

*Full Length Research Paper*

# Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages

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The demand for lemongrass (*Cymbopogon citratus*) is for its high citral content. Early or delayed harvesting of lemongrass affected essential oil and citral content. The objective of the study was to determine the effects of three maturity stages at harvest of lemongrass on essential oil, chemical composition and citral contents. The lemongrass plant was planted using a randomized complete block design with four replications, at the University Agriculture Park, Universiti Putra Malaysia. The plants were harvested at 5.5, 6.5, and 7.5 months after planting. After harvest, the essential oil, chemical composition and citral contents were analysed using gas chromatography-mass spectrometry (GC-MS) analysis. There were significant effects of maturity stages on essential oil and citral contents. Lemongrass harvested at 5.5 and 6.5 months after planting had significantly higher oil contents than those harvested at 7.5 months. A total of 65 compounds were detected from all the three stages of maturity. However, only 13 compounds were present at each of the maturity stage. Among 13 compounds, only 7 compounds ( $\beta$ -myrcene, 3-undecyne, neral, geranial, nerol, geranyl acetate and juniper camphor) had a concentration of greater than 1%. The citral content at 6.5 months after planting was higher by 11.4% than at 5.5 months after planting. The citral content decreased by 5.4% when lemongrass was harvested at 6.5 compared to at 7.5 months after planting. Citral content peaked at 6.7  $\pm$  0.3 months after planting. Thus, maturity stage at harvest influenced essential oil and citral contents of lemongrass. Therefore, lemongrass should be harvested at the appropriate level of maturity in order to achieve high quality essential oil and lower production cost.

**Key words:** Geranial, neral  $\beta$ -myrcene, hydrodistillation, freeze dry.

## INTRODUCTION

Lemongrass is an aromatic plant belonging to the Gramineae family (Akhila, 2010). It is a tall, clumped perennial grass growing to a height of 1 m. The leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm (Sugumaran et al., 2005). The leaf-sheath is tubular in shape and acts as a pseudostem. This plant produces flowers at matured stages of growth (Jaganath et al., 2000). Conversely, flowering has never

been observed under cultivation due to rapid harvesting time. The rhizome produces new suckers that extend vertically as tillers to form dense clumps. Lemongrass can tolerate a wide range of soils and climatic conditions. However, vigorous growth is obtained on well-drained sandy loam soil with high fertility and exposed to sunlight (Sugumaran et al., 2005).

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. Tillering increases in a sigmoidal-shaped curve until the maximum tiller number is reached where the main culms may be difficult to distinguish from the tillers. When most

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tillering has occurred, no more effective tillers are produced, and the late tillers will die due to competition effects (Moldenhauer and Slaton, 2006). Lemongrass is commonly cultivated as a ratoon crop. It is first harvested at 4 to 6 months after planting followed by subsequent harvests at 2 to 3 month interval (Joy et al., 2006). Harvesting is done by cutting at 20 cm above the ground level (Sugumaran et al., 2005).

Essential oils are natural products obtained from plants. They were formed by varied and complex volatile mixtures of chemical compounds, with predominance of terpene associated to aldehyde, alcohols and ketone which were deposited in various structure of the plant (Linares et al., 2005). Lemongrass contains mainly citral (Schaneberg and Khan, 2002) and 1 to 2% essential oil on a dry basis (Carlson et al., 2001). Essential oil and citral of lemongrass were detected to gather at parenchyma tissue cells, specifically in the adaxial surface of leaf mesophyll (Lewinsohn et al., 1998). Citral of lemongrass is a natural combination of two isomeric aldehydes, namely isomers geranial ( $\alpha$ -citral) and neral ( $\beta$ -citral) (Pengelly, 2004). Other unusual active components are limonene, citronellal,  $\beta$ -myrcene and geraniol (Schaneberg and Khan, 2002).

Essential oil and citral contents were influenced by factors such as temperature, light intensity, soil moisture, fertilizer, and maturity stage (Miyazaki, 1965). During maturity, the plant developed from the vegetative to the reproductive stage (Kays, 1999). Research reports showed that overall essential oil production is associated with the early growth stage in plants such as *Cymbopogon flexuosus* (Singh et al., 1989), *Cymbopogon martini* (Sangwan et al., 1982) and *Mentha* (Caskill and Croteau, 1995). In general, the yield of essential oil is highly correlated with the yield of biomass. The production of higher quality oil with high citral content (75%) is determined by the proportion of young leaves to older leaves, when harvested at a given point.

Normally, lemongrass oil is extracted by various ways such as the solvent, accelerated solvent, Soxhlet (Sargenti and Lancas., 1997), dense carbon dioxide (Carlson et al., 2001), solid-phase matrix (Pham-Tuan et al., 2001), and supercritical fluid (Schaneberg and Khan, 2002) extraction methods. However, the common procedure of extracting essential oil is by the hydrodistillation method (Kulkarni et al., 2003). Gas chromatography-mass spectrometry (GC-MS) has been the most applied analytical techniques for essential oil analysis (Masada, 1976) followed by the supercritical fluid extraction-gas chromatography (Liu et al., 1993). Due to the complexity of essential oil compositions, sophisticated instruments such as high performance liquid chromatography in combination with gas chromatography (HPLC-GC) (Mondello et al., 1996) is the preferred analysis. HPLC is effective for a broad class separation of a sample, which can be introduced into a GC for further high resolution separation.

The production of lemongrass have not been extensively studied (Linares et al., 2005) especially in Malaysia. There is also lack of information on best harvesting stage for lemongrass. Since the yield of essential oil and citral content are of importance, it is necessary for farmers to identify the proper harvesting time for lemongrass so as to obtain high quality essential oil, and lower production cost. The objective of this study was to determine the effects of maturity stage at harvest on the essential oil content, chemical composition and citral content of lemongrass.

## MATERIALS AND METHODS

### Field planting

The lemongrass was planted at the University Agricultural Park, Universiti Putra Malaysia, Serdang, Selangor. An experimental plot size of 20 × 32 m was used for planting the lemongrass. The planting materials used were lemongrass stalks of  $\pm 25$  cm in length. The lemongrass stalks were planted at 1 × 1 m spacing. The area was divided into 12 subplots with each subplot with spacing of 4 × 4 m and planted with 16 clusters of lemongrass. For each planting point, 4 stalks were planted directly into the soil by placing the basal part of the stalk within a depth of 2 cm. Lemongrass were harvested at 5.5, 6.5 and 7.5 months after planting.

### Agronomic practices

The plots were maintained by carrying out normal cultural practices. Sprinkler irrigation was set up in the field to water the whole area of lemongrass. Once a day, watering was done, except when it rained. The lemongrass was fertilized using a combination of single fertilizers at the rate of 300 kg N/ha, 100 kg P<sub>2</sub>O<sub>5</sub>/ha and 100 kg K<sub>2</sub>O/ha applied at 1.5, 3 and 4.5 months after planting. The fertilizer was evenly spread around each cluster. Manual weeding was carried out once a week. The lemongrass was harvested at 5.5, 6.5, and 7.5 months after planting. Four clusters of the lemongrass located in the middle of each subplot were harvested by digging them out of the soil together with their roots using a hoe. Then, the roots were cut off, and the remaining dirt on the leaf-stalk was cleaned. After the cleaning process, the lemongrass were tied in a bunch (30 stalk of lemongrass/bundle) and put in a plastic bag. Each plastic bag contained three bundles of lemongrass. The bags were placed into polystyrene boxes and then covered with ice to prevent water loss and reduce respiration rate. Then, the lemongrass was brought to the Postharvest Laboratory of the Faculty of Agriculture, Universiti Putra Malaysia, for laboratory analysis.

### Preparation of plant materials for extraction

The whole plant (leaf sheath and the blade) was used for extraction of the essential oil. Five hundred grams of plant samples were chopped into small pieces and crushed using a mortar and pastel to increase the surface area. Then, the plant samples were dried using a freeze dryer (Beta 1-8LD, Martin Christ, German) at -55°C, and 0.070 mbar for 48 h to remove the moisture content.

### Hydrodistillation of samples

Two hundred grams of the freeze dried sample were subjected to hydrodistillation. The hydrodistillation was carried out by using the

Clevenger equipment at 100°C for 6 h in an all glass Dean and Stark apparatus modified to allow lowest phase return (Sukari et al., 2008). Ten milliliters of the n-hexane were added, to trap the condensed oil, through the top of the condenser. Later and hexane was collected every hour. Then, new portion of hexane was added through the condenser. The mixtures were combined and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> for 24 h and then filtered. Finally, the hexane solution was evaporated or removed by using a rotary evaporator (Eyela N-1001, Tokyo Rikakikai, Japan) at 40°C to give a yellowish essential oil which was then stored at 4°C for further analysis. The yields were calculated based on dry weight of plant materials (Sukari et al., 2008).

#### Determination of essential oil content

The samples were removed after freeze drying and then weighed with a balance. The percentage yield of essential oil was determined using the formula described by Rao et al. (2005) where the amount of essential oil recovered (g) was determined by weighing the oil after moisture was removed. The essential oil percentage was calculated as follows:

$$\text{Percentage of essential oil} = \frac{\text{Essential oil weight} \times 100}{\text{Fresh sample weight} \times \text{air} - \text{dry sample factor}}$$

Where,

$$\text{Air} - \text{dry sample factor} = \frac{100 - \text{moisture content}}{100}$$

#### Determination of chemical constituents

The oil samples were analyzed using the GC-MS (QP5050A, Shimadzu, Japan), equipped with GC-17A, HP5MS (5% phenyl methylsilane). A capillary column BPX5 (30 m x 0.25 mm i.d. and 0.25 mm μm film thickness) was used for separation of the component. Helium gas was used as the carrier gas with a flow rate of 1.1 mL/min. Temperature program for oven was from 50 to 250°C at a rate of 5 min<sup>-1</sup> with holding time of 1 to 10 min. Injector temperature was 250°C, and the injection volume was 1.0 μL. The identification of components of the essential oil was based on comparison of Kovat's retention indices and mass spectra that corresponded with data (Adam, 1989) and mass spectra libraries (National Institute of Standards and Technology 98). The identification criterion employed was based on the selected compounds that represented more than 90% accuracy in the correlation of the mass spectrum with the patterns of the library. The quality for the perfumery of oil was evaluated on the basis of the addition of the phytoconstituents: β-myrcene, neral, citronellal, citronellol and geraniol.

#### Experimental design and statistical analysis

The experimental design used was the randomized complete block design with four replications of lemongrass. The treatments consisted of three maturity stages at harvest; 5.5, 6.5 and 7.5 months after planting. Data was analysed using analysis of variance (ANOVA), and the significant treatment means were separated by least significant difference (LSD) at 95% confidence level (SAS, version 9.1). The relationships between essential oil content and maturity stages at harvest were determined using regression analysis.

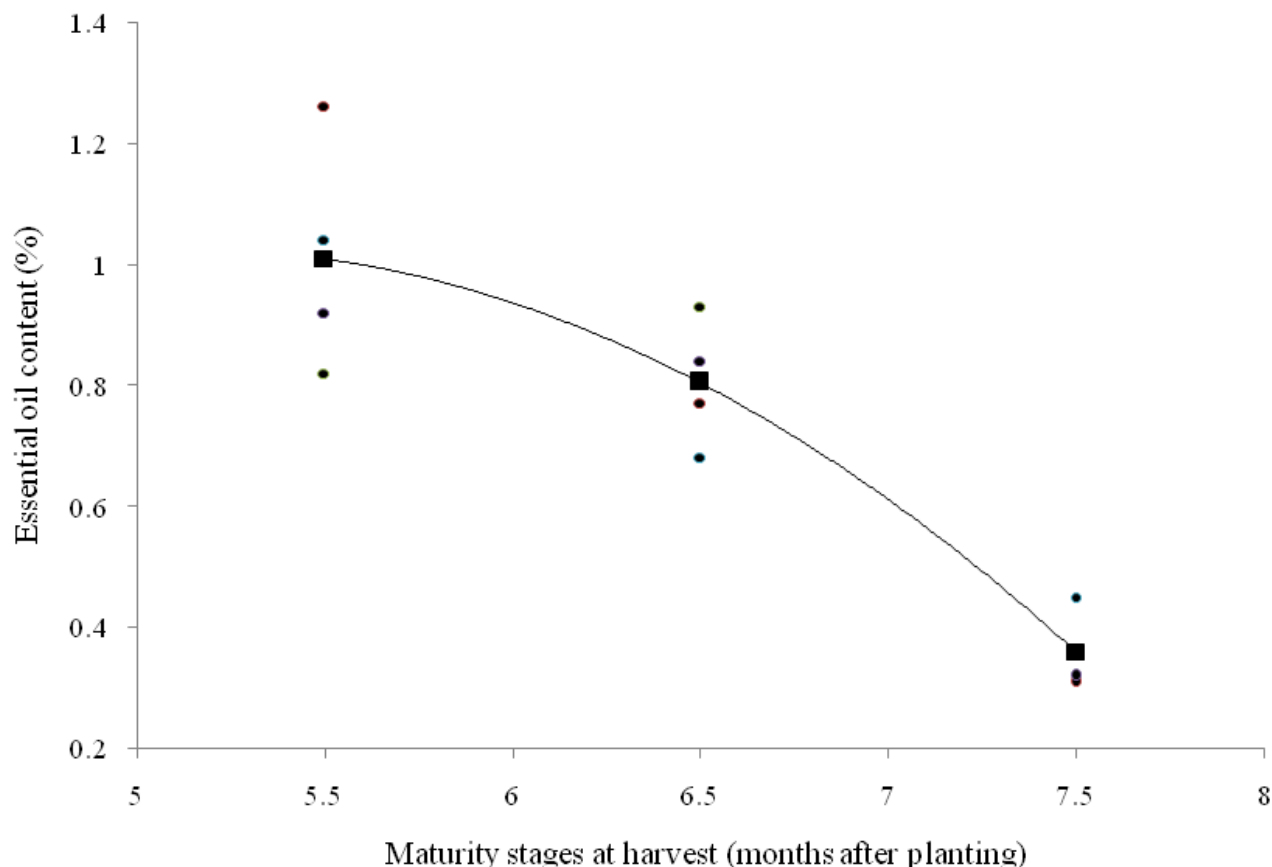
## RESULTS AND DISCUSSION

### Essential oil content (%)

Essential oil content is a crucial criterion in determining the quality of lemongrass oil. Figure 1 shows there was significant negative quadratic relationship between essential oil content and maturity at harvest of lemongrass. Essential oil content decreased with the increase in maturity stages at harvest. The result is in agreement with Ganjawala and Luthra (2007) who studied essential oil biosynthesis of *Cymbopogon flexuosus* (Nees ex Steud) incorporated with (2-14C) acetate. Their findings showed that the yield of essential oil at the initial leaf development of 10 to 15 days was higher (110 to 135 pmol/10 leaves) compared to the yield that was obtained at the end of leaf growth cycle of 40 to 50 days (40 to 50 pmol/10 leaves). Similarly, a study, conducted by Singh and Luthra (1987), on *Cymbopogon flexuosus*, showed that at 10 days after leaf emergence, the essential oil content was extremely low (10 to 20 mg/10 leaves), then gradually increased up to 80 mg/10 leaves by 20 days after leaf emergence. However, the essential oil remained constant and then decreased by 45 days after leaf emergence. A study on quality and quantity of essential oil showed that similar observation was reported in production of essential oil of lemongrass in relation to leaf age (Singh et al., 1989). At 10 days of leaf development, only 1 to 2 mg essential oil/leaf was obtained. However, the level of essential oil sharply increased (8 to 8.30 mg/leaf) at 20 to 40 days of leaf development then decreased as leaf age increased.

These results clearly indicated that only lemongrass leaves, which are still expanding, can quickly synthesize and increase essential oil. Observation from the current study showed that at 5.5 months after planting, lemongrass clumps were still dividing into slips containing 2 to 3 new tillers. Each tiller produced 4 to 5 new leaves, which had the ability to mobilize sucrose, to be converted to monoterpenes. At 7 months after planting, division of the clumps stopped, and the older leaves dried up, thus reducing the essential oil yield. According to Lommis and Croteau (1980), essential oil metabolism was controlled by the balance between photosynthesis and the utilization of photosynthate (sucrose) or the growth differentiation balance. In the growing lemongrass leaf, the sucrose or photosynthate was obtained from older leaves, breakdown of stored starch also from photosynthetic activity (Singh and Luthra, 1987). Increased photosynthetic activity resulted in excess of carbon export compared to import in the growing leaves (Giaquinta, 1978). Excess sucrose from metabolic processes was converted to monoterpenes and finally to accumulation of essential oil. Young lemongrass leaves have the ability to metabolize sucrose because of a high sink capacity (Ho and Baker, 1982). Sucrose mobilization is most rapid during the period of essential oil and citral accumulation.

Essential oil content varies considerably with growth



**Figure 1.** Relationships between essential oil content (%) and maturity at harvest (months after planting),  $y = -0.12x^2 + 1.24x - 2.15$ ,  $R^2 = 0.84$ . Each point (●) represents one replicate of lemongrass,  $n = 4$ .

stages, where the oil biogenesis is associated to rapid growth (Singh et al., 1989). Previous studies have reported that the essential oil content exhibited phase changes during 'Palmarosa' lemongrass (*Cymbopogon martini*) inflorescence development (Dubey and Luthra, 2001). The essential oil content was optimal at the unopened spikelets stage, and, decreased significantly with their development. The results show that immature inflorescence with unopened spikelets is active biogenetically, to accumulate essential oil. A similar result was reported by Mallavarapu et al. (1999) who studied the growth stage of the davana (*Artemisia pallens*) plant. Essential oil yield of davana plants increased with maturity stages, from flower heads emergence (0.98%) to anthesis (0.58%) and initiation of seed set (0.16%).

Other relevant factors that affect production of essential oil are leaf position and different plant parts of lemongrass. The amount and composition of the essential oil was affected by leaf position (Singh et al., 1989). A study on Japanese mint oil production found that the older leaves showed a significant decrease in percentage essential oil content indicating that leaf loss through senescence caused less loss of oil than of dry matter (Duriyaprapan and Britten, 1981). According to Ming et

al. (1996), there were differences in the amount of essential oil obtained from different plant parts of lemongrass grown in Brazil, whereby leaf-blade produced more essential oil (0.42%) compared to leaf-sheath (0.13%). Essential oil yield is higher in the lemongrass leaf-sheath (62.4%) compared to leaf-blade (59.6%) (Mirghani et al., 2010).

According to Lewinsohn et al. (1998), the oil cells of lemongrass were located inside the parenchymatous cells. Other studies on aromatic plants reported that essential oils accumulated inside the glandular trichomes (Werker et al., 1993; Serrato-Valenti et al., 1997), but it had been observed that the surface of lemongrass does not contain glandular trichomes (Lewinsohn et al., 1998). The parenchymatous cells embedded in the adaxial side of the leaf mesophyll were also present in leaf-sheath and leaf-blade of lemongrass (Lewinsohn et al., 1998). Taiz and Zeiger (2010) stated that parenchymatous cells are most abundant and consist of thin-walled, metabolically active cells that carry out a variety of functions in the plant, including photosynthesis and storage. Nevertheless, the essential oil content of lemongrass showed a wide range of variation due to genetic influence as well as agronomic and geoclimatic factors (Rao et al., 1980;

Patra et al., 1990).

### Chemical composition and citral content (%) of essential oil

The essential oil composition of lemongrass differed significantly at different harvesting stages. In this study, a total of 65 components were detected in the essential oil of lemongrass when harvested at 5.5, 6.5 and 7.5 months after planting. There were 44 compounds representing 98.64% of lemongrass essential oil when harvesting was done at 5.5 months after planting. When lemongrass was harvested at 6.5 months after planting, only 15 chemical compounds, representing 98.62% of essential oil, were detected. However, at 7.5 months after planting, 50 chemical compounds were detected representing 97.20% of the essential oil (Table 1). Only 13 compounds were always present at each maturity stage. Among the 13 compounds, only 7 compounds ( $\beta$ -myrcene, 3-undecyne, neral, geranial, nerol, geranyl acetate and juniper camphor) had concentrations greater than 1% (Table 1).

The content of geranial as a major compound in lemongrass oil was increased when the lemongrass was harvested at 5.5 (37.58%) to 6.5 (45.95%) months after planting. However, percentage composition of geranial decreased slightly when the plants were harvested at 7.5 (43.95%) months after planting. There was an increase of 8.37% and a reduction of 2% geranial in lemongrass harvested at 5.5 and 6.5 months after planting, respectively compared to 7.5 months after planting. Among the leading compounds in the lemongrass essential oil, neral appeared to be the second in content. Neral increased from 29.4% at 5.5 months after planting to 31.13% at 6.5 months after planting. Then, it decreased to 31.05% at 7.5 months after planting (Table 1). There was an increase of 1.69% and a reduction of 0.08% neral in lemongrass harvested at 5.5 and 6.5 months after planting, respectively compared to 7.5 months after planting. This result is in agreement with several studies that reported variations of 20 to 50% of geranial and 30 to 40% of neral in lemongrass chemical composition (Weiss, 1997; Pandey et al., 2003; Nath et al., 1994; Chandrashekar and Joshi, 2006). According to Schaneberg and Khan (2002), the essential oil of lemongrass contains mainly geranial and neral. Other isolated components, such as  $\beta$ -myrcene, ocimene,  $\beta$ -ocimene, linalool, citronellal, citronellol, caryophyllene and  $\beta$ -pinene, were present as minor components (Torres and Ragadio, 1996).

According to Niar (1977) the lemongrass essential oil is usually made up of citral at an average of 65 to 80%. Citral is a combination compound of bioactive isomers geranial and neral. There was a significant quadratic relationship between citral content and maturity stage at harvest (Figure 2). This indicated that there was an increase in citral content from 5.5 to 6.5 months

followed by a gradual decrease at 7.5 month after planting. Based

on the regression equation, the citral content was optimal at maturity stage of  $6.7 \pm 0.3$  months after planting.

This relationship is in agreement with the findings of Miyazaki (1965) who reported that citral content was always low in young plants but increased with the advance in age. Singh et al. (1989) reported that geranial content of *Cymbopogon flexuosus* at the earliest study period (10 to 20 days growth stage) increased to reach a maximum level of 8.1% by the 20 days growth stage, and then steadily decreased by 4.6% by the 50 days growth stage. Similarly, neral content increased by 6% from the 10 to 20 days of growth but decreased by 2.6% at the 50 days of growth stage. Other studies on *Cymbopogon flexuosus* leaves indicated that the enzyme, geraniol dehydrogenase, involved in geraniol-citral transformation, was most active in immature leaves (Singh et al., 1990, 1991). The level of citral decreased in leaves nearing maturity because of catabolism where the citral was converted into unidentified oil constituents or other chemical compounds (Singh et al., 1989).

Wijesekera (1981) indicated that the highest quantity of oil for *Cymbopogon citratus*, grown under tropical conditions of Guatemala and Brazil, was obtained six months after sowing. High quality lemongrass essential oil is primarily composed of more than 75% of citral. According to Inan et al. (2011), time of harvest is one of the key factors influencing the chemical composition, quality and quantity of the plant essential oil. The increase in the citral content of lemongrass might also be influenced by fertilizer application. Miyazaki (1965) reported that nitrogen deficiency affected increases in the citral content of lemongrass. He postulated that it was primarily due to the increase in leaf age caused by nitrogen deficiency.

### Conclusion

The essential oil and citral content showed significant differences when lemongrass was harvested at different maturity stages. The optimum percentage of essential oil was obtained when lemongrass was harvested at 5.5 months after planting. There were 65 chemical compounds detected in the essential oil of lemongrass. However, only 13 of the compounds were present in each maturity stage. The percentage citral content and percentage composition of geranial and neral were higher when lemongrass was harvested at 6.5 months after planting. The estimated optimum percentage of citral content was obtained at  $6.7 \pm 0.3$  months after planting. Thus, lemongrass should be harvested between 6.5 to 7.0 months after planting to achieve optimum essential oil with a high composition of citral.

### ACKNOWLEDGEMENT

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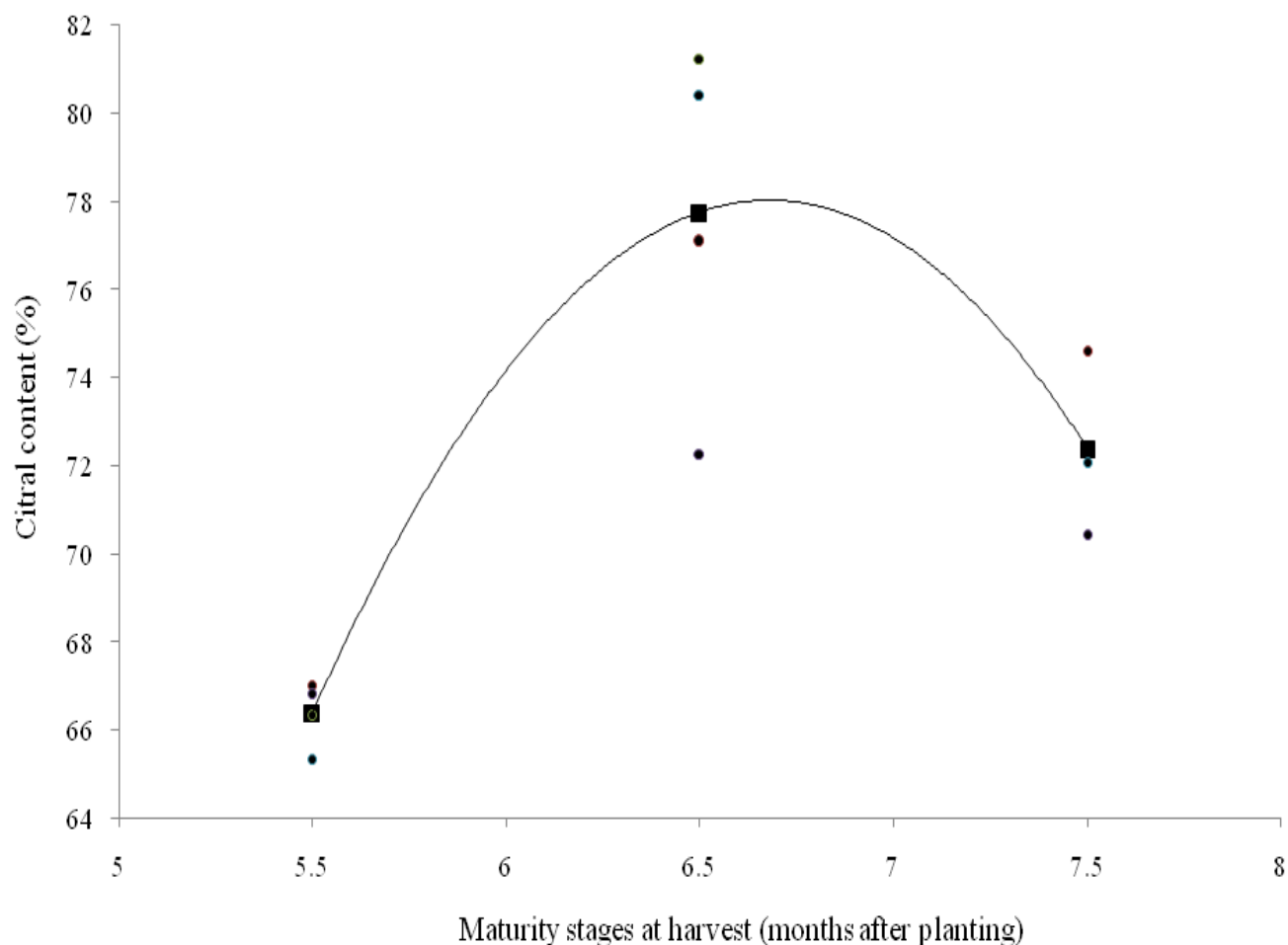
**Table 1.** Effects of maturity stages at harvest (5.5, 6.5 and 7.5 months after planting) on chemical composition of essential oil of lemongrass.

S/N	Compound	R.I.	Oil chemical composition (%)			Molecule formula
			5.5 <sup>a</sup>	6.5	7.5	
1	14-hexadiene,5-methyl-3-(1-methylidene)-	953	0.10	-	-	C10H16
2	$\beta$ -Myrcene	958	5.64	7.68	3.18	C10H16
3	Ocimene	958	0.34	0.26	0.20	C10H16
4	$\alpha$ -Pinene oxide	961	-	-	0.90	C16H16O
5	1,6-Octadiene, 2,6-dimethyl-	968	-	-	0.19	C10H18
6	$\beta$ -Ocimene	976	0.46	0.61	0.32	C10H16
7	1-Octyn-3-ol	977	-	-	0.15	C8H14O
8	Allo-ocimene	993	-	-	0.06	C10H16
9	Myrcenol	1064	0.09	-	-	C10H18O
10	Linalool	1082	0.87	0.60	0.58	C10H18O
11	Trans-chrysanthamal	1088	-	-	0.06	C10H16O
12	3,6,6-Trimethyl-cyclohex-2-enol	1114	0.20	-	-	C9H16O
13	Citronellal	1125	0.32	-	0.21	C10H18O
14	(-)-Isopinocampheol	1125	0.08	-	-	C10H18O
15	1-Pentanol,5-cyclopropylidene-	1128	-	-	0.05	C8H14O
16	3-undecyne	1132	6.08	2.07	1.46	C11H20
17	3-carvomenthenone	1158	-	-	0.01	C10H16O
18	(Z)-linalool oxide (furanoid)	1164	0.10	-	0.32	C10H18O2
19	Neral	1174	29.44	31.13	31.05	C10H16O
20	Geranial	1174	37.58	45.95	43.95	C10H16O
21	$\beta$ -Vatirenene	1179	-	-	0.10	C15H22
22	Citronellol	1179	0.51	0.35	0.40	C10H20O
23	Dextro-carvone	1190	-	-	0.15	C10H14O
24	Cycloisolongifolene	1197	0.35	-	0.07	C15H24
25	Trans-(-)-Carveol	1206	0.30	-	-	C10H16O
26	cis-Carveol	1206	0.16	-	0.12	C10H16O
27	Nerol	1228	3.73	3.50	3.14	C10H18O
28	Methyl n-nonyl ketone	1251	-	-	0.11	C11H22O
29	Oxiranmethanol,3-methyl-3(4-methyl-3-pentenyl)	1269	0.08	-	-	C10H18O2
30	Bicyclopentylone	1273	0.23	-	-	C10H16O
31	Geranic acid	1342	0.16	-	0.88	C10H16O2
32	Geranyl acetate	1352	2.16	1.81	1.06	C12H20O2
33	Isolongifolene, 4,5,9,10-dehydro-	1380	-	-	0.02	C15H20
34	Levo- $\beta$ -elemene	1398	0.34	-	0.22	C15H24
35	$\alpha$ -Gurjunene	1419	0.10	-	0.45	C15H24
36	$\beta$ -Maalinene	1432	0.43	-	-	C15H24
37	$\alpha$ -Bergamotene	1430	0.62	-	0.52	C15H24
38	$\gamma$ -Muurolene	1435	0.89	-	-	C15H24
39	$\alpha$ -Muurolene	1440	-	-	0.20	C15H24
40	(Z)- $\beta$ -Farnesene	1440	0.30	-	0.18	C15H24
41	$\alpha$ -Amorphene	1440	0.10	-	-	C15H24
42	$\beta$ -Sesquiphellandrene	1446	0.08	-	0.07	C15H24
43	$\alpha$ -Farnesene	1458	0.13	0.27	0.11	C15H24
44	$\delta$ -Cadinene	1469	-	0.18	0.95	C15H24
45	Valencene	1474	-	-	0.09	C15H24
46	$\alpha$ -Selinene	1474	-	-	0.18	C15H24
47	Valencene	1474	0.11	-	-	C15H24
48	$\alpha$ -Vulnesene	1490	0.23	-	0.41	C15H24
49	$\alpha$ -Guaiene	1490	0.14	-	0.10	C15H24

Table 1. Continue

50	Isocaryophyllene	1494	1.15	0.69	0.63	C15H24
51	$\beta$ -Caryophyllene oxide	1507	-	-	0.18	C15H24O
52	$\alpha$ -Elemol	1522	-	-	0.05	C15H26O
53	Germacrene D	1515	0.31	-	0.31	C15H24
54	Viridiflorol	1530	0.16	-	-	C15H26O
55	Humulene	1579	0.30	-	0.24	C15H24
56	$\tau$ -Cadinol	1580	0.18	-	0.20	C15H26O
57	$\alpha$ -Cardinol	1580	0.90	-	1.30	C15H26O
58	$\tau$ -Muurolol	1580	-	0.27	-	C15H26O
59	$\beta$ -Eudesmol	1593	0.11	-	0.19	C15H26O
60	1(5)-Guaien-11-ol	1614	0.19	-	0.16	C15H26O
61	Juniper camphor	1647	2.82	1.28	1.56	C15H26O
62	(E,E)-Farnesal	1656	-	-	0.10	C15H24O
63	trans,trans-Farnesal	1656	0.07	-	-	C15H24O
64	Pimelyl dihydrazide	2003	-	-	0.06	C7H16N4O2
65	Di-n-octyl phthalate	2832	-	1.97	-	C24H38O4

<sup>a</sup> Months after planting, R.I. = Retention index of gas chromatogram.



**Figure 2.** Relationships between citral content (%) and maturity at harvest (months after planting),  $y = -8.37x^2 + 111.76x - 295.21$ ,  $R^2=0.82$ . Each points (●) represents one replicate of lemongrass,  $n = 4$ .

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## REFERENCES

- Adams RP (1989). Identification of essential oil by Ion Trap Mass Spectrometry. San Diego, C.A: Academic.
- Akhila A (2010). Essential Oil-bearing Grasses: The genus *Cymbopogon*. Medical and aromatic plants-industrial profile. Taylor and Francis Group, L.L.C.
- Carlson LHC, Machado RAF, Spricigo CB, Pereira LK, Bolzan A (2001). Extraction of lemongrass essential oil with dense carbon dioxide. J. Supercritical Fluids. 21: 33-39.
- Caskill D, Croteau R (1995). Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (*Mentha piperita*) rely exclusively on plastid derived isopentenyl diphosphate. Planta. 197: 49-56.
- Chandrashekar KS, Joshi AB (2006). Chemical composition and antihelmintic activity of essential oils of three *Cymbopogon* species of South Canara, India. J. Saudi Chem. Soc. 10: 109-111.
- Dubey VS, Luthra R (2001). Biotransformation of geranyl acetate to geraniol during palmarosa (*Cymbopogon martinii*, Rox. wats. var. Motia) inflorescence development. Phytochem. 57(5): 675-680.
- Duriyaprapan S, Britten EJ (1981). The effects of age and location of leaf on quantity and quality of Japanese Mint oil production. J. Expt. Bot. 33(4): 810-814.
- Ganjawala D, Luthra R (2007). Essential oil biosynthesis and metabolism of geranyl aceta and geraniol in developing *Cymbopogon flexuosus* (Nees ex Steud) Wats Mutant cv. GRL-1 leaf. Am. J. Plant Physiol. 2(4): 269-275.
- Giaquinta R (1978). Source and sink leaf metabolism in relation to phloem translocation. Plant Physiol. 61: 380-385.
- Ho LC, Baker DA (1982). Regulation of loading and unloading: pathway and mechanisms. Plant Physiol. 56: 225-230.
- Inan M, Kirpik M, Kaya A, Kirici S (2011). Effects of harvest time on essential oil composition of *Thymbra spicata* L. growing in flora of Adiyaman. Adv. Environ. Biol. 5(2): 356-358.
- Jaganath IB, Ng LT (2000). Herbs: The garden pharmacy of Malaysia. Malaysia: MARDI.
- Joy PP, Baby PS, Samuel M, Mathew G, Joseph A (2006). Lemongrass: The fame of Cochin. India J. Arecanut, Species Med. Plants. 8(2): 55-64.
- Kays SJ (1991). Postharvest physiology of perishable plant products. Van Nostrand Reinhold, New York, pp. 55-88.
- Kulkarni RN, Baskaran K, Ramesh S (2003). Five cycles of recurrent selection for increased essential oil content in East Indian Lemongrass: response to selection, and effects on heritabilities of traits and inter trait correlations. Plant Breeding. 122: 131-135.
- Lewinsohn E, Dudai N, Tadmor Y, Katzir I, Ravid U, Putievsky E, Joel DM (1998). Histochemical localization of citral accumulation in lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf., Poaceae). Ann. Bot. 81: 35-39.
- Linares S, Gonzalez N, Gómez E, Usubillaga A, Darghan E, (2005). Effect of the fertilization, plant density and time of cutting on yield and quality of the essential oil of *Cymbopogon citratus* Stapf. Revist de la Facultad de AgronCa LUZ. 22: 247-260.
- Liu Z, Ostrovsky I, Farnsworth PB, Lee ML (1993). Instrumentation for comprehensive two-dimensional Supercritical Fluid-Gas Chromatography. Chromatographia, 35: 567-573.
- Loomis WD, Croteau R (1980). Biochemistry of terpenoids. In Stumpf P. K (Ed) The biochemistry of plants. Academic Press, New York. 4: 363-481.
- Mallavarapu GR, Kulkarni RN, Baskaran K, Rao L, Ramesh S (1999). Influence of plant growth stage on the essential oil content and composition in *davana* (*Artemisia pallens* Wall.). J. Agric. Food Chem. 47: 254-258.
- Masada Y (1976). Analysis of essential oils by GC and MS. John Willey and Sons, Chichester.
- Ming L, Figueiredo R, Machado S, Andrade R (1996). Yield of essential oil and citral content in different parts of lemongrass leaves (*Cymbopogon citratus* (D.C) Stapf.) Poaceae. In: Proceeding of the international symposium on medicinal and aromatic plants. Acta Hort. Leiden: ISHS, pp. 555-559.
- Mirghani ME, Parveen J, Liyana Y (2010). Comparison studies of chemical composition analysis from different parts of lemongrass (*Cymbopogon citratus*) essential oil. In: Proceeding of the 9th International annual symposium on sustainability science and management. pp. 232-237.
- Miyazaki Y (1965). Grass and oil yields from lemon-grass and the quality of oil. Japanese J. Trop. Agric. 37-41.
- Mondello L, Dugo G, Bartle, KD (1996). On-line HPLC-HRGC-MS for the analysis of natural complex mixtures. J. Chromatogr. Sci. 34:174-181.
- Moldenhauer K, Slaton N. (2006). Rice Production Handbook Publications. Retrieved October 7, 2009, from [http://www.uaex.edu/Other\\_Areas/publications/PDF/MP192/chapter1.pdf](http://www.uaex.edu/Other_Areas/publications/PDF/MP192/chapter1.pdf).
- Nair EVG (1977). Essential oil of East Indian lemongrass: Present position in India and scope of its development. Cultiv. Util. Med. Aromat. Plants, pp. 204-206.
- Nath SC, Saha BN, Bordoloi DN, Mathur RK, Leclercq PA (1994). The chemical composition of the essential oil of *Cymbopogon flexuosus* (Steud) Wats. growing in Northeast India. J. Essent. Oil Res. 6: 85-87.
- Pandey AK, Rai MK, Acharya D (2003). Chemical composition and antimycotic activity of the essential oils of corn mint (*Mentha arvensis*) and lemon grass (*Cymbopogon flexuosus*) against human pathogenic fungi. Pharma. Biol. 41: 421-425.
- Patra DD, Singh R, Singh DV (1990). Agronomy of *Cymbopogon* species. Current Res. Med. Arom. Plants, 12: 72-84.
- Pengelly A (2004). The Constituents of medicinal plants (Eds): An Introduction to the chemistry and therapeutics of herbal medicine. United Kingdom: CABI Publishing. pp. 85-103.
- Pham-Tuan H, Vercammen J, Sandra P (2001) A versatile robotic arm for static headspace sampling with SPME. LC-GC Europe, 14: 215-228.
- Rao BL, Verma V, Sobti SN (1980). Genetic variability in citral containing *Cymbopogon khasianus*. Indian Perf. 24: 13-16.
- Rao BRR, Kaul PN, Syamasundar KV, Ramesh S (2005). Chemical profiles of primary and secondary essential oils of palmarosa (*Cymbopogon martinii* (Roxb.) Wats. var. Motia Burk.). Indust. Crops Prod. 21(1): 121-127.
- SAS, Institute. (1990). SAS user's guide: Statistics, version 9. SAS Institute, North Carolina, Cary.
- Sangwan NK, Dhindsa KS, Malik OP, Sharma GD, Paroda RS (1982). Quantitative changes in levels of essential oil in *C. martinii* var motia during different growth stages and on ageing the harvested crop in the field and laboratory. In: Proc. Nat. Sem. Med. Arom. Plants., India.
- Sargenti SR, Lancas FM (1997). Supercritical fluid extraction of *Cymbopogon citratus* (DC.) Chromatographia, 46: 285-290.
- Schaneberg BT, Khan IA (2002). Comparison of extraction methods for marker compounds in the essential oil of lemongrass by GC. J. Agric. Food Chem. 50: 1345-1349.
- Serrato-Valenti G, Bisio A, Carnara L, Ciarallo G (1997). Structural and histochemical investigation of the glandular trichomes of *Salvia aurea* L. leaves and chemical analysis of this essential oil. Ann. Bot. 79: 329-336.
- Singh N, Luthra R (1987). Sucrose metabolism and essential oil accumulation during lemongrass (*Cymbopogon flexuosus* Stapf.) leaf development. Plant Sci. 57: 127-133.
- Singh N, Luthra R, Sangwan RS (1990). Oxidative pathways and essential oil biosynthesis in the developing lemongrass (*Cymbopogon flexuosus*) leaf. Plant Physiol. Biochem. 28: 703-710.
- Singh N, Luthra R, Sangwan RS (1991). Mobilization of starch and essential oil biogenesis during leaf ontogeny of lemongrass (*Cymbopogon flexuosus* Stapf.). Plant Cell Physiol. 32: 803-811.
- Singh N, Luthra R, Sangwan RS, Thakur RS (1989). Metabolism of monoterpenoids in aromatic plants. Current Res. Med. Arom. Plants. 11: 174-197.
- Sugumaran M, Joseph S, Lee KLW, Wong KW (2005). Herbs of



- Malaysia. Shah Alam: Federal Publication.
- Sukari MA, Mohd Sharif NW, Yap ALC, Tang SW, Neoh BK, Rahmani M, Ee GCL, Taufiq Yap YH, Yusof UK (2008). Chemical constituents variations of essential oils from rhizomes of four Zingiberaceae species. *The Malaysian J. Anal. Sci.* 12(3): 638-644.
- Taiz L, Zeiger E (2010). Plant tissue systems: dermal, ground, and vascular: dermal tissue. *Plant Physiology*, Fifth Edition Online. <<http://www.plantphys.net>> Accessed [August 12, 2011].
- Torres RC, Ragodio AG (1996). Chemical compositions of the essential oil of Philippines *Cymbopogon citratus* D.C Stapf. *Philippines J. Sci.* 125: 147-156.
- Weiss EA (1997). Lemongrass. In Weiss E. A. (Eds.) *Essential oil crops*. pp 86-103: Cambridge University Press, Cambridge.
- Werker E, Putievsky E, Ravid U, Dudai N, Katzir I (1993). Glandular hairs and essential oil in developing leaves of *Ocimum basilicum* L (Lamiaceae). *Ann. Bot.* 71: 43-50.
- Wijesekera R (1981). *Practical manual on: The essential oils industry*. Thailand Institute of Scientific and Technological Research. Central Institute of Medical and Aromatic Plants, India. United Nations Industrial Development Organization. Vienna Austria.