

Full Length Research Paper

Influence of plant age on the content and composition of essential oil of *Cymbopogon citratus* (DC.) Stapf.

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Received 3 September, 2014; Accepted 29 September, 2014

An experiment was carried out in Viçosa, Brazil to evaluate the influence of plant age (3, 6, 9 and 12 months) on the content and chemical composition of essential oils from lemon grass, using a completely randomized design with three replications. The plants were harvested between 7:00 and 8:30 a.m, and harvesting cut made at 20 cm height from the base of the leaf. The collected material was immediately taken to the laboratory where the leaves were selected, and subsequent determination of moisture content and other chemical analysis done. The essential oil extraction was carried out by hydrodistillation. The identification of the chemical components of the lemon grass volatile oil was performed by gas chromatography and mass spectrometry (GC-MS). The quantification of the chemical composition of essential oils was made by the gas chromatograph in conjunction with a flame ionization detector (GC-FID). Based on the results obtained, it can be concluded that plant age did not influence statistically, the essential oil content extracted from the lemongrass leaves. However this provided significant changes in its chemical composition.

Key words: Medicinal plants, lemongrass, volatile oils, monoterpenes.

INTRODUCTION

Cymbopogon citratus (D.C) Stapf is a native plant species from India and is widely distributed around the globe in tropical areas, including Brazil, where it has different popular names: lemongrass, holy-grass, catinga-grass, cidrão-grass, cidilho-grass, cidro-grass and ciri-grass. It belongs to the family Poaceae, also known as

Gramineae, which includes approximately 668 genera and about 9,500 species distributed universally, with some of them of great economic importance. Brazil is one of the countries where this plant is perfectly acclimatized (Negrelle and Gomes, 2007).

Tea from its leaves is used popularly in Brazil as an

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antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative and its essential oil is widely used for perfumes and cosmetics (Carlini et al., 1986). Its sedative and antispasmodic actions are attributed to the monoterpene citral, while the analgesic activity is due to myrcene. Currently, the essential oil of lemongrass has been the focus of research in fighting cancer and AIDS (Puatanachokchai et al., 2002; Wright et al., 2009). The oil from *C. citratus* were also found to possess anti-microbial, antifungal, insect repellent and antioxidative action (Velluti et al., 2004; Khadri et al., 2010; Singh et al., 2010; Sonker et al., 2014). For these numerous applications, the essential oil of lemongrass has demand in domestic and international markets, and their prices are considered extremely rewarding.

The most important component of the essential oil of *C. citratus* is citral (40 to 80%), while β -myrcene and geraniol compound make up the majority of the remaining percentage. Citral is a mixture of the isomers geranial (α -citral) and neral (β -citral) (Lewinsohn et al., 1998).

According to the United Nations Comtrade statistics, fragrance and flavor global market was estimated at US\$ 24 billion in 2011, growing at an annual rate of 10%. The major consumers in the multi-billion dollar global essential oils market are United States (40%), Western Europe (30%) and Japan (7%), with trade in essential oils and related products increasing at about 10% per year. Brazil has a prominent place in the production of essential oil, along with India, China and Indonesia. Brazil's position is largely due to her production of the essential oils of citrus (orange, lemon and lime), which are byproducts of the juice industry (Bizzo et al., 2009).

The essential oil production in medicinal plants can be affected by several factors such as plant spacing, cutting height, season, age, harvest time and drying method. These factors may influence directly the essential oil production and, therefore, its pharmacological properties. Some studies have stated the influence of these factors in medicinal plants like *Cymbopogon citratus* (Blank et al., 2007), *Juniperus excelsa* (Shanjani et al., 2010) and *Mikania Glomerata* (Rocha et al., 2014). However, studies on plant age of *C. citratus* are still rare. The plant age should be determined not only because of the plant mass to be harvested, but also because of the active ingredients content, without which the product attracts lower prices from end-users.

The objective of this study, therefore, was to evaluate the influence of different harvest dates on the quantity and quality of essential oil from lemongrass.

MATERIALS AND METHODS

Site selection and *C. citratus* cultivation

C. citratus leaves from plantations located in the experimental irrigation and drainage section of the Federal University of Viçosa, Viçosa-MG, Brazil, were used. The cultivation was carried out in a greenhouse measuring 52.5 m². The seedlings were propagated by

cuttings and transplanted in spacing of 0.30 m between rows and 0.30 m between plants as recommended by Blank et al. (2007). The greenhouse cover was constructed in semicircular shape, with 7.5 m length, 7 m width and a central height of 5 m and covered with low density polyethylene film of 150 μ m.

Irrigation

Different irrigation treatments were used. The treatments were:

1. Treatment 1 (T1) = 50% of ET_c;
2. Treatment 2 (T2) = 75% of ET_c;
3. Treatment 3 (T3) = 100% of ET_c;
4. Treatment 4 (T4) = 125% of ET_c.

Where ET_c = crop evapotranspiration

All treatments were irrigated twice a week. The applied irrigation were calculated by the software IRRIPPLUS® with the registration of daily data through a weather station, installed inside the greenhouse. The data obtained from weather station were relative humidity, maximum, average and minimum temperature, air velocity and solar radiation. Through these data, the daily evapotranspiration, the irrigation to be applied in each treatment and the time of irrigation were calculated. This was subjected to chemical analysis, but for only treatment 2 (T2 = 75%) because it obtained the greatest essential oil content. For this treatment of irrigation (T2 = 75%) the effect of plant age (3, 6, 9 and 12 months) in the content and quality of the essential oil was evaluated.

Statistical analyses

The design was completely randomized with three replications and results subjected to analysis of variance ($P \leq 0.05$). The treatment means were compared by Tukey test at 5% probability.

Sample collection

The species to be used were collected between 7:00 and 8:30 am and the cutting height was set at 20 cm from the basal end of the leaf. After harvesting, the fresh materials were transported to the appropriate laboratory where the leaves were selected, removing diseased and damaged parts and other extraneous plant parts. Three samples were randomly selected to evaluate the moisture content and perform the chemical analysis. Because of non-availability of a standard methodology for the determination of medicinal and aromatic plants in Brazil, the determination of moisture content was performed by gravimetric method as proposed by the ASAE (2000) for forage and similar plants. This was done by placing 25 g of the in natura leaves in a stove with forced air circulation at 103 + 2°C for 24 h, each done in triplicate.

Extraction procedures, analyses and identification

The extraction and analysis of chemical components of the essential oil of lemongrass were performed at the Laboratory of Analysis and Synthesis of Agrochemicals (LASA), located in the Department of Chemistry, Federal University of Viçosa. The essential oil was extracted by hydrodistillation utilizing clewenger equipment (modified). The flask was loaded with 90 g of in natura leaves of lemongrass. One liter of distilled water was added, which was the volume sufficient to cover the material. To facilitate the extraction, the leaves in natura were transversely cut every 2 cm. The extraction time was 90 min, counted from the moment of boiling, as determined in preliminary tests. The analysis of the chemical

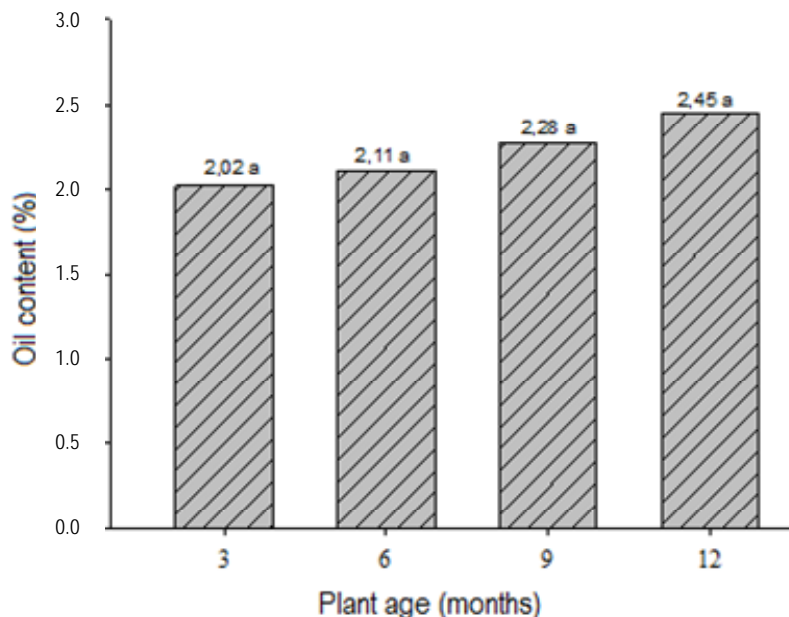


Figure 1. Content of essential oil of *Cymbopogon Citratus* obtained as a function of plant age. Mean of 3 treatments, followed by the same letter do not differ by Tukey test ($P < 0.05$).

chemical components of the essential oil of lemongrass consisted of identifying and quantifying the active ingredients of essential oil and followed the methodology described in Adams (1995).

The essential oil components of lemongrass was identified using a gaseous chromatograph system coupled to a mass spectrometer (Shimadzu GC-EM, GC-17A/QP-5000) and equipped with a capillary column DB-5 (30 m \times 0.25 mm (ID) \times 0.25 μ m film). Helium was used as the carrier gas at a flow rate of 1.8 ml/min, split ratio of 1:5 and the solvent cutting time of 5 min. The temperature of the injector was 220°C and that of the detector, 240°C. Initial temperature in the oven was kept at 40°C for 2 min, and was increased at a rate of 3°C per minute until 178°C. This temperature was maintained for 2 min, with total time of analysis of 50 min. Only ions at charge mass (m/z) ratios between 30 and 700 were detected by the mass spectrometer. The sample volume injected was 1 μ l, at a concentration of 10,000 ppm with hexane as a solvent.

The identification of components was conducted by comparing mass spectrometer obtained experimentally with those from the equipment database and comparison of the Kovats index for each component. Calculating this index required the injection of a mixture of hydrocarbons (C8 to C24), using the retention times of these as base for the calculation of Kovats index of the components of essential oils. For quantification of constituents of essential oils of lemongrass, we used the gas chromatograph coupled to a flame ionization detector (GC-FID), manufactured by Shimadzu, model QP 5000 and SPB-5 column of 0.25 mm thick, 30 m long, 0.25 mm internal diameter. Nitrogen was used as carrier gas at a flow rate of 1.8 ml min^{-1} , split ratio of 1:30, the solvent cutting time 5 min, injector temperature of 220°C and temperature in the flame ionization detector 240°C. The temperature of the column was programmed to start at 40°C and remain for 2 min, after an increase of 3°C per minute up to 178°C, when again it was maintained for 2 min, with a total analysis time of 50 min. The volume of sample injected was 1 μ l at a concentration of 10,000 ppm, using hexane as the solvent. The chemical constituents

present in the essential oil were quantified based on normalization method. The calculations were computer programmed and connected to the GC-FID.

RESULTS

Essential oil content

Figure 1 shows the percentages of essential oil and the statistical analysis obtained in different plant ages. It appears that plant age did not significantly influence the amount of essential oil extracted from the leaves of lemongrass.

Quality of the essential oil

Identification and quantification of the main chemical components of the essential oil from the leaves of lemongrass was performed to evaluate the influence of plant age on the quality of this oil. Figure 2 shows only one chromatogram of essential oil from lemon grass leaves, because no variation was observed in the presence of the chemical components identified in the oil, only variations in the concentrations of same. Table 1 presents the retention times and Kovats Index (KI) calculated and tabulated of the main chemical components of the essential oil from the leaves in natura of lemongrass. Statistical analysis of the main components of the essential oil of lemongrass for the plant age is show in Table 2.

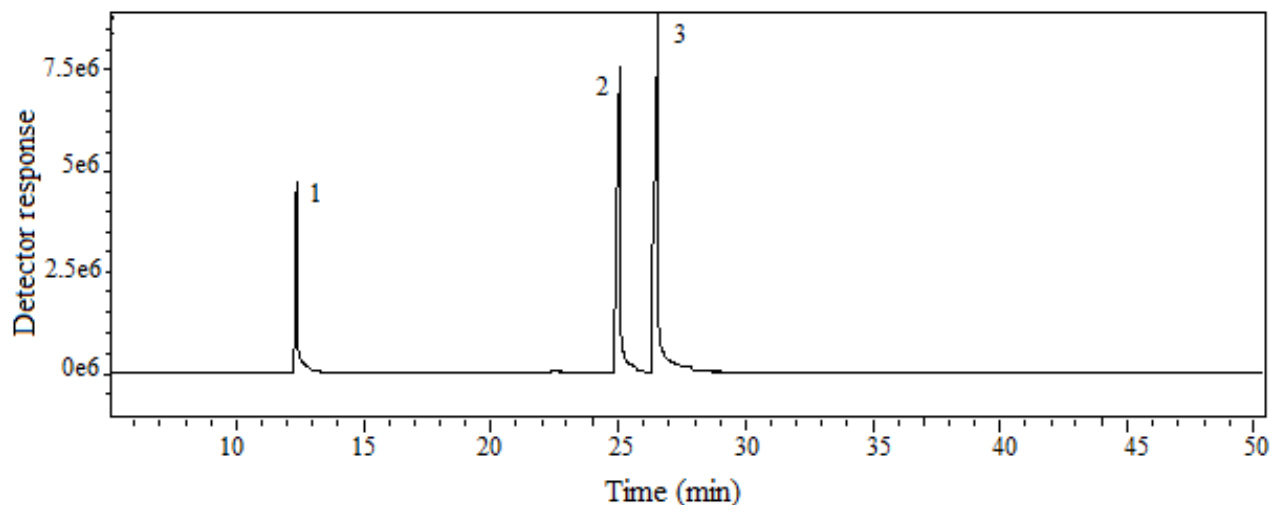


Figure 2. Chromatogram of essential oil of *Cymbopogon citratus* leaves in natura, obtained by gas chromatography. Compounds: 1 - myrcene; 2 - neral; 3 - geranial.

Table 1. Main components of the essential oil from the leaves in natura of *Cymbopogon citratus*, with their retention times and Kovats indexes calculated and tabulated.

Peak	Components	Retention time (min)	Kovats Index (calculated)	Kovats Index (tabulated)
01	Myrcene	12.53	988	991
02	Neral	25.01	1242	1240
03	Geranial	26.43	1272	1270

Table 2. Concentration of main chemical constituents of the essential oil extracted from in natura leaves of *Cymbopogon citratus*, depending on the plant age.

Plant age (months)	Components of essential oil (%)			
	Myrcene	Nera	Geranial	Citral
3	14.15 ^b	33.04 ^b	41.74 ^b	74.78 ^b
6	7.80 ^a	36.74 ^a	52.98 ^a	89.72 ^a
9	9.98 ^a	37.41 ^a	51.12 ^a	88.53 ^a
12	9.59 ^a	35.67 ^a	50.74 ^a	86.41 ^a

Means followed by different lowercase letters in the same column differ significantly by Tukey test at 5% probability.

DISCUSSION

Essential oil content

Opposite results were found by Leal et al. (2003) who observed gradual decrease in oil content of lemongrass with increasing plant age. Koshima et al. (2006) while evaluating different plant ages (6, 9, 12 and 15 months) on the amount of essential oil extracted from the leaves of the same medicinal species noted that the harvest,

when the plant age was 12 months, produced the least amount of essential oil. On the other hand, at 6 and 15 months, greater quantities of essential oils were produced. The authors were of the view that increased production of essential oil at 6 and 15 months was due to lower rainfall in samples taken in winter and autumn.

Most probably, irrigation was one of the reasons the results of the present study were different from those reported by Koshima et al. (2006). This study was conducted in a greenhouse with the same irrigation provided

throughout the year.

The appropriate plant age varies according to the plant organ, stage of development and the time of year. Analyzing the effects of two plant age on the content of essential oil from the leaves of *Melissa officinalis*, Meira et al. (2011) found that the essential oil content showed no significant difference between the periods evaluated, and the average oil content in the two cuts was 0.018%. Similarly, May et al. (2010), while evaluating the production of essential oil of *Rosmarinus officinalis* at different plant ages, found that the yield of essential oil was not affected over time. The variation in oil content depending on the age of the plant appears to be a factor that varies with the species. In a study of two plant ages (60 and 120 days after transplanting) of *L. alba*, Santos et al. (2004) reported that the most essential oil production occurred at 120 days. However for *C. citrates* a reduction in oil content with plant age was observed (Leal et al., 2003). Mint Innecco et al. (2003) have also observed that essential oil increased between 80 and 95 days after planting.

Quality of the essential oil

It can be observed in Table 2, that at the age of 3 months, the mycerne component was significantly higher compared to the other treatments. However, for the major component, citral (geranial + neral), the plants aged 6, 9 and 12 months showed higher concentrations compared to younger plants (3 months). Possibly the plant age influences the quality of the essential oil of medicinal plants because they have a higher concentration of active ingredients in certain periods of the year. The increase over the harvest dates may be due to the approximation of such a period. This variation can be also attributed to physiological and environmental factors. These results indicate that for the range of 1 year after the transplanting of lemongrass seedling, the ideal age to plant to harvest in order to produce the most important component of the essential oil of this species (citral) may vary between 6 to 12 months for growing conditions studied. According to Koshima et al. (2006), the concentration of citral essential oil of lemongrass varied depending on plant age (6, 9, 12 and 15 months), and is produced in higher concentrations at 6 and 15 months of age. Similar results were observed by Bezerra et al. (2008), who noted changes in the chemical composition of the essential oil of *Egletes viscosa* according to the plant age. Santos et al. (2012) studied the plant age (3, 6, 9 and 12 months) on the chemical composition of the essential oil of *A. zerumbet* and verified variations in chemical composition of this oil between six and nine months. However, May et al. (2010) evaluated the quality of the essential oil of *R. officinalis*, and concluded that the concentrations of active ingredients of essential oil of this species were not affected by the plant age.

Conclusion

The content of essential oil extracted from the leaves of *C. citratus* did not change significantly when measured at different harvest times, but there were statistical changes in its chemical composition.

ACKNOWLEDGMENTS

The authors thank JL Pereira for the technical support provided by the qualitative analysis of essential oil and CNPq and FAPEMIG for financial support.

Conflict of Interest

Authors have not declared any conflict of interest.

REFERENCES

- Adams RP (1995). Identification of essential oil components by Gas Chromatography mass spectroscopy. Allured Publishing Corporation. P 469.
- American Society of Agricultural Engineers (ASAE) (2000). Standards Engineering Practices Data: Moisture measurement-forages. ASAE S358.2. St. Joseph, MI: American Society of Agricultural Engineers. P 569.
- Bezerra AME, Filho SM, Oliveira LDM, Silveira ER (2008). Produção e composição química da macela em função da época de colheita. *Hortic. Braz.* 26(1):26-29.
- Bizzo HR, Hovell AMC, Rezende ECM (2009). Óleos essenciais no Brasil: aspectos gerais, desenvolvimento e perspectivas. *Quim. Nova.* 32(3):588-59.
- Blank AF, Arrigoni-Blank MF, Amancio VF, Mendonça MC, Santana Filho LGM, (2007). Densidades de plantio e doses de biofertilizante na produção de capim limão. *Hortic. Braz.* 25(3):343-349.
- Carlini EA, Contar JDP, Silva-Filho AR, Silveira-Filho NG, Frochtengarten ML, Bueno OF (1986). Pharmacology of lemongrass (*Cymbopogon citratus* Stapf) I. Effects of teas prepared from the leaves on laboratory animals. *J. Ethnopharmacol.* 17(1):37-64.
- Innecco R, Cruz GF, Vieira AV, Mattos SH, Chaves FCM (2003). Espaçamento, época e número de colheitas em hortelã rasteira (*Mentha x villosa* Huds). *Rev. Ciênc. Agron.* 34(2):247-251.
- Khadri A, Neffati M., Smiti S, Fale P, Lino ARL, Serralheiro MLM, Araujo MEM (2010). Antioxidant, antiacetylcholinesterase and antimicrobial activities of *Cymbopogon schoenanthus* L. Spreng (lemon grass) from Tunisia. *Food Sci. Technol.* 43(2):331-336.
- Koshima FAT, Ming LC, Marques MOM (2006). Produção de biomassa, rendimento de óleo essencial e citral em capim-limão, *Cymbopogon citratus* (D.C.) Staff, com cobertura morta nas estações do ano. *Rev. Braz. Plantas Med.* 8(4):112-116.
- Leal TCAB, Freitas SP, Silva JF, Carvalho AJC (2003). Produção de biomassa e óleo essencial em plantas de capim-cidreira (*Cymbopogon citratus* (Dc.) Stapf.) em diferentes idades. *Rev. Braz. Plantas Med.* 5(2):61-64.
- Lewinsohn E, Dudai N, Tadmor Y, Katzir I, Ravid U, Putievsky E, Joel DM (1998). Histochemical localization of citral accumulation in lemon grass leaves (*Cymbopogon citratus* (DC.) Stapf, Poaceae). *Ann. Bot.* 81:35-39.
- May A, Suguin OE, Martins AN, Barata LES, Pinheiro MQ (2010). Produção de biomassa e óleo essencial de alecrim (*Rosmarinus officinalis* L.) em função da altura e intervalo entre cortes. *Rev. Braz. Plantas Med.* 12(2):195-200.
- Meira MR, Manganotti SA, Martins ER (2011). Crescimento e produção de óleo essencial de *Melissa officinalis* L. nas condições climáticas

- de Montes Claros-MG. *Biotemas* 24(1):1-8.
- Negrille RRB, Gomes EC (2007). *Cymbopogon citratus* (DC.) Stapf: chemical composition and biological activities. *Rev. Braz. Plantas Med.* 9(1):80-92.
- Puatanachokchai R, Kishida H, Denda A, Murata N, Konishi Y, Vinitketkumnuen U (2002). Inhibitory effects of lemon grass (*Cymbopogon citratus*, Stapf) extract on the early phase of hepatocarcinogenesis after initiation with diethylnitrosamine in male Fischer 344 rats. *Cancer Lett.* 183(1):9-15.
- Rocha RP, Melo EC, Barbosa LCA, Radunz LL (2014). Effect of drying air temperature on the quality of essential oil from *Mikania glomerata*. *Eng. Agric.* 22(3):239-245.
- Santos MRA, Innecco R, Soares AA (2004). Caracterização anatômica das estruturas secretoras e produção de óleo essencial de *Lippia alba*(Mill.) N.E. Br. em função do horário de colheita nas estações seca e chuvosa. *Rev. Cien. Agron.* 35(2):377-383.
- Santos MRA, Jezler CN, Oliveira ARMF, Oliveira RA, Mielke MS, Costa LCB (2012). Harvest time and plant age on the content and chemical composition of the essential oil of *Alpinia zerumbet*. *Hortic. Braz.* 30(3):385-390.
- Shanjani PS, Mirza M, Calagari M, Adams RP (2010). Effects drying and harvest season on the essential oil composition from foliage and berries of *Juniperus excelsa*. *Indust. Crops Prod.* 32(2):83-87.
- Singh P, Shukla R, Kumar A, Prakash B, Singh S, Dubey NK (2010). Effect of *Citrus reticulata* and *Cymbopogon citratus* essential oils on *Aspergillus flavus* growth and aflatoxin production on *Asparagus racemosus*. *Mycopathologia* 170(3):195-202.
- Sonker N, Pandey AK, Singh P, Tripathi NN (2014) Assessment of *Cymbopogon citratus* (DC.) Stapf. essential oil as herbal preservatives based on antifungal, antiaflatoxin, and antiochratoxin activities and in vivo efficacy during storage. *J. Food Sci.* 79(4):628-634.
- Velluti A, Sanchis V, Ramos AJ, Marín S (2004). Effect of essential oils of cinnamon, clove, lemon grass, oregano and palmarosa on growth of and fumonisin B1 production by *Fusarium verticillioides* in maize. *J. Sci. Food Agric.* 84(10):1141-1146.
- Wright SC, Maree JE, Sibanyoni M (2009). Treatment of oral thrush in HIV/AIDS patients with lemon juice and lemon grass (*Cymbopogon citratus*) and gentian violet. *Phytomedicine* 16(2-3):118-124.