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Bioactivity of selected plant-derived essential oils against *Zabrotes subfasciatus* (Coleoptera: Bruchidae)

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ABSTRACT

The common bean *Phaseolus vulgaris* L. (Fabaceae) is an important vegetable protein source and constitutes a significant part of the diet in many tropical countries. The Mexican bean weevil *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) is one of the main pests affecting stored beans: it causes extensive qualitative and quantitative grain loss. We investigated the bioactivity of the essential oils extracted from *Chenopodium ambrosioides* L. (CA-EO), *Ocimum gratissimum* L. (OG-EO), and *Schinus terebinthifolius* Raddi (ST-EO) against *Z. subfasciatus*. After 12-h treatment, CA-EO and OG-EO at 20.0 μL/L of air killed 100% *Z. subfasciatus*, whereas ST-EO at 100.0 μL/L of air afforded 100% *Z. subfasciatus* mortality after 24 h. CA-EO provided the lowest 24 h LD_{50} (0.8 μL/L of air) and displayed efficient repellent activity against *Z. subfasciatus*. Our results demonstrate that CA-EO is a potentially economical and environmentally friendly alternative to manage *Z. subfasciatus* in stored beans.

1. Introduction

The global population is expected to be higher than 8.5 billion in 2025 (Babu et al., 2003), and this situation will clearly demand increased food production. The common bean *Phaseolus vulgaris* L. is consumed worldwide and constitutes an important calorie and protein source (Lagarda-Diaz et al., 2009). Several research groups have studied post-harvest grain loss (Alonso-Amelot and Avila-Núñez, 2011). When it comes to stored grains, the insect infestation can reduce the grain quantitative and qualitative contents (Madrid et al., 1990). Among the various insects that affect stored grains, the Mexican bean weevil *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) stands out (Costa et al., 2014). Weevil attack diminishes grain weight, alters the grain physical properties, and increases microorganism invasion (Baldin and Lara, 2008).

In this scenario, improved food storage strategies are crucial to mitigate losses and to meet the feeding needs of the growing world population. The battle against stored food infestation generally requires the use of chemical insecticides, which has undesirable consequences such as deleterious effects on the environment and on biodiversity (Moshi and Matoju, 2017). To overcome this issue, natural insecticides based on plant-derived products have been suggested as suitable approaches to weevil management (Jumbo et al., 2014). In this context, essential oils (EOs) have received special attention due to their natural biological functions like fungicidal, bactericidal, and insecticidal activities (Bakkali et al., 2008). These EOs act by disrupting the insect's neurophysiological functions: they target the γ-aminobutyric acid (GABA) receptors (Priestley et al., 2003) and octopaminergic system (Enan, 2001, 2005), and they inhibit acetylcholinesterase (López and Pascual-Villalobos, 2010).

Recently, the *Chenopodium ambrosioides* L., *Ocimum gratissimum* L., and *Schinus terebinthifolius* Raddi EOs have been shown to display interesting insecticidal activity against some insect pests (Denloye et al., 2010; Nguemtchouin et al., 2013), but their activities against *Z. subfasciatus* have not been reported yet. In this paper, we investigate the fumigant and repellent potential of the EOs extracted from these plant species against *Z. subfasciatus*.

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2. Material and methods

2.1. Insect colony

During this study, a *Z. subfasciatus* colony was maintained in a BOD chamber (25 ± 2 °C, 70% ± 10% RH) in order to obtain a sufficient number of insects to conduct the proposed bioassays. The insects were placed in clear 1-L glass flasks closed with a screw-on lid. This lid contained a circular opening that held a fine-mesh nylon screen, which allowed internal aeration. Each flask received approximately 0.3 kg of commercial Carioca bean seeds and approximately 200 unsexed adult insects. The seeds were periodically replaced, and emerging adult insects were used to infest new flasks and to keep the colony.

2.2. Essential oil extraction and chemical components

C. ambrosioides (Chenopodiaceae), *O. gratissimum* (Lamiaceae), and *S. terebinthifolius* (Anacardiaceae) were collected near the city of Patrocínio (18°56′35" S; 46° 59′31" O; 972 m), State of Minas Gerais, Brazil, in March 2011. The plants were identified by Prof. Rosângela de Oliveira Araújo. Voucher specimens (HUFU 61679, HUFU 61682 and HUFU 61673, respectively) were deposited at the Herbarium of the Federal University of Uberlândia, Minas Gerais, Brazil (Herbarium HUFU).

Fresh leaves of each plant species were submitted to hydrodistillation in a Clevenger-type apparatus for 3 h. To this end, 1500 g of plant material was divided into three 500-g samples, and 500 mL of distilled water was added to each sample. The EOs were collected manually; traces of water remaining in the oils were removed with anhydrous sodium sulfate, followed by filtration. The EOs were stored in amber bottles and kept in the refrigerator at 4 °C. The *C. ambrosioides* (CA-EO), *O. gratissimum* (OG-EO), and *S. terebinthifolius* (ST-EO) EO yields were calculated from the weight of the leaves and expressed as the average of triplicate analysis.

2.3. Fumigant toxicity

The activities of the EOs (CA-EO, OG-EO, and ST-EO) against *Z. subfasciatus* were evaluated by fumigation. For this purpose, CA-EO, OG-EO, ST-EO samples were dissolved in Tween 80. A filter paper was impregnated with eight graded doses of each EO to provide fumigant concentrations of 2.5, 5.0, 10.0, 20.0, 40.0, 60.0, 80, and 100.0 μL/L of air. For the most active EO, CA-EO, the concentration of 1.25 μL/L of air was also tested. The filter papers were attached to the undersurface of the screw cap of a 50-mL glass vial (fumigation chamber). Five adult one-day-old *Z. subfasciatus* couples and 10 g of beans were transferred to the vials. Five replicates of each treatment and controls were set up, and the percentage adult mortality was recorded 12, 24, 48, and 72 h after treatment. The insects were considered dead when no leg or antennal movements were observed. Tween 80 was used as negative control; the insecticide based on aluminum phosphide (Gastoxin- B 57®, Bequisa Co, São Vicente, Brazil) was used as positive control at the dose recommended by the manufacturer (2 g/m^3 of air).

2.4. Repellent activity

The EO repellent activities were assessed by using the methodology described by Procópio and co-workers (Procópio et al., 2003). The experimental apparatus consisted of five circular plastic containers - the central container was connected to the other four containers by plastic cylinders (12-cm long, 1-cm diameter). Two lateral containers were filled with beans treated with 50, 100, and 500 μL of one of the EOs/kg of bean. Two other lateral containers (controls) were filled with untreated beans. In the central container, ten one-day-old adult couples were released. The total number of insects per container was recorded after 24 h. Ten replicate assays were carried out for each EO in a completely randomized design.

2.5. Statistical analysis

The data obtained from the biological assays (percentage mortality and repellent activity) were submitted to one-way ANOVA. The normality and the homogeneity of the variances were verified through Shapiro-Wilk and Levene tests, respectively (Winer et al., 1991). Differences between treatment means were determined by using Tukey's Studentized (HSD) test (Ogendo et al., 2008). The software SAS was employed (SAS Institute, 2001). To estimate the LD_{50} values, the relationship between the applied EO concentration and the percent insect mortality was determined by probit (softwares Poloplus Probit and Logit Analysis version 1.0) regression analysis of transformed data. The Abbott formula was used to calculate the control efficiencies (Abbott, 1985).

The repellent activity index (RI) was calculated as proposed by Lin et al. (1990): RI = 2G/(G + P), where G = number of insects in the container with treated beans and P = number of insects in the container with untreated beans. The classification interval (CI) was calculated by the formula $CI = 1 \pm t_{(n-1;\ \alpha\ =\ 0.05)} \times SD/\sqrt{n}$, where t = tabulated value, SD = standard deviation, and n = number of repetitions. Comparison between the RI and the CI values indicated the EO activity: RI = CI corresponded to neutral activity, RI > CI maximum referred to attraction activity, and RI < CI minimum corresponded to repellent activity.

Table 1
CA-EO, OG-EO, and ST-EO fumigant activity (percentage adult mortality) against *Z. subfasciatus*.

EO	Tested doses (μL/L of air)	Exposure time			
		12 h	24 h	48 h	72 h
CA-EO	1.25	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	2.5	8.2 ± 0.8 ab	72.5 ± 2.1 b	100.0 ± 0.0 b	100.0 ± 0.0 b
	5.0	14.0 ± 1.7 ab	80.0 ± 3.3 b	100.0 ± 0.0 b	100.0 ± 0.0 b
	10.0	22.5 ± 3.6 c	98.2 ± 2.2 c	100.0 ± 0.0 b	100.0 ± 0.0 b
	20.0	76.5 ± 5.8 d	100.0 ± 0.0 c	100.0 ± 0.0 b	100.0 ± 0.0 b
	40.0	100.0 ± 0.0 d	100.0 ± 0.0 c	100.0 ± 0.0 b	100.0 ± 0.0 b
	60.0	100.0 ± 0.0 d	100.0 ± 0.0 c	100.0 ± 0.0 b	100.0 ± 0.0 b
	80.0	100.0 ± 0.0 d	100.0 ± 0.0 c	100.0 ± 0.0 b	100.0 ± 0.0 b
	100.0	100.0 ± 0.0 d	100.0 ± 0.0 c	100.0 ± 0.0 b	100.0 ± 0.0 b
OG-EO	2.5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	5.0	0.0 ± 0.0 a	80.3 ± 1.4 b	80.5 ± 3.6 b	88.2 ± 4.4 b
	10.0	0.0 ± 0.0 a	86.2 ± 3.6 b	86.5 ± 3.8 b	88.2 ± 4.2 b
	20.0	96.1 ± 3.5 b	100.0 ± 0.0 b	100.0 ± 0.0 b	100.0 ± 0.0 b
	40.0	92.5 ± 6.5 b	98.0 ± 4.4 b	100.0 ± 0.0 b	100.0 ± 0.0 b
	60.0	92.5 ± 3.5 b	100.0 ± 0.0 b	100.0 ± 0.0 b	100.0 ± 0.0 b
	80.0	90.0 ± 4.3 b	100.0 ± 0.0 b	100.0 ± 0.0 b	100.0 ± 0.0 b
	100.0	90.0 ± 4.3 b	98.2 ± 5.8 b	100.0 ± 0.0 b	100.0 ± 0.0 b
ST-EO	2.5	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	5.0	0.0 ± 0.0 a	10.3 ± 0.9 a	18.2 ± 1.4 a	48.3 ± 3.1 b
	10.0	0.0 ± 0.0 a	6.2 ± 1.1 a	20.2 ± 0.7 a	48.3 ± 3.3 b
	20.0	16.3 ± 6.8 abc	88.0 ± 3.8 b	94.5 ± 7.1 b	100.0 ± 0.0 c
	40.0	8.2 ± 3.4 ab	88.0 ± 3.3 b	96.5 ± 5.6 b	100.0 ± 0.0 c
	60.0	30.0 ± 2.1 c	96.0 ± 2.9 bc	100.0 ± 0.0 b	100.0 ± 0.0 c
	80.0	30.0 ± 1.7 c	96.0 ± 4.5 bc	100.0 ± 0.0 b	100.0 ± 0.0 c
	100.0	24.6 ± 3.3 bc	100.0 ± 0.0 c	100.0 ± 0.0 b	100.0 ± 0.0 c

For each exposure period, the percentages with the same letters do not differ by the Tukey Test ($P > 0.05$).