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Full Length Research Paper

Effect of season on yield and composition of the essential oil of *Eucalyptus citriodora* Hook. leaf grown in sub-tropical conditions of North India

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Seasonal variation of the essential oil of *Eucalyptus citriodora* leaves were analysed by Gas chromatography mass spectrometry (GC-MS) analysis in the trees grown at subtropical conditions of North Indian plains for commercial cultivation and determination of proper harvesting time. At this condition, oil yield ranged between 1.0 to 2.1% during different months. Oil yield was observed to be high during April to September, when temperature was high and the yield was low during November to March, when the temperature was relatively low. However, during rainy season, when both temperature and humidity was high, oil yield was also observed to be quite high (1.8 to 2.1%). At this condition, the major constituent of oil was citronellal (69.7 to 87.4%) followed by citronellol (5.1 to 9.9%), linalool (2.1 to 6.4%), isopulegol (0.9 to 3.1%) and citronellyl acetate (0.4 to 1.2). Concentration of citronellal decreased during the summer and rainy seasons, while concentration of rest of the major constituents increased during this period. Yield of oil and concentrations of citronellal was observed to be comparable to the plants grown commercially in South India but concentration of rest of the constituents decreased considerably.

Key words: *Eucalyptus citriodora,* seasonal variation, essential oil composition, citronellal, citronellyl acetate, linalool, citronellol.

INTRODUCTION

Eucalyptus citriodora Hook. (Family- Myrtaceae), commonly known as 'Lemon-Scented Eucalyptus' or 'Lemon-Scented Gum', is an evergreen tree native to Queensland, Australia (Chen et al., 2007). This tree is highly valued for its citronellal rich essential oil extracted from its leaves. It has been introduced in different countries including India for commercial cultivation. Its oil is widely used in a number of perfumery formulations, toiletries and as disinfectants. Citronellal obtained from the oil is used mainly for the production of synthetic menthol and citronellol. The leaves reported to possess antiseptic properties and are used in the treatment of various skin diseases. Oil of *E. citriodora* reported to possess antibacterial, antifungal, ascaricidal and insect repellent activities (Low et al., 1974; Husain et al., 1988; Ramezani et al., 2002; Singh et al., 2002; Verbel et al., 2009; Luqman et al., 2008). The oil is also observed to be phytotoxic and has potential to be used as herbicide (Batish et al., 2004, 2006a, b, 2007, 2008; Singh et al., 2005, 2006).

In traditional medicine, essential oil is used as antispasmodic and to relieve joint pains (Buchman et al., 1979). This species has been introduced in India during the middle of 19th century (Shiva et al., 1987). It has been observed that the location, season, nature of soil, age of plants and planting density greatly influence the yield of leaves and oil (Singh et al., 1976; Sefidkon et al., 2009). Harvesting of leaves for economic recovery of essential oil during February to June, before the onset of monsoon has been recommended in tropical weather conditions

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(Muralidharan et al., 1974; Nair et al., 1974).

Leaf oil of *E. citriodora* plants, growing in different parts of the world, is characterized by their major constituent's citronellal and citonellol (Zini et al., 2003). Chemical compositions of decanted and recovered oils of *E. citriodora* leaves were examined. The decanted oil was observed to be rich in citronellal, citronellol, citronellyl acetate and β-caryophyllene, respectively. On the other hand, recovered oil was rich in isopulegol, borneol, menthol, neral and eugenol. Citronellal, the major constituent of the decanted oil, was absent in the recovered oil (Rao et al., 2003).

However, the oil isolated from the leaves of the plants growing in Egypt, was reported to be rich in 3- hexen-1-ol and cis-geraniol (Abd El Mageed et al., 2011). So far, commercial cultivation of *E. citriodora* has not been undertaken in the sub-tropical north Indian plains. For the determination of proper harvesting time for higher yield and quality of oil, plants of *E. citriodora* were domesticated at this condition and monthly variation in its leaf oil yield and its constituents has been studied in five year old trees.

MATERIALS AND METHODS

Plant material

The fresh leaves of *E. citriodora* were collected every month (January 2009 to December 2009) from the trees domesticated at the experimental field of CIMAP, Lucknow and hydro-distilled in Clevenger-type apparatus for 3 h to extract the essential oil. The moisture from the oil was removed by anhydrous sodium sulphate, then measured and stored at 4°C prior to analysis.

Gas chromatography (GC) analysis

For GC, a Perkin-Elmer Auto System XL gas chromatograph was used fitted with an Equity-5 column (60 m x 0.32 mm i.d., film thickness 0.25 µm; Supelco Bellefonte, PA, USA). The oven column temperature ranged from 70 to 250°C, programmed at 3°C/min, with initial and final hold time of 2 min, using H_2 as carrier gas at 10 psi constant pressure, a split ratio of 1:30, an injection size of 0.03 µL neat, and injector and detector (FID) temperatures were 250 and 280°C, respectively.

Gas chromatography mass spectrometry (GC/MS) analysis

GC/MS utilized a Perkin-Elmer Auto System XL GC interfaced with a Turbo mass Quadrupole mass spectrometer fitted with an Equity-5 fused silica capillary column (60 m x 0.32 mm i.d., film thickness 0.25 µm; Supelco Bellefonte, PA, USA). The oven temperature program was the same as described in capillary GC; injector, transfer line and source temperatures were 250°C; injection size 0.03 µL neat; split ratio 1:30; carrier gas He at 10 psi constant pressure; ionization energy 70 eV; mass scan range 40 to 450 amu. Duplicate analysis was performed. Quantitative results are mean data derived from GC analysis.

Identification of compounds

Characterization was achieved on the basis of retention time, Kovats Index, relative retention index using a homologous series of n-alkanes (C_8-C_{25} hydrocarbons, Polyscience Corp. Niles IL), coinjection with standards in GC-FID capillary column (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature data (Adams et al., 2001). The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

RESULTS AND DISCUSSION

E. citriodora oil is extensively used in the treatment of various diseases and also has perfumery value (Husain, 1988). Its oil is usually rich in citronellal and citronellol (Zini et al., 2003). However, Egyptian oil was found to be rich in 3- hexen-1-ol and cis-geraniol (Abd El Mageed et al., 2011). For development of its commercial cultivation, plants of *E. citriodora* were grown at sub-tropics of North India and their oil was evaluated.

Total yield of essential oil from the leaves of *E. citriodora* during different months of the year 2009 along with the weather data of the experimental area have been shown in Table 1. The constituents of essential oils have been shown in Table 2. A total of twenty nine compounds have been identified in the oil by GC and GC–MS, which constituted 91.9 to 98.5% of the oil. Yield of the oil from the leaves varied between 1.0 to 2.1% in different months. Oil yield was low (1.0 to 1.1%) during the months of November to March when the temperature was relatively low (min. temp 7 to 16°C; max. temp 23 to 30°C). While, oil yield was high (1.5 to 2.1%) during April to October, when the temperature was relatively high (min. temp 18 to 27°C; max. temp 31 to 40°C). However, during rainy season (July to September), when both temperature and humidity was high, oil yield was observed to be quite high (1.8 to 2.1%).

At the sub-tropical conditions also, the major constituents of the oil were observed to be citronellal (69.7 to 87.4%) followed by citronellol (5.1 to 9.9%), linalool (2.1 to 6.4), isopulegol (0.9 to 3.1%) and cironellyl acetate (5.1 to 9.9%). Rest of the constituents of the oil was very low in their quantity. Concentration of major oil constituent citronellal was observed to be maximum (87.4%) during the month of January. Its concentration gradually decreased with the advent of summer season. In May and June, its concentration became 72 to 73%, while during rainy season (July to August), its concentration decreased further (69 to 71%).

With the advent of autumn, concentration of citronellal again picked up and became quite high (83 to 87%) during winter season, when the day length was also short (Table 2 and Figure 1). On the other hand, it is interesting to note that concentration of rest of the constituents including citronellol, linalool, isopulegol and citronellyl acetate reduced during the winter season and became high during summer and rainy seasons (Figure 2). Concentration of most of the minor constituents like α-thujone, α-pinene, sabinene, myrcene, α-terpenene, p-

Table 1. Weather data of 2009 in experimental area during collection of *Eucalyptus citriodora.*

Months	Temperature (Min) (mean ± SD)	Temperature (Max) (mean ± SD)	Average day length	Average rainfall	Oil yield (%)
Jan	07.21 ± 1.439	23.20 ± 1.097	10.40	0.00	1.0
Feb	12.83 ± 1.138	24.71 ± 2.743	11.20	0.47	1.0
Mar	15.74 ± 2.556	26.63 ± 4.705	12.00	0.00	1.0
Apr	22.98 ± 2.020	37.48 ± 1.311	12.40	0.00	1.7
May	25.45 ± 1.573	38.31 ± 3.179	13.20	1.25	1.6
Jun	25.90 ± 1.674	39.47 ± 4.249	14.00	0.00	1.6
Jul	27.00 ± 0.831	32.25 ± 2.392	13.20	5.64	1.8
Aug	26.55 ± 0.830	31.76 ± 2.017	12.00	14.24	1.9
Sep	25.57 ± 1.027	32.91 ± 2.457	12.00	5.11	2.1
Oct	18.43 ± 2.054	31.87 ± 1.487	11.20	0.00	1.5
Nov	14.87 ± 2.704	29.49 ± 2.126	10.40	0.24	1.1
Dec	09.49 ± 1.180	24.65 ± 1.871	10.00	0.00	1.0

Table 2. Chemical composition of essential oils [%] of *Eucalyptus citriodora* during different months.

Compounds	RI	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
α -thujene	932	0.1	0.1	01.	0.1	0.2	0.2	0.1	t	t	t	t	t
α-pinene	936	0.1	0.2	0.3	0.3	0.3	0.2	0.2	0.1	0.1	t	t	t
Sabinene	967	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.1	0.1	0.2	0.2	0.2
Myrcene	980	t	0.1	0.1	0.2	0.2	0.2	0.1	t	t	0.1	t	t
α-hellandrene	997	t	t	t	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
α –terpenene	1011	-	-	-	0.1	0.37	0.1	0.1	0.1	0.1	0.1	0.1	t
p-cymene	1015	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.1	0.1	0.1	0.1	0.1
Limonene	1024	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	t	t
1,8-cineole	1031	0.1	0.1	0.1	0.2	0.2	0.5	0.8	0.6	0.3	0.3	0.1	0.1
Z-β-ocimene	1034	t	t	t	0.1	0.2	0.1	0.1	0.1	t	t	t	t
E-β-ocimene	1043	0.1	0.2	0.4	0.4	0.8	0.4	0.4	0.3	0.3	0.3	0.3	0.3
Linalool	1085	2.1	2.4	2.7	2.9	3.5	5.1	6.4	5.7	5.0	3.6	3.2	3.1
Citronellal	1135	87.4	85.6	84.2	81.9	73.2	72.6	71.1	69.7	73.7	81.9	83.2	83.8
Isopulegol	1143	1.0	0.9	1.2	1.3	1.9	2.5	3.1	2.9	2.6	1.8	1.6	1.3
Borneol	1152	0.2	0.2	0.3	0.3	0.5	0.5	0.6	0.6	0.6	0.5	0.5	0.4
Menthol	1161	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2
α-terpeneol	1180	-	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.3	0.1	0.2	0.1
Citronellol	1208	5.5	5.7	6.1	7.8	7.9	8.7	9.9	9.4	9.2	6.1	5.1	5.2
Nerol	1222	0.2	0.2	0.1	0.1	1.01	0.1	0.1	0.1	0.1	0.2	0.4	0.2
Geraniol	1240	0.1	0.1	0.2	0.3	0.9	0.1	0.1	0.1	0.1	0.2	0.2	0.3
Geranial	1250	0.1	t	t	t	-	0.1	-	-	0.1	0.1	0.1	0.1
Unidentified	1275	0.2	0.2	0.3	0.3	1.1	0.8	0.6	0.6	0.6	0.2	0.2	0.2
Citronellyl acetate	1331	0.4	0.5	0.8	0.8	0.8	0.8	0.9	1.2	0.6	0.6	0.6	0.4
Eugenol	1342	t	t	-	0.1	0.3	0.1	t	t	-	t	-	t
Geranyl acetate	1365	t	t	-	0.1	0.2	0.1	t	-	-	-	-	t
β-caryophyllene	1425	-	t	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	-	-
Aromadendrene	1446	-	t	t	0.1	0.1	0.2	0.2	0.2	0.3	0.2	0.1	0.1
Cadinene-γ	1509	-	-	-	-	t	t	t	-	-	t	-	-
Cadinene-δ	1516	-	-	-	-	0.1	t	t	-	-	0.1	-	-
Caryophyllene oxide	1571	0.1	0.1	t	t	t	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total identified compounds (%)		98.2	97.2	98.5	98.4	94.28	94.0	95.9	91.9	94.2	97.1	96.4	96.1

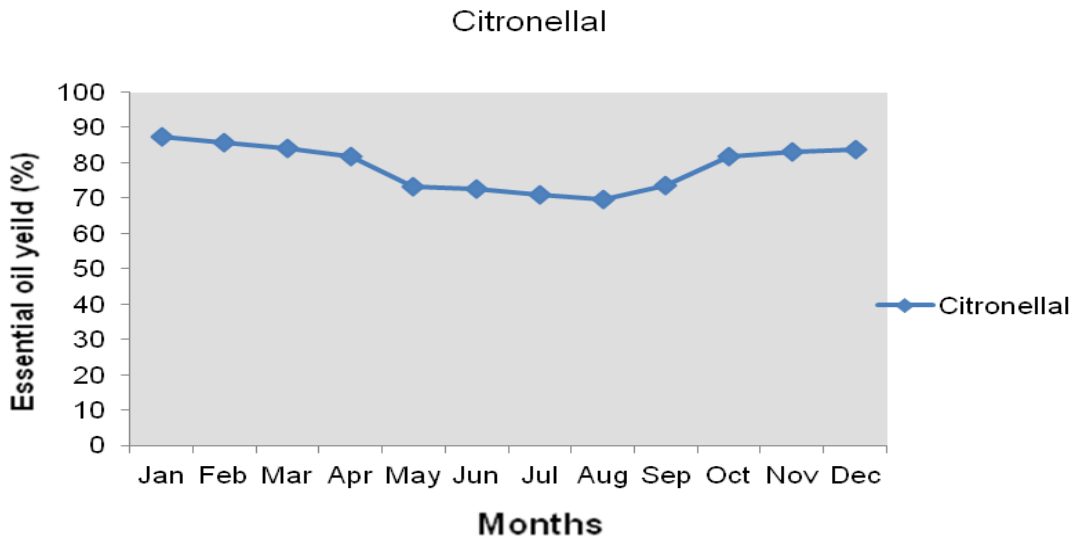

Figure 1. Seasonal variations in oil yield of *Eucalyptus citriodora* throughout the year.

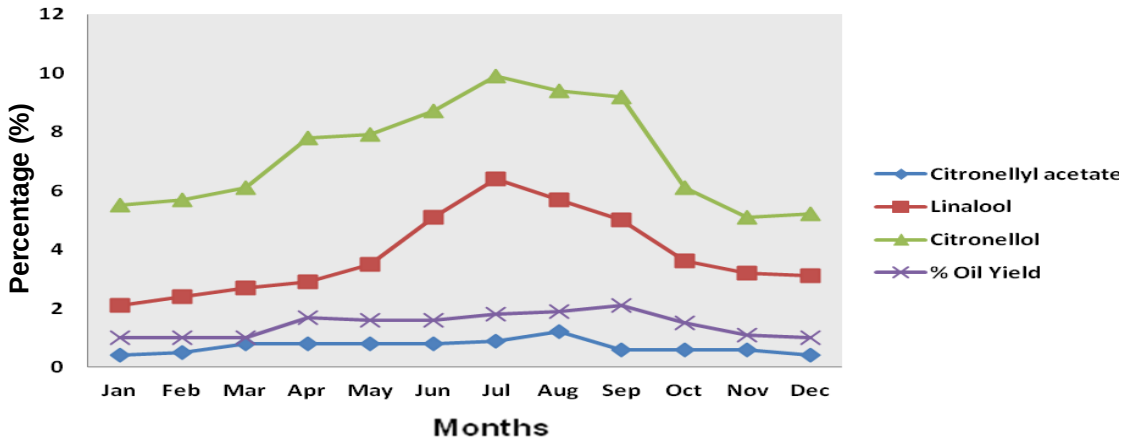

Figure 2. Variation in the content of different major oil constituents of *Eucalyptus citriodora* throughout the year.

cymene, limonene, Z-β-ocimene, E-β-ocimene, menthol, geraniol, eugenol, geranyl acetate and β-caryophyllene also increased during summer season. While concentrations of some minor constituents like 1, 8-cineole and borneol increased during rainy season.

Growing of *E. citriodora* at sub-tropical conditions showed that at this situation where there is wide variation in temperature and humidity, there is comparable yield of oil almost equivalent to commercial cultivation at South India that is, between 1.0 to 2.1% and the main constituent citronellal between 69.7 to 87.4%. However, concentration of citronellol and geraniol reduced considerably. Therefore, for higher yield of oil, the leaves should be harvested during rainy season (July to September) and for the higher yield of citronellal, it should be harvested during winter months (November to March) particularly in the month of January.

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