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A study on developmental changes in essential oil content and composition in *Cymbopogon flexuosus* cultivar Suvarna

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ABSTRACT *Cymbopogon flexuosus* (Steud.) cv. Suvarna produces an essential oil with unique lemon like aroma which is broadly used in flavors, fragrance, perfumery and pharmaceuticals. Here we report changes in essential oil content and compositions during *C. flexuosus* cv. Suvarna leaf development, leaf positions and leaf age. Essential oils were isolated by hydro-distillation of leaves harvested at six different developmental stages (10-50 days), 1st-5th leaf positions and three parts (basal, middle and apical) of the leaves. Analyses of essential oils by gas chromatography and GC-MS have identified 20 different terpenoids constituents with citral as the major monoterpene. The study showed that essential oil content was highest in early stages (10-20 days) of leaf development, then declined substantially. Similar pattern of essential oil content was observed between the 1st-5th leaf positions and the basal-apical parts within a leaf. Essential oil compositions were also markedly fluctuated. Percentage together of geranyl acetate and geraniol declined rapidly from 32% to 4% on 10th and 20th days, respectively; while percentage of citral (geranial + neral) increased correspondingly from 56% to 81% on 10th and 20th days, respectively. Similar changes in monoterpene composition were observed in leaf positions and leaf age (basal to apical part). Thus the study revealed that accumulation of essential oil depends on the developmental stages of the concerned plant parts and changes in essential oil content is also reflected by compositional changes in oil constituents.

Acta Biol Szeged 59(2):119-125 (2015)

KEY WORDS

citral
Cymbopogon
essential oil
geranyl acetate
geraniol
GC-MS
ontogeny

Introduction

Cymbopogon flexuosus is an esteemed member of the genus *Cymbopogon* (Poaceae). It is popularly known as lemongrass and locally Cochin grass or Malabar grass. Lemongrass is tufted perennial grass, with numerous stiff stems arising from a short, rhizomatous rootstock (Weiss 1997). The rhizome produces new suckers that extend vertically as tillers to form dense clumps. In lemongrass, tiller growth usually begins at the apical meristem where cell division occurs, followed by production of axillary buds and the emergence of new tillers (Ganjewala and Gupta 2013). In India, lemongrass is grown in Kerala, Assam, Maharashtra and Uttar Pradesh. Apart from India, lemongrass is also cultivated in large scale in Brazil, Mexico, Dominica, Haiti, Madagascar, Indonesia and China (Ganjewala et al. 2008). Lemongrass has been used in

medicine in India for more than 2000 years but its use for distillation is about only 100 years old.

Cymbopogon species biosynthesize and accumulate essential oils predominantly in the young and rapidly expanding leaves. The surface of the lemongrass leaves does not contain glandular trichomes such as those present in many other aromatic plants. Lemongrass leaves accumulates essential oils in specific oil cells that are present in parenchymal tissues (Lewinsohn et al. 1998; Luthra et al. 2007). The essential oil isolated from aerial parts (leaves) of lemongrass is yellow to reddish-brown in color and the odor is powerful lemon like. Lemongrass essential oil has wide applications in flavor, fragrance, perfumery and pharmaceutical industries (Ganjewala et al. 2008; Ganjewala et al. 2012; Ganjewala and Gupta 2013). It is also used for the synthesis of vitamin A and ionones (b-ionones, methyl ionone, etc.); synthetic citral, derived from conifer turpentine is normally used for these purposes (Dawson 1994). Besides, *Cymbopogon* oils possessed a number of useful bioactivities such as antimicrobial, allelopathic, anthelmintic, anti-inflammatory, anticancer, antioxidant, insect and mosquito repellent (Ganjewala 2009; Ganjewala

Submitted September 9, 2015; Accepted November 11, 2015

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and Gupta 2013; Ganjewala et al. 2014). Essential oils are defined as complex mixture of cyclic and acyclic monoterpenoids which are mainly derived through secondary transformation such as stereo-specific isomerization, acetylation, deacetylation, cyclization and dehydrogenation of geranyl diphosphate (GPP) the universal precursor of monoterpenes. The best known monoterpenoids constituents of lemongrass essential oil are citral (existing as racemic mixture of two isomers geranial and neral), geranyl acetate and geraniol and others are citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, α -carophyllene, methylheptenone (Khanuja et al. 2005; Ganjewala et al. 2008; Ganjewala and Gupta 2013). The monoterpenes are C₁₀ compounds derived from GPP synthesized by fusion of two isopentenyl diphosphate (IPP) produced from the MEP pathway (Gershenzon et al. 2000). Essential oil biosynthesis and accumulation is a highly complex process regulated and controlled by complex interplay of n-number of factors. However, essential oil accumulation is mainly dependent on the developmental stages of the concerned organ/plants parts (Ganjewala et al. 2008). Besides several other factors *viz.*, genetics, inter or intra-specific variation, geographical origin, pre- and post harvesting processes greatly influence essential oil content and compositions. Some of the previously published reports have documented variation in essential oil content and compositions in *Cymbopogon* species (Ganjewala et al. 2008). In the present work we studied essential oil content and composition accompanying leaf development in *C. flexuosus* cv. Suvarna which is a drought resistant cultivar developed by Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India.

Materials and Methods

Plant material

Slips of *Cymbopogon flexuosus* (lemongrass) cv. Suvarna were procured in 2011 from Central Institute of Medicinal and Aromatic Plants (CIMAP; Lucknow, India). It was grown at the Organic Farm House (Amity University, Uttar Pradesh, Noida Campus) following standard agronomical practices. The plants were tagged at the time of emergence of leaves. The plants grow to maturity from 10 to 50 days. Leaves were harvested at six different stages of development first at 5 days intervals from 10-20 days and then 10 days intervals from 20 to 50 days and used for isolation of essential oils. A fully grown lemongrass plant is a cohort of five overlapping leaves. The innermost (1st) leaf is the youngest one while the outermost (5th) leaf is a fully matured. To study the effects of leaf positions, fully matured tillers (25 samples) were harvested and leaves were separated for isolation of essential

oils. Lemongrass leaf grows in a manner typical of monocotyledons: there is an age gradient between the proximal and the distal parts of the leaves. To study the effects of leaf age, immature (15 d old) leaves were collected and cut into three equal parts (basal, middle and apical) and used for isolation of essential oil.

Weight measurements

For the determination of fresh weight (FW) and dry weight (DW) a known numbers of leaves were weighed immediately after harvest and then oven dried till constant weight.

Isolation of essential oil

Leaves (100 g) were cut into small pieces and hydro-distilled for 2-3 h in mini Clevenger apparatus to obtain essential oils (Clevenger 1928). Essential oils were collected in small-stopper vials containing anhydrous sodium sulphate to remove the traces of moisture. Essential oils were isolated in triplicates from the freshly harvested leaves; these essential oil extracts were pooled for gas chromatographic analysis.

Gas chromatographic (GC) analysis of essential oils

Essential oil compositions were analyzed by gas chromatography (GC) (Shimadzu, GC 2010). The GC was equipped with flame ionization detector (FID) stainless steel fused capillary column RTX 5 MS (30 m x 320 μ m i.d., film thickness 0.25 μ m) packed with diphenyl dimethyl polysiloxane on Chromosorb WAW (80-100 mesh). The column temperature isothermal was 200 °C, injector and detector (FID) temperature 250 °C and 260 °C, respectively. Essential oil (100 μ l) was injected while nitrogen used as a carrier gas at a flow rate of 40 ml/min. Major essential oil constituents were identified by co-injecting commercial standards (Sigma-Aldrich) and quantified using a Varian integrator (Model 4400).

Gas chromatography-mass spectrometry (GC-MS)

Essential oils were also subjected to gas chromatography-mass spectrometry (GC-MS) analyses using a Varian 240 GC-MS system. The GC-MS was equipped with a DB-5 (Optima-5) capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μ m); coated with 5% diphenyl/95% polydimethylsiloxane. Oven temperature was 90-260 °C at a rate of 3 C/min, transfer line temperature 320 °C. Carrier gas was helium with a flow rate of 1.21 ml/min, the split ratio was 1:10, the ionization energy was 70 eV, and mass range of 35-400 AMU (atomic mass unit).

Table 1. Essential oil compositions of *C. flexuosus* cv. Suvarna immature (10 d) and mature (50 d) leaves.

Compounds	Essential oil constituents (%)		Kovat Index (KI)
	10 d	50 d	
Geranial	37.10	53.80	1000.90
Neral	19.00	30.90	603.44
Geranyl acetate	24.00	0.61	1000.29
Geraniol	7.00	2.20	1000
Dodecanoic acid	5.31	0.22	1101.25
Citronellol	1.26	0.10	900.78
Citronellal acetate	0.50	0.20	1000.1
Myristolic acid	1.91	2.16	701.07
Caryophyllene oxide	1.12	1.65	1798.12
Neroplomacrol	1.27	0.14	1495.14
Isoamyl acetate	0.53	0.60	1001.63
Linalool	0.89	1.50	999.4
Isogeranial	0.97	1.70	1000
Bergamotene	0.50	0.78	1001.21
Geranic acid	0.10	0.85	1495.4
Triacotane	0.27	0.50	1200.30
Myrcene	0.35	0.20	800.12
β -caryophyllene	0.50	0.65	1414.15
Limonene	0.44	0.08	1000.0

Statistical analysis

The result are presented as mean \pm SEM, n=3. Analysis of data was done by IBM SPSS (statistical package for social science) version 19.0.

Results

Chemical composition of essential oil

The GC and GC-MS analyses revealed the presence of 20 terpenoids constituents in essential oils samples isolated from leaves harvested at 10 and 50 d, respectively (Table 1). Citral, an acyclic monoterpene aldehyde was identified as the major constituent. It is a racemic mixture of two isomers, geranial (citral a) and neral (citral b). The percentages of citral (a + b) in essential oil from 10 d and 50 d old leaves were 56% and 85%, respectively. The percentages of geranyl acetate and geraniol from 10 d old leaves were 25% and 7%, respectively; this is much higher than their concentrations in the essential oil from 50 d old leaves. Other constituents recognized in both the essential oils were geranyl acetate (3.80 and 0.50%), geraniol (3.43 and 1.0%), dodecanoic acid (5.31 and 0.22%), myristolic acid (1.91 and 2.16%), caryophyllene oxide (1.12 and 1.65%), neroplomacrol (1.27 and 0.14%), linalool (0.89 and 1.50%) isogeranial (0.97 and 1.70%), citronellol (1.26 and 0.10%) and β -caryophyllene (0.50 and 0.65%). Besides,

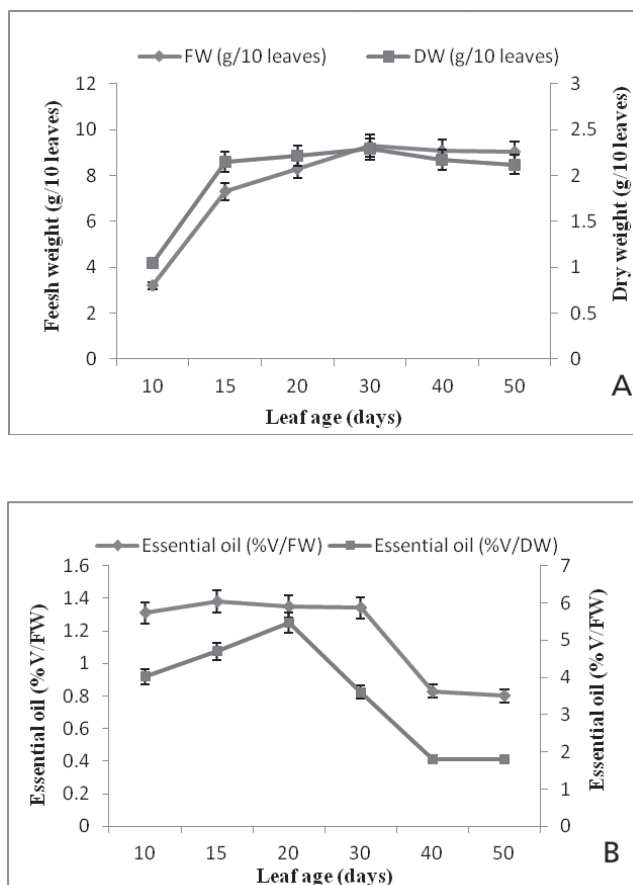


Figure 1. (A) Changes in the fresh and dry weights of leaves, and (B) their essential oil content during *C. flexuosus* cv. Suvarna leaf development.

few more constituents present were geranic acid (0.10 and 1.70%), bergamotene (0.50 and 0.78%), citronellal acetate (0.50 and 0.20%). The remaining nine constituents were present in trace amounts (>0.010% - 0.10%).

Developmental changes in essential oil content and composition

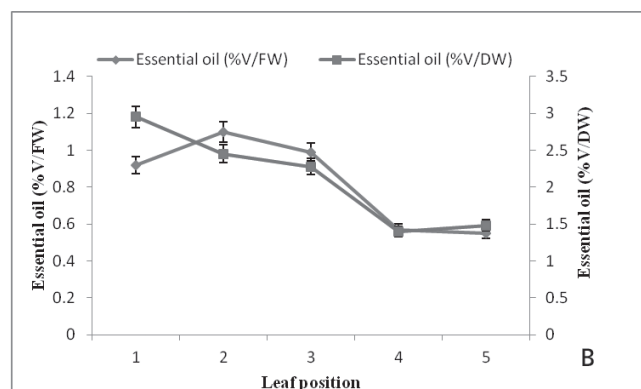
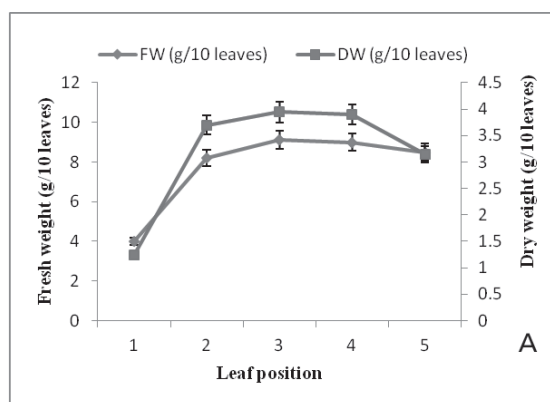
Essential oil contents were measured at six stages of leaf development from 10 to 50 days. Essential oil content (expressed as %V/FW and %V/DW) tend to increase rapidly during early stages (10 to 20 days) of leaf development and then declined considerably in late stages (30 and 50 days) of leaf development. It was observed that FW and DW of leaves also rapidly increased from 10 to 30 days and then declined slowly until the 50th day of the leaf development (Fig. 1).

Essential oil composition changed dramatically as leaves grow older (Table 2). Essential oil from 10-days old leaves showed in addition to citral (56%) presence of relatively high

Table 2. Developmental changes in essential oil composition in *C. flexuosus* cv. Suvarna.

Compounds	Leaf age (days)					
	10	15	20	30	40	50
	% of total monoterpene					
Geraniol	7.0	4.3	2.9	2.2	1.8	1.68
Geranyl acetate	24.2	2.1	0.5	0.61	0.38	0.37
Geranial	37.1	52.9	52.6	53.9	53.8	53.8
Neral	19.0	27.7	29.0	30.3	30.9	30.9
Others	12.8	13.0	13.0	13.0	13.1	13.3

amount of geranyl acetate (24%) and geraniol (7%). The relative proportions of geranyl acetate + geraniol rapidly declined from 32% to 3.5% from the 10th to the 20th day, respectively (Table 2). Levels of these compounds continuously declined until the 50th day. In parallel, levels of citral (geranial + neral) increased substantially from 56% to 81% between the 10th and 20th days; thereafter it remained more or less similar. The decrease in percentage of geranyl acetate and geraniol was corresponding to an increase in percentage of citral. Other

**Figure 2.** (A) Changes in the fresh and dry weights of leaves, and (B) essential oil content depending on the leaf position in *C. flexuosus* cv. Suvarna.

oil constituents did not show any significant changes in their percentage.

Effects of leaf positions and age on essential oil content and compositions

Essential oil content and compositions were influenced by leaf position and leaf age. The study revealed that essential oil content (expressed as %V/FW and %V/DW) was found to be highest between 1st to 2nd leaf positions when leaves were young and rapidly growing (Fig. 2). However, essential oil content declined rapidly from 3rd to 4th leaf positions and become stable in the 5th leaf position. Fresh weight and dry weight of leaves increased significantly from 1st to 3rd leaf positions then declined in subsequent leaf positions (Fig. 2).

Analyses of essential oil composition by GC and GC-MS revealed that relative proportion of major oil constituents markedly changed during 1st-5th leaf positions (Table 3). The total accumulated percentage of geranyl acetate and geraniol, declined substantially from 32% to 5% in the 1st and 2nd leaf positions, respectively; then gently declined reaching the minimum level (1.3%) in the 5th leaf position. In contrast, percentage of citral (geranial + neral) increased substantially in a corresponding manner from ~55% to 81%, in the 1st and 2nd leaf positions, respectively; then slowly increased up to 85% between the 2nd to 4th leaf positions. However, very little decrease in the percentage of citral was observed in the 5th leaf position.

To test whether the developmental stages of the leaf is responsible for the changes observed in essential oil content and composition, 15 days-old leaves were cut equally into three parts (basal, middle and apical) and investigated for essential oil content and composition. Essential oil content (%V/FW and %V/DW) measured was highest in the basal part (Table 4). Analyses of essential oils by GC and GC-MS revealed variation in composition of essential oils isolated from the basal, middle and apical parts. The proportion of geranyl acetate + geraniol in basal part was 9% which declined to 4.6% in apical part while the proportion of citral increased correspondingly.

Table 3. Effects of leaf position on essential oil composition in *C. flexuosus* cv. Suvarna.

Compounds	Leaf positions				
	1	2	3	4	5
	% of total monoterpene				
Geraniol	8.0	3.2	4.5	2.1	1.8
Geranyl acetate	24.0	1.9	0.4	0.5	0.3
Geranial	39.1	53.8	52.8	53.7	51.8
Neral	16.1	27.2	29.1	31.4	31.3
Others	12.8	13.9	13.2	12.3	14.8

Table 4. Effects of leaf age on essential oil composition in *C. flexuosus* cv. Suvarna.

	Leaf parts		
	Basal	Middle	Apical
Essential oil content			
%V/FW	1.38±0.12	1.38±0.14	0.82±0.20
%V/DW	4.74±0.65	5.01±1.1	2.14±0.39
Compounds	% of total monoterpene		
Geraniol	5.2	3.3	3.0
Geranyl acetate	3.9	1.7	1.6
Geranial	53.9	52.8	53.2
Neral	28.1	28.2	31.3
Others	9.0	14.1	11.0

Discussion

The genus *Cymbopogon* comprises more than 145 species all of which are aromatic in nature. Most *Cymbopogon* species provides an essential oil which is characterized by the presence of citral, an acyclic monoterpene aldehyde. In the present work *C. flexuosus* cv. Suvarna yielded an essential oil which is highly rich in citral (80-85%); there were total of twenty monoterpenes present in Suvarna essential oil (Table 1). However, composition of essential oils relies completely on the type and age of the accumulating tissue/part (Ganjewala et al. 2008; Rao et al. 2010). Marked differences could be seen in the percentages of monoterpene constituent of essential oils from immature (10 days-old) and matured (50 days-old) leaves (Table 1). Essential oil composition of *C. flexuosus* cv. Suvarna reported here is consistent with previously published reports (Ganjewala et al. 2008; Patra et al. 1997; Lewinsohn et al. 1998; Baser and Buchbauer 2009; Rohloff 1999). Citral because of its characteristic lemon like aroma is of considerable interest in the flavor, fragrance and perfumery (Ganjewala et al. 2012).

Accumulation of essential oil is dependent on growth and developmental stages of concerned parts/organs/or tissues (Luthra et al. 1991; Ganjewala et al. 2008; Smitha and Rana 2015). Here, in *C. flexuosus* cv. Suvarna we noticed similar observation about essential oil content which rapidly changes with leaf development, leaf position (ontogeny) and leaf age. Therefore, these results are in line with previously published reports favoring developmental and ontogenic regulation of essential oil accumulation in plants. The results suggested, that in *C. flexuosus* cv. Suvarna only young and rapidly expanding leaves are biogenetically active to synthesize and accumulate substantial amount of essential oil. At any stage of the leaf development the net accumulation of essential oil is

the results of the anabolic and catabolic processes. Decline in essential oil content after 30 days may be attributed partially to very significant loss of the essential oil through catabolism as reported previously (Croteau 1987; Luthra et al. 1991; Grehenzon et al. 2000). The patterns of accumulation of essential oils studied here match with the previously published reports in garden sage (*Salvia officinalis*) (Croteau et al. 1981), dill (*Anethum graveolens*) (Porter et al. 1983), caraway (*Carum carvi*) (Bouwmeester et al. 1998), palmarosa (Dubey et al. 2000) and lemongrass (*Cymbopogon flexuosus*) (Singh et al. 1989; Ganjewala et al. 2008; Ganjewala and Gupta 2013). However, in peppermint and in other members of Lamiaceae essential oil and monoterpene levels increased with leaf age. Several other factors, such as extensive overhead irrigation harvesting time or the sampling method influenced the detected essential oil content (Srivastava and Luthra 1991).

The changes in essential oil content are often reflected by quantitative fluctuations in the monoterpene composition (Luthra et al. 1991; Rohloff 1999; Dubey et al. 2000; Ganjewala et al. 2008). In the present study the percentage of geranyl acetate and geraniol declined sharply during 10-20 days period of leaf development while that of citral (geranial + neral) increased correspondingly (Table 2). Similar compositional changes in these monoterpene constituents were also observed between 1st-5th leaf positions and leaf age (Table 3 and 4). These results suggested that geranyl acetate and geraniol are metabolically associated with citral formation. Geranyl acetate is first hydrolyzed by geranyl acetate esterase to produce the geraniol (Dubey and Luthra 2001; Ganjewala and Luthra 2009); in turn the geraniol is oxidized into citral by the action of geraniol dehydrogenase (Sangwan et al. 1993). Higher percentage of geranyl acetate (monoterpene acetate) in early stages of the leaf development observed here is consistent with the previous reports (Porter et al. 1984; Dubey et al. 2000; Ganjewala and Luthra 2009). Previously, compositional changes in monoterpenes has been reported from *Micromeria fruticosa* in which the percentage of some compounds (e.g., limonene, isopulegone and (+)-pulegone) in the oil decreased with leaf maturation while compounds such as neoisopulegone, isopulegol and pulegol increased (Dudai et al. 1992; Telci et al. 2006). Similar developmental changes in essential oil yield and compositions have also been reported from *Mentha*, *C. citratus*, *Nepeta cataria* and *Salvia officinalis* (Rohloff 1999; Gershenzon et al. 2000; Mohammadi and Saharkhiz 2011; Prins et al. 2013; Lakuš et al. 2013).

In conclusion, in *C. flexuosus* essential oil content and composition are greatly influenced by the leaf developmental stages, leaf positions and leaf age. However, there are several other factors like seasons, genetic, biotic and abiotic and geographical factors which may influence the essential oil content and composition.

Acknowledgements

The corresponding author would like to thank Council of Scientific and Industrial Research (CSIR, Government of India, New Delhi) for providing financial support (Grant No. 1235/EMR-II/2010). The technical support from AIRF, JNU (New Delhi, India) is also duly acknowledged. Finally, special thanks to founder Dr. Ashok K Chauhan and Chancellor Mr. Atul Chauhan (Amity University Uttar Pradesh, Noida, India) for providing the necessary support and facilities.

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