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Toxicity and antifeedant activity of essential oils from three aromatic plants grown in Colombia against *Euprosterna elaeasa* and *Acharia fusca* (Lepidoptera: Limacodidae)

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ABSTRACT

Objective: To determine the biological effects of essential oils (EOs) isolated from *Cymbopogon nardus*, *Cymbopogon flexuosus* and *Cymbopogon martinii* grown in Colombia against two Lepidoptera larvae, common pests in the oil palm.
Methods: Specimens were captured in the field and the antifeedant activity and dermal contact lethality of EOs were measured against *Acharia fusca* and *Euprosterna elaeasa* (Lepidoptera: Limacodidae) at various concentrations 0.002-0.600 μL/cm^2 and 0.002-8 μL/g, respectively.
Results: All EOs exhibited strong antifeedant and toxicity activity toward *Acharia fusca* and *Euprosterna elaeasa* larvae. *Cymbopogon martinii* oil was the most active against both pest insect species, although all tested EOs were better than the synthetic repellent IR3535 on both insects.
Conclusions: Colombian EOs have potential for integrated pest management programs in the oil palm industry.

1. Introduction

All the organs of the African oil palm (*Elaeis guineensis* Jacquin 1763) can be attacked by insects. Although this species was originally found in West Africa, the majority of the pests of economic importance that attacks the plant are from Tropical America, which adapted to the new crop[1-5]. The leaves constitute the main source of food for a diverse number of insect pests. Most of these belong to the order Lepidoptera but also include various species of Coleoptera and some Orthoptera[2,6].

In Colombia, the Lepidoptera insects attack the majority of African oil palm crops[5,7]. All of them are phytophagous in the larval stages and are considered as the most important pests of agricultural crops, by feeding on the leaves and the parenchyma. These negatively affect the competitiveness of oil palm sector, by causing declines in yield, an increase in the use of agricultural inputs and then increasing costs[2,8].

Euprosterna elaeasa Dyar (Lepidoptera: Limacodidae) (*E. elaeasa*) and *Acharia fusca* Stoll (Lepidoptera: Limacodidae) (*A. fusca*) highlight as insect crops that cause extensive defoliation in the palm areas[5,6]. The main damage is caused by the larvae. In fact, larva one specimen of *E. elaeasa* can consume during its larval stage, 50 cm^2 of leaflet, leaving just the midrib, and an entire colony can cause up to 80% defoliation whereas a larva of *A. fusca* can consume 350 cm^2 of foliage throughout their lives[1,5]. These pests are commonly controlled using chemical insecticides, but over time, insects have acquired some physiological and behavioral resistance. This has forced many plantations to increase the doses of insecticides and application frequencies, with serious implications in terms of production costs, environmental pollution and natural agroecosystem imbalance[6].

Over the recent years, essential oils (EOs) have long been touted as attractive alternatives to synthetic chemical insecticides for pest management. This arises from the fact

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that these botanical mixtures reputedly pose little threat to the environment or to human health[9]. A significant number of authors have studied the antifeedant effect of EOs in Lepidoptera[10-14], as well as their toxicity on larvae[15-17], even though they have also been used as oviposition deterrents[18].

In this study, EOs from three species of the Colombian flora were tested for toxicity and antifeedant activity against *A. fusca* and *E. elaeasa*, two common defoliators of African oil palm plantation in Colombia.

2. Materials and methods

2.1. EOs

Cymbopogon nardus (C. nardus), *Cymbopogon flexuosus (C. flexuosus)* and *Cymbopogon martinii (C. martinii)* EOs were obtained from plant material (300 g in 0.3 L of water), by microwave assisted hydrodistillation and were characterized as previously reported[19] at the Research Center of Excellence, CENIVAM, Industrial University of Santander, Bucaramanga. The oils were provided by Dr. Elena Stashenko, and stored at −4 °C until used for conducting experiments. Each extraction was repeated in triplicate. The chemical composition of the EOs were presented in the supplementary information.

2.2. Test procedures

2.2.1. Experimental units

Third instar larva specimens of *A. fusca* and *E. elaeasa* were collected directly from oil palm plantations in the municipality of Maria La Baja, Bolivar-Colombia (9°58′52″ N, 75°17′55″ W) where used for the assays (Figure 1). Organisms were stored in glass containers covered with a plastic mesh with a diet of fresh oil palm leaflets at (26±2) °C, relative humidity of 70%-85% and photoperiod 10:14 h (light: dark) and kept under these conditioning until used for assays, within 96 h after collection.

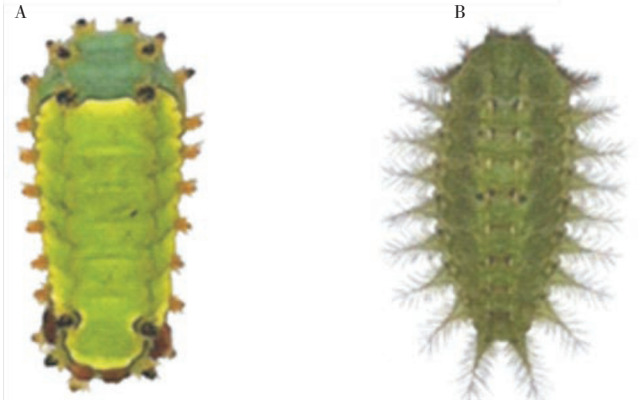

Figure 1. Third instar larva of *A. fusca* (A) and *E. elaeasa* (B).

2.2.2. Antifeedant assay

The antifeedant activity was assessed through the binary choice method described by Wellsow *et al*. using leaves of oil palm impregnated with EOs[20]. The leaves were cut disc shaped of 2 cm in diameter and weighed using an analytical balance to the nearest 0.1 mg (Ohaus Pioneer). EOs were dissolved in acetone and 60 μL of respective solutions where applied on the leaves to produce final concentrations of 0.002, 0.020, 0.200, 0.400 and 0.600 μL/cm^2 on 2 cm discs. A commercial repellent formulation (Stay off Colombia), which contains a 150 mL/L solution [ethyl 3-(N-acetyl-N-butylamino) propionate] (IR3535), was employed as positive control. Ten larvae were individually placed in Petri dishes (9 cm×1.2 cm) with a single treated or vehicle control (60 μL acetone) leaf disc. After 12 h, the remained leaf fraction was weighted and used to calculate the feed-rate using formula[21]:

FI (%)=[1-(T/C)]×100

Where T=consumption on treated dish; C=consumption of control dish. An FI=100% indicates complete feeding inhibition. Three replicates were used for each tested concentration of EO (n=30), and the assays were repeated twice.

2.2.3. Contact toxicity assays

The contact toxicity of the EOs was evaluated using a topical application test[14,17]. Dilutions of the tested EOs (0.1-30.0 mL/L) were prepared using acetone as a solvent. Each larva was individually weighed using an analytical balance (Ohaus Pioneer) and received 40 μL of solution per treatment, with acetone alone as the control. Doses used were between 0.02 μL/g and 8.00 μL/g of larva, and solutions were applied topically to the dorsal surface of the larvae using a micropipette. After 24 h exposure dead larvae were counted and data tabulated for mortality assessment. To determine whether the larva was alive or dead, the palpation method was utilized (the larva was touched with a soft painting brush; if it makes any movement, it is considered alive, otherwise it is considered dead)[17]. Five replicates were used for each tested concentration of EO (n=50), and each assay was repeated twice.

2.3. Statistical analysis

The results are presented as mean±SE. The sign obtained in the calculation of FI (%) was employed to qualify the antifeedant (positive) or phagostimulant (negative) action of the EO. FI_{50} and median lethal dose (LD_{50}) of EOs and their confidence intervals at 95% were calculated using Probit Analysis[22]. Normal distribution and equality between variances were checked by Kolmogorov-Smirnov's and Bartlett's tests, respectively. Comparisons of the FI (%) and mean mortality between evaluated EOs and positive control