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Review

Contact and fumigant toxicity and repellency of *Eucalyptus citriodora* Hook., *Eucalyptus staigeriana* F., *Cymbopogon winterianus* Jowitt and *Foeniculum vulgare* Mill. essential oils in the management of *Callosobruchus maculatus* (FABR.) (Coleoptera: Chrysomelidae, Bruchinae)

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ABSTRACT

The weevil *Callosobruchus maculatus* (Fabr.) is considered the most important pest of cowpea, *Vigna unguiculata* (L.) Walp., stored in tropical and subtropical countries. Secondary compounds extracted from plants can be used in its control, as a potential alternative to synthetic insecticides. Therefore, the present study aimed to: (a) make the chromatographic and mass spectrometry analyses of the essential oils of *Eucalyptus citriodora* Hook, *Eucalyptus staigeriana* F., *Cymbopogon winterianus* Jowitt and *Foeniculum vulgare* Mill; (b) to evaluate the contact and fumigant toxicity; (c) test the repellent effect. The oils' main compounds were: *E. citriodora* (citronellal 89.59%; citronellyl acetate 3.34%; 1,8-cineole 2.87%), *E. staigeriana* (limonene 28.75%; geranial 15.20%; neral 12.16%), *C. winterianus* (geranial 21.83%; citronellal 10.94%) and *F. vulgare* (limonene 41.82%; (E)-anethole 17.91%; α-pinene 11.13%). The LC_{50s} of *F. vulgare*, *E. citriodora*, *C. winterianus* and *E. staigeriana* in contact tests were estimated at 178.13, 298.17, 328.42 and 345.57 ppm cowpea grains, respectively. According to regression analyses, the higher the oil concentration, the lower the number of laid eggs and emerged insects. In fumigation tests with adults, LC_{50s} ranged from 2.58 to 7.85 μL/L of air, while the toxicity ratios ranged from 1.25 to 3.04. In all concentrations tested, the *E. citriodora* and *C. winterianus* oils were repellent to adult *C. maculatus*; *F. vulgare* was classified as neutral, while *E. staigeriana* was neutral at lower than 558 ppm concentrations and repellent at higher concentrations. Regarding the essential oils tested, the percentage of oviposition reduction varied from 6.3 to 100%, while emergence percentages varied from 0.9 to 100%.

1. Introduction

The cowpea grain [(*Vigna unguiculata* (L.) Walp.)] stands out in the North and Northeast of Brazil for its high economic and social significance, as one of the main source of protein and energy for the rural population (Marsaro Jr. and Vilarinho, 2011). It is part of the diet of 27.5 million people, with an average annual consumption of 20 kg/person, in addition to creating 2.4 million jobs (Santos et al., 2009). However, one of the main problems that occur during storage is the attack of insect pests, notably the weevil *Callosobruchus maculatus* (Fabr., 1775) (Sanon et al., 2002). The losses arise from larvae penetration and feeding within the grains, which leads to weight loss, as well as lower nutritional value, germination potential and cleanliness (Barbosa et al., 2002). In addition, the mite infestation and infection by microorganisms, especially fungi, contribute to the increase of the grain mass temperature, affecting the product's quality (Sari et al., 2003).

Chemical control with Protect synthetic insecticides (organophosphates and pyrethroids) and fumigants (phosphine) is a common practice used to control pests of stored grains. However, due to the accumulation of residues in grains, the selection of resistant insect population and other side effects, alternative approaches in Integrated Pest Management (IPM) have been considered. In this

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context, several plants and constituent bioactive substances, also called insecticides of plant origin or botanical insecticides, have been tested and considered promising for the control of agricultural and urban pests (Arruda and Batista, 1998; Martinazzo et al., 2000; Kemabonta and Odebiyi, 2005). Essential oils have, as main constituents, monoterpenes, sesquiterpenes and low molecular weight aromatic compounds (Knaak and Fiuza, 2010). They can be obtained by various methods, including hydrodistillation, maceration, solvent extraction and supercritical gases. However, the method most used is steam distillation (Craveiro et al., 1981). The separation of complex mixtures of various chemical substances is carried out using chromatographic techniques (Rodrigues et al., 2005).

Essential oils can be used as fumigants and contact insecticides, in the control of stored grain pests, and as repellents (Kheradmand et al., 2010; Paranagama and Gunasekera, 2011), opening new perspectives for the management of these pests, through behavioral approaches.

Thus, considering the good results with the use of insecticides of plant origin in stored grains pest control, this study aimed to: (a) perform the chromatographic analysis and mass spectrometry of the *Eucalyptus citriodora*, *Eucalyptus staigeriana*, *Cymbopogon winterianus* Jowitt and *Foeniculum vulgare* Mill essential oils, (b) to evaluate the toxicity by contact and fumigation, and (c) test the repellent effect in *C. maculatus.*

2. Material and methods

The experiments were conducted at the Laboratory of Agricultural Entomology, Department of Agronomy, Phytosanitary Area, Rural Federal University of Pernambuco (UFRPE) at 28.5 ± 1.6 °C, 52.6 ± 7.4% relative humidity and 12 h photophase. The insects were reared for several generations in cowpea cv. Evergreen grains packed in glass containers closed with perforated plastic lids lined on the inside with a fine cloth to allow gas exchange. They were confined for three days for oviposition, before being removed. The containers were stored until the emergence of the F_1 generation.

Clean and dry grains, used for experiments, were placed in plastic bags and kept in a freezer at −10 °C for seven days, to eliminate possible insect infestation from the field. Then, the grains were transferred to glass flasks and kept in the laboratory for 10 days in order to reach the equilibrium moisture content. *Eucalyptus citriodora* and *E. staigeriana* oils were obtained from the Department of Forest Sciences ESALQ/USP, and *C. winterianus* and *F. vulgare* were obtained from the UFPB-Bananeiras. The extraction the oils was made by hydrodistillation through a modified Clevenger-type equipment, separated from the water, dried with anhydrous Na_2SO_4 and stored at low temperature in dark hermetically closed containers.

2.1. Gas chromatography and mass spectrometry

The chromatographic analysis (GC) of essential oils was performed in the Analytical Center of the Chemistry Department, Federal University of Pernambuco (UFPE), using a Hewlett Packard 5890 SERIES, equipped with ionization detector (FID) and a J&W Scientific DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 mm). The column's temperature was programmed at 40 °C for 2 min. The temperature increased 4 °C/min up to 220 °C and 20 °C/min up to 280 °C, being maintained for 10 min. The injector and detector temperatures were 250 °C and 280 °C, respectively. Hydrogen was used as the carrier gas, with a flow rate of 1.5 mL min^{-1}. A solution of 1.5 μL of 200 μg/mL of the essential oil in hexane was administered. The analysis was conducted by GC/MS of the oils, using a Shimadzu QP5050 equipment with the same column and temperatures used in the experiment with GC. Helium was used as a carrier gas at 1.5 mL min^{-1} flow. Then, 1 μL of 200 μg/mL solution in n-hexane was applied. The mass spectrum was obtained for each compound at 70 eV, and the reading speed was 0.5 scan s^{-1} *m*/*z* 40–650. The identification of the essential oils chemical composition was performed based on the comparison of retention rates experimentally obtained and found in the literature (Adams, 2007), as well as the comparison of the compounds mass spectra with those from the NIST library. The retention index was obtained by applying an oil sample with a C_{11}–C_{24} linear hydrocarbons mixture.

2.2. Contact toxicity tests

Preliminary tests were performed to define each oil concentration. The *E. citriodora* (189, 210, 252, 315, 420 and 462), *E. staigeriana* (311.55, 381.3, 465 and 558), *F. vulgare* (127.5, 153, 225.5, 276.25 and 340) and *C. winterianus* (207.5, 260.75, 456.5 and 601.75 ppm) oils were tested individually, as well as control for each sample, in a completely randomized design with four replications. Each treatment consisted of 20 g of cowpea cv. Sempre Verde infested with eight female *C. maculatus*, 0–48 h of age, packed in 250 mL glass containers with a perforated lid, coated with thin fabric (voile) to allow gas exchange. The oils were added to the grains with an automatic pipettor, in glass containers, and subjected to manual agitation for 2 min. After 48 h from the experiment assembly, mortality percentage was evaluated. Eggs were counted at 12 days and the insects hatched 32 days after confinement.

The lethal concentrations (LC_{50} and LC_{90}) of essential oils were estimated using the POLO-PC program (LeOra Software, 1987). Toxicity ratios (TR) were calculated using the following formula: TR = LC_{50} and/or LC_{90} of the oil with less toxicity/LC_{50} and/or LC_{90} of the other oils, individually. Mortality data, number of eggs and emerged insects were subjected to regression analysis by the SAS program version 2.8 (SAS Institute, 2001).

2.3. Fumigant toxicity tests

Fumigation chambers (adapted from Aslan et al. 2004) composed of glass containers with a volume of 2.5 L were used in the evaluation of the fumigant effect of the essential oils. Preliminary tests were carried out to define the concentration of each oil. The *E. citriodora* (4.0, 4.8, 5.4, 6.2, 7.0, 8.0), *E. staigeriana* (4.8, 5.4, 6.0, 8.0, 8.8), *F. vulgare* (2.6, 3.0, 3.4, 3.8, 4.0, 4.8) and *C. winterianus* (2.0, 3.0, 4.0, 6.0, 8.6, 12.4, 12.8 μL/L air) oils were tested individually, as well as a control for each sample. Strips of filter paper (5 × 2 cm) were impregnated with the oils, with the aid of an automatic pipettor, and fixed to the lower surface of containers' lids. To avoid the direct contact of the insects with the oils, a porous fabric was used between the lids and the containers. The containers were hermetically sealed with aluminum foil and adhesive tape in order to prevent the escape of vapors. After 48 h of the experiment assembly, the percentage of mortality was evaluated. The lethal concentrations (LC_{50} and LC_{90}) of essential oils were estimated using the POLO-PC program (LeOra Software 1987). Toxicity ratios (TR) were calculated using the following formula: TR = LC_{50} and/or LC_{90} of the oil with less toxicity/LC_{50} and/or LC_{90} of the other oils, individually.

2.4. Repellent effect of essential oils

Eucalyptus citriodora (210, 252, 336 and 462), *E. staigeriana* (232.5, 372, 511.5 and 558), *F. vulgare* (127.5, 212.5, 276.25 and 340) and *C. winterianus* (207.5, 332, 456.5 and 622.5 ppm) oils were tested. Bioassays were conducted in arenas made of two 120 mL plastic containers connected to a central plastic box through plastic tubes. In one of the boxes, 20 g of cowpea grains