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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.

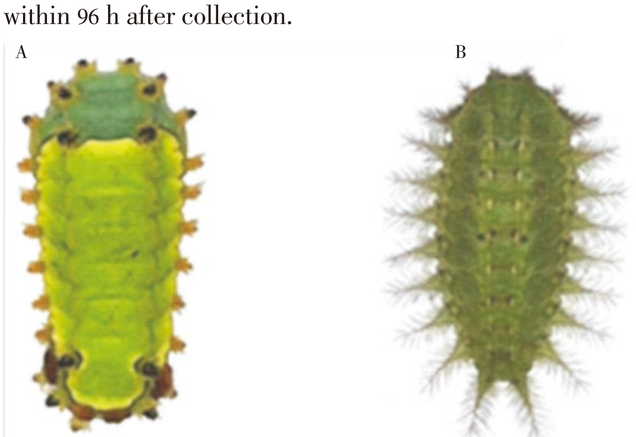

Figure 1. Third instar larva of *A. fusca* (A) and *E. elaeasa* (B).

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 $\mu L/cm^2$ 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]:

$$FI\ (\%)=[1-(T/C)]\times 100$$

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 were performed using ANOVA, with Dunnett's post-test used to compare treated with control group, Tukey's post-test to compare between the concentrations of the EOs and t-test to compare between the concentrations of EOs for both pest insects. Statistical analysis was performed with Statgraphics Plus 5.1[23], and Graph pad Prism 5 for Windows[24].

3. Results

3.1. Antifeedant activity of EOs

The results of the antifeedant activity assays for tested EOs are presented in Table 1. Data showed that at all tested concentrations, the EOs presented antifeedant properties against both examined organisms, with a clear dose-dependent activity (Tables 1 and 2). The maximum feed rate inhibitions were obtained for *C. martinii* at the highest tested concentration (0.600 μL/cm^2) with values of 98% and 88% for *A. fusca* and *E. elaeasa*, respectively.

Table 1

Feed rate inhibition (%) on *A. fusca* and *E. elaeasa* exposed to EOs of three different leaves and IR3535.

Pest insect	Concentration (μL/cm^2)	*C. nardus*	*C. flexuosus*	*C. martinii*	IR3535
A. fusca	0.002	20±3	18±6	26±10	7±4
	0.020^a	38±3^d	25±6	59±4^{bcd}	21±4
	0.200^a	51±3^d	37±4	64±1^{bd}	36±3
	0.400^a	73±4^d	54±2	84±4^{bcd}	43±3
	0.600^a	80±3^d	69±3	98±1^{bcd}	63±6
E. elaeasa	0.002	14±7	9±4	20±5	6±7
	0.020	39±6	28±6	43±3	28±4
	0.200	49±6	41±3	51±5	38±7
	0.400	65±4	58±3	64±6	44±5
	0.600^a	84±1^d	71±3	88±2^{bd}	65±6

Values are mean±SE, n=6. a: Significant difference between activities of EOs at a particular concentration, ANOVA (P<0.05); b: Significant difference when compared to *C. martinii*, Tukey's post-test (P<0.05); c: Significant difference between pest insects for the activity elicited by an EO at a particular concentration, t-test (P<0.05); d: Significant difference between the activity of an EO and the positive control (IR3535); ANOVA, Dunnett's post-test (P<0.05).

Table 2

FI_{50} at 12 h after of *A. fusca* and *E. elaeasa* exposed with three EOs and positive control (IR3535) at five concentrations.

EO	*A. fusca*				*E. elaeasa*			
	FI_{50} (μL/cm^2)	95% CL (μL/cm^2)	Slope	χ^2	FI_{50} (μL/cm^2)	95% CL (μL/cm^2)	Slope	χ^2
C. nardus	0.19	0.13-0.25	2.52±0.36	55.53	0.24	0.18-0.29	2.75±0.36	65.07
C. flexuosus	0.36	0.29-0.45	2.19±0.34	43.86	0.34	0.28-0.42	2.51±0.35	56.39
C. martinii	0.08	0.02-0.13	3.42±0.43	77.34	0.20	0.14-0.26	2.62±0.36	58.70
IR3535	0.45	0.37-0.55	2.31±0.35	46.67	0.42	0.35-0.53	2.18±0.34	42.58

Data of slope are expressed as mean±SE. n=300 larvae; CL: Confidence limit; χ^2: *Chi*-square.

At concentrations between 0.020 and 0.600 μL/cm^2, there were statistical differences between the antifeedant properties of tested EOs against *A. fusca* (0.020 μL/cm^2, F=16.15; P=0.000 2; 0.200 μL/cm^2, F=22.26; P<0.000 1; 0.400 μL/cm^2, F=21.53; P<0.000 1; 0.600 μL/cm^2, F=34.67; P<0.000 1; Table 1). However, for *E. elaeasa*, these differences occurred only at 0.600 μL/cm^2 (F=20.57; P<0.0001; Table 1). Post-test analysis revealed that for some concentrations at which there were statistical differences between EOs, for *A. fusca*, *C. martinii* showed significant differences against *C. nardus* and *C. flexuosus*; whereas for *E. elaeasa*, the only detected difference was observed between *C. martinii* and *C. flexuosus* at 0.600 μL/cm^2. On the other hand, when comparing *A. fusca vs. E. elaeasa*, only the EO of *C. martinii* presented greater activity on the first, and this happened at 0.020, 0.400 and 0.600 μL/cm^2 (T=3.54; P=0.005; T=2.97; P=0.01; T=4.29; P=0.002; Table 1). The activities of tested EOs were compared to that elicited by the commercial repellent IR3535. For *A. fusca*, only the oils from *C. martinii* and *C. nardus* were significantly greater than the positive control at concentrations greater than 0.002 μL/cm^2, whereas for *E. elaeasa*, such difference was registered for *C. martinii* at the greatest tested concentration.

Finally, based on the FI-values (Table 2), the antifeedant properties of the EOs against *A. fusca* decreased in the order *C. martinii*≈*C. nardus*>*C. flexuosus*, whereas for *E. elaeasa* it was *C. martinii*≈*C. nardus*>*C. flexuosus*. In both cases, the EO isolated from *C. flexuosus* was the least potent.

3.2. Contact toxicity of EOs

The results of the contact toxicity assays for examined EOs are shown in Figure 2. All EOs showed toxicity activity against *A. fusca* and *E. elaeasa*, with a clear dose-dependent toxicity (Table 3). The maximum mortality percentage obtained for *A. fusca* was reached with *C. martinii* 70%, whereas for *E. elaeasa* it was 63%, also with the same EO at the highest applied concentration.

Table 3

Lethal doses (LD_{50}) at 24 h after of *A. fusca* and *E. elaeasa* were exposed with three EOs and positive control (IR3535) at five concentrations.

EO	*A. fusca*				*E. elaeasa*			
	LD_{50} (μL/g)	95% CL (μL/g)	Slope	χ^2	LD_{50} (μL/g)	95% CL (μL/g)	Slope	χ^2
C. nardus	7.35	6.47-8.61	0.18±0.02	77.5	4.83	4.33-5.52	0.36±0.04	95.5
C. flexuosus	6.70	5.99-7.64	0.21±0.02	100	5.52	4.83-6.57	0.30±0.04	66.3
C. martinii	4.34	3.73-5.05	0.19±0.02	93.0	4.00	3.60-4.52	0.37±0.04	114
IR3535	>8	-	-	-	7.03	6.02-8.85	0.33±0.05	46.1

Data of slope are expressed as mean±SE. n=500 larvae; CL: confidence limit; χ^2: *Chi*-square.

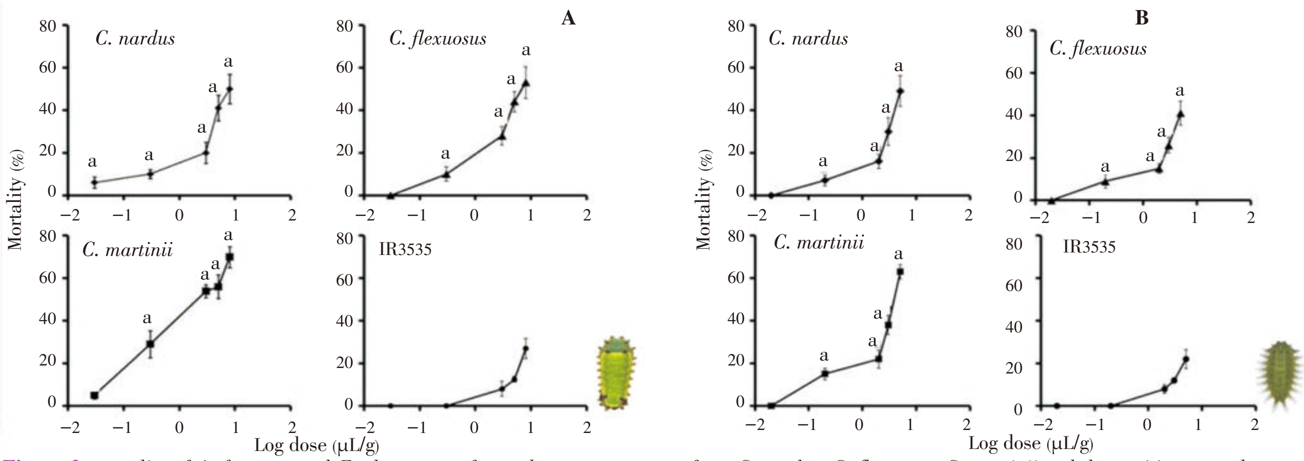

Figure 2. Mortality of *A. fusca* (A) and *E. elaeasa* (B) after 24 h exposure to EOs from *C. nardus*, *C. flexuosus*, *C. martinii* and the positive control (IR3535).
a: Significant difference between activities of EOs and the positive control (P<0.05). Error bars represent the SE.

Based on LD_{50} values (Table 3), the dermal toxicity of EOs against *A. fusca* decreased in the order *C. martinii*>*C. flexuosus*≈*C. nardus*. However, for *E. elaeasa*, although the LD_{50} was lower for *C. martinii*, the variability of the data was greater within EOs, with clear overlapping between confidence intervals. Interestingly, the positive control, IR3535, was not only less potent but also presented lower efficacy than the examined EOs.

After 24 h exposure to EOs, *A. fusca* and *E. elaeasa* larvae depicted characteristic behavioral changes, consisting of extreme agitation, random walking and wandering, dieresis and convulsions, finally leading to paralysis and death. However, only *A. fusca* larvae exhibited discoloration, changing their body color to a dark brown (Figure 3). Larvae treated with vehicle-control did not show any change.

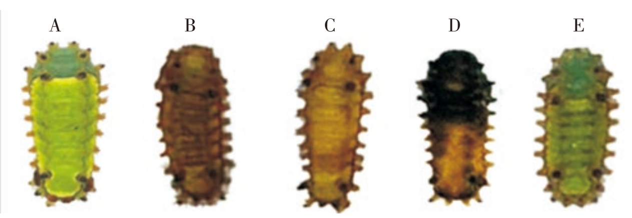

Figure 3. Representative specimens of *A. fusca* larva exposed for 24 h to vehicle-control and EOs from *C. nardus*, *C. flexuosus*, *C. martinii* and IR3535.
A: Vehicle-control; B: *C. nardus*; C: *C. flexuosus*; D: *C. martinii*; E: IR3535.

4. Discussion

Plants with insecticidal properties have been traditionally used for crop protection, but only recently, the potential for the development of products utilized in pest management applications has been recognized[25]. In general, EOs are mostly considered nontoxic to vertebrates[26]. On the other hand, they act as broad spectrum pesticides due to their diverse mode of action, including repellency and antifeedant activity, disruption of molting and cuticle, as well as retardation of growth and fecundity[27,28]. Recent reports indicated a strong antifeedant effect of plant derivatives and recommended their widespread use as they showed great environmental safety[29-31]. However, it should be kept in mind that some EOs may posse neurotoxic effects, evident from their rapid action against some pest insects[26].

The present study demonstrated that the EOs isolated from *C. nardus*, *C. flexuosus* and *C. martinii*, exhibited strong toxicity and antifeedant activity toward *A. fusca* and *E. elaeasa* larvae. The EO from *C. martinii* was the most active against both species, whether it was evaluated as a larvicidal or as a feeding deterrent. In terms of acute toxicity and antifeedant properties, tested EOs were better than the synthetic repellent IR3535 on both insects.

The EOs extracted from the genus *Cymbopogon* have been evaluated by numerous authors as repellents and insecticides for protecting crop as well as for preventing from mosquito bites[32-36], making this genus a great source of natural repellents of worldwide popularity[37-39]. The composition of these oils has been previously published[19], and some of their components, such as citronellal and citronellol have been reported for their ability as contact insecticides, repellents and antifeedant chemicals[12,14,17,37,40-44].

It should be pointed that synergistic effects of complex mixtures such as EOs are thought to be important in plant defense against herbivore predators. Plants usually present defenses as a set of compounds, thus, complex EOs may be more efficient than individual pure compounds[45,46].

Although several extracts and EOs isolated from this genus have been evaluated against other insect species. This is the first report showing the use of EOs to control

A. fusca and *E. elaeasa*. These promising results should encourage the development of field tests to validate these results, with the aim of to be included together with other effective control options, in the management of defoliator insect in crop of African oil palm.

Conflict of interest statement

We declare that we have no conflict of interest.

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