ESSENTIAL OIL YIELD IN *EUCALYPTUS CITRIODORA* IN RELATION TO FOLIAGE AGE AFTER POLLARDING AND TREE AGE

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In a series of field experiments in subtropical south China, *Eucalyptus citriodora* leaf biomass, essential oil yield and composition were measured in relation to tree age, different pruning treatments and foliage age following pollarding. The dominant compounds in the essential oil included citronellal (76.9%), citronellol (7.5%), isopulegol (6.4%) and neoisopulegol (3.1%). The 3- and 5-year-old trees yielded the most essential oil (4.4 and 4.7% respectively) and citronellal (82.6 and 82.5% respectively), and yield decreased with tree age. One year following the pruning treatments, foliage biomass was three times greater from 1, 2 and 3 m high stumps than from coppiced trees and 0.5 m high stumps. Oil yield was highest for trees pollarded at 1 m (equivalent to 5.9% from dry leaves) and lowest from coppiced trees (3.2%). All pollarding treatments yielded more citronellal (79.3–81.9%) than coppiced trees (66.0%). Oil yield and citronellal content were higher (5.9 and 77.7% respectively) when harvested at 12 months than at 6 months, 2, 3, 4 and 5 years after pollarding. Based on our results, harvest of fresh *E. citriodora* leaves should begin 3–5 years after planting, with a 1-year harvest interval, and pollarding above 1.0 m for optimal oil yield.

Keywords: Citronellal, rotation cycle, coppice, foliage biomass, gas chromatography-mass spectrometry

INTRODUCTION

Eucalyptus citriodora (Myrtaceae), commonly called lemon-scented eucalyptus, is a fast-growing ornamental tree species planted for timber and oil production. The essential oil extracted from its leaves is used in food, pharmaceutical, daily-use chemical and fragrance products (Tripathi et al. 2008, Bello et al. 2013). The oil has been used for sterilisation, as an insect repellent (Singh et al. 2011), and for its anti-inflammatory and acaricidal activities (Clemente et al. 2010, Gbenou et al. 2012). The main components of *E. citriodora* oil are citronellal, neoisopulegol, isopulegol, citronellol, citronellyl acetate, and β-caryophyllene (Zini et al. 2001, de Araujo-Filho et al. 2018).

Essential oil yields of 2.3 to 5.9% have been reported from dried leaves, depending on the growing location (Moudachirou et al. 1999). Productivity is influenced by genetic factors (Doran & Matheson 1994) and other factors such as ontogeny and age of leaf and environmental effects (Nishimura et al. 1984, Jesús et al. 2010, Brilli et al. 2013). Further, the chemical composition of essential oil has been shown to vary with harvesting time (Moudachirou et al. 1999, Tolba et al. 2015). Leaf essential oil content is higher during the wet season (April to September) in subtropical north India (Manika et al. 2012), and higher in leaves from older trees and in leaves collected in autumn than in summer (de Andrade & Gomes 2000). Silvicultural practices such as pruning also influence leaf and essential oil productivity in *E. citriodora* (Wirthensohn & Sedgley 1998). Pollarded trees in general produce more leaves than coppiced trees (Muralidharan & Mascarenhas 1995) but for *E. citriodora* in Dehradun, northern India, essential oil yields were slightly better for coppiced rather than pollarded trees on a 6-month or 1-year harvest cycle (Shiva et al. 1988).

The influence of pollarding height, tree and foliage age on the composition and yield of *E. citriodora* essential oil are not well understood. In this study, leaf biomass, essential oil yield and composition were measured in relation to tree age, different pruning treatments and foliage age following pollarding, with the aim of identifying silvicultural practices supporting optimal production of this essential oil.

MATERIALS AND METHODS

Study location

The study sites are located in north-east Wuming, Nanning, Guangxi Zhuang Autonomous Region (22°59'–23°33' N, 107°49'–108°37' E). The climate in this region is subtropical monsoon with a dry season from September to April accounting for an average of 473 mm of precipitation, and rainy season from May to August with 767 mm of precipitation, adding up to an average annual total of 1240 mm. The average annual temperature ranges from 20 to 22 °C (National Meterological Center).

Essential oil extraction and analysis

For all experiments, the leaves were harvested in November (winter), when oil yield peaks for *E. citriodora* in subtropical China (Miguel et al. 2005). About 500 g of fresh leaves were collected from 50 *E. citriodora* trees chosen randomly from a 25-year-old pure species stand (planted in 1992) at the study location. Tree spacing was 2 m × 3 m. The leaves were cut into 2–3 cm pieces, added to 800 mL water in a 2–L round-bottom flask and subjected to steam distillation for 1 hour using a conventional steam distillation apparatus to extract the essential oil (following Tolba et al. 2015). *E. citriodora* oil on the top of the condensed water was collected, dried overnight in a desiccator and stored at 5 °C. Quantitative and qualitative analysis of *E. citriodora* oil was carried using a gas chromatograph attached to a mass spectrometer (Wang et al. 2014) to determine the main components and their contents. The essential oil content of dried leaves (%) was then calculated as follows:

$$\text{Essential oil yield of dried leaves (\%)} = \frac{\text{Fresh leaf oil weight}}{(1 - \text{fresh leaf water content})} \times 100$$

Qualitative analysis conditions of GC-MS

The column was a ZB-5 silica capillary column (30 m × 0.25 mm, 0.25 µm) coupled with a mass selective detector. The column temperature was initially held at 70 °C for 5 min and was programmed to rise from 70 to 80 °C at 2 °C min^{-1}, 80 to 85 °C at 1 °C min^{-1}, 85 to 120 °C at 5 °C min^{-1}, and 120 to 220 °C at 10 °C min^{-1}. Maximum temperature was maintained for a further 3 min before cooling. The injector and detector temperatures were 250 °C. The carrier gas was high purity He flowing at 1.0 mL min^{-1}. For GC–MS the detection and electron impact system was used with an ionisation energy of 70 eV and a scan range from 45–350 amu. The sample size (1% ethanol solution) was 0.5 µL with a splitting ratio of 100:1.

Quantitative analysis conditions of GC

GC analyses of essential oil samples were performed on an gas chromatograph fitted with a flame ionisation detector, using a ZB-WAX silica capillary column (60 m × 0.25 mm, 0.25 µm). The column temperature was raised linearly from 70 to 220 °C at a rate of 2 °C min^{-1}. The carrier gas was N_2 flowing at 1.0 mL min^{-1}. The injector and detector temperatures were 200 and 250 °C respectively. The sample size was 0.5 µL with a splitting ratio of 100:1.

Quantifying essential oils from trees of different ages

Fresh leaves were harvested from each of 20 *E. citriodora* trees that were 1, 3, 5, 10, 15, 20 and 25 years old (i.e. the stand was planted in a 5-year succession from 1992–2012, and in 2014 and 2016). Plant spacing was 2 m × 3 m in this pure species stand. Essential oils were extracted from the leaves using the procedure described earlier, then determined and quantified by GC.

Quantifying essential oils from coppiced and pollarded trees

The trees evaluated in this study were 15 years old and were planted in 2002 as a pure-species stand (20 rows of 20 trees in 1.5 m × 1.5 m spacing). Fifty trees were randomly selected for each of five pruning treatments: coppicing (at ground level),

and pollarding tree stems at heights of 0.5, 1, 2 and 3 m. One year later, fresh leaves were clipped off the regrowth and the trees pruned to the prescribed coppice or pollarding treatment for subsequent studies (not covered in this paper). The essential oils were extracted from the leaves using the procedure described earlier, then determined and quantified by GC.

Quantifying essential oil production over time, after pollarding

The trees evaluated in this study were 15 years old and were planted in 2002 as a pure-species stand (20 rows of 20 trees in 1.5 m × 1.5 m spacing). All 400 trees were pollarded to 1 m height. Fresh leaves were collected from a random sample of 50 trees after 6 months and on year 1, 2, 3, 4 and 5 after that one-time pollarding. The essential oils were extracted from the leaves using the procedure described earlier, then determined and quantified by GC.

Statistical analysis

Statistical analyses were carried out using SPSS 19.0 software. Comparisons were made using *t*-test. The significan thresholds were 0.05 (marked by *) and 0.01 (**). Results are given as mean (± standard deviation).

RESULTS AND DISCUSSION

Composition of *E. citriodora* essential oil

Twenty-one compounds were identified, which represented 97.2% of total compounds in the essential oil of *E. citriodora*. The dominant compounds included citronellal (76.9%), citronellol (7.5%), isopulegol (6.4%) and neoisopulegol (3.1%), which comprised 93.9% of oil yield (Table 1). The contents of citronellyl acetate (1.1%) and *p*-menthane-3,8-diol (1.0%) made up 2.1% while the rest of the compounds

Table 1 Quantification of the compounds detected in *Eucalyptus citriodora* essential oil

No.	Retention time (min)	Compound	Formula	Similarity (‰)	Molecular weight	Relative content (%)
1	6.78	β-Pinene	$C_{10}H_{16}$	892	136	0.11
2	7.17	β-Myrcene	$C_{10}H_{16}$	821	136	0.06
3	8.88	Limonene	$C_{10}H_{16}$	852	136	0.02
4	8.89	D-Limonene	$C_{10}H_{16}$	841	136	0.01
5	9.01	1,8-Cineole	$C_{10}H_{18}O$	860	154	0.09
6	9.18	trans-β-Ocimene	$C_{10}H_{16}$	852	136	0.13
7	15.66	Isopulegol	$C_{10}H_{18}O$	934	154	6.37
8	16.13	Citronellal	$C_{10}H_{18}O$	889	154	76.87
9	16.31	Neoisopulegol	$C_{10}H_{18}O$	930	154	3.09
10	19.84	Citronellol	$C_{10}H_{18}O$	807	154	7.46
11	23.70	*p*-menthane-3,8-diol	$C_{10}H_{20}O_2$	870	172	1.03
12	23.96	Citronellyl acetate	$C_{10}H_{22}O_2$	882	198	1.11
13	24.92	2-Methylbenzyl acetate	$C_{10}H_{12}O_2$	782	164	0.15
14	25.46	Caryophyllene	$C_{15}H_{24}$	927	204	0.50
15	26.14	Humulene	$C_{15}H_{24}$	859	204	0.03
16	26.58	β-copaene	$C_{15}H_{24}$	748	204	0.04
17	26.83	γ-Elemene	$C_{15}H_{24}$	810	204	0.05
18	28.08	Spathulenol	$C_{15}H_{24}O$	780	220	0.02
19	28.16	Caryophylene oxide	$C_{15}H_{24}O$	799	220	0.03
20	28.22	Globulol	$C_{15}H_{26}O$	759	222	0.02
21	31.40	Phthalic acid, diisobutyl ester	$C_{16}H_{20}O_4$	836	276	0.02

(each <1%) comprised the remainder (Table 1). The four dominant compounds identified and used as indicators of oil quality in our study were the same ones reported as major *E. citriodora* essential oil compounds previously (Singh et al. 2011).

Essential oil yield in relation to tree age

Essential oil yield was highest from the 3- and 5-year-old trees (4.4 and 4.7% respectively), which were not significantly different in yield, and thereafter negatively correlated with tree age for the 10- to 25-year-old trees in this study (Figure 1). It was noted that oil yield from the 15-year-old trees was sharply lower than all other tree ages including the 20- and 25-year-old trees and this could be due to variation in planting stock or growth conditions for these succession plantings over the 25 year period.

The most important compound citronellal was highest from the 3- and 5-year-old trees (82.6 and 82.5% respectively), which were not significantly different (Figure 1b). Citronellal yield decreased with tree age—the 25-year-old-trees had a yield of 55.5%. On the other hand, citronellol yield increased with tree age—the 3- and 25-year-old trees had yields of 4.3 and 12.5% respectively (Figure 1d). Based on our results, 3- to 5-year-old trees appeared to be an optimal age range for harvesting high quality oil.

Essential oil yield in relation to pruning height

One year following the pruning treatments, foliage biomass was three times greater from 1, 2 and 3 m high stumps, than from coppiced trees and 0.5 m high stumps (Figure 2). Oil yield was highest for trees pollarded at 1 m, although not significantly different from trees pollarded at 0.5, 2 and 3 m. Oil yield was lowest from coppiced trees (3.2%). Oil yield from the fresh leaves of trees pollarded at 1 m was 5.9% which was similar to that obtained from *E. citriodora* in Benin (Moudachirou et al. 1999) and Congo-Brazzaville (Loumouamou et al. 2009). All pollarding treatments yielded high quantities of citronellal (77.7–81.9%), significantly greater than yield from coppiced trees (66.0%). The low foliage biomass from coppiced *E. citriodora* found in our study is in contrast with a previous study indicating that pollarded trees gave slightly less oil yields than coppiced trees (Shiva et al. 1988). While coppiced stems initially grew faster than uncut or seedling trees (Blake 1980) and produced more biomass in the first year of growth (Harrington & Fownes 1995), several
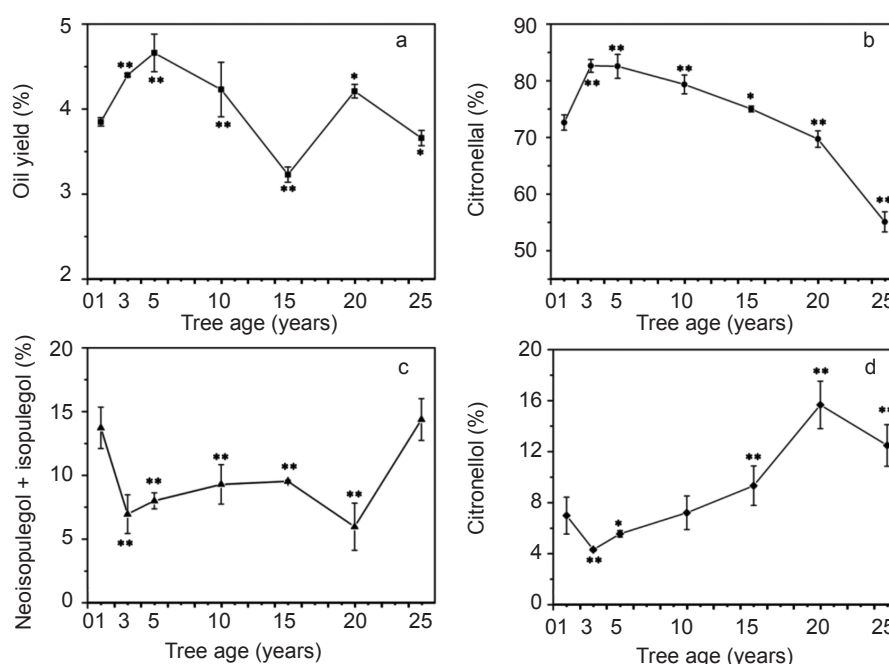

Figure 1 Mean percentage yield (± standard deviation, n = 20) of (a) *Eucalyptus citriodora* essential oil, and its major compounds: (b) citronellal, (c) neoisopulegol and isopulegol, and (d) citronellol from trees of different ages; * = p < 0.05, ** = p < 0.01 (*t*-test)
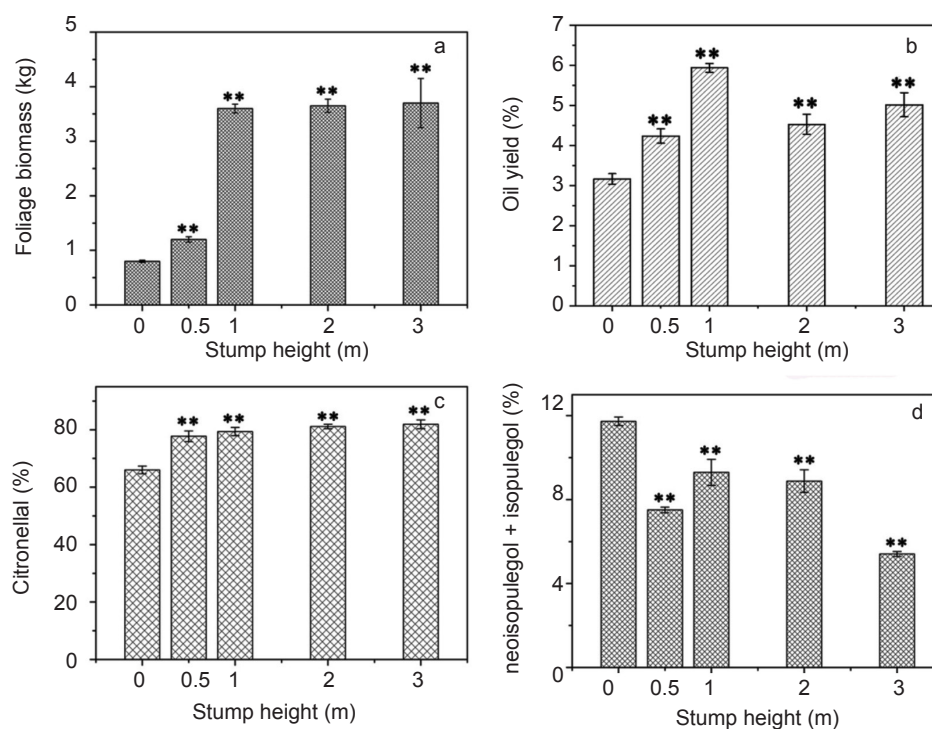
Figure 2　　Mean values (± standard deviation, n = 50) of (a) foliage biomass and (b) oil yield of *E. citriodora*, and of major compounds: (c) citronellal, and (d) neoisopulegol and isopulegol in relation to coppicing and pollarding; * = p < 0.05, ** = p < 0.01 (*t*-test)

authors have reported decreased stand volume, stump loss and sprout mortality that reduced productivity in coppiced eucalypt trees (Whittock et al. 2003, Zhou et al. 2017).

Essential oil yield up to 5 years after pollarding

For this field trial where all the trees were pollarded at 1 m, oil yield was significantly higher when harvested at 12 months (5.9%)—almost double that of the other harvest times (Figure 3a). Citronellal content was also highest at 12 months after pollarding (77.7%) while the other harvest times yielded citronellal in the range of 66.4–75.0% (Figure 3b). Citronellol content was highest in the fourth year after pollarding (16.6%), but the content at 12 months (10.0%) was still good. Our results partially concurred with the report by Shiva et al. (1988) that essential oil and citronellal yield was best 12 months after pruning but differed from Shiva et al. (1988) in that yield was significantly less at 6 months. Our results indicated that oil should be harvested while foliage was young (12 months) as essential oil content appeared to decrease with age.

CONCLUSIONS

The major components of *E. citriodora* essential oil were citronellal, citronellol, isopulegol and neoisopulegol, and their contents and oil yield changed with tree age, coppicing or pollarding height, and foliage age after pollarding. Essential oil yield and citronellal content appeared to be highest in 3- and 5-year-old trees, was higher in pollarded trees (stump heights 0.5–3.0 m) than in coppiced trees, and was best in year-old foliage of pollarded trees. Based on our results, for optimal oil yield, we recommend that harvest of *E. citriodora* leaves should begin in the 3rd to 5th year after planting with a 1-year harvest interval and pollarding above 1.0 m.
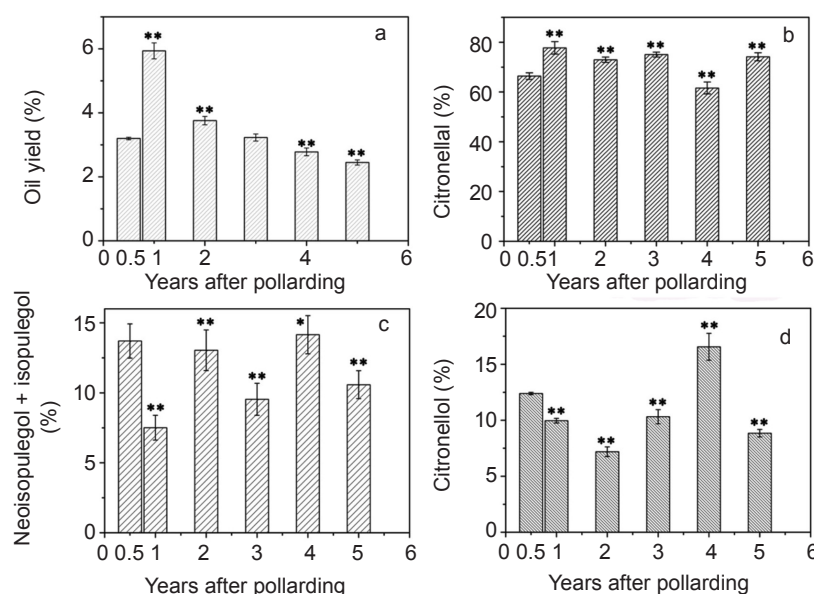
Figure 3 Mean percentage (± standard deviation, n = 50) of *E. citriodora* (a) essential oil yield and its major compounds: (b) citronellal, (c) neoisopulegol and isopulegol, and (d) citronellol, after pollarding (1 m stump height); *= p < 0.05, ** = p < 0.01 (*t*-test)

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