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Changes in the essential oil composition of *Majorana hortensis* Moench. cultivated in India during plant ontogeny

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Abstract: The essential oil content and composition of "sweet marjoram" (*Majorana hortensis* Moench.) cultivated in the Kumaon region of the western Himalayas was studied at different ages of the crop. The samples were taken after 60, 90, 120 and 150 days of transplanting. The essential oil contents varied from 0.20 to 0.70 %. The essential oil was analyzed by GC and GC–MS. Twenty eight components, representing 96.53–98.44 % of the oil, were identified. The major essential oil constituents, *viz.*, *cis*-sabinene hydrate (37.05–47.49 %), terpinen-4-ol (14.45–16.22 %) and *trans*-sabinene hydrate (5.81–6.97 %) showed considerable variation in their concentrations in relation to crop age.

Keywords: *Majorana hortensis*; Lamiaceae; crop age; essential oil content; GC–MS.

INTRODUCTION

Majorana hortensis Moench. commonly known as "sweet marjoram" is a member of the family Lamiaceae. It is a perennial herb native to Cyprus and eastern Mediterranean countries.[1] The plant is propagated by seeds and tender stem cuttings. This plant is characterized by a strong, sweet, spicy pleasant odour. The leaves are used fresh or dried and are highly esteemed as a condiment for seasoning food products. The aerial parts of the plants are used for the isolation of oil, which has many uses in the flavour, perfumery and pharmaceutical industries. In the food industry, it is mainly used as a spice in sausages, but its use in baked goods, processed vegetables, condiments, soups, snack foods and gravies has also been reported.[2] In addition to this, marjoram is well known for its medicinal[3] and insecticidal values.[4] The plant is also reported to possess anticancer,[5] antioxidant[6] and antifungal properties.[7,8]

The essential oil composition of marjoram was investigated by a number of workers in different countries[9–19] but it has been the subject of limited invest-

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tigation from India.[20,21] The time of harvest or harvesting stage, in general, is in close relation to the yield and quality of the essential oil and it varies from place to place and from plant to plant. [22–25] Therefore, it is essential to determine the proper harvesting time for aromatic plants to obtain a better yield and quality of the essential oil. A review of the literature revealed that there are no reports on the effect of crop age on the yield and composition of the essential oil of *M. hortensis* from the western Himalayas, India. Therefore, in the present investigation, the essential oils obtained from crops of different age were compared for yield and chemical composition.

EXPERIMENTAL

Plant material

M. hortensis rooted cuttings were transplanted in the experimental field of the Central Institute of Medicinal and Aromatic Plants (CIMAP), Resource Centre, Purara, Uttarakhand, on the 15th December, 2007, and the crop was raised following normal agricultural practices. Sampling was started after 60 days (early vegetative stage) following transplanting and then taken every month at 30 days interval until 150 days (flowering stage). The soil pH, organic carbon, available nitrogen, available phosphorus and available potassium of the experimental site were 6.5, 0.35 %, 175, 7 and 135 kg ha $^{-1}$, respectively. The site is located between the coordinates 28° 60' to 31°29' N, 77°49' to 80°60' E and at an altitude of 1250 m in Kattyur Valley. Climatologically, the site falls into a sub-temperate zone (1200–1700 m) of the western Himalaya, with the monsoon usually breaking in June and continuing up to September. A voucher specimen of the plant was deposited in the Herbarium of CIMAP, Resource Centre, Purara.

Isolation of the essential oils

Freshly harvested samples were immediately subjected to hydro-distillation in a Clevenger type apparatus for 3 h for extraction of the essential oil. The oils were collected, dehydrated with anhydrous sodium sulphate, measured and kept cool in the dark prior to analysis.

Gas chromatography (GC)

GC analyses of the oil samples was realised using either a Perkin-Elmer Auto XL GC or a Nucon gas chromatograph model 5765 equipped with FIDs and two different stationary phases, PE-5 (50 m×0.32 mm, 0.25 µm film coating) and BP-20 (coated with a Carbowax 20M, 30 m×0.32 mm×0.25 µm film thickness) fused silica capillary columns, respectively. Hydrogen was the carrier gas at a flow rate of 1.0 mL/min. The temperature of the columns was programmed from 100 to 280 °C at 3 °C/min (for the PE-5) and from 70 to 230 °C at 4 °C/min (for the BP-20). The injector and detector temperatures were 220 and 300 °C on the PE-5 and 200 and 230 °C on the BP-20 columns, respectively. The injection volume was 0.02 µL neat and the split ratio was 1:30.

Gas chromatography – mass spectrometry (GC–MS)

The GC–MS spectra were recorded on a Perkin–Elmer Auto System XL GC coupled to a turbo mass spectrometer, using a fused silica capillary column, PE-5 (50 m×0.32 mm, film thickness 0.25 µm). The column temperature was programmed 100–280 °C at 3 °C/min,

using helium as the carrier gas at a constant pressure of 69 kPa. The MS conditions were: EI mode, 70 eV; ion source temperature, 250 °C.

Identification of compounds

The identification was realised based on the retention time, the Kovats Index, an MS Library search (NIST & Wiley), the *n*-alkane (C_9–C_{22}) hydrocarbon pattern (Nile, Italy) and by comparing the mass spectra with MS literature data.[26,27] The relative amounts of the individual components were calculated based on the GC peak areas without using correction factors.

RESULTS AND DISCUSSION

The essential oil yield and terpenoids composition together with the crop age/phenological stage are presented in Table I. The *M. hortensis* crop harvested at 150 days, flowering stage, produced a higher yield of essential oil (0.70 %) than those harvested at 120 days (flower initiation; 0.66 %), 90 days (late vegetative stage; 0.32 %) and 60 days (early vegetative stage; 0.20 %). Apparently, the dynamics of the essential oil content in marjoram is metabolically regulated during the vegetative and flowering stages of crop growth. In most aromatic plants, the essential oil preferentially accumulates during the flowering stage, probably due to its ecological role in attracting pollinators and as a defence mechanism. A similar variation in the essential oil content was registered for other aromatic crops.[23–25] Twenty-eight compounds, comprising 96.53–98.44 % of the total, were identified with the help of GC and GC–MS (Table I). The chemical formulae of the major components, compounds **1**–**3**, are shown in Fig. 1.

TABLE I. Composition (%) of the essential oil of Sweet marjoram (*Majorana hortensis* Moench.) at different ages/stages from India (t = trace (<0.1 %))

Compound[a]	*KI*[b]	*KI*[c]	Phenological stage/crop age[d], days				Detection[e]
			EVS	LVS	FI	FS	
			60	90	120	150	
α-Pinene	1022	935	1.03	0.85	0.73	1.35	A,B,C
β-Pinene	1105	980	0.12	0.10	0.06	0.09	A,B
Sabinene	1119	974	4.59	4.98	5.01	7.64	A,B,C
β-Myrcene	1163	989	1.05	1.30	1.44	2.22	A,B
α-Terpinene	1177	1021	2.01	2.89	2.81	5.85	A,B
Limonene	1194	1030	0.99	1.07	0.99	1.51	A,B
1,8-Cineole	1204	1035	0.96	1.16	0.80	0.86	A,B
γ-Terpinene	1244	1062	4.06	5.20	5.77	8.66	A,B,C
(*E*)-*β*-Ocimene	1244	1047	0.27	0.23	–	–	A,B
p-Cymene	1271	1025	2.81	1.13	0.64	0.52	A,B,C
α-Terpinolene	1079	1090	0.96	1.15	1.19	2.33	A,B
3-Octanol	–	993	0.64	0.67	0.15	t	A,B
1-Octen-3-ol	1411	978	t	0.16	–	t	A,B
trans-Sabinene hydrate[t]	1463	1068	6.08	5.81	6.12	6.97	A,B
Camphor	1506	1146	0.38	0.52	1.48	0.42	A,B,C
cis-Sabinene hydrate[t]	–	1097	44.90	47.89	44.79	37.05	A,B
Linalool	1538	1098	1.30	1.20	1.27	0.81	A,B,C

TABLE I. Continued

Compound[a]	*KI*[b]	*KI*[c]	Phenological stage/crop age[d], days				Detection[e]
			EVS	LVS	FI	FS	
			60	90	120	150	
Linalyl acetate	–	1257	0.16	0.06	0.04	0.04	A,B
Bornyl acetate	1558	1285	–	–	t	t	A,B
β-Caryophyllene	1594	1419	2.04	1.56	2.11	1.18	A,B
Terpinen-4-ol	1604	1177	14.45	14.76	16.22	15.36	A,B
α-Humulene	1669	1462	0.24	0.20	0.26	0.17	A,B
α-Terpineol	1701	1188	2.88	3.28	3.85	3.68	A,B
Piperitone	–	1252	2.84	1.87	1.67	0.50	A,B
Geraniol 1830		1237	0.61	0.40	t	t	A,B,C
β-Caryophyllene oxide	1995	1584	1.16	t	t	0.76	A,B
Thymol	2209	1313	–	–	–	t	A,B
Carvacrol	2243	1315	–	–	–	t	A,B
Class composition							
Monoterpene hydrocarbons	–	–	15.08	17.77	18.0	29.65	–
Oxygenated monoterpenes	–	–	74.56	76.95	76.24	65.69	–
Sesquiterpene hydrocarbons	–	–	2.28	1.76	2.37	1.35	–
Aliphatic compounds	–	–	0.64	0.83	0.15	t	–
Phenolic monoterpenes	–	–	2.81	1.13	0.64	0.52	–
Others	–	–	1.16	t	t	0.76	–
Total terpenoids/ %	–	–	96.53	98.44	97.40	97.97	–
Oil content/ % of fresh weight	–	–	0.2	0.32	0.66	0.70	–

[a]Compounds are listed in the order of elution from a polar column (BP-20); [b]*KI* literature (BP-20); [c]*KI* calculated (PE-5); [d]EVS = early vegetative stage, LVS = late vegetative stage, FI = flower initiation and FS = flowering stage (bloom); [e]A = Kovats index, B = GC–MS, C = co-injection with authentic sample; [f]cis/trans related to methyl *vs.* isopropyl groups

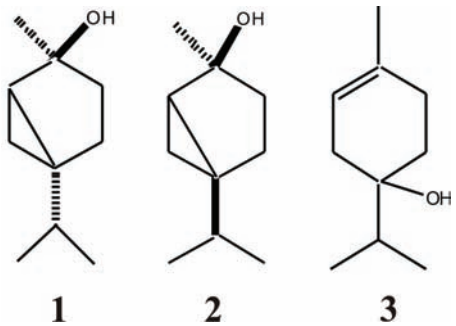

Fig. 1. The chemical formulae of the major components identified in the essential oil.

The obtained results cle arly indicate that there wer e considerable variations in the qualitative co mposition of the oils obtained from crops of different ages. The oils were dom inated by oxygenated monoterpenes (65.69–76.95 %) with the maximum at 90 (late vegetative stage) and 120 days (flower initiation). On the other hand, monoterpene hydrocarbons were found to be increased with advancement of the crop age (15.08 –29.65 %) and attain ed the maximum at 150 day s (flowering stage). The major components of these oils were *cis*-sabinene hydrate (**1**, 37.0 5–47.89 %), *trans*-sabinene hydrate (**2**, 5.8 1–6.97 %), terpinen-4-ol (**3**, 14.45–16.22 %), sabinene (4.59–7.64 %), α-terpinene (2.01–5.85 %), γ-terpinene (4.06–8.66 %) and α-terpineol (2. 88–3.85 %). The percent of *cis*-sabinene hyd rate (47.89 %) and 3-o ctanol (0.67 %) were found to b e higher in 90 -day followed by in 6 0-day old crops (44 .90 and 0 .64 %, respectively), while their lowest percent were recorded at the flowering stage, *i.e*., after 150 days (37.05 % and trace, respectively). However, the amount of terpinen-4-ol and α-terpineol reached higher values during the flower initiation stage, *i.e*., at 120 days (16.22 and 3.85 %, respectively) an d started to decline as the flowering advanced. The am ounts of *trans*-sabinene hydrate (6.97 %), α-pinene (1.35 %), sabinene (7.64 %), myrcene (2.22 %), α-terpinene (5.85 %), limonene (1.51 %), γ-terpinene (8.66 %) and α- -terpinolene (2.33 %) were higher at 1 50 da ys than a t an y ot her crop age. Furthermore, the registered pe rcents of β-pinene (0.12 %), (*E*)-β-ocimene (0.27 %), *p*-cymene (2.81 %), linalool (1.30 %), lina lyl acetate (0.16 %), piperitone (2.84 %), geraniol (0.61 %) and β-caryophyllene oxide (1.16 %) were relatively higher in the crop harvested after 60 days after transplanting.

The experiments performed under c ontrolled as well as under field conditions indicated the infl uence of environmental factors, such as light, tem perature, *etc*. on the monoterpene metabolism of aromatic crops, which resu lted in marked variations in their essential oil com positions.[28–31] Therefore, thes e variations in the essential oil content and com position of *M. hortensis* might be due to variation in enzyme levels an d their pool sizes in response to changi ng weather conditions during different months.

CONCLUSIONS

The sweet marjoram grown in the hill y tracks of northern India belongs to the *cis*-sabinene hy drate chemotype. Based on the e ssential oil content, the flowering stage (150 days) could be considered as the best harvesting time for sweet marjoram, but the flavour compounds of marjoram consist mainly of light oxygenated com pounds; reports concerning t his oil alwa ys refer to *cis*-sabinene hydrate,[32] the content of whic h was lowest at da y 150. Therefore, it is judicious to harvest and distil *M. hortensis* crop at the flower initiation stage, *i.e*., at day 12 0 under the sub-temperate conditions of north India in order to obtain a good yield of quality oil.

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И З В О Д

ПРОМЕНА САСТАВА ЕТАРСКОГ УЉА ТОКОМ РАСТА БИЉКЕ *Majorana hortensis* Moench. ИЗ ИНДИЈЕ

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Испитиван је састав етарског уља „слатког мајорана“ (*Majorana hortensis* Moench.) гајеног у области Кумаон у западним Хималајима, у различитим периодима раста. Узорци су сакупљани после 60, 90, 120 и 150 дана од пресађивања. Количина етарског уља у биљци је варирала од 0,20 до 0,70 %. Етарско уље је анализирано методама GC и GC–MS. Идентификовано је двадесет осам састојака који су чинили укупно 96,53–98,44 % етарског уља. Главни састојци етарског уља су били *cis*-сабинен-хидрат (37,05–4 7,49 %), терпинен-4-ол (14,45––16,22 %) и *trans*-сабинен-хидрат (5,81– 6,97 %). Њихов удео се знатно мењао са старењем биљке.

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