Chemical Composition and Phytotoxicity of Volatile Essential Oil from Intact and Fallen Leaves of *Eucalyptus citriodora*

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A total of 23 volatile constituents was identified and characterized by GC and GC-MS in the volatile essential oil extracted from intact (juvenile and adult) and fallen (senescent and leaf litter) leaves of lemon-scented eucalyptus (*Eucalyptus citriodora* Hook.). The leaves differed in their pigment, water and protein content, and C/N ratio. The oils were, in general, monoterpenoid in nature with 18 monoterpenes and 5 sesquiterpenes. However, a great variability in the amount of essential oils and their individual constituents was observed in different leaf tissues. The amount was maximum in the senescent leaves collected from the floor of the tree closely followed by that from juvenile leaves. In all, 19 constituents were identified in oil from juvenile and senescent leaves compared to 23 in adult leaves and 20 in leaf litter, respectively. Citronellal, a characteristic monoterpene of the oil reported hitherto was found to be more (77–78%) in the juvenile and senescent leaves compared to 48 and 54%, respectively, in the adult leaves and leaf litter. In the adult leaves, however, the content of citronellol – another important monoterpene – was very high (21.9%) compared to other leaf types (7.8–12.2%). Essential oil and its two major monoterpenes *viz.* citronellal and citronellol were tested for their phytotoxicity against two weeds (*Amaranthus viridis* and *Echinochloa crus-galli*) and two crops (*Triticum aestivum* and *Oryza sativa*) under laboratory conditions. A difference in the phytotoxicity, measured in terms of seedling length and dry weight, of oil from different leaves and major monoterpenes was observed. Oil from adult leaves was found to be most phytotoxic although it occurs in smaller amount (on unit weight basis). The different toxicity of different oil types was due to the relative amount of individual monoterpenes present in the oil, their solubility and interactive action. The study concludes that oil from senescent and juvenile leaves being rich in citronellal could be used as commercial source of citronellal whereas that from adult leaves for weed management programmes as it was the most phytotoxic.

Key words: Monoterpenes, Growth Inhibition, Chlorophyll Content

Introduction

Essential oils are the volatile oils that occur in plants and provide them with a characteristic odor, flavor and a number of other properties. They are complex mixtures, principally composed of terpenes that are synthesized within plants as secondary metabolites. Within the plant, essential volatile oils act as protectants whereas outside they are involved in a variety of ecological functions such as attractants, herbivore deterrents, stress tolerant and even chemical signals (Langenheim, 1994; Holopainen, 2004; Peñuelas and Llusià, 2004). Besides, they are commercially important and find large scale use in food flavoring and perfumery industry. They possess a wide spectrum of biological activities and their antibacterial, antifungal, insecticidal and pesticidal properties have made them highly sought secondary metabolites (Vokou, 1999; Isman, 2000). In addition, the volatile oil has also been implicated in allelopathic interactions suppressing the germination and growth of other plants (Lorber and Muller, 1976; Kohli, 1990; Angelini *et al*., 2003; Barney *et al*., 2005). This property of essential oils is being exploited for their use as bioherbicide (Dudai *et al*., 1999; Tworkoski, 2002; Singh *et al*., 2005). Moreover, these are generally regarded as safer compounds because of their biodegradable nature (Isman, 2000) and thus may serve as an excellent ecofriendly tool for weed management. It is thus worthwhile to explore the various sources of vola-

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tile oils and their allelopathic nature with a view to use them as novel herbicides.

The genus *Eucalyptus* L'Hér. (family Myrtaceae), a native of Australia and commonly known as gum tree, is represented by around 800 species that are distributed throughout the world (Brooker and Kleinig, 2004). Eucalyptus trees are generally tall with evergreen fragrant foliage containing volatile essential oil. The oil is of commercial importance and rank high in quality as well as in quantity and is used in perfumery and pharmaceutical industry. It is composed of a variety of volatile monoterpenes such as cineole, citronellol, citronellal, limonene, linalool, and α-terpinene (Brooker and Kleinig, 2004). The amount and composition of oil, however, varies with species, metabolic stages besides location and climatic conditions.

Eucalyptus was first introduced in India around 1792 and is now cultivated in about 32.6 Mha mainly for its commercial importance and under various afforestation programmes (FAO, 2001). The most common species planted across the country include Tasmanian blue gum (*Eucalyptus globulus* Labill.), river red gum (*E. camaldulensis* Dehnh.), forest red gum (*E. tereticornis* Sm.) and lemon scent eucalyptus (*E. citriodora* Hook.). Among these, *E. citriodora* – a tall graceful tree with a crown of drooping foliage, is planted in plains of North India in gardens, parks, roadsides and in farmers' fields under various forestry and agroforestry programmes. Depending upon age, the tree displays various morphological phases (Chalchat *et al.*, 2000). The oil of the tree (extracted from leaves, stem, and buds) is rich in citronellal that is commercially very important. Besides, it possesses a wide spectrum of biological activities such as fungicidal (Ramezani *et al.*, 2002), insecticidal (Isman, 2000), nematicidal (Pandey *et al.*, 2000) and phytotoxic (Singh *et al.*, 2005). For commercial and other purposes, however, the oil is extracted from the juvenile and adult foliage. Being evergreen, the leaves of the tree keep falling throughout the year after senescence and are replaced by young and juvenile foliage. As a result, the floor around *E. citriodora* is covered by a matrix of foliage that includes freshly fallen senescent leaves (yellow in colour) and aged undecomposed leaf litter (brown in colour). However, nothing is known about the content and chemical nature of the volatile oil of these leaf types that may also serve as an important bioresource for commercial exploitation and understanding their role in plant growth inhibition thereby regulating vegetation under and near the tree. A study was therefore undertaken to explore the variability in the content and chemical constituents of the volatile oil of lemon-scented eucalyptus (*E. citriodora*) leaves at different stages, *i.e.* intact (juvenile and adult leaves) and fallen (senescent leaves and brown leaf litter), and to determine their role as plant growth suppressant with a view to understand their role in managing vegetation dynamics.

Material and Methods

Collection of material

Nearly 25-year-old trees of lemon-scented eucalyptus (*Eucalyptus citriodora* Hook.) growing on the campus of Panjab University, Chandigarh, India were selected for collection of plant material used in the present study. Juvenile and adult intact leaves were plucked from the trees whereas freshly fallen senescent leaves (yellow in color) and leaf litter (brown coloured non-decayed leaves) were collected from the tree floor. The four types of leaves not only differed in appearance and colour but also in their pigment, water and protein content, and C/N ratio. The data on these parameters are presented in Table I. Amounts of chlorophyll *a*, *b*, total chlorophyll and even carotenoids differed among various leaf types. The differences were particularly evident

Table I. Pigment, protein and water content, and C/N ratio in *E. citriodora* leaves at four stages under study.

Leaf stage	Chl *a* [μg/mg DW]	Chl *b* [μg/mg DW]	Chl *a*/*b*	Total Chl [μg/mg DW]	Carotenoids [μg/mg DW]	Protein content [mg/g DW]	Water content (%)	C/N ratio
Juvenile	1.24 ± 0.26	1.14 ± 0.33	1.089 ± 0.28	2.43 ± 0.12	1.21 ± 0.19	209.1 ± 14.89	57.70 ± 1.96	39.01
Adult	1.82 ± 0.03	0.97 ± 0.12	1.863 ± 0.08	2.81 ± 0.15	0.65 ± 0.14	79.4 ± 1.24	50.03 ± 1.42	39.73
Senescent	0.20 ± 0.02	0.38 ± 0.05	0.508 ± 0.04	0.60 ± 0.07	0.21 ± 0.02	124.6 ± 19.83	38.46 ± 2.18	67.40
Leaf litter	0.15 ± 0.01	0.42 ± 0.03	0.363 ± 0.02	0.57 ± 0.09	0.09 ± 0.01	184.3 ± 4.43	25.63 ± 1.80	54.24

Data presented as mean ± SE; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*.

between intact and fallen leaves and their amounts were higher in intact leaves. Chlorophyll *a* and total chlorophyll contents were found to be maximum in adult leaves and minimum in leaf litter (Table I). Chlorophyll *b* and carotenoids contents were, however, higher in juvenile leaves compared to adult leaves. Water content was maximum in juvenile followed by adult, senescent leaves and least in leaf litter. Amount of total proteins varied among leaf types and was maximum in juvenile leaves compared to adult leaves. This was also supported by the higher amount of N and a lesser C/N ratio in the juvenile leaves (Table I). The highest amount of proteins in juvenile leaves is not surprising owing to greater synthesis and higher metabolic rate during the juvenile stage, which gets slowed down as the leaf matures.

Extraction of oil

Essential oil was obtained from all the four leaf types by hydro-distillation in a Clevenger's apparatus. Nearly 250 g leaves were mixed with 1 l of distilled water in a 2 l round bottom flask fitted with a condenser. The contents in the flask were boiled for 2.5 h, and thereafter the volatile oil was collected from the nozzle of the condenser. It was dried over sodium sulphate, its amount measured and stored at 4 °C for further analysis and use.

Analyses of essential oil

GC analyses were accomplished with a Shimadzu GC-14B gas chromatograph with a flame ionization detector and using a Supelco wax column (60 m × 0.25 mm i.d., film thickness 0.25 μm), working with the following temperature programme: 70 °C for 4 min, ramp at 4 °C/min to 220 °C for 5 min; carrier gas, N_2. Relative amounts of different constituents were determined by computer-based calculation of peak area normalization without any correction factor. Peaks obtained were compared with data obtained from GC-MS.

GC-MS analyses were performed with a Q Mass 910 Perkin-Elmer mass spectrophotometer equipped with fused silica (BP 21) capillary columns (30 m × 0.25 mm i.d., film thickness 0.25 μm). Analytical conditions were as follows: injector and detector temperatures were 230 °C and 250 °C, respectively; oven temperature was programmed from 40 °C (isothermal for 7 min) to 190 °C (isothermal for 20 min) at 5 °C/min; carrier gas, helium.

The compounds were identified on the basis of computer matching of mass spectra using the library search system HP-5872 (Hewlett-Packard), consulting data bases *viz.* Wiley 275 and NBS 75K libraries (McLafferty, 1989), NIST 98 (Stein, 1990) and compilation by Adams (1995).

Growth inhibition studies

The comparative growth inhibitory impact of all the four types of leaf essential oil and their two major monoterpenes (citronellal and citronellol) was studied against four test species – two crops *viz.* wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) and two weeds *viz.* redroot pigweed (*Amaranthus viridis* L.) and barnyard grass [*Echinochloa crus-galli* (L.) Beauv.]. Certified seeds of the crop species were procured from Punjab Agricultural University, Ludhiana, India, whereas those of weeds were locally collected from farmer's fields.

To elucidate comparative phytotoxicity of different essential oil types and their two major monoterpenes, seeds of each test plant were divided into 7 groups (four for essential oil, two for monoterpenes and one for control) of 100 each for each test concentration of oil or monoterpene. Seeds of crops and *A. viridis* were imbibed in distilled water for 8 h whereas those of *E. crus-galli* for 24 h. Imbibed seeds (25 per Petri dish; 4 replicates) were then placed in 15 cm diameter Petri dishes lined with a single layer of Whatman filter circle wetted with 7 ml of distilled water. The filter paper was treated with respective essential oil or monoterpene or distilled water (to serve as control) so as to have a concentration of 0.125 mg/ml or 0.250 mg/ml. Petri dishes were sealed with a paraffin film to avoid volatilization of essential oil. They were placed in an environmentally controlled growth chamber at (24 ± 3) °C, a 16 h/8 h light/dark photoperiod, photon flux density of approx. 150 μmol m^{-2} s^{-1} and relative humidity of around 75% for 8 d. After 8 d, length and dry weight of emerged seedlings of all the test plants in all the treatments were measured.

Estimation of pigment content, total soluble proteins, water content and C/N ratio

Leaf pigments were extracted from leaves (25 mg) in dimethyl sulphoxide (4 ml) at 60 °C for 1 h (Hiscox and Israelstam, 1979) and their amount was determined spectrophotometrically at

470, 645 and 663 nm. Amounts of chlorophyll *a* and *b* and total chlorophyll were estimated using the equation of Arnon (1949) whereas carotenoid content was determined after Lichtenthaler and Wellburn (1983). They were expressed on the basis of dry weight as suggested by Rani and Kohli (1991). Protein content was determined from dried leaf samples (freed of water, pigment, oils and fatty compounds, etc.) as per Lowry *et al.* (1951). Water content was determined by oven drying of the leaf samples at 80 °C for 72 h. Elemental analysis (C and N) was done on a CHN elemental analyzer using dried powder of each leaf type.

Statistical analysis

Significance of observed values of seedling length, dry weight and chlorophyll content in response to the oil treatment was determined over control at $p < 0.05$ and 0.01 by one-way ANOVA followed by separation of means applying Dunnett's test using SPSS package (version 10).

Results and Discussion

The amount of volatile oil was found to be maximum in the senescent leaves followed by juvenile leaves of *E. citriodora*. Compared to (4.80 ± 0.15) ml and (4.73 ± 0.23) ml volatile oil in senescent and juvenile leaves, adult leaves contained only (2.66 ± 0.16) ml of volatile oil per 100 g leaves on dry weight basis. Between juvenile and senescent leaves the difference in the volatile oil content was little and statistically insignificant. In the leaf litter, however, the least content of oil [(2.36 ± 0.04) ml/100 g leaves] was determined and it was nearly half of that found in the juvenile or senescent leaves. Such variability in the content of essential oils (secondary metabolites) is not surprising as the amount of secondary metabolites changes with aging or metabolic state of the plant (Einhellig, 1996). Kong *et al.* (2002) reported that the amount of volatile oils in *Ageratum conyzoides* increases under stress conditions. The greater amount of essential oil in senescent leaves may be related to the physiological stage of the leaves during which growth stimulating hormones like gibberellins and cytokinins decrease. Since the biosynthesis of these hormones and terpenoid-rich essential oils occurs via the mevalonic acid pathway this may result in shift of precursors towards the synthesis of the latter. Alternatively, in the juvenile leaves the maximum amount could be attributed to a higher rate of biosynthetic activity.

The GC and GC-MS analyses of the oil revealed the presence of most of the monoterpenoid compounds and five sesquiterpenes. There were 23 constituents in the oils (18 monoterpenes and 5 sesquiterpenes) that appeared between 5 to 41 min. These represented a combination of hydrocarbon alcohols, cyclic ethers, acyclic aldehydes and acyclic monoterpene esters. The sesquiterpenes identified from the oils were β-carophyllene, aromadendrene, γ-eudesmol, β-eudesmol and α-eudesmol (Table II). In contrast to 19 constituents identified in oil from juvenile and senescent leaves, 23 and 20 were identified in adult leaf and leaf litter oil, respectively. In general, the relative amount of citronellal and citronellol constituted the bulk followed by isopulegol and its isomer isoisopulegol, though their relative amount varied. In all the four types of foliage citronellal was identified to be the major monoterpene followed by citronellol. Similar to the amount of volatile oils, the content of the monoterpene citronellal was also nearly same in juvenile and senescent leaves being nearly 78 and 77%, respectively (Table II). However, it was only 48 and 54% in adult leaves and leaf litter, respectively. In contrast to citronellal, the amount of citronellol was maximum in adult leaves (22%) and minimum in leaf litter (8%), and almost similar (11–12%) in juvenile and senescent leaves (Table II). In addition, other major constituents of the oil were isopulegol and isoisopulegol, and linalool in senescent leaves (Table II). The amount of isoisopulegol was maximum (12.7%) in adult leaves whereas linalool content was quite high in senescent leaves compared to the other types of foliage. β-Carophyllene, the cyclic sesquiterpene identified in all the four types of foliage, was the maximum in juvenile leaves followed by adult leaves (Table II). However, the content of all the eudesmols (α-, β-, γ-) was maximum in leaf litter oil and γ-eudesmol accounted for about 10.6% of the oil components (Table II). The variations in the constituents and their respective amounts in the four types of leaf oils could be due to the difference in the metabolic stage of leaves.

The essential oil from different leaf stages of *E. citriodora* and two major monoterpenes (citronellal and citronellol) exhibited different inhibitory activity against test crops and weeds. Seedling length and seedling weight of test plants were se-

Table II. Chemical characterization of the volatile oils extracted from various leaf stages of *E. citriodora* as revealed by GC and GC-MS analysis.

Constituent	RT [min]	Relative amount (%)			
		JL[a]	AL[a]	SL[a]	LL[a]
α-Pinene	6.25	0.04 ± 0.007	0.10 ± 0.009	0.09 ± 0.006	0.22 ± 0.012
Myrcene	8.30	–	0.01 ± 0.001	–	0.32 ± 0.017
Limonene	10.14	–	0.01 ± 0.001	–	–
1,8-Cineole	11.29	–	0.01 ± 0.001	–	–
Linalool	11.64	1.39 ± 0.019	0.21 ± 0.009	2.77 ± 0.024	Traces
trans-Rose oxide	14.96	0.04 ± 0.006	0.04 ± 0.004	0.03 ± 0.008	Traces
trans-Pulegol	15.59	0.07 ± 0.008	0.01 ± 0.001	0.21 ± 0.007	0.71 ± 0.011
Citronellal	19.70	77.67 ± 3.481	48.33 ± 4.873	76.90 ± 3.872	53.87 ± 4.673
Isopulegol	21.71	1.62 ± 0.108	5.81 ± 0.207	2.14 ± 0.167	4.86 ± 0.219
Isoisopulegol	21.93	4.29 ± 0.184	12.69 ± 0.742	5.14 ± 0.192	8.94 ± 0.276
α-Terpineol	22.72	0.32 ± 0.018	0.54 ± 0.024	0.61 ± 0.021	0.25 ± 0.017
Isolimonene	23.10	0.07 ± 0.002	0.45 ± 0.016	0.22 ± 0.019	0.46 ± 0.031
β-Citronellene	24.29	0.05 ± 0.003	4.81 ± 0.104	1.46 ± 0.097	3.24 ± 0.176
Linalool acetate	25.11	0.32 ± 0.011	0.04 ± 0.006	0.04 ± 0.004	0.09 ± 0.005
Citronellol	26.84	12.17 ± 1.019	21.87 ± 1.871	10.98 ± 1.348	7.81 ± 0.973
Geraniol	28.19	0.34 ± 0.018	0.40 ± 0.024	–	–
Eugenol	31.29	0.21 ± 0.011	0.17 ± 0.007	0.02 ± 0.001	0.33 ± 0.016
Citronellyl acetate	32.13	0.05 ± 0.001	0.16 ± 0.009	0.14 ± 0.007	0.22 ± 0.010
β-Caryophyllene	32.50	0.54 ± 0.097	0.46 ± 0.041	0.14 ± 0.008	0.35 ± 0.019
Aromadendrene	34.97	0.01 ± 0.001	0.34 ± 0.012	0.01 ± 0.001	0.21 ± 0.009
γ-Eudesmol	36.52	0.01 ± 0.001	1.09 ± 0.117	0.50 ± 0.011	10.58 ± 0.488
β-Eudesmol	38.80	0.02 ± 0.001	0.78 ± 0.026	0.31 ± 0.012	3.16 ± 0.378
α-Eudesmol	40.08	–	0.46 ± 0.018	0.20 ± 0.009	1.89 ± 0.134
Total		99.23	98.79	99.42	97.59

Data presented as mean ± SE. [a] JL, juvenile leaf; AL, adult leaf; SL, senescent leaf; LL, leaf litter.

verely reduced in response to all essential oils and monoterpenes tested. Among the test species inhibitory effect on seedling length was higher in the weedy species (*A. viridis* and *E. crus-galli*) than the crop species (*T. aestivum* and *O. sativa*) and *A. viridis* was affected the most (Table III). A higher effect on *A. viridis* was primarily due to the very small size of the seeds compared to the other test plants. Likewise, seedling dry weight of test plants was also reduced by the treatment of essential oil from *E. citriodora* or citronellal and citronellol (Table III). Among the four oil types, the adult leaf oil was most inhibitory whereas the leaf litter oil was least (Table III). The inhibitory effect of monoterpenes on all the test plants except *A. viridis* was, in general, more than that of leaf oils (Table III). In general, the adult leaf oil was most phytotoxic and leaf litter oil was least. The inhibitory effect of juvenile and senescent leaf oil, though of same magnitude, was lesser than adult leaf oil. The growth inhibitory activity study indicates that volatile oil from adult leaves holds a good potential for practical utilization for weed management purposes.

Essential oil from adult leaves contained higher amounts of monoterpenes with an alcoholic group such as citronellol, isopulegol, isoisopulegol compared to juvenile and senescent leaf volatile oil that were rich in citronellal (containing aldehyde group). Oils with a higher proportion of alcoholic monoterpenes have relatively higher solubility in water compared to citronellal which is insoluble in water. Probably, this was the reason that volatile oil from adult foliage was more inhibitory in activity compared to juvenile or senescent leaf oil.

It is thus evident from the above results that although the composition of essential oil from different leaves of *E. citriodora* was more or less the same, yet there was a great variation in the relative amount of constituent monoterpenes. The juvenile and senescent leaves with a relatively higher amount of citronellal (>75%) could serve as an important source for commercial exploitation in the perfumery, where required amount of citronel-

Table III. Effect of volatile essential oils from four leaf stages of *E. citriodora* and their major monoterpenes on seedling length (SL) and seedling weight (DW) of test plants.

Treatment	Conc. [mg/ml]	*A. viridis*		*E. crus-galli*		*T. aestivum*		*O. sativa*	
		SL [cm]	DW [mg]	SL [cm]	DW [mg]	SL [cm]	DW [mg]	SL [cm]	DW [mg]
Control	0	6.39 ± 0.31	0.73 ± 0.08	11.40 ± 0.35	1.27 ± 0.15	17.31 ± 0.89	14.68 ± 1.93	7.43 ± 0.11	4.98 ± 0.39
Juvenile leaf oil	0.125	4.57 ± 0.24*	0.55 ± 0.06*	9.06 ± 0.37*	0.97 ± 0.05*	13.41 ± 0.49*	12.52 ± 0.49*	6.15 ± 0.24*	4.04 ± 0.27*
	0.250	0	0	6.52 ± 0.19**	0.86 ± 0.02*	10.25 ± 1.02**	10.79 ± 1.05*	5.24 ± 0.19*	3.28 ± 0.24**
Adult leaf oil	0.125	2.05 ± 0.16**	0.44 ± 0.09**	6.41 ± 0.27**	0.91 ± 0.03*	12.46 ± 0.49*	12.25 ± 0.87*	4.68 ± 0.34**	3.82 ± 0.14*
	0.250	0	0	4.63 ± 0.31**	0.65 ± 0.05**	8.77 ± 0.76**	10.25 ± 0.73*	2.37 ± 0.28**	3.10 ± 0.31**
Senescent leaf oil	0.125	4.65 ± 0.47*	0.55 ± 0.09*	8.35 ± 0.26*	1.01 ± 0.12*	13.88 ± 0.48*	13.06 ± 0.41*	5.73 ± 0.17*	4.07 ± 0.54*
	0.250	0	0	6.01 ± 0.16**	0.83 ± 0.14*	11.41 ± 0.82**	11.70 ± 0.46*	4.65 ± 0.37**	3.42 ± 0.41**
Leaf litter oil	0.125	5.36 ± 0.34*	0.62 ± 0.11	9.26 ± 0.31*	1.11 ± 0.18*	14.55 ± 0.42*	13.15 ± 1.05*	6.14 ± 0.48*	4.53 ± 0.36*
	0.250	0.33 ± 0.09**	0.06 ± 0.01**	8.04 ± 0.25*	0.99 ± 0.07*	12.59 ± 0.73*	11.93 ± 1.07*	5.64 ± 0.45*	4.03 ± 0.24*
Citronellal	0.125	2.89 ± 0.21**	0.42 ± 0.08**	5.21 ± 0.13**	0.68 ± 0.07**	8.79 ± 0.47**	8.79 ± 0.19**	3.95 ± 0.19**	2.68 ± 0.24**
	0.250	2.30 ± 0.19**	0.35 ± 0.09**	1.96 ± 0.12**	0.30 ± 0.11**	5.12 ± 0.39**	4.08 ± 0.34**	1.77 ± 0.24**	0.52 ± 0.12**
Citronellol	0.125	2.41 ± 0.17**	0.48 ± 0.05*	7.17 ± 0.45**	0.74 ± 0.18**	6.67 ± 0.18**	6.65 ± 0.67**	4.31 ± 0.16**	2.85 ± 0.29**
	0.250	2.07 ± 0.28**	0.43 ± 0.07**	1.92 ± 0.18**	0.27 ± 0.08**	3.57 ± 0.24**	3.97 ± 0.28**	2.09 ± 0.11**	1.49 ± 0.34**

Within a column * and ** represent significant difference from control at $p < 0.05$ and 0.01 applying Dunnett's test.

lal is around 65–80% (Sohounloue *et al.*, 1996). In contrast, the oil from adult leaves was the most phytotoxic and suppressed the weed growth, and thus holds a great potential for future weed management purposes. Further, adult leaves can provide a good source of citronellol and isoisopulegol – other important monoterpenes.

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Adams R. P. (1995), Identification of Essential Oils Components by Gas Chromatography and Mass Spectrometry. Allured Publishers Corp., Carol Stream, Illinois.

Angelini L. G., Carpanese G., Cioni P. L., Morelli I., Macchia M., and Flamni G. (2003), Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. J. Agric. Food Chem. **51**, 6158–6164.

Arnon D. I. (1949), Copper enzymes in isolated chloroplasts: Polyphenylperoxidase in *Beta vulgaris*. Plant Physiol. **24**, 1–15.

Barney J. N., Hay A. G., and Weston L. A. (2005), Isolation and characterization of volatiles from mugwort. J. Chem. Ecol. **31**, 247–265.

Brooker M. I. H. and Kleinig D. A. (2004), Field Guide to Eucalypts. Vol. 3. Northern Australia. Blooming Books, Victoria, Australia.

Chalchat J.-C., Garry R.-P., Sidibe L., and Harama M. (2000), Aromatic plants of Mali (V): Chemical composition of essential oils of four *Eucalyptus* species implanted in Mali: *Eucalyptus camaldulensis, E. citriodora, E. toreliana* and *E. tereticornis*. J. Essent. Oil Res. **12**, 695–701.

Dudai N., Mayer A. M., Putievsky E., and Lerner H. R. (1999), Essential oil as allelochemicals and their potential use as bioherbicides. J. Chem. Ecol. **25**, 1079–1089.

Einhellig F. A. (1996), Interactions involving allelopathy in cropping systems. Agron. J. **88**, 886–893.

FAO (Food and Agricultural Organization) (2001), Global Forest Resources Assessment 2000 Main Report. FAO Forestry Paper 140. Food and Agricultural Organization, Rome, Italy.

Hiscox J. D. and Israelstam G. F. (1979), A method for extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. **57**, 1332–1334.

Holopainen J. L. (2004), Multiple functions of inducible plant volatiles. Trends Plant Sci. **9**, 529–533.

Isman M. B. (2000), Plant essential oils for pest and disease management. Crop Prot. **19**, 603–608.

Kohli R. K. (1990), Allelopathic Potential of *Eucalyptus*, Project Report. Department of Environment, New Delhi, India, p. 199.

Kong C., Hu F., and Xu X. (2002), Allelopathic potential and chemical constituents of volatile oil from *Ageratum conyzoides* under stress. J. Chem. Ecol. **28**, 1173–1182.

Langenheim J. H. (1994), Higher plant terpenoids: Phytocentric overview of their ecological roles. J. Chem. Ecol. **20**, 1223–1280.

Lichtenthaler H. K. and Wellburn W. R. (1983), Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem. Soc. Trans. **11**, 591–592.

Lorber P. and Muller W. H. (1976), Volatile growth inhibitors produced by *Salvia leucophylla*: effects on seedling root tip ultrastructure. Am. J. Bot. **63**, 196–200.

Lowry O. H., Rosebrough L. J., Farr A. L., and Randall R. J. (1951), Protein estimation with folin-phenol reagent. J. Biol. Chem. **193**, 265–275.

McLafferty F. W. (1989), Registry of Mass Spectral Data, 5th ed. John Wiley and Sons, New York.

Pandey R., Kalra A., Tandon S., Mehrotra N., Singh H.N., and Kumar S. (2000), Essential oils as potent sources of nematicidal compounds. J. Phytopathol. **148**, 501–502.

Peñuelas J. and Llusià J. (2004), Plant VOC emissions: making use of the unavoidable. Trends Ecol. Evol. **19**, 402–404.

Ramezani H., Singh H. P., Batish D. R., and Kohli R. K. (2002), Antifungal activity of the volatile oil of *Eucalyptus citriodora*. Fitoterapia **73**, 261–262.

Rani D. and Kohli R. K. (1991), Fresh matter is not an appropriate relation unit for chlorophyll content: experience from experiments on effects of herbicides and allelopathic substances. Photosynthetica **25**, 655–657.

Singh H. P., Batish D. R., Setia N., and Kohli R. K. (2005), Herbicidal activity of volatile oils from *Eucalyptus citriodora* against *Parthenium hysterophorus*. Ann. Appl. Biol. **146**, 89–94.

Sohounloue D. K., Dangou J., Gnomossou B., Garneau F.-X., Gagon H., and Jean F.-I. (1996), Leaf oils of three *Eucalyptus* species from Benin: *E. torelliana* F. Muell., *E. citriodora* Hook. and *E. tereticornis* Smith. J. Essent. Oil Res. **8**, 111–113.

Stein S. E. (1990), Mass Spectral Database and Software, Ver. 3.02. National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA.

Tworkoski T. (2002), Herbicide activity of essential oil. Weed Sci. **50**, 425–431.

Vokou D. (1999), Essential oils as allelochemicals: Research advances in Greece. In: Allelopathy Update. Vol. 2. Basic and Applied Aspects (Narwal S.S., ed.). Science Publishers, Enfield, NY, pp. 47–63.