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Productivity and essential oil composition of rosemary (*Rosmarinus officinalis* L.) harvested at different growth stages under the subtropical region of north India

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ABSTRACT

Productivity and essential oil composition of rosemary (*Rosmarinus officinalis* L.) were evaluated under the subtropical region of north India. The crop was planted in mid-November and harvested at six different stages. The crop harvested at 225 days after transplanting yielded 88.03 L ha⁻¹ essential oil which was at par with the yield obtained at 210 days (83.34 L ha⁻¹). The essential oils extracted from whole aerial parts and leaves were analysed using gas chromatography–flame ionization detector and gas chromatography–mass spectrometry techniques. A total of 30 constituents, representing 96.2–98.2% of the total oil composition were identified. Major constituents of the oil were camphor (23.9–35.8%), 1,8-cineole (18.0–23.9%), α -pinene (4.5–14.4%), verbenone (6.5–12.4%), camphene (2.5–6.9%), limonene (2.1–2.8%), bornyl acetate (1.1–4.1%), α -terpineol (1.9–3.6%) and β -pinene (2.1–3.3%). In conclusion, cultivation of rosemary as a seasonal crop can produce good-quality essential oil in the subtropical region of north India.

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Introduction

Rosmarinus officinalis L., commonly known as ‘rosemary’, belongs to Lamiaceae family. It is a dense, evergreen, aromatic shrub, naturally distributed in the Mediterranean countries, Northern Africa, Argentina, Brazil, England, Mexico and the USA (1,2). It is commercially cultivated in Spain, Dalmatia, Turkey, Egypt, Italy, Greece, France, Northern Africa, Portugal and Yugoslavia for the production of fresh or dry leaves and essential oil, which finds extensive application in perfume, cosmetic and pharmaceutical industries (3–5). Rosemary finds uses in folk medicine due to its carminative, stomachic and spasmodic properties. The leaves of rosemary are used for culinary purpose and also being used in foodstuffs for the control of *Salmonella* infections (6–8). Rosemary is used as a food-flavouring agent and known medicinally for its powerful antimutagenic, antibacterial, antioxidant and chemopreventive properties (9). In addition to the therapeutic use, the essential oil is extensively used in the cosmetic industry for producing various cologne waters, bathing essences, hair lotions and shampoos. The leaves of the rosemary are an essential spice of the French, Italian and Spanish food (10).

The chemical composition of rosemary oil has been the subject of considerable study. The major

constituents of the rosemary essential oil are α -pinene, 1,8-cineole and camphor, associated with variable amounts of camphene, limonene, borneol, verbenone, bornyl acetate, α -terpineol, etc. (11). In addition to the essential oil, rosemary also contains significant amounts of phenolic acids, phenolic diterpenes and pentacyclic triterpenes (12). The yield and chemical composition of the rosemary essential oil generally depends on a number of factors such as plant part (13), stage of the plant (14,15), extraction methods (16,17), season of harvesting (4,18), drying procedure (19) and storage conditions (20,21).

Rosemary is an emerging aromatic crop of India (14,22–24). A review of the literature revealed that no attempts have been made to date to explore the possibility of the commercial cultivation and production of rosemary in the foothills of Uttarakhand (subtropical climate), where the period of October to June is suitable for growing this crop. Therefore, considering the commercial potential and availability of sufficient natural resources, rosemary was introduced as a seasonal crop (winter–summer) to the foothills of Uttarakhand, India. In the present research, the effects of the harvesting date on the yield (herb, leaves and essential oil) and essential oil composition of rosemary were investigated. The outcome of this research can help to promote the cultivation of rosemary and open new avenue for income

generation for farmers in the subtropical region of north India.

Experimental

Transplanting of the crop and experimental site

One-month-old rooted plants of rosemary (*Rosmarinus officinalis* var. *CIM-Hariyali*) were transplanted at 50 cm inter- and intra-row spacing in the experimental field at CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre Pantnagar during the winter season (November 2014 and 2015). The experiment was laid down in a randomized block design with six treatments [harvesting stages, T₁: 15 April (150 days); T₂: 1 May (165 days); T₃: 15 May (180 days); T₄: 1 June (195 days); T₅: 15 June (210 days); T₆: 1 July (225 days)] and three replications. The crop was grown following normal agricultural practices. The experimental site is located at coordinates 29° N, 79.38° E and at an altitude of 243.84 m in foothills of north India. The climate of this site is subtropical and humid. The maximum temperature ranges between 35°C and 45°C and the minimum between 2°C and 5°C. Soil can be characterized as mollisol, with neutral pH.

Extraction of the essential oil

Freshly harvested aerial parts and leaves (100 g) of rosemary were subjected to hydrodistillation in a Clevenger's type apparatus for 3 h for isolation of essential oil. Essential oil was measured directly in the extraction burette, and content (%) was calculated as volume (mL) of essential oil per 100 g of fresh plant material. The oil samples were dehydrated over anhydrous Na₂SO₄ and kept in a cool and dark place until further analysis.

Gas chromatography and gas chromatography–mass spectrometry

Gas chromatography (GC) analysis of essential oil was carried out on Nucon Gas Chromatograph (model

5765) equipped with DB-5 (30 m × 0.25 mm; 0.25 µm film thickness) fused silica capillary column and flame ionization detector (FID). Hydrogen was used as carrier gas at 1.0 mL min⁻¹. Temperature programming was done from 60°C to 230°C at 3°C min⁻¹. The injector and detector temperatures were 220°C and 230°C, respectively. The injection volume was 0.02 µL neat with a split ratio of 1:40. Gas chromatography–mass spectrometry (GC-MS) analysis was done using a Clarus 680 GC interfaced with a Clarus SQ 8C mass spectrometer of PerkinElmer fitted with Elite-5 MS fused-silica capillary column (5% (phenyl)-polymethylsiloxane stationary phase; 30 m × 0.25 mm internal diameter, film thickness 0.25 µm). Identification of the essential oil constituents was carried out on the basis of retention index (RI), determined with reference to homologous series of *n*-alkanes (C₇–C₃₀) and MS Library search (NIST and WILEY), and by comparing RI and mass spectral data with the literature (25). The relative amounts of individual components were calculated based on the relative % peak areas (FID response), without using the correction factor.

Results and discussion

The growth and yield attributes of rosemary harvested at different stages in the subtropical region of north India are presented in Table 1. The plant height and canopy of rosemary ranged from 30.87 to 60.0 cm and 30.40 to 59.70 cm, respectively, with significantly higher values reported at T₆ followed by T₅. Branches per plant were significantly higher for T₆ (11.0 plant⁻¹), which was at par with T₅ and T₄. On the other hand, the essential oil content was found to be significantly higher (1.02%) at T₃, which was at par with T₂ and T₄. Moreover, the fresh herb (whole aerial parts), fresh leaves and essential oil yields were found to increase with the increase of crop duration. The fresh herb and essential oil yields ranged from 10.44 to 94.99 q ha⁻¹ and 10.28 to 88.03 L ha⁻¹, respectively. The fresh herb and essential oil yields were

Table 1. Growth and yield attributes of rosemary (*Rosmarinus officinalis* var. *CIM-Hariyali*).

Harvesting time	Plant height (cm)	Canopy (cm)	Branch/plant	Essential oil content (%) ^a	Fresh herb yield (q ha ⁻¹)	Fresh leaf yield q ha ⁻¹	Essential oil yield (L ha ⁻¹) ^a
T ₁	30.87	30.40	7.0	0.98	10.44	7.94	10.28
T ₂	35.23	31.20	9.0	1.00	23.26	17.66	23.26
T ₃	38.67	39.27	9.0	1.02	29.96	21.55	30.59
T ₄	43.87	43.63	10.0	1.00	50.14	39.40	50.14
T ₅	53.90	48.50	11.0	0.98	84.88	61.99	83.34
T ₆	60.00	59.70	11.0	0.93	94.99	73.88	88.03
SEm (0.05)	0.65	1.22	0.35	0.02	7.16	3.57	6.77
CD (0.05)	2.04	3.85	1.10	NS	22.58	11.26	21.36

T₁: 15 April (150 days); T₂: 1 May (165 days); T₃: 15 May (180 days); T₄: 1 June (195 days); T₅: 15 June (210 days); T₆: 1 July (225 days); NS: non-significant.

^aEstimated from hydrodistillation of fresh herb (whole aerial parts).

significantly higher for T₆ and were at par with T₅. Under hill conditions, this variety is grown as a perennial crop and produces 53.72, 170.87 and 359.47 L ha⁻¹ essential oil in the first, second and third years of crop, respectively (22). Further, Mishra et al. (2009) reported essential oil yield 55.5 and 32.73 kg ha⁻¹ for the first- and second-year crop, respectively, under hill conditions (23). Moreover, in the semiarid tropical conditions, the essential oil yield of rosemary was 110.94 kg ha⁻¹ (14). Thus, the production potential of rosemary in the subtropics (94.99 q ha⁻¹ fresh herb and 88.03 L ha⁻¹ essential oil) was quite promising as compared with other locations of India.

The essential oils of fresh whole aerial parts and leaves obtained from the various treatments were analysed using GC-FID and GC-MS. The results are summarised in Table 2. The representative GC profile of the oil is shown in Figure 1. Major constituents of the essential oil of fresh whole aerial parts were camphor (23.9–33.2%), 1,8-cineole (20.4–23.9%), α -pinene

(8.5–14.4%), verbenone (6.9–10.1%), camphene (3.5–6.9%), limonene (2.3–2.8%), bornyl acetate (1.1–3.4%), α -terpineol (1.9–2.8%), β -pinene (2.5–3.2%), borneol (1.7–2.5%) and linalool (0.9–2.0%). The amount of camphor was found to be higher at T₁ (33.2%), followed by T₂ (32.4%); however, it was recorded lowest at T₆ (23.9%). Likewise, other constituents, such as bornyl acetate (3.4%), borneol (2.5%), linalool (2.0%) and γ -terpinene (1.2%) were also recorded relatively higher at T₁. The next major component of the oil, 1,8-cineole, reached its higher level at T₂ (23.9%), followed by T₄ (22.9%). However, α -pinene and camphene reached their higher value at T₆ (14.4% and 6.9%, respectively), followed by T₅ (12.8% and 6.2%, respectively). Further, verbenone content recorded higher at T₅ (10.1%), followed by T₃ (9.6%) and T₆ (8.9%).

Moreover, major constituents of the leaf oil were camphor (24.8–35.8%), 1,8-cineole (18.0–22.7%), α -pinene (4.5–13.5%), verbenone (6.5–12.4%), camphene (2.5–6.6%), bornyl acetate (1.2–4.1%), α -terpineol

Table 2. Effect of harvesting time on the essential oil composition of *Rosmarinus officinalis* var. *CIM-Hariyali* grown in the subtropical region of north India.

S.no.	Compound (%)	RI	Whole aerial parts						Leaves					
			T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
1.	α -Thujene	925	0.1	0.2	0.4	0.1	0.1	0.1	0.2	t	0.1	0.1	0.4	0.3
2.	α -Pinene	934	8.5	6.6	12.7	10.6	12.8	14.4	4.5	5.8	11.1	8.3	13.5	12.5
3.	Camphene	945	4.4	3.5	5.9	5.5	6.2	6.9	2.5	3.2	5.0	3.9	6.6	6.2
4.	β -Pinene	973	3.1	2.5	3.2	2.9	2.7	2.6	2.1	2.6	3.3	2.2	2.8	2.3
5.	β -Myrcene	990	0.7	0.9	1.1	1.2	1.2	1.3	0.6	0.8	1.1	1.0	1.4	1.1
6.	α -Phellandrene	1000	0.7	1.1	1.5	1.5	1.8	1.9	0.7	1.2	1.8	1.4	2.0	1.8
7.	α -Terpinene	1017	0.1	0.4	0.5	0.5	0.7	0.8	0.2	0.4	0.5	0.5	0.7	0.7
8.	<i>p</i> -Cymene	1020	0.3	t	0.5	0.9	0.7	0.7	0.2	t	0.4	0.4	0.7	0.6
9.	Limonene	1026	2.3	2.8	2.3	2.6	2.5	2.5	2.3	2.6	2.5	2.1	2.5	2.3
10.	1,8-Cineole	1028	20.4	23.9	20.6	22.9	22.3	22.1	20.0	22.7	22.1	18.0	21.7	20.4
11.	(<i>E</i>)- β -Ocimene	1044	0.3	0.7	0.8	0.8	1.0	1.0	0.3	0.8	0.9	0.8	1.0	1.0
12.	γ -Terpinene	1052	1.2	0.1	0.5	0.3	0.2	0.2	1.5	0.3	0.6	0.4	0.2	0.2
13.	Terpinolene	1082	0.4	0.7	0.8	0.7	0.9	0.9	0.4	0.7	0.9	0.8	0.9	0.8
14.	Linalool	1097	2.0	1.4	1.3	1.1	0.9	1.0	2.3	1.5	1.4	1.1	0.8	0.8
15.	Chrysanthenone	1128	0.8	0.2	0.7	0.7	0.7	0.6	0.9	0.3	0.8	0.7	0.6	0.6
16.	Camphor	1147	33.2	32.4	24.5	27.7	24.9	23.9	35.8	32.0	27.4	30.5	24.8	25.1
17.	δ -Terpineol	1164	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.2
18.	Borneol	1166	2.5	2.3	1.7	2.0	1.8	1.9	2.8	2.3	2.0	2.4	1.9	2.1
19.	Terpinen-4-ol	1174	0.7	1.5	0.8	1.0	0.9	0.9	0.8	1.4	0.8	1.1	0.9	0.9
20.	α -Terpineol	1190	2.8	2.8	2.1	2.3	1.9	2.1	3.6	3.0	2.1	2.6	1.9	2.0
21.	Myrtenol	1196	0.1	0.1	0.2	0.3	0.2	0.2	0.1	0.2	0.3	0.3	0.3	0.3
22.	Verbenone	1210	6.9	8.3	9.6	8.4	10.1	8.9	8.5	9.1	6.5	12.4	9.2	11.2
23.	Citronellol	1222	0.4	0.5	0.3	0.3	0.3	0.3	0.5	0.1	0.3	0.4	0.2	0.3
24.	Bornyl acetate	1282	3.4	2.8	2.1	1.8	1.5	1.1	4.1	3.3	2.5	2.4	1.4	1.2
25.	Eugenol	1351	t	t	0.1	0.1	0.1	0.1	t	0.1	0.1	0.1	0.1	0.1
26.	α -Copaene	1373	0.1	t	-	t	t	t	0.1	t	0.1	t	t	0.1
27.	(<i>E</i>)-Caryophyllene	1418	0.4	0.4	1.1	0.5	0.5	0.6	0.8	0.8	1.0	0.8	0.6	0.9
28.	α -Humulene	1448	0.4	0.4	1.0	0.4	0.5	0.5	0.7	0.8	0.9	0.8	0.6	0.8
29.	γ -Cadinene	1512	0.1	0.1	0.3	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3
30.	Caryophyllene oxide	1579	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1
Class composition														
	Monoterpene hydrocarbons		21.8	19.5	29.7	26.7	30.1	32.6	15.3	18.4	27.8	21.5	32.0	29.2
	Oxygenated monoterpenes		73.4	76.4	64.1	68.8	65.7	63.2	79.7	76.2	66.5	72.2	63.9	65.1
	Sesquiterpene hydrocarbons		1.0	0.9	2.4	1.0	1.1	1.3	1.8	1.8	2.2	1.8	1.4	2.1
	Oxygenated sesquiterpenes		0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1
	Others		0.3	t	0.6	1.0	0.8	0.8	0.2	0.1	0.5	0.5	0.8	0.7
	Total identified (%)		96.7	96.9	97	97.7	97.8	98	97.2	96.7	97.2	96.2	98.2	97.2

RI: Retention Index determined on DB-5 gas chromatography column (30 m $\times$ 0.25 mm) using *n*-alkanes series; T₁: 15 April (150 days); T₂: 1 May (165 days); T₃: 15 May (180 days); T₄: 1 June (195 days); T₅: 15 June (210 days); T₆: 1 July (225 days); t: trace (<0.05%).

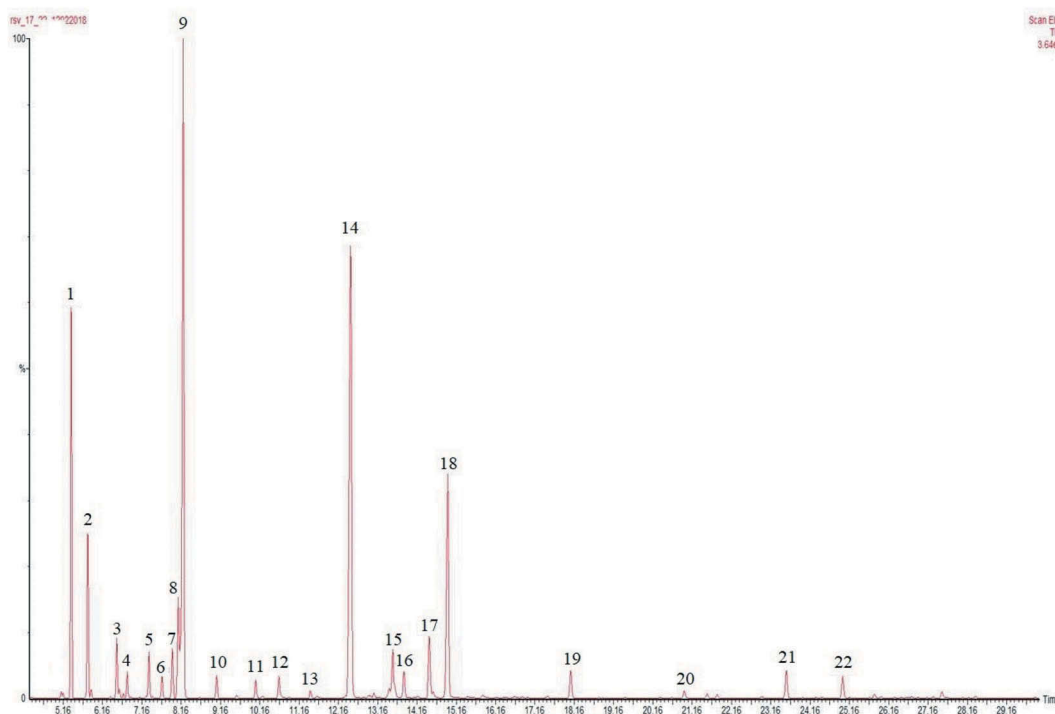


Figure 1. Chromatographic profile (total ion chromatogram) of rosemary (*Rosmarinus officinalis* L.) essential oil [Peak: **1**: α -pinene; **2**: camphene; **3**: β -pinene; **4**: β -myrcene; **5**: α -phellandrene; **6**: α -terpinene; **7**: *p*-cymene; **8**: limonene; **9**: 1,8-cineole; **10**: γ -terpinene; **11**: terpinolene; **12**: linalool; **13**: chrysanthenone; **14**: camphor; **15**: borneol; **16**: terpinen-4-ol; **17**: α -terpineol; **18**: verbenone; **19**: bornyl acetate; **20**: eugenol; **21**: (*E*)-caryophyllene; **22**: α -humulene].

(1.9–3.6%), β -pinene (2.1–3.3%), limonene (2.1–2.6%), borneol (1.9–2.8%) and linalool (0.8–2.3%). The content of camphor was found to be higher at T_1 (35.8%), followed by T_2 (32.0%); however, it was recorded minimal at T_5 (24.8%). Likewise, other constituents, namely bornyl acetate (4.1%), α -terpineol (3.6%), borneol (2.8%), linalool (2.3%) and γ -terpinene (1.5%), were also recorded relatively higher at T_1 . The next major component of the oil, 1,8-cineole, reached its higher level at T_2 (22.7%), followed by T_3 (22.1%). However, α -pinene and camphene reached their higher value at T_5 (13.5% and 6.6%, respectively), followed by T_6 (12.5% and 6.2%, respectively). Further, the content of verbenone was recorded higher at T_4 (12.4%), followed by T_6 (11.2%) and T_5 (9.2%).

It was evident from the data that harvesting stage and type of material processed influenced the yield and content of different components in the essential oil. In general, contents of camphor, borneol, bornyl acetate, α -terpineol, linalool and γ -terpinene were higher in the early harvested crops as compared with the late harvested crop; however, its reverse was true for α -pinene, camphene, β -myrcene, α -phellandrene and (*E*)- β -ocimene. Moreover, the content of verbenone was recorded relatively higher in the leaf oil (6.5–12.4%) as

compared with the whole aerial parts (6.9–10.1%). Earlier, the essential oil composition of *R. officinalis* var. *CIM-Hariyali* grown in the hilly region has been evaluated. The chemical composition varied due to plant parts (13), season (18) and drying procedure (19). Comparison of the chemical composition clearly indicated that verbenone, an important component in high-quality rosemary essential oil (26), was substantially higher in the examined oil from the subtropical region (whole aerial herb: 6.9–10.1%; leaf: 6.5–12.4%) as compared with the hilly region (4.06–6.0%) (18,22). These variations are possible due to various extrinsic factors, including soil and climatic conditions, which considerably affect the yield and composition of active plant principles.

Conclusions

Rosmarinus officinalis var. *CIM-Hariyali* introduced in the subtropical region of north India as a seasonal crop yielded up to 94.99 q ha⁻¹ fresh herb and 88.03 L ha⁻¹ essential oil. The major components of the essential oil were camphor, 1,8-cineole, α -pinene and verbenone. In terms of the contents of major components, including verbenone, oil produced in the subtropics was superior

over the hilly region. The study suggested that rosemary can be grown as a seasonal crop (mid-November to mid-June) in the subtropical region of north India.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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