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Toxicity and repellency of essential oils of *Lippia alba* chemotypes and their major monoterpenes against stored grain insects

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ABSTRACT

The insects *Sitophilus zeamais* and *Tribolium castaneum* cause losses in stored grains and are considered pests of wide distribution and global significance. In the present study, we evaluated the toxicity and repellency of essential oils of different *Lippia alba* genotypes (carvone chemotypes LA-13 and LA-57 and citral chemotypes LA-10 and LA-44) and their major monoterpenes, carvone and citral, on *S. zeamais* and *T. castaneum*. Toxicity bioassays by exposure of the insects on treated filter paper were performed to determine the concentration and lethal time. Repellency tests were performed using the most toxic compounds according to the toxicity bioassays. The carvone chemotypes were more toxic than the citral chemotypes for both species: for *S. zeamais*, the LC_{50} values were 15.2 μL/mL (LA-13) and 16.7 μL/mL (LA-57) and for *T. castaneum*, the LC_{50} values were 28.7 μL/mL (LA-13) and 19.7 μL/mL (LA-57). Isolated carvone (LC_{50} = 8.8 μL/mL) was more toxic than citral. For *S. zeamais*, the monoterpene citral had the lowest lethal time (LT_{50} = 6 h), whereas for *T. castaneum*, the monoterpenes carvone and citral showed a more rapid toxicity (LT_{50} = 7.3 h). The compounds tested were highly repellent to *T. castaneum*; however, no repellency's effect was observed against *S. zeamais*, except for LA-13 chemotype. The essential oils from the carvone chemotype and the monoterpene carvone have potential for the development of natural insecticides against stored grain insects *S. zeamais* and *T. castaneum*.

1. Introduction

Plants have a wide variety of chemical compounds that have been studied as alternatives to conventional insecticides due to their bioactive potential (Silva et al., 2007; Rattan, 2010; Thormar, 2012). Among these compounds, the essentials oils (EOs) of plants – mixtures of different compounds, mostly of high volatility – have shown lethal and sublethal effects on various organisms (Isman, 2000). The efficiency of EOs for controlling insects is due in large part to their complexity and potential for synergistic or additive effects between their components (Berenbaum, 1985). The advantage of using plant chemical compounds in pest management has been justified primarily by their rapid degradation (e.g., high volatility), which mitigates environmental contamination and effects on non-target organisms (Isman, 2006).

Conventional control of stored grain pests is a global concern because some of these insects cause significant economic losses. *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) are widely distributed and infest silos and warehouses (Ferrari-Filho et al., 2011; Chu et al., 2012; Gueye et al., 2012) causing quantitative and qualitative losses in the production of stored grains (Padin et al., 2002). The presence of insecticide residues in stored grains may pose a threat to human health. Therefore, several studies have evaluated the potential of plant EOs as an alternative for controlling stored grain insects by different routes of exposure (Liu and Ho, 1999; Bouda et al., 2001; García et al., 2005; Nerio et al., 2009; Paes et al., 2012).

The essential oils of the medicinal plant *Lippia alba* (Verbenaceae) have shown bioactive potential against insects (Fontenelle et al., 2007) including stored grain pests (Caballero-Gallardo et al., 2011; Verma et al., 2000). The monoterpenes carvone and citral are the most prevalent compounds in the EO of *L. alba*. Carvone is an unsaturated monoterpenoid ketone, and due to the presence of a chiral center, some plants can synthesize it as enantiomers

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including (*R*)-(−)-carvone. Citral is a mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*-citral, citral A) and neral (*cis*-citral, citral B). Genotypes of *L. alba* have been characterized as different chemotypes due to variation in the concentration of its constituents (Hennebelle et al., 2006; Jannuzzi et al., 2010). Small variations in the concentrations of the EO constituents can trigger distinct responses by insects. Therefore, different EO chemotypes can exhibit lethal and sublethal effects of differing efficacy.

Aiming to broaden the knowledge in the insecticide activity of essential oils of *L. alba* and their chemical compounds against stored grain insects, and identify the better chemotype, with focus in a standardization of insecticide natural compounds, our goal was to evaluate the toxicity and repellency of EOs from *L. alba* genotypes belonging to the carvone chemotype (LA-13 and LA-57) and citral chemotype (LA-10 and LA-44), and their major isolated monoterpenes (carvone and citral) against the stored grain insect species *S. zeamais* and *T. castaneum*.

2. Material and methods

2.1. Plant material

Leaves of *L. alba* genotypes LA-10, LA-13, LA-44 and LA-57 were collected at the Active Germplasm Bank of the Federal University of Sergipe located at the Research Farm "Campus Rural da UFS" (Table 1). Defoliation was performed manually between 2 pm and 4 pm, and leaves were placed in a forced air oven at 40 °C for five days.

2.2. Extraction and analysis of EOs

The EOs were extracted by hydrodistillation using a Clevenger apparatus. Each sample consisted of 75 g of dried leaves distilled for 120 min.

GC analyses were performed using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler AOC-20i (Shimadzu). Separations were accomplished using an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl–95%-dimethyl polysiloxane) of 30 m × 0.25 mm i.d., 0.25 μm film thickness, at a constant helium (99.999%) flow rate of 1.2 mL/min. Injection volume of 0.5 μL (5 mg/mL) was employed, with a split ratio of 1:10. The oven temperature was programmed from 50 °C (isothermal for 1.5 min), with an increase of 4 °C/min, to 200 °C, then 10 °C/min to 250 °C, ending with a 5 min isothermal at 250 °C.

The MS and FID data were simultaneously acquired employing a detector splitting system; the split flow ratio was 4:1 (MS:FID). A 0.62 m × 0.15 mm i.d., restrictor tube (capillary column) was used to connect the splitter to the MS detector; a 0.74 m × 0.22 mm i.d., restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were acquired in the full scan mode (m/z of 40–350) at a scan rate of 0.3 scan/s using the electron ionization (EI) with an electron energy of 70 eV. The injector temperature was 250 °C and the ion-source temperature was 250 °C. The FID temperature was set to 250 °C, and the gas supplies for the FID were hydrogen, air, and helium at flow rates of 30, 300, and 30 mL/min, respectively. Quantification of each constituent was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas and they were arranged in order of GC elution.

Identification of individual components of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in NIST21, NIST107 and WILEY8 mass spectral library of the GC-MS data system. A mixture of hydrocarbons (C_9H_{20}–$C_{19}H_{40}$) was injected under these same conditions and identification of constituents was then performed by comparing the spectra obtained with those of the equipment data bank and by the Kovats index, calculated for each constituent as previously described (Adams, 2007). Retention indices were obtained with equation proposed by Vandendool and Kratz (1963).

2.3. Insects

Specimens of *S. zeamais* and *T. castaneum* were obtained from corn grain in the municipality of Aracaju, state of Sergipe (SE), Brazil. For insect rearing, corn kernels were first washed and kept in a freezer (−10 °C) for 15 days to eliminate possible insecticide residues and organisms present. The kernels were transferred to plastic containers (25 cm high; 12 cm diameter) where the insects were placed. The plastic containers were kept away from light at the Laboratory of Entomology of the Federal University of Sergipe, located in the municipality of São Cristovão (SE), Brazil.

2.4. Toxicity bioassays

Treatments consisted in EOs of the four *L. alba* genotypes and their isolated compounds, carvone (Sigma–Aldrich) and citral (Sigma–Aldrich). On control group was used only the solvent acetone.

Each experimental plot consisted of a glass Petri dish (6 cm diameter × 1.5 height) containing 10 adult insects. A 0.4 mL aliquot of each treatment was applied to filter paper (6 cm diameter) (Unifil, cod. 501.009). After the solvent evaporated, 10 specimens of *S. zeamais* or *T. castaneum* were added per plot. The Petri dishes were placed in a biochemical oxygen demand (BOD) incubator at 25 ± 5 °C and photoperiod of 12 h.

2.4.1. Lethal concentration

The experimental design was completely randomized with four replicates. Three concentrations (1, 10 and 100 μL/mL) of each treatment were used in the initial tests. The initial test results provided parameters for selecting the EOs' concentrations that would cause 0–100% mortality for use in subsequent tests. Considering this mortality range and its respective concentrations, a more narrow response range was used for each treatment.

The number of dead and living individuals was registered up to 48 h after application. Insects were considered dead in all treatments when they did not respond to stimuli.

2.4.2. Lethal time

The completely randomized experimental design was used with four replications. In these tests, we used the concentration of 10 μL/mL.

The number of dead and living individuals was evaluated after 6, 12, 24, 48, 72, 96, 120 and 144 h after application. Insects were

Table 1
Identification and origin of *Lippia alba* genotypes from the Active Germplasm Bank of the Federal University of Sergipe.

Genotype code	Chemotype	Municipality/ State of origin	Provenance	Voucher Number
LA-13	Carvone	Fortaleza-Ceará	Federal University of Ceará	13488
LA-57	Carvone	Rio Real-Bahia	Federal University of Sergipe	13469
LA-10	Citral	Brasília-Distrito Federal	University of Brasília	13495
LA-44	Citral	Brasília-Distrito Federal	University of Brasília	14788