

Chemical variation and insecticidal activity of *Lippia javanica* (Burm. F.) Spreng essential oil against *Sitophilus zeamais* Motschulsky

John F. Kamanula^a, Steven R. Belmain^{b*}, David R. Hall^b, Dudley I. Farman^b,
David J. Goyder^c, Brighton M. Mvumi^d, Friday F. Masumbu^a and Philip C.
Stevenson^{b,c}

^a Chemistry Department, Mzuzu University, P/Bag, 201, Luwinga, Mzuzu 2, Malawi

^b Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime,
Kent ME4 4TB, United Kingdom

^c Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, United Kingdom

^d Department of Soil Science & Agricultural Engineering, Faculty of Agriculture, University
of Zimbabwe, P.O. Box MP167, Mount Pleasant, Harare, Zimbabwe

* Corresponding author: s.r.belmain@gre.ac.uk

Abstract

Lippia javanica (Burm. F.) Spreng is used commercially as a herbal tea and medicinal plant in sub-Saharan Africa. Here we investigated the chemical variation and pesticidal potential of *L. javanica* essential oils against a major stored product pest, *Sitophilus zeamais* Motschulsky. We identified two morphologically distinct varieties of *L. javanica* growing at different locations in Malawi. Perillaldehyde was the major constituent in oil of *L. javanica* var. *javanica* while myrcenone (ipsdienone) was the major compound in oils of *L. javanica* var. *whytei*. Myrcene, linalool, carvone, β -caryophyllene and germacrene D were identified as

the other most significant components in oils from both varieties. The yields of oil and the chemical composition also varied significantly with time of harvest during the season in both cases. In contact toxicity tests against *S. zeamais*, oils from both varieties were active. However, whereas perillaldehyde, linalool and carvone, components of the oil of *L. javanica* var. *javanica* were all toxic against adult *S. zeamais*. Myrcenone, the main component of oil from *L. javanica* var. *whytei*, was not. The oil from *L. javanica* var. *javanica* also showed some fumigant toxicity against *S. zeamais*. The high efficacy of *L. javanica* oil against *S. zeamais* suggests it is suitable to be used as a botanical insecticide to control *S. zeamais* in stored maize. However, further research is required to optimise and standardise the variety and harvest time to be recommended and to evaluate its activity against *S. zeamais* and other storage insect pests under farm conditions before it will be adopted by farmers more widely.

Key words: chemotype, myrcenone, ipsdienone, perillaldehyde, botanical pesticide, pest management

1. Introduction

The genus *Lippia* (Lamiales: Verbenaceae) contains more than 200 species, many of which are aromatic (Terblanche and Kornelius, 1996). The species of attention to this study, *Lippia javanica* (Burm. f.) Spreng. (fever tea), is a perennial, erect, woody shrub that can grow up to 2 m high (Viljoen *et al.*, 2005), and its leaves produce a strong smell when crushed or rubbed (Van Wyk and Gericke, 2000). Current uses for this species include ethno-medicinal such as treatment of respiratory disorders as leaf infusions or inhalants, and as remedies of the digestive system diseases such as cholera, diarrhoea, and dysentery using leaf or root decoction or infusions (Maroyi, 2017; Van Wyk *et al.*, 1997). A combination of *L. javanica* with leaves of *Artemisia afra* Jacq. ex Willd. is reportedly a good remedy for fever

and influenza (Hutchings, 1996). *Lippia javanica* is also used for ethno-veterinary purposes including control of ectoparasites such as ticks in cattle and bed bugs in fowl runs when applied as crushed leaves and twigs alone or mixed with water (Maroyi, 2017; Nyahangare *et al.* 2015).

Lippia javanica grows in most parts of Africa, including Nigeria, Ethiopia, South Africa, Botswana, Tanzania, Zimbabwe, Malawi, Swaziland, Mozambique and Kenya (Chagonda and Chalchat, 2015; Le Roux, 2004; Muzemu *et al.*, 2011; Fernandes, 2005; Viljoen *et al.*, 2005). The species is well suited to a broad range of climates and ecological zones and hence is appropriate for wide-scale use and even propagation, with potential as a commercial plant for tea and as a medicine. However, this use is complicated by phenotypic variation associated with its occurrence as two morphologically distinct varieties, *L. javanica* var. *javanica* and *L. javanica* var. *whytei* (Fernandes, 2005). Chemical variation within the genus is also reported with potential impacts on use (Soro *et al.*, 2016). For example, Manenzhe *et al.* (2004) reported that *L. javanica* essential oil contained several terpenoids of which 3-methyl-6-(1-methylethylidene)-cyclohexen-2-en-1-one (piperitenone) was the major component. Elsewhere 2-methyl-6-methylene-2,7-octadien-4-one (ipsdienone, myrcenone), myrcene and (*E*)- and (*Z*)-tagetenone were reported to be present in *L. javanica* oil (Mwangi *et al.*, 1991, 1992; Terblanche and Kornelius, 1996; Velasco-Negeureula *et al.*, 1993). Chagonda *et al.* (2000) showed linalool content to vary from 1.8 to 27 % in the leaves of *L. javanica* collected from the same location in Zimbabwe while Ngassapa *et al.* (2003) reported geranial and neral chemotypes of *L. javanica* from Tanzania. Viljoen *et al.* (2005) identified three distinct chemotypes characterised by content of carvone and limonene (chemotype 1), myrcenone and myrcene (chemotype 2) and piperitenone and limonene (chemotype 3). Other components identified in these chemotypes included germacrene D, (*Z*)- β -ocimene, (*E*)- β -ocimene, linalool, verbenone, and bicyclogermacrene. The chemical structures of some

monoterpenoids and sesquiterpenoids identified in *L. javanica* oil (Viljoen *et al.*, 2005 and this study) are presented in Figure 1. Although the chemical variability of *L. javanica* oil has been previously reported, it remains unknown whether this is due to differences in variety and whether this could subsequently affect its efficacy.

Figure 1 here

In Malawi, smallholder farmers produce large quantities of maize, but most are unable to store it to the next season mainly due to insect pests. *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), for example, is a major pest and able to disperse from stores where it has infested the grain to the maize crops in the field and infest the next crop before harvest (Haines, 1991). This can lead to significant losses of maize grain in stores as well as in the field (Longstaff, 1981). In northern Malawi, some small-scale farmers use dry *L. javanica* leaves to protect maize grain from insect attack (Kamanula *et al.*, 2011).

Understanding chemical variability is critical to assessing the potential of plants for pest management as well as the factors that underpin these differences (Belmain *et al.*, 2012; Stevenson *et al.*, 2012). In plant oils such as that of *L. javanica*, these variations are attributed to factors including geographical, cultivation, age and part of the plant material analysed, season, environment and genetics (Argyropoulou *et al.*, 2007; Bernath *et al.*, 2005; Folashade and Omoregie, 2012; Marzoug *et al.*, 2011; Verma *et al.*, 2010; Viljoen *et al.*, 2005;).

In this study we investigated the biological activity of *L. javanica* against a major stored products pest, *S. zeamais*, and determine how chemical variation influenced consistency in the efficacy of the species and informs its commercial potential. The specific objectives were (1) to investigate the effect of variety and time of leaf harvesting on the oil

content and chemical composition of *L. javanica* leaf oil, and (2) to evaluate the efficacy of *L. javanica* oil against adult *S. zeamais* under laboratory conditions.

2. Materials and Methods

2.1. Collection and identification of plant material

Mature leaves of *L. javanica* were collected from three locations in northern Malawi. Location 1 was Nchenachena extension planning area (EPA) in Rumphu district (10°45'S, 34°02'E), Locations 2 and 3 were Chikangawa Forest (11°35'S, 33°48'E) and Jenda (12°22'S, 33°33'E) (Champhira EPA in Mzimba district), respectively. Leaf samples were collected in October, December, February, April, June and August 2010/2011 (Year 1) and 2011/2012 (Year 2). The identity of the species was confirmed at the Royal Botanic Gardens, Kew, UK, where voucher specimens are deposited. *Lippia javanica* from Nchenachena was identified as *L. javanica* var. *javanica* (Stevenson04113) while the *L. javanica* from Chikangawa Forest and Jenda was identified as *L. javanica* var. *whytei* (Stevenson04112 and Stevenson04111, respectively). The distance between Nchenachena and Jenda is about 235 km.

2.2. Determination of moisture content

The moisture content of leaves of *L. javanica* was determined from three separate samples per collection location. Leaves were weighed (10 g) on an analytical balance (AAA 160 L, Adam Equipment, Johannesburg, South Africa) and transferred into a round-bottomed flask (250 mL). Toluene (100 mL, Associated Chemical Enterprises Ltd, Johannesburg, South Africa) was added to the leaves in each flask and distilled for 1 h 30 min using a Dean and Stark apparatus. The aqueous layer was transferred to a vial and weighed to give the moisture content (% w/w).

2.3. *Extraction of essential oils*

Lippia javanica leaves were subjected to hydro-distillation using a Clevenger-type apparatus and the distillation process was replicated three times per sample. The distillation flask (2 L) was charged with *L. javanica* leaves (100-150 g, depending on the freshness of the leaves). Distilled water was added (400 mL) and the oil was distilled for 3 h. Both water and oil were transferred into a clean vial (10 mL) and the oil was separated from water and transferred into a second vial (5 mL) using a Pasteur pipette. Anhydrous sodium sulphate was added to dry the oil. The dried oil was then transferred into a pre-weighed vial (5 mL) and weighed using an analytical balance (AAA 160 L, Adam Equipment). The yield of oil as percentage of fresh weight was calculated and the moisture content value was used to calculate the yield (%) of oil on dry matter basis. The density of the oils was 0.884 ± 0.001 g/cm³.

2.4. *Synthetic chemicals*

β -Pinene, myrcene, ocimene (2:1 *E/Z*), (\pm)-linalool, (\pm)-carvone, perillaldehyde, β -caryophyllene and verbenone were obtained from SigmaAldrich (Gillingham, Dorset, UK).

A fraction enriched in germacrene D (65%) was obtained by chromatography of ylang-ylang essential oil (Holland and Barrett, Nuneaton, Warwks, UK) on silica gel eluted with hexane.

A reference sample of myrcenone was prepared by oxidation of 2-methyl-6-methylene-2,7-octadien-4-ol (ipsdienol; SEDQ, Barcelona, Spain) with pyridinium chlorochromate in dichloromethane followed by chromatography on silica gel eluted with 5% diethyl ether in hexane. For bioassays, myrcenone was isolated from the essential oil from *L. javanica* var. *whytei* harvested in Chikangawa by chromatography on silica gel eluted with a gradient of diethyl ether in petroleum spirit (b.p. 40-60 °C). From 5 g oil was obtained 1.68 g

material containing 93% myrcenone after kugelrohr distillation at 140 °C and 25 mm pressure. This had mass spectrum and H¹ NMR spectrum consistent with those that reported by Reece *et al.* (1968).

2.5. *Identification of volatile constituents by gas chromatography-mass spectrometry (GC-MS)*

GC-MS identification of volatile constituents in *L. javanica* leaf oil was carried out on a Varian CP-3800 GC coupled to a Varian Saturn 2200 ion-trap mass spectrometer (Varian Inc., Walnut Creek, California, US) operated in the electron impact (EI) mode at 70 eV. The ion trap and manifold temperatures were 170 and 80 °C, respectively while transfer line temperature was maintained at 240 °C. The GC was fitted with a fused silica capillary column, (30 m x 0.25 mm x 0.25 µm dia.) coated with the non-polar phase VF-5 (5% diphenyl-, 95% dimethyl-polysiloxane; Varian). The injector temperature was 220 °C and the oven temperature was programmed from 40 °C held for 2 min, then at 10 °C/min to 240 °C, and held for 5 min. The injection (1 µL) was in splitless mode with helium (1.0 mL/min) used as a carrier gas. A series of *n*-alkanes (C9-C24) was used for the determination of Kovats Indices (Liolios *et al.*, 2009; Wannan *et al.*, 2009). Peaks were identified using the National Institute of Standards and Technology (NIST) mass spectral library (Dawidowicz *et al.*, 2008; Okey *et al.*, 2009; Yang *et al.*, 2013), Kovats Indices (www.pherobase.com) and comparison with authentic standards.

2.6. *Quantification of compounds by gas chromatography with flame ionization detection (GC-FID)*

Quantification of volatile constituents in *L. javanica* oil was performed on a HP 6850 gas chromatograph (Agilent Technologies equipped with flame ionization detector (FID) and

fitted with a fused silica capillary column (30 m x 0.32 mm i.d. x 0.25 µm film thickness) coated with non-polar HP5 (Agilent), essentially identical to that used in GC-MS analyses. Carrier gas was helium (2.4 mL/min) and injector and detector temperatures were 250 °C. The oven temperature was programmed from 50 °C for 2 min, then at 10 °C/min to 250 °C and then held for 5 min. Sample injection volume was 1 µL in splitless mode. Data were processed using EZchrom software (Elite v 3.0; Agilent). To quantify the amount of constituents in the oil, standard solutions (0, 0.05, 0.10, 0.20, 0.50 and 1.0 mg/mL) for the calibration curves were prepared from pure synthetic compounds (perillaldehyde, linalool, limonene) in dichloromethane (Pesticide Residue Grade; Fisher Scientific, Loughborough, Leicestershire, UK). These three compounds were chosen for quantification because our preliminary results (GC-MS) showed these compounds to be present in *L. javanica* hexane leaf extract. After running the standards on GC-FID, regression equations ($y = mx + c$) obtained from standard calibration curves were used for the calculation of the amount of perillaldehyde, linalool and limonene in *L. javanica* oil.

2.7. *Collection and identification of S. zeamais*

Initial stocks of *S. zeamais* used in this study were obtained from farmers' infested maize grain in Champhira and Nchenachena in northern Malawi and their taxonomic identification was confirmed according to Haines (1991). Cultures were maintained on maize kernels at 25°C.

2.8. *Bioassay of contact toxicity against adult S. zeamais*

Test solutions (200 µL) was pipetted into a screw-top glass vial (8 mL) with a micropipette (Finnpipette F2, Fisher Scientific, 20-200 µL). The vial was turned manually to coat the whole inner surface of the vial. The vials were then opened and left to dry (1 h) in a fume hood at

room temperature (26 ± 3 °C). Control vials were set up in which *n*-hexane (200 μ L) was added. After drying, adult, 7-14 days old, *S. zeamais* of both sexes were put in each of the treated and control vials and the vials were then closed (Belmain *et al.*, 2012). Treatments were replicated ten times, and the treated and untreated vials containing insects were left in a constant temperature room (27 ± 3 °C, 65 ± 5 % RH, 12L:12D photoperiod). Percentage mortality was recorded. Mortality data were expressed as percentage of the total number of insects introduced after 24 and 48 h of exposure. Mortality data were \log_{10} -transformed to correct for heterogeneity of treatment variances before being subjected to one-way ANOVA using SPSS v.16. The lethal concentrations (LC_{50s}) were determined using probit analysis (PROC PROBIT) (Finney, 1971).

Stock solutions (50 mg/mL) of oil from *L. javanica* var. *javanica* harvested in Nchenachena during October 2010, perillaldehyde, limonene and linalool were prepared separately in *n*-hexane (Pesticide Residue Grade, Fisher Scientific) followed by a series of dilutions to 10, 5, 2.5, 1, 0.5 and 0.05 mg/mL. A combination of perillaldehyde and linalool (10 mg/mL total), major components of the oil from *L. javanica* var. *javanica*, was also prepared followed again by serial dilutions.

Similar solutions were prepared of oil from *L. javanica* var. *whytei* harvested in Chikangawa during August 2012. Also tested were Mixture 1 containing equal amounts of β -pinene, myrcene, limonene, linalool, myrcenone, carvone, verbenone and β -caryophyllene, Mixture 2 containing all compounds in Mixture 1 except myrcenone and limonene, carvone and myrcenone. All these compounds were identified in *L. javanica* var. *whytei* oil from Chikangawa and Jenda.

2.9. Bioassay of fumigant toxicity against adult *S. zeamais*

A stock solution (100 mg/mL) was prepared by dissolving essential oil (1.0 g) from *L. javanica* var. *javanica* harvested in Nchenachena during June 2011 in *n*-hexane (10 mL). From the stock solution, serial dilutions were made in 5 mL volumetric flasks to give 50, 20, 10 and 5 mg/mL solutions for bioassay, based on preliminary work. Each solution (100 μ L) was pipetted using a micropipette (Finnpipette F2, 20-200 μ L) onto a filter paper (Whatman No. 1, 2.5 cm diameter) and the filter papers were allowed to dry in a fume hood for 50 min. Unsexed, 7-14 day old adult *S. zeamais* (20) were introduced into a screw-top glass vial (27 cm³) (Sahaf *et al.*, 2008). A treated or untreated filter paper was attached to the underside of the cap by clear seal tape and the cap was screwed on tightly giving doses of 370, 185, 74, 37, 19 and 0 μ g/cm³ for the five solutions and blank respectively. Each treatment was replicated 10 times. Mortality counts were recorded at 72, 96 and 120 h based on preliminary counts at 24 and 48 h where all insects were still alive. Percentage mortality and LC₅₀ values were calculated as above.

3. Results

3.1 Extraction and identification of volatile constituents in essential oil of *L. javanica* var. *javanica*

Hydrodistillation of *L. javanica* var. *javanica* leaves that had been collected from Nchenachena in October 2010 produced a yellow distillate; with a yield of 1.19 ± 0.17 % (dry matter) and moisture content of 13.55 ± 2.88 %. Analysis of the oil by GC-MS and GC-FID for comparison of Kovats Indices (KI) and mass spectra with literature data and NIST library showed perillaldehyde (4-(1-methylethenyl)-1-cyclohexene-1-carboxaldehyde) to be the major constituent (44.4 %) of the oil with limonene (24.1 %) the next most abundant. Other constituents included myrcene, (*E*)-ocimene, linalool, piperitenone, β -caryophyllene and

germacrene D (Table 1). In total, 15 compounds were identified representing 96 % of the total oil constituents.

Table 1 here

3.2. *Effect of variety on oil content and chemical composition of essential oil of L. javanica*

Lippia javanica var. *javanica* and *L. javanica* var. *whytei* oils which were collected during August 2011 from Nchenachena and Chikangawa respectively showed distinct variations in their oil content and chemistry indicating varietal and spatial significance for harvesting material. The *L. javanica* var. *whytei* yielded 13.8 ± 0.4 g/kg dry matter while *L. javanica* var. *javanica* leaves gave 8.4 ± 0.8 g/kg dry matter.

The results of GC-MS and GC-FID analyses of *L. javanica* oils from these two varieties are presented in Table 2. Oil from *L. javanica* var. *javanica* was characterized by a high percentage of perillaldehyde (63.25 ± 2.96 %) and limonene (16.69 ± 1.40 %) whereas *L. javanica* var. *whytei* oil contained myrcenone (54.52 ± 2.68 %) as the major constituent. Other compounds that were identified in both oils included β -pinene, myrcene, germacrene D and β -caryophyllene (Table 2). The percentage of myrcene in *L. javanica* var. *whytei* oil was twice that in *L. javanica* var. *javanica* oil. Verbenone, linalool and carvone were detected in *L. javanica* var. *whytei* oil only (Table 2).

Table 2 here

3.3. Effect of leaf harvesting time on oil content and chemical composition of essential oil of *L. javanica*

The amount of oil from *L. javanica* var. *javanica* collected in Nchenachena and of *L. javanica* var. *whytei* from Chikangawa and Jenda varied significantly ($F = 9.705$; $P < 0.001$) with time of leaf harvesting (Table 3). The highest amounts of oil in *L. javanica* var. *whytei* from Chikangawa (20.14 ± 1.20 g/kg dry matter) and Jenda (19.28 ± 0.83 g/kg dry matter) were obtained in February of Year 1 while the lowest amounts (10.76 ± 0.37 and 9.29 ± 0.82 g/kg dry matter, respectively) were recovered in June of Year 1. The amount of oil distilled in December Year 1, April, June and August Year 1 in Chikangawa did not differ significantly ($P > 0.05$). In Nchenachena, the maximum (13.60 ± 1.90 g/kg dry matter) and minimum (4.86 ± 0.56 g/kg dry matter) amount of oil was extracted in February and August Year 2, respectively. Surprisingly, there was no significant difference ($F = 2.394$; $P = 0.100$) in the amount of *L. javanica* oil distilled from Nchenachena in Year 1 (Table 3).

Table 3 here

Besides variations in oil content, *L. javanica* essential oils from Nchenachena and Chikangawa showed chemical variations with respect to leaf harvesting time. The relative concentration of perillaldehyde, the major component of Nchenachena oil, was highest (63.25 %) in August and lowest (1.11 %) in April (Table 4). In contrast, the highest concentration (16.95 %) of germacrene D in Nchenachena *L. javanica* oil was recorded in April while the lowest percentage (2.60 %) was observed in August. Other constituents in Nchenachena oil whose concentrations were highest in April year 2 included piperitenone, β -caryophyllene, bicyclogermacrene and spathulenol (Table 4). In Chikangawa *L. javanica* oil, limonene, (*Z*)-ocimene, verbenone, perillaldehyde, spathulenol and caryophyllene oxide were

detected in August only. The concentrations of carvone and myrcene, the second and third major compounds in Chikangawa *L. javanica* oil, did not change significantly ($P > 0.05$) with respect to harvesting time.

Table 4 here

Figure 2 shows the actual amounts of perillaldehyde, linalool and limonene present in *L. javanica* var. *javanica* oil extracted from leaves collected in October, December, February, April, June and August of Year 1. The results showed great variations in the amount of perillaldehyde present in the oil. In October, the amount of perillaldehyde was 355.88 ± 19.04 mg/g oil. In December the same year it decreased until it reached the lowest amount in April (Figure 2). In June, however, perillaldehyde increased dramatically to 680.07 ± 8.25 mg/g oil, until it reached 693.35 ± 14.12 mg/g oil in August. The limonene content was consistent from October to April but reached highest amount (262.70 ± 10.09 mg/g oil) in June. Linalool was detected in December, February and April only (Figure 2).

Figure 2 here

3.4. Contact toxicity against adult *S. zeamais*

The toxicities of *L. javanica* var. *javanica* essential oil, perillaldehyde (its major constituent), limonene, linalool and a mixture of perillaldehyde and linalool were evaluated against unsexed adult *S. zeamais*. The results showed that the crude oil, linalool, perillaldehyde and a mixture of perillaldehyde and linalool were toxic to adult *S. zeamais*. The toxicities were dose- and time-dependent. After 48 h exposure, the highest concentration (10 mg/mL) of *L. javanica* var. *javanica* essential oil, linalool, perillaldehyde and a mixture

of perillaldehyde and linalool, caused 85, 100, 99.5 and 100 % mortality of adult *S. zeamais*, respectively (Figure 3). No mortality of adult *S. zeamais* was observed for any concentration of limonene. For *L. javanica* oil, no significant ($P \geq 0.905$) differences in mortality of adult *S. zeamais* were observed for concentrations 0 to 2.5 mg/mL. However, 2.5 mg/mL of perillaldehyde, linalool and a mixture of perillaldehyde + linalool, caused 61, 16 and 86 % mortality of *S. zeamais* after 48 h exposure, respectively. Comparison of concentrations 5 and 10 mg/mL of perillaldehyde, linalool and the mixture showed no significant ($P > 0.05$) differences in mortality of adult *S. zeamais* after 48 h. However, overall, perillaldehyde and the mixture of perillaldehyde + linalool were more toxic ($P = 0.001$) to the test insects than the oil or linalool.

Figure 3 here

The LC_{50} values for *L. javanica* oil, perillaldehyde, linalool and a mixture of perillaldehyde + linalool were 6.22, 1.07, 1.82 and 0.85 mg/mL, respectively. No significant ($P = 0.063$) difference in mortality of *S. zeamais* was observed between *L. javanica* oil and linalool.

The results of contact toxicity of *L. javanica* var. *whytei* oil, Mixture 1 (β -pinene, myrcene, limonene, linalool, myrcenone, carvone, verbenone and β -caryophyllene), Mixture 2 (all compounds in Mixture 1 except myrcenone and limonene), carvone and myrcenone after 48 h are presented in Figure 4. All these compounds were identified in *L. javanica* var. *whytei* oil from Chikangawa and Jenda.

Figure 4 here

The oil, carvone, Mixture 1 and Mixture 2 were active ($LC_{50} = 2.96 \pm 1.02$; 1.44 ± 0.51 ; 0.57 ± 0.13 and 2.66 ± 0.44 mg/mL, respectively) against adult *S. zeamais*. At the lowest concentration (0.5 mg/mL), Mixture 2 caused 63.6 % mortality of the test insects compared to 0.5 % mortality for Mixture 1. *L. javanica* oil and carvone were also not active at this concentration. However, at the highest concentration (10 mg/mL) 83 % mortality was recorded for the *L. javanica* var. *whytei* oil and 100 % mortality for Mixture 1, Mixture 2 and carvone. Myrcenone, the major constituent isolated from *L. javanica* var. *whytei* oil, was not active against adult *S. zeamais*, with the highest concentration (10 mg/mL) causing only 6.5 % mortality of the test insects after 48 h of exposure, similar to the effect of limonene above. Exclusion of myrcenone and limonene from Mixture 2 in this experiment increased the toxicity ($LC_{50} = 0.57$ mg/mL) of the mixture against adult *S. zeamais* after 48 h of exposure. The results suggest that the presence of myrcenone and limonene in Mixture 1 and *L. javanica* oil diluted the concentrations or interfered with the activity of the other components.

3.5. Fumigant toxicity of *L. javanica* var. *javanica* oil from *Nchenachena* against adult *S. zeamais*

Lippia javanica var. *javanica* oil exhibited fumigant toxicity against adult *S. zeamais* which increased with dose. The highest dose ($370 \mu\text{g}/\text{cm}^3$ air) caused 60 % mortality after 72 h (Figure 5). However, after 120 h, the cumulative mortality increased only slightly to 68 % suggesting that *S. zeamais* are less sensitive to this oil by fumigant exposure than direct contact. The LD_{50} fumigant toxicity values for the 72 and 120 h were 254 and $216 \mu\text{g}/\text{cm}^3$ air, respectively.

Figure 5 here

4. Discussion

Essential oils from plants and their constituents can be useful alternatives to conventional insecticides and fumigants due to their low mammalian toxicity, high degradation rate, high volatility and local availability (Isman, 2000; Konstantopoulou *et al.*, 1992; Rajendran and Sriranjini, 2008). The volatility of essential oils makes them less persistent on treated stored food commodities with low long term residues (Koul *et al.*, 2008). This study has shown that both variety and time of leaf harvesting affect the quantity, chemistry and biological efficacy of *L. javanica* oil and these variables must be considered when using or promoting the use of this material for pest control. If these parameters are not controlled for and understood, this might lead to key properties being overlooked in the development of new pest control technologies (Ali *et al.*, 1985; Emara and Shalaby, 2011; Stashenko *et al.*, 2004).

The two varieties of *L. javanica* reported by Fernandes (2005) as *L. javanica* var. *javanica* and var. *whytei*, are not mentioned in other work on this species, even though we have shown this distinction has a major influence on chemistry and biological activity of the species. *L. javanica* is morphologically highly variable with respect to the number of spikes per axil and length of peduncle. Fernandes (2005) separated two varieties based on length of peduncle and spike. In *L. javanica* var. *javanica* peduncles are usually slender and greater than 1 cm long and longer than spikes which were usually small. Var. *whytei* (which elsewhere corresponds to *Lippia whytei* Moldenke), has shorter peduncles (< 1 cm long) which are typically shorter than the spikes which are longer, thicker and purple when in fruit. Additionally, var. *javanica* spikes occur in lax axillary groups on peduncles of unequal length, whereas in var. *whytei* spikes occur in compact groups on short equal peduncles. Variation between the varieties is broadly continuous and, although they appear different, the

two varieties are not separated formally. Records indicate that var. *javanica* is more abundant in the south while var. *whytei* is more common in the north of Southern and Eastern Africa.

We found that the chemistry of *L. javanica* var. *javanica* from Nchenachena differed dramatically from *L. javanica* var. *whytei* from Chikangawa and Jenda with the cyclised monoterpenes perillaldehyde and limonene dominating the essential oil of the former and the acyclic myrcenone dominating the oil from the latter along with myrcene. Viljoen *et al.* (2005) reported four distinct chemotypes of *L. javanica* from six different sites in Swaziland, based on the major components of the oils, although these were not related to botanical variation among the samples. The major components reported differed from our studies with carvone and limonene dominating the chemistry of some chemotypes while myrcenone and myrcene were dominant in other chemotypes. A further chemotype reportedly contained limonene and piperitenone. The *L. javanica* var. *whytei* reported in the present study resembles two chemotypes identified by Viljoen *et al.*, (2005), and it would be useful to know if this related to the variety studied. *Lippia javanica* var. *whytei* is more abundant in Southern Africa according to Fernandes (2005). These earlier data together with our data show that variation in *Lippia* chemistry is common and maybe dramatic and must be understood before promoting plant materials for personal use or commercialization.

Viljoen *et al.*, (2005) did not report perillaldehyde, possibly because they did not encounter *L. javanica* var. *javanica*. However, this compound was reported as a major constituent of *L. javanica* oil by Omolo *et al.* (2004) suggesting they were working with *L. javanica* var. *javanica*. Omolo *et al.*, (2004) also reported the repellent effect of perillaldehyde against mosquitoes, *Anopheles gambiae* Giles (Diptera: Culicidae), showing the wide variety of potential uses this compound and this species have in pest control.

Understanding chemical variability is critical to assessing the potential of plants for pest management as well as the factors that underpin these differences (Belmain *et al.*, 2012;

Stevenson *et al.*, 2012). The oils from *L. javanica* var. *javanica* and *L. javanica* var. *whytei* had similar contact toxicities against *S. zeamais*, but whereas a mixture of the two components from the former, perillaldehyde and linalool, was even more toxic, the major component from the latter, myrcenone, showed no toxicity at the doses tested.

In plant oils such as those from *L. javanica* these variations are attributed to factors including geographical cultivation, age and part of the plant material analysed, season, environment and genetics (Argyropoulou *et al.*, 2007; Bernath *et al.*, 2005; Folashade and Omoregie, 2012; Marzoug *et al.*, 2011; Verma *et al.*, 2010; Viljoen *et al.*, 2005). None of these authors reported taxonomic associations between chemotypes. *Lippia citriodora* oil is reported to have highly variable concentrations of limonene and geranial across seasons (Argyropoulou *et al.*, 2007) while Emara and Shalaby (2011) reported seasonal influence on the essential oil content of *Eucalyptus* species. The data in the present study also shows dramatic chemical variation across the year.

A survey of farmers in Zambia and Malawi revealed that farmers in these areas use a variety of pesticidal plants to protect their crops from insect attack including *L. javanica* (Kamanula *et al.*, 2011; Nyirenda *et al.*, 2011). However, the level of adoption of these pesticidal plants was low, which could be attributed to a number of factors, but variable efficacy of the plant species as a result of variation in the chemistry could reinforce low adoption (Sarasan *et al.*, 2011; Stevenson *et al.*, 2012) while scarcity of the plant material and promotion of plant species that are not effective may also influence farmer decisions (Stevenson *et al.*, 2012). The chemistry of *L. javanica* essential oil in this study was shown to vary with time and plant variety. Perillaldehyde, the major constituent in *L. javanica* var. *javanica*, and also biologically active, was shown to be at its highest concentration (63.25 %) during August and lowest (1.11 %) during April. Thus *L. javanica* oil extracted from leaves harvested during April may have lower efficacy and farmers using materials harvested at the

least optimal time of year might experience little benefit and be dissuaded from using plants as pesticides in future (Stevenson *et al.*, 2014). Thus, it is critical to ensure that promotion of plant materials is accompanied by robust chemical data that can inform how best to apply material for pest control.

5. Conclusions

This study has shown that two morphologically distinct varieties of *L. javanica* occur in Southern Africa as *L. javanica* var. *javanica* and *L. javanica* var. *whytei*, and these correspond to two distinct chemotypes. The two varieties showed variations in the chemical composition of their essential oils in that oil from *L. javanica* var. *javanica* was characterized by perillaldehyde and limonene as main components, while the *L. javanica* var. *whytei* was characterized by a high percentage of myrcenone. Furthermore there were major variations in the amounts and chemistry of the oils from *L. javanica* with respect to time of harvesting, and both of these factors will influence the success with which farmers benefit from them as a pest control technology.

The guidelines for farmers or producers of essential oils to determine the optimum harvesting time for aromatic and other oil producing plants are scant or unclear (Abdalla *et al.*, 2008). Our data could fill this gap if used as a guideline for the harvesting of *L. javanica* leaves in the study areas. For commercial and small-scale production of *L. javanica* essential oil our data suggest that growers should focus on the *javanica* variety, and in Malawi, harvest in February (rainy season) because of the higher oil yield. However, for the purposes of isolating perillaldehyde to be used as a botanical pesticide, the optimum time for harvesting would be June to August. Further investigation on the use of oils of the two varieties of *L. javanica* for the control of field and storage insect pests should be explored.

Acknowledgements

We are grateful to Prof. Rick Hodges from the Natural Resources Institute, University of Greenwich, UK for confirming the identity of *S. zeamais*. Research was funded by three European Union Development Fund projects: the SAPP project (ICART/CRARF/GC/005/069/ACP/SAD/1-11); the ADAPPT project (ACP/FED/2009/217/064); and the OPTIONs project (ACP/FED/2013/329/272) <http://www.acp-st.eu/>. Further funding was obtained through a DelpHE project funded by the British Council-DFID. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Abdalla, A.E.M., El-Difrawy, E.A., Abdelneem, Y.F., 2008. A study on the effect of harvesting time on quality of Egyptian olive oil. *Alex. J. Food Sci. Technol. Special Volume Conference*, 61-74.
- Ali, N., Hussain, S.A., Rashid, A., 1985. Effect of harvesting time on seed and oil yield in *Poorbi raya*. *Pakistan J. Agric. Res.* 6, 255-258.
- Argyropoulou, C., Daferera, D., Tarantilis, P.A., Fasseas, C., Polissiou, M., 2007. Chemical composition of the essential oil from leaves of *Lippia citriodora* H.B.K. (Verbenaceae) at two developmental stages. *Biochem. Syst. Ecol.* 35, 831-837.
- Belmain, S.R., Amoah, B.A., Nyirenda, S.P., Kamanula, J.F., Stevenson, P.C., 2012. Highly variable insect control efficacy of *Tephrosia vogelii* chemotypes. *Agric. Food Chem.* 60, 10055-10063.
- Bernath, J., Nemath, E., Cracker, L.E., Gardener, Z.E. (ed.), 2005. Changes in essential oil quantity and quality influenced by ontogenetic factors. *Bioprospecting and Ethnopharmacology. Acta Hort.* 675, ISHS.

- Chagonda, L.S., Makanda, C.D., Chalchat, J., 2000. Essential oils of wild and cultivated *Lippia javanica* (Spreng) and *L. oatessi* (Rolfe) from Zimbabwe. *J. Essent. Oil Res.* 12, 1–6.
- Chagonda, L.S., Chalchat, J., 2015. Essential oil composition of *Lippia javanica* (Burm.f.) Spreng chemotype from Western Zimbabwe. *J. Essent. Oil Bear. Pl.* 18, 482-485.
- Dawidowicz, A.L., Rado, E., Wianowska, D., Mardarowicz, M., Gawdzik, J., 2008. Application of PLE for the determination of essential oil components from *Thymus vulgaris* L. *Talanta* 76, 878-884.
- Emara, Kh. S., Shalaby, E.A., 2011. Seasonal variation of fixed and volatile oil percentage of four *Eucalyptus* spp. related to lamina anatomy. *Afr. J. Plant Sci.* 5, 353-359.
- Finney, D.J., 1971. Probit analysis, Cambridge University Press, pp. 333.
- Fernandes, R. (2005). Verbenaceae. In: Pope, G.V. & Martins, E.S. (eds.) *Flora Zambesiaca: Mozambique, Malawi, Zambia, Zimbabwe, Botswana.* 8(7): 6-61. The Royal Botanic Gardens, Kew.
- Folashade, K.O., Omoregie, E.H., 2012. Essential oil of *Lippia multiflora* Moldenke: A review. *J. Appl. Pharm. Sci.* 2, 15-23.
- Haines, C.P. (ed.), 1991. *Insects and Arachnids of tropical stored products: Their biology and identification.* A Training Manual, 2nd edition, Natural Resources Institute, UK.
- Hutchings, A., (1996). *Zulu Medicinal Plants.* Natal University Press, Pietermaritzburg, RSA.
- Isman, M.B. (2000). Plant essential oils for pest and disease management. *Crop Prot.* 19, 603-608.
- Kamanula, J., Sileshi, G.W., Belmain, S.R., Phosiso, S., Mvumi, B.M., Nyirenda, G.K.C., Nyirenda, S.P., Stevenson, P.C., 2011. Farmers' insect pest management practices and pesticidal plant use in the protection of stored maize and beans in Southern Africa. *Int. J. Pest. Manage.* 57, 41-49.

- Konstantopoulou, L.L., Vassilopoulou, L., Mavragani-Tsipidou, P., Scouras, Z.G., 1992. Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. *Experientia* 48, 616-619.
- Koul, O., Walia, S., Dhaliwal, G.S., 2008. Essential oils as green pesticides: Potential and constraints. *Biopestic. Int.* 4, 63-84.
- Le Roux, L. (2004). *Lippia javanica* (Burm. f.) Spreng. Lowveld National Botanical Garden.
- Liolios, C.C., Gortzi, O., Lalas, S., Tsaknis, J., Chinou, I., 2009. Liposomal incorporation of carvacrol and thymol isolated from the essential oil of *Origanum dictamnus* L. and in vitro antimicrobial activity. *Food Chem.* 112, 77-83.
- Longstaff, B.C., 1981. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): a critical review. *Prot. Ecol.* 2, 83-130.
- Manenzhe, N.J., Potgieter, N., van Ree, T., 2004. Composition and antimicrobial activities of volatile components of *Lippia javania*. *Phytochemistry* 65, 2333-2336.
- Maroyi, A., 2017. *Lippia javanica* (Burm.f.) Spreng.: Traditional and commercial uses and phytochemical and pharmacological significance in the African and Indian Subcontinent. *Evidence-Based Complimentary and Alternative Medicine Volume* 2017, Article ID 6746071, pp. 34.
- Marzoug, H.N., Romdhane, M., Lebrihi, A., Mathieu, F., Couderc, F., Abderraba, M., Khouja, M.L., Bouajila, J., 2011. *Eucalyptus oleosa* essential oil: Chemical composition, antimicrobial and antioxidant activities of the oils from different plant parts (stems, leaves, flowers and fruits). *Molecules* 16, 1695-1709.
- Muzemu, S., Mvumi, B.M., Nyirenda, S.P.M., Sileshi, G.W., Sola, P. Kamanula, J.F., Belmain, S.R., Stevenson, P.C., 2011. Pesticidal effects of indigenous plants extracts against rape aphids and tomato red spider mites. *Afr. Crop Sci. Conf. Proc.* 10, 169-171.

- Mwangi, J.W., Addae-Mensah, I., Munavu, R.M., Lwande, W., 1991. Essential oils of Kenyan *Lippia* species part III. Flavour Frag. J. 6, 221–224.
- Mwangi, J.W., Addae-Mensah, I., Muriuki, G., Munavu, R.H., Lwande, W., Hassanali, A., 1992. Essential oil of *Lippia* species in Kenya IV. Maize weevil (*Sitophilus zeamais*) repellency and larvicidal activity. Int. J. Pharmacogn. 30, 9–16.
- Ngassapa, O., Runyoro, D.K.B., Harvala, E., Chinou, I.B., 2003. Composition and microbial activity of essential oils of two populations of Tanzania *Lippia javanica* (Burm.f.) Spreng. (Verbenaceae). Flavour Frag. J. 18, 221-224.
- Nyahangare, E.T., Mvumi, B.M., Mutibvu, T., 2015. Ethnoveterinary plants and practices used for ecto-parasite control in semi-arid smallholder farming areas of Zimbabwe. J. Ethnobiol. Ethnomed. 11:30 DOI 10.1186/s13002-015-0006-6.
- Nyirenda, S.P., Sileshi, G.W., Belmain, S.R., Kamanula, J.F., Mvumi, B.M., Sola, P., Nyirenda, G.K.C., Stevenson, P.C., 2011. Farmers' ethno-ecological knowledge of vegetable pests and pesticidal plant use in Malawi and Zambia. Afr. J. Agric. Res. 6, 525-1537.
- Okey, F., Aslim, B., Ozturk, S., Altundag, S., 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chem. 112, 874-879.
- Omolo, M.O., Okinyo, D., Ndiege, I.O., Lwande, W., Hassanali, A., (2004. Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*. Phytochemistry 65, 2797-2802.
- Rajendran, S., Sriranjini, V., 2008. Plant products as fumigants for stored-product insect control. J. Stored Prod. Res. 44, 126-135.
- Reece, C.A., Rodin, J.O., Brownlee, R.G., Duncan, W.G., Silverstein, R.M., 1968. Synthesis of the principal components of the sex attractant from male *Ips confusus* frass: 2-

methyl-6-methylene-7-octen-4-ol, 2-methyl-6-methylene-2,7-octadien-4-ol, and (+)-cis-verbenol. *Tetrahedron* 24, 4249-4256.

Sahaf, B.Z., Moharramipour, S., Meshkatalasadat, M.H., 2008. Fumigant toxicity of essential oil from *Vitex pseudo-negundo* against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.). *J. Asia-Pacific Entomol.* 11, 175-179.

Sarasan, V., Kite, G.C., Sileshi, G.W., Stevenson, P.C., 2011. Applications of phytochemical and in vitro techniques for reducing over-harvesting of medicinal and pesticidal plants and generating income for the rural poor. *Plant Cell Rep.* 30, 1163-1172.

Soro, L.C., Munier, S., Pelissier, Y., Grosmaire, L., Yada, R., Kitts, D., Atchibri, A.L.O-A, Guzman, C., Boudard, F., Menut, C., Robinson, J.C., Poucheret, P., 2016. Influence of geography, seasons and pedology on chemical composition and anti-inflammatory activities of essential oils from *Lippia multiflora* Mold leaves. *J. Ethnopharmacol.* 194, 587-594.

Stashenko, E.E., Jaramillo, B.E., Martinez, J.R., 2004. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Colombia, and evaluation of its in vitro antioxidant activity. *J. Chromatogr. A* 1025, 93-103.

Stevenson, P.C. Nyirenda, S.P., Mvumi, B.M., Sola, P., Kamanula, J.F., Sileshi, G., Belmain, S.R., 2012. *Pesticidal plants: A viable alternative insect pest management approach for resource-poor farming in Africa*. In: Opende Koul, G. S. Dhaliwal, S. Khokhar and Ram Singh (eds.), *Biopesticides in Environment and Food Security: Issuers and Strategies*, Scientific Publishers, Jodhpur, India, pp. 212-238.

Stevenson, P.C., Arnold, S.E.J., Belmain, S.R., 2014. *Pesticidal plants for stored product pest in smallholder farming in Africa*: In: D. Singh (ed.), *Advances in Plant Biopesticides*. Springer Verlag pp. 212-238.

- Terblanche, F.C., Kornelius, G., 1996. Essential oil constituents of the genus *Lippia* (Verbenaceae). *J. Essent. Oil Res.* 8, 471–485.
- Van Wyk, B.E., Van Oudshoorn, B., Gericke, N., 1997. *Medicinal Plants of South Africa*, first ed. Briza Publications, Pretoria.
- Van Wyk, B.E., Gericke, N., 2000. *People's plants. A guide to useful plants of southern Africa*. Pretoria, South Africa: Briza Publications.
- Velasco-Negeureula, A., P´erez-Alonso, M.J., Guzm´an, C.A., Zygadlo, J.A., Ariza-Espinar, L., Sanz, J., Garc´ia-Vallejo, M.C., 1993. Volatile constituents of four *Lippia* species from C´ordoba (Argentina). *J. Essent. Oil Res.* 5, 513–524.
- Verma, R.S., Rahman, L., Verma, R.K., Chauhan, A., Yadav, A.K., Singh, A., 2010. Essential oil composition of Menthol Mint (*Mentha arvensis*) and Peppermint (*Mentha piperita*) cultivars at different stages of plant growth from Kumaon region of western Himalaya. *OAJMAP* 1, 13-18.
- Viljoen, A.M., Subramoney, S., van Vuuren, S.F, Baser, K.H.C., Demirci, B., 2005. The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. *J. Ethnopharmacol.* 96, 271-277.
- Wannes, W.A., Mhamdi, B., Marzouk, B., 2009. Variations in essential oil and fatty acid composition during *Myrtus communis* var. *italica* fruit maturation. *J. Food Chem.* 112, 621-626.
- Yang, K., Liu, Q.Z., Liu, Z.L., Du, S.S., 2013. GC-MS analysis of insecticidal essential oil of aerial parts of *Paederia scandens* (Lour) Merrill (Rubiaceae). *Trop. J. Pharm Res.* 11, 461-467.

Tables

Table 1

Chemical composition of essential oil from leaves of *Lippia javanica* var. *javanica* collected in Nchenachena in October 2010.

Compound	KI ^a	Peak area (%)	Identification method ^b
Myrcene	991	1.71	MS/KI/Co-injection
Limonene	1029	24.10	MS/KI/Co-injection
(<i>E</i>)-ocimene	1049	0.64	MS/KI/Co-injection
Terpinolene	1088	6.43	MS/KI
Linalool	1101	0.56	MS/KI/Co-injection
Isopulegol	1147	2.37	MS/KI
Myrcenone	1150	1.12	MS/KI/Co-injection
Carvone	1246	3.05	MS/KI/Co-injection
Perillaldehyde	1277	44.43	MS/KI/Co-injection
Piperitenone	1344	2.24	MS/KI
β -Caryophyllene	1422	2.59	MS/KI/Co-injection
Germacrene D	1486	3.88	MS/KI/Co-injection
Bicyclogermacrene	1502	0.73	MS/KI
Spathulenol	1579	1.25	MS/KI
Caryophyllene oxide	1586	0.64	MS/KI

^a KI = Kovats index on HP5 column

^b MS mass spectrum matching with NIST library

Table 2

Effect of variety on chemical composition of essential oil from *Lippia javanica* var. *javanica* and *L. javanica* var. *whytei* collected from Nchenachena and Chikangawa respectively in August 2011

Compound	KI ^a	Peak area (% ± SE; N = 3)	
		Nchenachena (var. <i>javanica</i>)	Chikangawa (var. <i>whytei</i>)
β-pinene	972	0.73 ± 0.00	1.72 ± 1.11
Myrcene	992	2.96 ± 0.00	5.59 ± 0.23
Limonene	1030	16.69 ± 1.40	1.44 ± 0.08
(Z)-Ocimene	1039	0.10 ± 0.00	0.46 ± 0.26
(E)-Ocimene	1049	0.52 ± 0.09	0.26 ± 0.03
Linalool	1101	not detected	2.41 ± 0.09
Myrcenone	1150	0.13 ± 0.00	54.52 ± 2.68
Verbenone	1206	not detected	1.08 ± 0.58
Carvone	1245	not detected	5.41 ± 1.03
Perillaldehyde	1277	63.25 ± 2.96	0.11 ± 0.00
β-Caryophyllene	1420	1.56 ± 0.07	1.37 ± 0.06
Germacrene D	1485	2.60 ± 0.10	2.94 ± 0.09
Bicyclogermacrene	1501	0.64 ± 0.02	0.51 ± 0.00
Spathulenol	1578	1.00 ± 0.09	1.09 ± 0.00
Caryophyllene oxide	1586	0.43 ± 0.04	1.04 ± 0.08

^a KI = Kovats index on HP5 column

Table 3

Effect of harvesting time on the quantity of *L. javanica* leaf oils extracted from Nchenachena, Chikangawa and Jenda provenances in Year 1 (2010/11) and Year 2 (2011/12) of the study.

Month/year	Mean yield of oil (g/kg dry matter; $N = 3$) ^a			
	Nchenachena (var. <i>javanica</i>)		Chikangawa (var. <i>whytei</i>)	Jenda (var. <i>whytei</i>)
	Year 1	Year 2	Year 1	Year 1
October	11.86	9.72 ^{abc}	15.26 ^b	15.98 ^{cd}
December	11.47	11.43 ^{bc}	12.43 ^{ab}	13.35 ^{bc}
February	11.11	13.60 ^c	20.14 ^c	19.28 ^e
April	8.31	6.76 ^{ab}	13.84 ^{ab}	17.24 ^{de}
June	11.76	11.76 ^{bc}	10.76 ^a	9.29 ^a
August	8.43	4.86 ^a	13.79 ^{ab}	10.64 ^{ab}
Mean	10.49	9.69	14.37	14.30
<i>P</i>	0.100	0.001	0.000	0.000
<i>F</i>	2.394	9.705	14.040	34.141

^a Means in a column followed by different letters are significantly different at $\alpha = 0.05$ by Tukey test.

Table 4Effect of harvesting time on the chemical composition of *L. javanica* oil from Nchenachena and Chikangawa during Year 1 (2010/2011)

Compound	Mean proportion (%; <i>N</i> = 3)										
	Nchenachena (var. <i>javanica</i>)						Chikangawa (var. <i>whytei</i>)				
	Oct	Dec	Feb	Apr	Jun	Aug	Oct	Dec	Feb	Apr	Aug
β -pinene	-	-	-	-	-	0.7	-	-	-	-	1.7
Myrcene	1.7	2.3	1.2	1.1	-	3.0	10.2	8.5	7.2	6.1	5.6
Limonene	24.1	25.6	22.9	16.9	29.1	16.7	-	-	-	-	1.4
(<i>Z</i>)- β -ocimene	-	0.9	0.8	0.8	-	0.1	-	-	-	-	0.5
(<i>E</i>)- β -ocimene	0.6	0.7	1.0	1.7	0.7	0.5	0.9	-	-	-	0.3
Terpinolene	6.4	3.8	3.0	3.5	-	-	-	-	-	-	-
Linalool	0.6	1.7	2.5	2.7	-	-	2.3	2.8	2.1	2.0	2.4
Myrcenone	1.1	1.0	-	1.7	-	0.1	51.7	58.1	63.3	52.4	54.5
Verbenone	-	-	-	-	-	-	-	-	-	-	1.1
Carvone	3.1	3.7	0.5	0.6	-	-	9.6	10.4	6.9	7.7	5.4
Perillaldehyde	44.4	28.1	8.7	1.1	61.8	63.3	-	-	-	-	0.1

Piperitenone	2.2	7.3	32.6	25.2	-	-	-	-	-	-	-
β -caryophyllene	2.6	3.3	4.3	5.4	1.5	1.6	1.6	2.3	1.8	1.8	1.4
Germacrene D	3.9	5.8	9.6	17,0	3.3	2.6	4.0	4.8	4.8	4.4	2.9
Bicyclogermacrene	0.7	1.7	2.8	5.2	0.8	0.6	0.9	-	-	-	0.5
Spathulenol	1.3	2.1	1.4	2.4	-	1.0	-	-	-	-	1.1
Caryophyllene oxide	0.6	0.8	0.9	0.8	-	0.4	-	-	-	-	1.0

Figures

Figure 1. Some monoterpenoids and sesquiterpenoids identified in essential oils from *Lippia javanica* var. *javanica* and *L. javanica* var. *whytei*.

Figure 2. Amounts of perillaldehyde, linalool and limonene in *Lippia javanica* var. *javanica* leaves collected in Nchenachena during October, December, February, April, June and August 2010/11

Figure 3. Contact toxicity against *Sitophilus zeamais* of *Lippia javanica* var. *javanica* oil from Nchenachena, perillaldehyde, linalool, perillaldehyde+linalool and limonene after 48 h.

Figure 4. Contact toxicity against *Sitophilus zeamais* of essential oil of *Lippia javanica* var. *whytei* from Chikangawa, Mixture 1 (β -pinene, myrcene, limonene, linalool, myrcenone, carvone, verbenone and β -caryophyllene), Mixture 2 (all compounds in Mixture 1 except myrcenone and limonene), carvone and myrcenone against adult *Sitophilus zeamais* after 48 h.

Figure 5. Fumigant toxicity of *Lippia javanica* var. *javanica* oil against adult *Sitophilus zeamais* after 72, 96 and 120 h.