Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1054 (2004) 67–72
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Essential oil yield and quality of methyl eugenol rich *Ocimum tenuiflorum* L.f. (syn. *O. sanctum* L.) grown in south India as influenced by method of harvest

S.K. Kothari [a,*], A.K. Bhattacharya [a], S. Ramesh [b]

[a] *Central Institute of Medicinal and Aromatic Plants (CIMAP), Field Station, Boduppal, Uppal (PO), Hyderabad 500039, India*
[b] *Central Institute of Medicinal and Aromatic Plants (CIMAP), Field Station, Allalasandra, GKVK Post, Bangalore 560065, India*

Available online 24 April 2004

Abstract

A field experiment carried out during 2001–2002 under semi-arid conditions of Hyderabad, India investigated the effect of three different methods of harvesting at full bloom stage, on essential oil yield and quality of methyl eugenol rich sacred/holy basil (*Ocimum tenuiflorum* L.f.; Lamiaceae). The harvest methods were: harvesting of primary branches, secondary branches and shoot biomass cut at 30 cm above ground level. Four harvests at 102, 192, 287 and 360 days after transplanting of the crop were taken in 1 year in each method of harvest. Harvesting of secondary branches led to maximum plant height and number of secondary branches per plant compared to harvesting of primary branches or shoot biomass cut at 30 cm above ground during second, third and fourth harvests. On the contrary, secondary branch harvest gave least biomass yield in all the four harvests. But due to higher essential oil content, secondary branch harvest gave 25.2 and 15.4% higher total (sum total of all four harvests) essential oil yield (kg/ha per year) over primary branches and shoot biomass cut at 30 cm above ground methods of harvesting, respectively. A similar treatment difference was observed in respect of oil composition studied in the first harvest. Harvesting shoot biomass at 30 cm above ground produced oil containing highest amount of methyl eugenol. The content of methyl eugenol decreased in the order of shoot biomass cut at 30 cm above ground > primary branch > secondary branch treatments. A reverse trend was observed, however, in respect of (*E*)-cinnamyl acetate, eugenol and β-elemene constituents of the oil. Little variability was, however, observed among the treatments in respect of 24 other constituents of the oils.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Essential oils; *Ocimum tenuiflorum* L.f.; Methyl eugenol

1. Introduction

Ocimum tenuiflorum is ranked among few wonder herbs for having enormous medicinal potentialities which act as panacea for a number of ailments and diseases. Due to its manifold curative uses, the plant is considered as highly sacred, worth worshipping and hence was given the name of "Sacred Tulsi" or "Holy Basil" in India. It is a perennial shrub and primarily it occurs in two colors, green (*Lakshmi*/*Sri Tulsi*) and purple (*Krishna Tulsi*). It has several chemotypes, i.e. morphologically indistinguishable plants differing in their chemical constituents. Its oil possesses the pleasant odor characteristic of the plant, with an appreciable note of clove. The chemical composition of the oil of *O. tenuiflorum* has been the subject of previous studies [1–9]. The essential oil has either phenolic constituents like eugenol, thymol or sesquiterpene alcohols as major oil constituents and terpene compounds as minor constituents.

Chemotypes of *O. tenuiflorum* containing methyl eugenol as a major or minor constituent of essential oil has been reported earlier from India [10,11] and Thailand [1]. Recently, a chemotype of *O. tenuiflorum* containing higher essential oil concentration and which is rich in methyl eugenol (>70%) has been isolated, developed as variety *Kanchan* (CIM HY-1) and is being released for commercial cultivation [12,13]. Methyl eugenol is used quite widely in perfume compositions of the carnation type and in bouquets of oriental character [14]. It is also used as a flavoring agent in jellies, baked goods, non-alcoholic beverages, chewing gum, candy, pudding, relish and ice cream [15,16]. As a flavoring agent, it has spicy, ginger like undertones and its odor is

* Corresponding author. Tel.: +91-40-27201131/27202602.
E-mail address: cimaphyd@rediffmail.com (S.K. Kothari).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.03.019

musty-tea like warm and mildly spicy [17]. It has also been used as a powerful male insect attractant for insect surveillance and control program [18,19].

Earlier a large variability in volatile constituents from leaves, stem and inflorescence oil of *O. tenuiflorum* has been shown [8,20]. In order to maximize productivity of methyl eugenol rich *O. tenuiflorum*, effect of the method of harvest on growth, biomass yield and content, yield and quality of essential oil was studied.

2. Materials and methods

2.1. Edapho-climatic conditions

A field experiment was conducted in 2001–2002 at the Research Farm of the Central Institute of Medicinal and Aromatic Plants, Field Station, Hyderabad (542 m above m.s.l., 17°20′N and 78°3′E), India. The mean annual rainfall is about 760 mm of which 80% is received between June and September (south–west monsoon). The average temperature is 29 °C, and varies from 22 to 35 °C, the highest (44 °C day temperature) being in May and the lowest (12 °C night temperature) in January. The winter season is characterized by mild, cool dry weather. The experimental location has a semi-arid tropical climate.

The soil of the experimental site was well drained, red sandy-loam (alfic ustochrept) in texture, having organic carbon 0.3%, pH 7.3 and available N, P and K at 60.3, 9.5 and 142.5 kg/ha, respectively.

2.2. Experimental plan and isolation of essential oil

The experiment was laid out in a randomized block design with three harvest method treatments (harvesting of primary branches, secondary branches and shoot biomass cut at 30 cm above ground) replicated six times on individual plots measuring 3 m × 6 m. Primary branches harvesting consisted of biomass of primary and secondary branches, leaves and inflorescences. Secondary branch harvest constituted biomass of secondary branches, leaves and inflorescences. Shoot biomass cut at 30 cm above ground consisted of biomass of primary and secondary branches, leaves and inflorescences. Each plot received uniform dose of *Neem* (*Azadirachta indica*) seed cake (0.5 t/ha), single superphosphate (40 kg P_2O_5/ha) and muriate of potash (40 kg K_2O/ha) as a basal dose, which was incorporated with 5 cm top soil using hand hoe. *O. tenuiflorum* var. *Kanchan* seeds were sown in the nursery in second week of June 2001 and 6-week-old seedlings were transplanted at 60 cm row-to-row and 45 cm plant-to-plant spacing on 10 July 2001. The field was irrigated immediately after planting for early establishment of the seedlings. Thereafter, the field was irrigated 13 times during the course of investigation. Nitrogen at 120 kg/ha was applied in the form of urea, spreading over four harvests in 1 year. The crop received five hand weedings at 25 and 45 days after transplanting (DAT) before first harvest and subsequently one each after first, second and third harvests. The crop was harvested at full bloom stage as per treatments four times in 1 year corresponding to 102, 192, 287 and 360 DAT. At each harvest, observations were recorded on plant height, plant spread and number of primary and secondary branches, on five randomly selected plants in each experimental plot. Plot-wise biomass yields were recorded at each harvest (I–IV harvests). Essential oil content in plant tissue samples (3 treatments × 6 replications = 18) was determined following hydrodistillation of the samples (200 g each mixed with 750 ml water) in 2 l capacity round bottom flasks in Clevenger apparatus [21] for 4 h at each harvest. The essential oil samples were dehydrated with anhydrous sodium sulfate and stored at 0 °C in air-tight containers. Gas chromatography (GC), GC–mass spectrometry (MS) analyses were carried out for the first harvest oil samples only. For this study, the oil samples of all the six replications were pooled treatment-wise for gas chromatographic and GC–MS analyses.

2.3. Statistical analysis

The data on plant height, plant spread, number of primary branches/plant and number of secondary branches/plant were subjected to statistical analysis employing analysis of variance (ANOVA) technique as applicable to randomized block design [22]. Multivariate analysis was carried out to study the interaction effect (methods of harvest × harvest numbers) in respect of biomass yield, essential oil content and essential oil yield [22].

2.4. GC analysis

The oils were analyzed using a Perkin-Elmer (Italy) gas chromatograph (Model 8500) equipped with flame ionization detector (FID), GP-100 printer-plotter and an electronic integrator using BP-1 (SGE, USA) (25 m × 0.5 mm i.d. × 0.25 μm film thickness) capillary column coated with polydimethylsiloxane. Nitrogen was used as carrier gas at 10 psi inlet pressure with a flow rate of 0.4 ml/min (linear velocity 14 cm/s). Temperature was programmed from 60 to 220 °C at a ramp rate of 5 °C/min with a final hold time of 10 min. Injector and detector were maintained at 250 and 300 °C, respectively. Samples (0.1 μl) were injected neat with a split ratio 1:80.

2.5. GC–MS analysis

GC–MS analysis of the oil samples were carried out on a Shimadzu (Japan), Model QP-2000, equipped with a capillary column OV-1 (Ohiovalley, USA) (50 m × 0.25 mm id × 0.25 μm film thickness). Carrier gas used was helium at a flow rate 2 ml/min with temperature programming 100 °C (6 min) to 250 °C at 10 °C/min. Samples (0.1 μl) were injected neat with 1:50 split ratio. Mass spectra were recorded

over 40–400 amu range at 1 scan/s with ionization energy 70 eV and ion source temperature 250 °C.

2.6. Identification of essential oil constituents

The compounds of the essential oils were identified by comparing the retention times of the chromatogram peaks with those of authentic compounds run under identical conditions, by comparison of relative retention indices [23] (Retention indices were computed from gas chromatograms by logarithmic interpolation between *n*-alkanes. The homologous series of *n*-alkanes C_8–C_{22}, Poly Science Inc., Niles, USA, were used as standard.) with literature data [24], peak enrichment on co-injection with authentic compounds, comparison of mass spectra of the peaks with those of standard compounds reported in literature [25]. Quantitative data was obtained by electronic integration of peak areas (FID) without the use of response correction factors.

3. Results and discussion

3.1. Crop growth and morphology

Method of harvest significantly influenced crop growth in respect of plant height, plant spread and number of secondary branches/plant particularly during second, third and fourth harvests (Table 1). Harvesting of secondary branches of *O. tenuiflorum* led to maximum plant height, plant spread and number of secondary branches/plant during second, third and fourth harvests. The primary branch harvest treatment produced short statured plants with fewer number of secondary branches/plant compared to secondary branch harvest and shoot biomass harvested at 30 cm above ground treatments. The effect of method of harvest on number of primary branches/plant was relatively less pronounced compared to number of secondary branches/plant.

3.2. Biomass yield

In general, irrespective of methods of harvest, biomass yield was higher in first harvest and declined gradually in second, third and fourth harvests (Table 2). As envisaged, method of harvest affected biomass yield. Harvesting of secondary branches produced significantly lower biomass yield than other two methods of harvest. The treatments on harvesting of primary branch and shoot biomass cut at 30 cm above ground did not show any significant differences in respect of biomass yield. Considering total biomass yield (sum total of all the four harvests), shoot biomass cut at 30 cm above ground gave maximum biomass yield and harvesting of secondary branches produced minimum biomass yield (Table 3). Harvesting of secondary branches produced 20.5 and 23.4% lower biomass yield compared to harvesting of shoot biomass at 30 cm above ground and primary branch treatments, respectively.

Table 1
Growth and morphology of *O. tenuiflorum* in different harvests as influenced by method of harvest

Method of harvest	Harvests															
	Plant height (cm)				Plant spread (cm)				No. of primary branches per plant				No. of secondary branches per plant			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Primary branches	67.6 a	63.6 a	64.4 a	61.1 a	44.5 a	52.8 a	47.6 a	44.0 a	9.1 a	12.6 b	10.1 a	8.1 a	68.3 a	94.6 a	119.3 a	165.3 a
Secondary branches	65.3 a	74.6 b	79.2 b	73.8 b	48.1 a	63.1 b	63.8 b	66.0 b	8.6 a	13.8 b	10.7 a	9.8 b	63.5 a	127.3 b	153.6 b	225.0 b
Shoot biomass cut at 30 cm above ground	66.5 a	69.6 ab	76.6 b	72.3 b	45.3 a	48.5 a	49.6 a	50.0 a	8.8 a	10.0 a	10.8 a	10.5 b	65.8 a	115.1 b	148.6 b	214.4 b
L.S.D. ($P = 0.05$)	N.S.	8.1	7.5	8.0	N.S.	6.2	6.5	7.1	N.S.	2.1	N.S.	1.6	N.S.	14.5	16.1	25.2

Letters with similar alphabets within a column are not significantly ($P = 0.05$) different.

Table 2
Biomass yield, oil content and oil yield of *O. tenuiflorum* in different harvests as influenced by method of harvest

Method of harvest	Harvests											
	Biomass yield (t/ha)				Essential oil content (% w/w)				Essential oil yield (kg/ha)			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Primary branches	6.8	5.1	4.5	2.6	0.49	0.50	0.58	0.66	33.3	25.5	26.1	17.1
Secondary branches	5.0	3.6	3.7	2.8	0.76	0.79	0.90	1.00	38.0	28.4	33.3	28.0
Shoot biomass cut at 30 cm above ground	6.5	5.3	4.8	3.1	0.51	0.53	0.60	0.67	33.1	28.0	28.8	20.8
L.S.D. ($P = 0.05$) (method of harvest × harvest number)	0.6				0.08				3.2			

Table 3
Total biomass and essential oil yield as influenced by method of harvest

Method of harvest	Total biomass yield (t/ha per year)	Total essential oil yield (kg/ha per year)
Primary branches	19.0 b	102.0 a
Secondary branches	15.1 a	127.7 b
Shoot biomass cut at 30 cm above ground	19.7 b	110.7 a
L.S.D. ($P = 0.05$)	2.1	13.1

Letters with similar alphabets within a column are not significantly ($P = 0.05$) different.

3.3. Essential oil content

Contrary to biomass yield, essential oil content in general, was lower in first harvest and increased gradually in subsequent harvests to reach maximum in fourth harvest. In general, higher essential oil content was associated with lower biomass yield. The variation in biomass yield in different harvests was due to seasonal variation. June–September being rainy season, the weather conditions were more favorable for crop growth and thus resulted in highest biomass yield in the first harvest. Harvesting of secondary branches

Table 4
Oil composition (area counts) of *O. tenuiflorum* as influenced by method of harvest

S. no.	Constituents	RI	Area counts			Mode of identification
			Primary branch	Secondary branch	Shoot biomass cut at 30 cm above ground	
1	(*Z*)-3-Hexenol	837	2506 (0.19)	3022 (0.24)	2430 (0.16)	a, b
2	Ethyl 2-methyl butyrate	856	262 (0.02)	122 (0.01)	911 (0.06)	a, b
3	α-Pinene	931	2242 (0.17)	1015 (0.08)	1361 (0.09)	a, b, c, d
4	β-Pinene	981	392 (0.03)	130 (0.01)	1519 (0.10)	a, b, c, d
5	Myrcene	987	923 (0.07)	625 (0.05)	1671 (0.11)	a, b, c, d
6	Limonene	1020	556 (0.05)	635 (0.05)	1065 (0.07)	a, b, c
7	(*E*)-β-Ocimene	1039	14510 (1.10)	13979 (1.11)	16402 (1.08)	a, b, c, d
8	γ-Terpinene	1057	919 (0.07)	760 (0.06)	1373 (0.09)	a, b, c
9	*trans*-Linalool oxide^{+}	1074	266 (0.02)	504 (0.04)	751 (0.05)	a, b
10	Linalool	1083	664 (0.05)	752 (0.06)	1367 (0.09)	a, b, c, d
11	Eugenol	1325	96029 (7.28)	106793 (8.48)	66216 (4.36)	a, b, c
12	Methyl eugenol	1375	989709 (75.03)	918951 (72.97)	1190681 (78.40)	a, b, c, d
13	β-Elemene	1379	33505 (2.54)	35388 (2.81)	26274 (1.73)	a, b
14	(*E*)-Cinnamyl acetate	1412	50785 (3.85)	60071 (4.77)	30678 (2.02)	a, b
15	β-Caryophyllene	1417	57644 (4.37)	34506 (2.74)	121042 (7.97)	a, b, c
16	Isoeugenol	1432	1311 (0.10)	631 (0.05)	1822 (0.12)	a, b, c
17	α-Guaiene	1439	1327 (0.10)	630 (0.05)	1061 (0.07)	a, b
18	α-Humulene	1449	8574 (0.65)	756 (0.06)	8201 (0.54)	a, b,d
19	β-Selinene	1485	791 (0.06)	999 (0.08)	1370 (0.09)	a, b
20	α-Muurolene	1489	528 (0.04)	1385 (0.11)	1366 (0.09)	a, b
21	δ-Cadinene	1522	927 (0.07)	1007 (0.08)	1367 (0.09)	a, b
22	Nerolidol^{++}	1550	662 (0.05)	1637 (0.13)	911 (0.06)	a, b
23	Caryophyllene oxide	1570	5936 (0.45)	8060 (0.64)	7897 (0.52)	a, b
24	α-Guaiol	1600	661 (0.05)	504 (0.04)	761 (0.05)	a, b
25	τ-Cadinol	1625	1847 (0.14)	3778 (0.30)	2886 (0.19)	a, b
26	β-Eudesmol	1639	2242 (0.17)	2519 (0.20)	–	a, b
27	α-Bisabolol	1669	400 (0.03)	–	–	a, b
28	(*E*,*Z*)-Farnesol	1695	5276 (0.40)	6549 (0.52)	6227 (0.41)	a, b

Figures in parenthesis are percent values; RI: retention index on BP-1 column; a: retention times; b: retention index; c: peak enrichment; d: mass spectra; +: furanoid form; ++: correct isomer not identified.

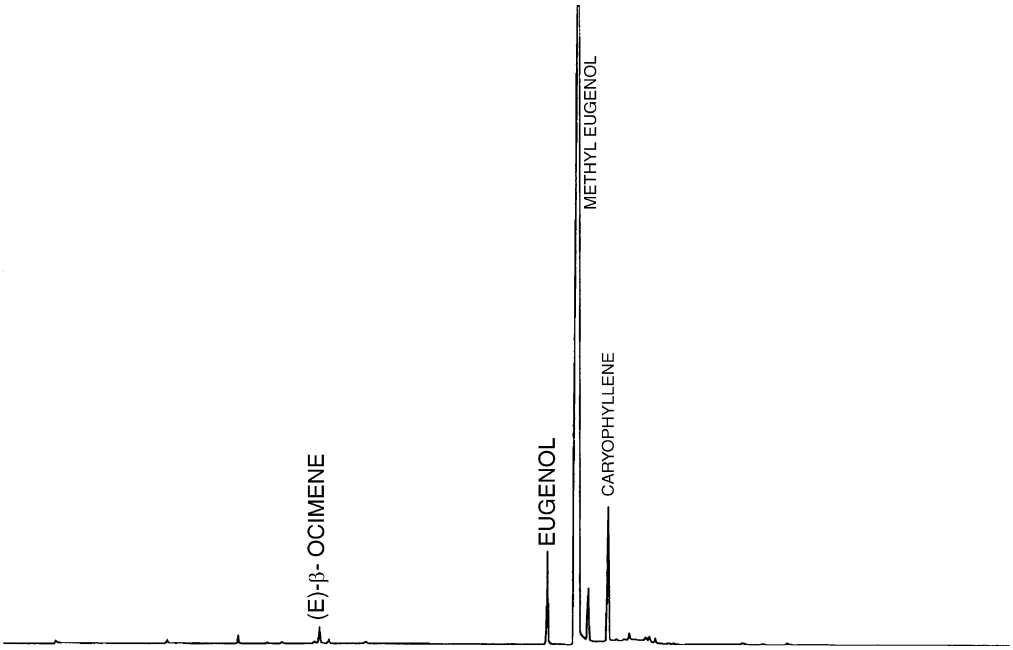

Fig. 1. GC profile of *Ocimum tenuiflorum* oil harvested at 30 cm above ground at first harvest.

recorded highest essential oil content among the three different methods of harvest. Essential oil content in different treatments was in the order of secondary branch > shoot biomass harvested at 30 cm above ground > primary branch. The higher essential oil content in the secondary branch harvest treatment is attributed to relatively lower contribution of thick branches (without leaf and containing very negligible quantity of oil) to total biomass yield [20]. The essential oil content did not vary appreciably between the treatments on harvest of primary branch and shoot biomass cut at 30 cm above ground.

3.4. Essential oil yield

Like biomass yield, the essential oil yield irrespective of method of harvest was in general higher in first harvest and lower in fourth harvest (Table 2). Method of harvest significantly influenced essential oil yield. Maximum essential oil yield was recorded in the secondary branch harvest treatment during all the four harvests. Considering the total essential oil yield (sum total of four consecutive harvests), the secondary branch harvest treatment produced 25.2 and 15.4% higher essential oil yield, compared to primary branch and shoot biomass cut at 30 cm above ground harvest methods (Table 3).

3.5. Essential oil composition

A GC chromatogram of the essential oil representing shoot biomass cut at 30 cm above ground treatment in the first harvest is shown in Fig. 1. Like essential oil yield, treatment differences were observed in respect of essential oil composition (Table 4). Maximum methyl eugenol content in essential oil was observed in the shoot biomass cut at 30 cm above ground treatment, contrary to essential oil yield. Methyl eugenol content in different harvest treatments were in the order of shoot biomass cut at 30 cm above ground > primary branch > secondary branch. In previous studies, marked variation in methyl eugenol content in essential oils from whole herb, leaf, stem and inflorescence of *O. tenuiflorum* was observed [20]. Further, decline in eugenol and methyl eugenol in *O. tenuiflorum* was observed with progressive maturation of leaf [3]. Alteration in composition of biomass (in respect of leaf, stem and inflorescence weight) due to different harvest treatments therefore, is likely to influence oil composition. Although secondary branch harvest treatment showed lower methyl eugenol in essential oil, because of high essential oil yield in this treatment, it produced maximum methyl eugenol yield (93.2 kg/ha), compared to primary branch or shoot biomass cut at 30 cm above ground harvest treatments. The other constituents of essential oil like eugenol, (*E*)-cinnamyl acetate and β-elemene declined in the reverse order such that the lowest content was observed in the 30 cm above ground harvest treatment. Little variability was observed among the treatments in respect of 24 other minor and trace constituents of oil.

The results showed that in *O. tenuiflorum* harvesting of secondary branches gave highest yield of essential oil and methyl eugenol compared to other methods of harvesting.

Acknowledgements

The authors are thankful to the Director, CIMAP, Lucknow and Scientist-in-Charge, CIMAP, Field Station, Hyderabad for providing necessary facilities and Dr. G.R.

Mallavarapu, Senior Scientist (retired), CIMAP for his help in identification of the oil constituents.

References

[1] B.M. Lawrence, J.W. Hogg, S.J. Terhune, N. Pichitakul, Flavor Ind. 3 (1972) 47.
[2] B.M. Lawrence, R.H. Powell, D.M. Peele, in: Proceedings of the VIIIth International Congress of Essential Oils, Cannes, Fedarom Grasse, 1980, pp. 111–117.
[3] B.B. Dey, M.A. Choudhuri, Biochem. Physiol. Pflanz. 178 (1983) 331.
[4] M.S. Malik, M. Rafique, R. Ahmed, Pakistan J. Sci. Ind. Res. 29 (1986) 111.
[5] L. Laakso, T. Seppanen-Laakso, B. Hermann-Wolf, K. Knobloch, Planta Med. 56 (1990) 527.
[6] P. Pushpangadan, B.L. Bradu, in: K.L. Chaddha, R. Gupta (Eds.), Medicinal and Aromatic Plants: Advances in Horticulture, vol. 11, Malhotra Publishing House, New Delhi, India, 1995, pp. 628–657.
[7] J.A. Pino, A. Rosado, M. Rodriguez, D. Garcia, J. Essent. Oil Res. 10 (1998) 437.
[8] M.I.L. Machado, M.G.V. Silva, F.J.A. Matos, A.A. Craveiro, J.W. Alencar, J. Essent. Oil Res. 11 (1999) 324.
[9] M.L. Maheshwari, B.M. Singh, R. Gupta, M. Chien, Indian Perfum. 31 (1987) 137.
[10] M.P. Philip, N.P. Damodaran, Indian Perfum. 29 (1985) 49.
[11] A.K. Vimalan, K. Sadanandan, M.P. Philip, N. Natrajan, M. Murugesan, N.P. Damodaran, in: Proceedings of the 11th International Congress of Essential Oils, Fragrances and Flavours, New Delhi, India, 1989, pp. 181–188.
[12] S.K. Kothari, A.K. Bhattacharya, C.P. Singh, K. Singh, K.V. Syamasundar, S. Ramesh, S. Kumar, J. Med. Arom. Pl. Sci. 22(4A) and 23(1A) (2001) 385.
[13] S.K. Kothari, A.K. Bhattacharya, C.P. Singh, K. Singh, B.R. Rajeswara Rao, K.V. Syamasundar, S. Ramesh, S.N. Garg. S. Kumar, M.P. Darokar, S.P.S. Khanuja, J. Med. Arom. Pl. Sci. (2004) in press.
[14] E. Guenther, The Constituents of Essential Oils, The Essential Oils, vol. 2, Robert E. Krieger Publishing Company, Malabar, FL, USA, 1984, pp. 519–520.
[15] Anonymous, Opinion of the Scientific Committee on Food on Methyl Eugenol (4-Allyl-1,2-Dimethoxybenzene), Health and Consumer Protection Directorate General, European Commission, Brussels, Belgium, 2001.
[16] Anonymous, Council of European Committee of Experts on Flavouring Substances, Publication Datasheet on Methyl Eugenol, Document RD 4.14/2-45 Submitted by the Delegation of Italy for the 45th Meeting in Zurich, 1999.
[17] W.A. Poucher, in: A.J. Jouhar (Ed.), The Raw Materials of Perfumery, Poucher's Perfumes, Cosmetics and Soaps, vol. 1, ninth ed., Chapman & Hall, London, UK, 1991, pp. 148–149.
[18] J.M. Cowley, N. Z. Entomol. 13 (1990) 81.
[19] T.E. Shelly, A.M. Dewire, Ann. Entomol. Soc. Am. 87 (1994) 375.
[20] S.K. Kothari, A.K. Bhattacharya, S. Ramesh, S.N. Garg, S.P.S. Khanuja, J. Essent. Oil Res. (2004) in press.
[21] J.F. Clevenger, J. Am. Pharm. Assoc. 17 (1928) 345.
[22] W.G. Cochran, G.M. Cox, Experimental Designs, Asia Publishing House, New Delhi, India, 1959.
[23] E. Kovats, Adv. Chromatogr. 1 (1965) 229.
[24] N.W. Davies, J. Chromatogr. 503 (1989) 1.
[25] R.P. Adams, Identification of Essential Oils by Ion Trap Mass Spectroscopy, Academic Press, New York, 1989.