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Chemical composition and insecticide activity of lemon verbena essential oil

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Abstract: Essential oil extracted from the leaves of Lemon verbena, *Lippia citriodora* Kunth (Verbenaceae) was tested for fumigant toxicity in the laboratory against two stored-product insects *Tribolium confusum* Jacquelin du Val and *Callosobruchus maculatus* (F.). The chemical composition of the isolated oil was examined by GC-MS. The major compounds were citral (11.3%), limonene (10.6%), neral (7.9%), 4-phenyl undecan-4-ol (7.7%), α-curcumene (6.5%), α-cedrol (4.5%) and caryophyllene oxide (4.5%). Furthermore, lesser amounts of the other components include carveol (3.7%), linalool (3.5%), α-pinene (3.2%), caryophyllene (2.8%) and geranyl acetat (1.8%) were existed in the essential oil. In the fumigant toxicity set at 27 ± 1 °C and 65 ± 5% R. H., darkness condition and 24 h exposure time, considerable differences in mortality of insect to essential oil vapor were observed. *C. maculatus* (LC_{50} = 10.2 µl/l air) was significantly more susceptible than *T. confusum* (LC_{50} = 497.8 µl/l air). These results suggested that essential oil of *L. citriodora* could be used as a potential control agent against stored-product insects.

Keywords: Essential oil, GC-Mass, Insecticide, *Lippia citriodora*

Introduction

A key pest of cowpea is the cowpea weevil, *Callosobruchus maculatus* (F.), a bruchid that infests both pods in the field and seeds in storage. Tanzubil (1991) found that this insect can damage 100% of stored seeds causing weight losses of up to 60%.

The confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) is among the most common and destructive insect species in flour mills and related areas (Rees, 1995).

The most commonly used method of controlling stored-product pests is the application of insecticides which cause several problems such as development of insecticide resistance to pest insects, environmental pollution and undesirable effects on non-target organisms, especially humans. These chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for mammalian health (Isman, 2006). The use of plant materials can lead to the identification of new bio-insecticides for the benefit of tropical agriculture. Essential oils for instance have been widely tested and have given promising results under laboratory conditions (Shaaya *et al.*, 1997; Isman, 2000; Rajendran and Sriranjini, 2008).

The genus *Lippia* L. (Verbenaceae) is widely distributed in tropical, subtropical, central and South America, as well as in Africa. This genus consists of approximately 250 species of herbs,

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shrubs and small trees (Terblanche´ and Kornelius, 1996; Braga *et al*., 2005). *Lippia citriodora* Kunth is indigenous to South America and was introduced into Europe at the end of the 17th century. According to the literature, limonene is the component found to occur in higher quantities in essential oils of the genus *Lippia*, followed by *p*-cymene, *α*-pinene, camphor, *β*-caryophyllene, linalool and thymol in a decreasing order (Pascual *et al*., 2001; Terblanche´ and Kornelius, 1996). Various biological activities have been reported for some species of *Lippia*, such as virucidal (Ocazionez *et al*., 2010), antibacterial (Ghaemi *et al*., 2006) and antifungal activity (Tatsadjieu *et al*., 2009) and insecticidal properties (Amer and Mehlhorn, 2006; Zoubiri and Baaliouamer, 2011).

In the present study, the chemical constituents of essential oil from *L. citriodora* leaves were determined, and the insecticidal activity of the essential oil was tested against the adult stages of the stored-products pests, *T. confusum* and *C. maculatus*. Based on our knowledge, no study has been reported previously relating to the activity of the tested oil as fumigants against these stored product insects.

Materials and methods

Insects

C. maculatus and *T. confusum* were reared on bean grain and wheat flour mixed with yeast (10: 1, w/w), respectively in plastic containers (20 cm length, 14 cm width and 8 cm height, covered by a fine mesh cloth for ventilation). The culture was maintained in the dark in growth chamber set at 27 ± 1 °C and 65 ± 5 R.H. All experiments were carried out under the same environmental conditions.

Extraction and analysis of essential oil

The leaves of *L. citriodora* collected from Iranshahr (27° 15' N, 60° 40' E; alt. 590 m), located in Sistan and Baluchestan province, Iran, from June to July, 2010. The plant material was dried naturally on laboratory benches at room temperature (23-27 °C) until crisp. The dried materials were stored at -24 °C and then hydrodistilled to extract its essential oil. Essential oil was extracted from the plant samples using a Clevenger-type apparatus where the plant material is subjected to hydrodistillation. Conditions of extraction were 50 g of samples, 1:10 plant material: water volume ratio, and 4 h distillation. The oil was dehydrated with anhydrous sodium sulphate (10 min) and immediately stored in airtight glassware in refrigerator at 4 °C. The yield of the oil was 0.8% of dry leaves.

The essential oil was analyzed on a gas chromatograph mass spectrometer (GC-mass) (Shimadzu-17A-QP5050, Japan). The GC column was DB-5 (30 m × 0.25 mm i. d, 0.25 µm film thickness). The column oven temperature was set at 60 °C for 3 min, and then increased to 260 °C at a rate of 5 °C/min. Injector and detector temperatures were 230 and 245 °C, respectively. The GC mass analysis was carried out with the same characteristics as used in GC. The ionization energy was 70 ev with a scan time of 1s and mass range of 40-500 amu. Unknown essential oil was identified by comparing its GC retention time to that of known compounds and by comparison of its mass spectra, either with known compounds or published spectra.

Fumigant Toxicity

To determine the fumigant toxicity of the oil, filter paper (2cm diameter) was impregnated with 3, 10, 15 and 20 µl of oils without using any solvent for *T. confusum* and impregnated with 0.5, 0.75, 1.25 and 1.5 µl of oils for *C. maculatus*. Then the filter paper was attached to the under surface of the screw cap of a 60 ml glass vial to generate concentrations of 50, 166.66, 250 and 333.33 µl/l air for *T. confusum* and concentrations of 8.33, 12.5, 20.83 and 25 µl/l air for *C. maculatus.* The cap was screwed tightly on the vial containing ten adults (1-7 days old with undefined sex) of each species of insect separately. Each concentration and control was replicated four times. Mortality was determined 3, 6, 9, 12 and 24 h after exposure in serial time method. When no sign of leg or antennal movement were observed, insect were considered as dead.

Another experiment was designed to assess 50% lethal concentration after 24h exposure to essential oil (LC_{50}). The concentrations of the essential oils were chosen based on range-finding tests (to cause mortality between 20 and 80%) and five different concentrations, each with four replicates and ten individuals per each replicate, were used for each bioassay. The volume of glass vials were 60 and 250 ml for *T. confusum* and *C. maculatus*, respectively .The number of dead and live insect in each bottle was counted 24h after initial exposure to the essential oil. The mortality was determined as described in previous experiment. The treatment bottles were monitored for 48 h after recording the data and no affected insect recovered. Data obtained from each dose response bioassay were subjected to probit analysis (Finney, 1971). LC_{50} values were determined using the statistical software SPSS version 16 (George and Mallery, 2008).

Results

The chemical compositions of essential oil of the *L. citriodora* leaves are presented in the Table 1. The analysis of *L. citriodora* essential oil revealed that citral (11.3%), limonene (10.6%), neral (7.9%), 4-phenyl undecan-4-ol (7.7%), *α*-curcumene (6.5%), *α*-cedrol (4.5%) and caryophyllene oxide (4.5%) were the main products.

In all cases, significant differences were observed in mortality of insect exposed to essential oil vapor in different concentrations and exposure times. The mortality increased with increasing concentrations and exposure time (Figure 1). Results indicated that the oil was significantly more toxic against *C. maculatus* than *T. confusum*, as inferred by the confidence intervals of LC_{50} (Table 2). Based on LC_{50} (Table 2) and fumigant toxicity experiments (Figure 1), although the oil of *L. citriodora* displayed strong insecticidal activity against *C. maculatus* (LC_{50} = 10.2 µl/l air), but revealed poor activity against *T. confusum* (LC_{50} = 497.8 µl/l air).

Table 1 Chemical composition of essential oils of *Lippia citriodora* leaves.

Compounds	Concentration (%)
Citral	11.32
Limonene	10.6
Neral	7.86
4-Phenyl undecan 4-ol	7.72
α- Curcumene	6.54
α- Cedrol	4.54
Caryophyllene oxide	4.46
α- Terpineol	4.06
Carveol	3.71
Linalool	3.54
α- pinene	3.15
Caryophyllene	2.84
Neoalloocimene	2.81
Duvatriendiol	2.69
Trans Caryophyllene	1.99
Carotol	1.84
Geranyl acetat	1.78
4- Terpineol	1.67
α- Terpineol Acetate	1.6
Nerolidol	1.56
Rose furan epoxide	1.56
Copaene	1.43
Trans Limonene oxide	1.40
β- Bourbonene	1.38
α- Cadinol	1.38
Khusinol	1.20
Cis carveol	1.02
α- Cedrane	1.02
Trans Sabinene Hydrat	0.8
Verbenol	0.42
Other compounds	2.1

Table 2 Efficiency of essential oil extracted from *Lippia citriodora* leaves against *Tribolium confusum* and *Callosobruchus maculatus* adults.

Insects	LC_{50} (95% CL)	χ^2 (df)	P-value	Slope ± SE
T. confusum	497.83 (445.9-560.6)	0.73 (3)	0.87	4.02 ± 0.89
C. maculatus	10.17 (8.89-11.83)	2.10 (3)	0.55	3.21 ± 0.59

LC_{50}: lethal concentration (µl/l air), CL: confidence limits (µl/l air).

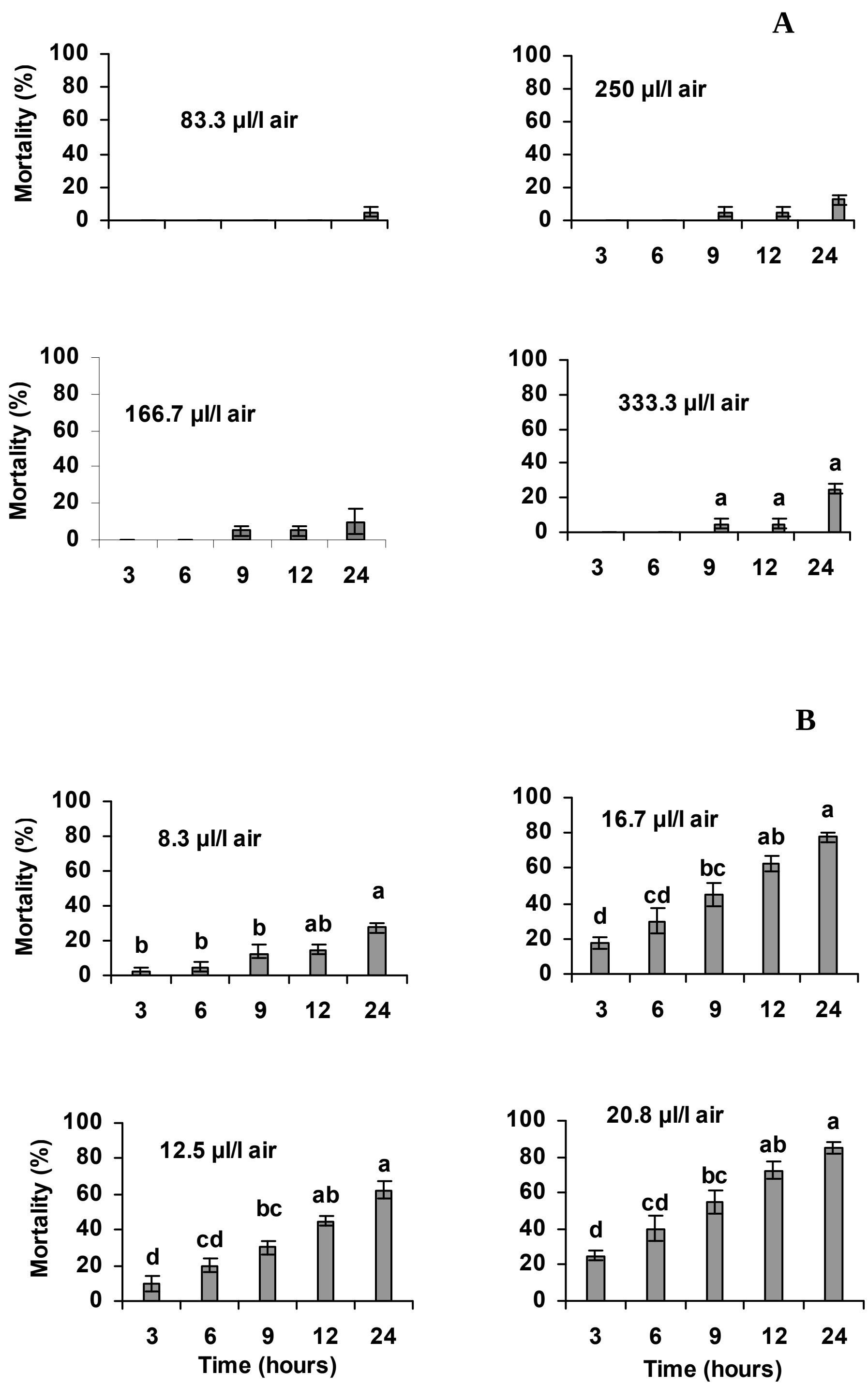

Figure 1 Cumulative percentage mortality of *Tribolium confusum* **(A)** and *Callosobruchus maculatus* **(B)** exposed to various concentration of essential oils from *Lippia citriodora* leaves, at various periods of time.

Discussion

The analysis of *L. citriodora* essential oil revealed that citral (11.3%), limonene (10.6%), neral (7.9%), 4-phenyl undecan-4-ol (7.7%), *α*-curcumene (6.5%), *α*-cedrol (4.5%) and caryophyllene oxide (4.5%) were the main components of the oil. Furthermore, lesser amounts of the other components include carveol (3.7%), linalool (3.5%), *α*-pinene (3.2%), caryophyllene (2.8%) and geranyl acetate (1.8%) were existed in essential oil of this plant.

The main components in the essential oils of *L. citriodora* leaves collected from Armenia (Colombia), were geranial (18.9%), neral (15.6%), limonene (10.7%), 1,8-cineol (5.0%), spathulenol (4.7%), geraniol (2.7%), trans-β-caryophyllene (2.3%), nerol (2.0%) and sabinene (1.9%) (Olivero-Verbel *et al*., 2009). The main constituents of the essential oil extracted from fresh leaves of *L. citriodora* from Greek origin, were geranial, neral and limonene (Argyropoulou *et al*., 2007). Also, GC-MS analysis of essential oils revealed that 1, 8-Cineole (23.66%), *α*-curcumene (14.83%), geranial (13.74%), limonene (13.40%) and caryophyllene oxide (6.60%) were the main components of essential oils of *L. citriodora* leaves, respectively (Meshkatalsadat *et al*., 2010). ar-curcumene (14.1%) and caryophyllene oxide (6.6%), isoaromadendrene epoxide (4.0%), allo-aromadendrene oxide (3.3%), 1, 8-cineole (4.2%), *E*-citral (4.7%), *Z*-citral (4.4%), 6-methyl-5-hepten-2-one (7.1), *β*-caryophyllene (3.9%) and limonene (3.8%) were the main components of *L. citriodora* essential oil (Alavi *et al*., 2011).

According to the literature, geranial, neral and limonene were the component found to occur in higher quantities in essential oils of the *L. citriodora* (Argyropoulou *et al*., 2007; Olivero-Verbel *et al*., 2009; Terblanche′ and Kornelius, 1996). Similarly, in our study, limonene and neral were the major compounds. Furthermore, citral, *α*-curcumene and caryophyllene oxide were available in oil of the species that was before reported in *L. citriodora* essential oil collected from the other localities of Iran (Meshkatalsadat *et al*., 2010; Alavi *et al*., 2011). However, our results did not show the presence of geranial and spathulenol, which have been mentioned in other studies concerning *L. citriodora* (Meshkatalsadat *et al*., 2010; Alavi *et al*., 2011; Olivero-Verbel *et al*., 2009).

Results of fumigant toxicity experiment, showing that *C. maculatus* (LC_{50} = 10.2 µl/l air) was significantly more susceptible to the tested plant product than *T. confusum* (LC_{50} = 497.8 µl/l air). Different susceptibility of stored product insect species to the essential oils has previously been reported (Lee *et al*., 2003; Negahban *et al*., 2007).

Based on our knowledge, no study has been previously reported on the insecticidal activity of the oils of *L. citriodora* against *C. maculatus* and *T. confusum*. However, the insecticidal efficacy of *L. citriodora* oil has been reported against other insects.

Concentrations of 5, 50 and 500 µl/l air of *L. citriodora* showed no mortality on *Sitophilus granarius* (L.) (Col.: Curculionidae) after 24 h fumigation. But, 120 h fumigation with concentration of 5 µl/l air of *L. citriodora* caused 50% mortality of the insect (Zoubiri and Baaliouamer, 2011). Amer and Mehlhorn (2006) reported LC_{50} values ranging between 10 and 100 ppm for *L. citriodora* on various species of mosquito larvae. Also, Massebo *et al*. (2009) reported LC_{50} values of 47.1 and 56.4 ppm for *L. citriodora* on *Aedes aegypti* (L.) and *Anopheles arabiensis* Patton larvae, respectively.

Although *L. citriodora* oil showed higher toxicity on *T. confusum* (LC_{50} = 497.9 µl/l air, Table 1) compared to *S. granarius* (LC_{50} > 500 µl/l air), but mosquito larvae were more susceptible than *T. confusum*. However, *C. maculatus* was most susceptible insect compared to others. In addition to insect species variation, the difference could be related with variability of chemical composition of essential oil of the plant species. In our study, citral and neral were the major compounds of *L. citriodora* oil, while in the study of Zoubiri and Baaliouamer (2011), these compounds were not found in *L. citriodora* essential oil.

Monoterpenes in general have been well documented to be active as fumigants, repellents or insecticides toward stored products insects (Isman, 2006). The insecticidal activity of the essential oils investigated in the present study may be attributed to their major constituents of monoterpenes. Insecticidal activity of major constitutes of the test oil, such as citral (Jeon *et al*., 2009), caryophyllene oxide and neral (Cheng *et al*., 2009), limonene (Tripathi *et al*., 2003) were before reported.

In conclusion, according to the results of this study the essential oil from *L. citriodora* could become an interesting alternative to conventional chemical control strategies. However further studies need to be conducted to evaluate the safety of these oils before practical use in stored- product insect control.

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ترکیب شیمیایی و فعالیت حشره‌کشی اسانس به‌لیمو

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چکیده: اسانس برگ به‌لیمو *Lippia citriodora* Kunth (Verbenaceae) از نظر سمیت تنفسی در شرایط آزمایشگاهی روی دو آفت محصولات انباری شپشه آرد *Tribolium confusum* Jacquelin du Val و سوسک چهارنقطه‌ای حبوبات *Callosobruchus maculatus* (F.) بررسی شد. ترکیب شیمیایی اسانس به روش اسپکترومتری جرمی تعیین گردید. ترکیبات اصلی موجود در اسانس شامل سیترال (۱۱/۳ درصد)، لیمونن (۱۰/۶ درصد)، نرال (۷/۹ درصد)، ۴- فنیل آن دکان ۴- اُل (۷/۷ درصد)، آلفا کورکومن (۶/۵ درصد)، آلفا سدرول (۴/۵ درصد) و کاریوفیلن اکساید (۴/۵ درصد) بود. همواره تفاوت‌های مشخصی در میزان تلفات حشرات در غلظت‌ها و زمان‌های اسانس‌دهی مختلف مشاهده گردید. در سوسک چهارنقطه‌ای حبوبات حساسیت بیشتری به اسانس برگ به‌لیمو (LC_{50} = 10.2 µl/l air) نسبت به شپشه آرد (LC_{50} = 497.8 µl/l air) نشان داد. نتایج نشان داد اسانس به‌لیمو دارای پتانسیل بالقوه جهت کنترل آفات انباری می‌باشد.

واژگان کلیدی: اسانس، اسپکترومتری جرمی، حشره‌کشی، به‌لیمو