) Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *Journal of Chromatography A* **503**, 1-24

El-Wahab A, Mohamed (2009) Evaluation of spearmint (*Mentha spicata* L.) productivity grown in different locations under upper Egypt conditions. *Research Journal of Agriculture and Biological Sciences* **5 (3)**, 250-254

Hadjiakhoondi A, Aghel N, Zamanizadeh-Nadgar N, Vatandoost H (2000) Chemical and biological study of *Mentha spicata* L. essential oil from Iran. *DARU* **8 (1&2)**, 19-21

Khanuja SPS (2007) Aroma vision 2020: Technology path of cultivating today for a fragrant tomorrow, *National Convention and Seminar on Business Enabling of Aromatic Plants and Products*, November 21-22, Dehradoon, India

Kokkini S, Karousou R, Lanaras T (1995) Essential oils of spearmint (carvone-rich) plants from the Island of Crete (Greece). *Biochemical Systematics and Ecology* **23**, 287-297

Patra NK, Kumar B (2005) Improved varieties and genetic research in medicinal and aromatic plants (MAPs). In: *Proceeding of 2nd National Interactive Meet on Medicinal and Aromatic Plants*, CIMAP Lucknow, pp 53-61

Patra NK, Tanveer H, Khanuja SPS, Shasanay AK, Singh HP, Singh VR, Kumar S (2001) A unique interspecific hybrid spearmint clone with growth properties of *Mentha arvensis* L. and oil qualities of *Mentha spicata* L. *Theoretical and Applied Genetics* **102**, 471-476

Shah SC, Gupta LK (1989) Response of *Mentha* species to different harvesting intervals. *Progressive Horticulture* **21**, 148-150

Sharma S, Tyagi BR (1991) Character correlation, path coefficient and heritability analyses of essential oil and quality components in corn mint. *Journal of Genetics* **45**, 257-262

Singh AK, Raina VK, Naqvi AA, Patra NK, Kumar B, Ram P, Khanuja SPS (2005) Essential oil composition and chemoarrays of menthol mint (*Mentha arvensis* L. f. *piperascens* Malinvaud ex. Holmes) cultivars. *Flavour and Fragrance Journal* **20**, 302-305

Spencer A, Hamill JD, Rhodes MJC (1990) Production of terpenes by differentiated shoot cultures of *Mentha citrata* transformed by *Agrobacterium tumefaciens* T37. *Plant Cell Reports* **8**, 601-604

Verma RS, Rahman L, Verma RK, Chanotiya CS, Chauhan A, Yadav A, Yadav AK, Singh A (2010a) Changes in the essential oil content and composition of *Origanum vulgare* L. during annual growth from Kumaon Himalaya. *Current Science* **98 (8)**, 1010-1012

Verma RS, Rahman L, Verma RK, Chauhan A, Singh A, Chanotiya CS, Yadav A, Singh AK, Kukreja AK, Khanuja SPS (2010b) Essential oil composition of the inflorescence of *Artemisia capillaris* Thunb. collected at different stages of flowering from Kumaon region of Western Himalaya. *Journal of Essential oil Research* **22 (4)**, 340-343

Verma RS, Rahman L, Verma RK, Chauhan A, Yadav AK, Singh A (2010c) Essential oil composition of menthol mint (*Mentha arvensis* L.) and peppermint (*Mentha piperita* L.) cultivars at different stages of plant growth from Kumaon region of western Himalaya. *Open Access Journal of Medicinal and Aromatic Plants* **1 (1)**, 13-18

Verma RS, Verma RK, Chauhan A, Yadav AK (2010d) Changes in the essential oil composition of *Majorana hortensis* Moench. cultivated in India during plant ontogeny. *Journal of the Serbian Chemical Society* **75 (4)**, 441-447

Virmani OP, Gauniyal AK, Kumar VS (1987) A high yielding superior strain of *Mentha viridis* '*Supriya'* developed. *CIMAP Newsletter* **14**, 2-3

Virmani OP, Gauniyal AK, Kumar VS (1988) A high yielding variety of *Mentha citrata* 'Kiran' developed. *CIMAP Newsletter* **15**, 1-2

Zheljazkov VD, Cantrell CL, Astatkie T, Ebelhar MW (2010a) Peppermint productivity and oil composition as a function of nitrogen, growth stage, and harvest time. *Agronomy Journal* **102 (1)**, 124-128

Zheljazkov VD, Cantrell CL, Astatkie T, Ebelhar MW (2010b) Productivity, oil content, and composition of two spearmint species in Mississippi. *Agronomy Journal* **102 (1)**, 129-133