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Potential use of clove and cinnamon essential oils to control the bean weevil, *Acanthoscelides obtectus* Say, in small storage units

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ABSTRACT

The bean weevil, *Acanthoscelides obtectus* Say, causes severe post-harvest losses in the common bean, *Phaseolus vulgaris* L. The control of these insects is either non-existent or relies heavily on the use of conventional insecticides, which increase the risks associated with pest resistance, hazards to human health and environmental contamination. Protecting grains with alternative chemical control options that alleviate the concerns outlined above are urgently needed, and essential oils of plants have been presented as a suitable alternative to fill this void. Therefore, this investigation evaluated the non-fumigant applications of clove, *Syzygium aromaticum* L., and cinnamon, *Cinnamomum zeylanicum* L., essential oils adequately control *A. obtectus* on common beans. The oils were tested for insecticidal (lethal toxicities, disturbances on reproductive traits and persistence of action) and repellent activities. Both oil types showed similar toxicity (clove LD = 43.6 μL/kg beans; cinnamon LD_{50} = 46.8 μL/kg beans), steadily decreased the growth rate of *A. obtectus* in a dose-dependent manner, and similarly lost their insecticidal activity over the time. Additionally, the clove oil delayed bean weevil emergence, whereas cinnamon oil repelled the bean weevil. These results indicate clove and cinnamon essential oils as desirable tools for protecting stored beans against *A. obtectus* in small storage facilities, promoting environmentally friendly pest control strategies.

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1. Introduction

The bean weevil *Acanthoscelides obtectus* (Say) is insect pest of a neotropical origin that cosmopolitanly feed on wild and cultivated common beans *Phaseolus vulgaris* (L.) (Abate and Ampofo, 1996; Alvarez et al., 2005, 2006; Jovanović et al., 2007; Paul et al., 2009; Southgate, 1978; Thakur, 2012; Vilca Mallqui et al., 2013). Infestations of *A. obtectus* begins in the field and continues as beans are moved to storage facilities, where the pest causes major losses (Baier and Webster, 1992). In conjunction with the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman), *A. obtectus* have been responsible for serious losses on stored beans in Latin American countries, such as Ecuador and Brazil (Castillo and Gallegos, 1995; Oliveira et al., 1979; Teixeira and Zucoloto, 2012; Vilca Mallqui et al., 2013). When left untreated, the *A. obtectus* populations grow exponentially and can completely destroy the stored crops within a few months (Gołębiowski et al., 2008), which consequently might constrain small farmers (especially subsistence producers) to sell their production early after harvest or, even worse, extinguish their bean seeds saved on-farm from previous harvest.

Currently, the management of *A. obtectus* on storage facilities is either non-existent (by small farmers) or relies on the application of synthetic insecticides (in big storage facilities), such as phosphine, pyrethroids and organophosphates (Daglish et al., 1993; Oliveira et al., 2013). However, the application of these compounds has recently been under intense scrutiny due to concerns about pest resistance, hazards to human health and environmental contamination (Daglish, 2008; Subramanyam and Hagstrum, 1995), which have led to an environmental movement that seeks sustainable alternatives in pest control (Dayan et al., 2009; Isman, 2006, 2008; Rattan, 2010; Regnault-Roger et al., 2012).

Because plant products such as essential oils apparently do not pose the same risks as traditional insecticides, they have been suggested as a suitable alternative for controlling mites and

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insect pests worldwide (Castillo et al., 2009; Fang et al., 2010; Konstantopoulou et al., 1992; Morais et al., 2006; Murungi et al., 2013; Pascual-Villalobos and Robledo, 1998; Pavela, 2011), including *A. obtectus* (Papachristos et al., 2004; Regnault-Roger et al., 1993). Essential oils exert insecticidal effects or disrupt insect development (Konstantopoulou et al., 1992; Regnault-Roger and Hamraoui, 1994; Weaver et al., 1991) by interfering with the insect nervous system, notably acting on the GABAnergic (Bloomquist et al., 2008; Priestley et al., 2003; Tong and Coats, 2012) and aminergic (Enan, 2001, 2005a, 2005b; Kostyukovsky et al., 2002) transmissions, and by inhibiting acetylcholinesterases (Keane and Ryan, 1999; López and Pascual-Villalobos, 2010; Ryan and Byrne, 1988).

Despite the previously described studies of *Cinnamomum* (Chang and Cheng, 2002; Cheng et al., 2004; Fichi et al., 2007b; Regnault-Roger and Hamraoui, 1994; Regnault-Roger et al., 1993; Yang et al., 2005) and *Eugenia* (=*Syzygium*) (Fichi et al., 2007a; Kim et al., 2003a; Park and Shin, 2005; Yang et al., 2003) oils on insects and mites, only few studies have addressed (Regnault-Roger and Hamraoui, 1994, 1995) their fumigant potential to control *A. obtectus* on common beans. Here, we determined the chemical composition (*via* gas chromatography) of clove, *Syzygium aromaticum* L., and cinnamon, *Cinnamomum zeylanicum* L., essential oils and evaluated their insecticidal (lethal toxicities, disturbances on reproductive traits, persistence of action) and repellent activities on *A. obtectus* in a non-fumigant manner.

2. Material and methods

2.1. Insects

The original population of *A. obtectus* was field-collected from small farms in the Viçosa region (Minas Gerais State, Brazil) during the year 2012. The population started with at least 500 individuals and was multiplied and reared under laboratory conditions (27 ± 2 °C, 75 ± 5% relative humidity, 12 h of scotophase). Whole common beans (*P. vulgaris*) were obtained from the local market with the common name "Feijão Rainha" (landrace Manteigão). In order to avoid possible infestations from the field and to reduce any potential insecticide residual effect, the bean grains were kept a temperature of −10 °C for 14 days prior to be offered to *A. obtectus*. The bean grains had a water content of 11.8% and were offered *ad libitum*.

2.2. Essential oil extraction

Cinnamon bark and dried flower buds of clove were purchased from the local spice store, and their distilled oil were obtained as described in Jham et al. (2005). Briefly, cinnamon bark and dried flower buds of clove were ground to pass through a 1 mm sieve, and the obtained powder was extracted at room temperature by constant percolation with hexane until all of the hexane-soluble components were removed. The essential oils were collected by hydrodistillation for 6 h. The distillate was extracted twice with dichloromethane, including water-soluble or dispersed components, and dried over anhydrous sodium sulfate, and the dichloromethane was evaporated using a rotary evaporator under vacuum. The distilled oils were stored in airtight screw-capped vials at −10 °C until use.

2.3. Gas chromatography (GC)

Chemical compositions of the essential oils were determined at the Laboratório de Análises e Síntese de Agroquímicos of the Federal University of Viçosa (Viçosa, Minas Gerais, Brazil) and followed the methods described in Demuner et al. (2011). Gas chromatography (GC) analyses were carried out with a GC-17A Series instrument (Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector (FID). The chromatographic conditions were as follows: fused silica capillary column (30 m × 0.25 mm) with a DB-5 bonded phase (0.25 μm film thickness); the carrier gas was N_2 at a flow rate of 1.8 mL/min; the injector temperature was 220 °C; and the detector temperature was 240 °C. The column temperature was programmed to start at 40 °C (isothermal for 2 min), with an increase of 3 °C/min to 240 °C; isothermal at 240 °C for 20 min; injection of 1.0 μL (1% w/v in CH_2Cl_2); split ratio 1:10; column pressure 118 kPa. The analyses were conducted in triplicate, and the amount of each compound was expressed as a relative percentage of the total area of the chromatograms.

2.4. Insecticidal activity

2.4.1. Lethal toxicity

Dose–mortality bioassays were conducted to determine the lethal doses of the cinnamon and clove essential oils to *A. obtectus*. We used pure doses of each essential oil (*i.e.*, without dilution in any solvent) in order to mimic situations faced by small farmers. The essential oils were applied with a 25 μL Hamilton syringe (Hamilton, Reno, NV, USA) to 200 g beans that were placed in 0.8 L glass jars. After the application, the jars were manually shaken for 60 s, ensuring a complete distribution of the essential oils. Twenty-five unsexed 1–3 day old *A. obtectus* adults were placed in each jar, and the jars were kept under controlled conditions (27 ± 2 °C, 75 ± 5% relative humidity; only scotophase) for 24 h. Mortality was assessed after this exposure period, and the insects were considered dead if they were unable to move for a distance at least equal to their body length. Five doses (20; 60; 80; 120 and 140 μL/kg of beans) of cinnamon oil and seven doses (15; 45; 60; 90; 105; 120 and 150 μL/kg of beans) of clove oil were used in the bioassays. In each bioassay a control treatment (without oil application) was also used. Four replicates were used for each dose, and the doses were calculated as μL of essential oil/kg of beans.

2.4.2. Essential oil effects on the biological development

The developmental rate (instantaneous rate of increase – r_i) and population growth were used to estimate the essential oil effects toward *A. obtectus* biological development. Experiments on the instantaneous rate of increase and grain loss were performed using 0.8 L capacity glass jars containing 200 g insecticide-free beans. Bean masses were treated with lethal doses of clove (in μL/kg beans: $LD_{10} = 13.5$; $LD_{30} = 27.0$; $LD_{50} = 43.6$; $LD_{70} = 70.5$ and $LD_{90} = 141.0$) and cinnamon (in μL/kg beans: $LD_{10} = 17.9$; $LD_{30} = 31.5$; $LD_{50} = 46.8$; $LD_{70} = 69.3$ and $LD_{90} = 141.0$) essential oil, which were based on the dose–mortality results previously obtained. Twenty-five adult insects were released in each jar and left to colonize the bean mass for 45 days (one generation) under controlled conditions (27 ± 2 °C, 75 ± 5% relative humidity, 24 h of scotophase). After this period, the total number of (adult) live insects and the bean weight in each experimental unit were recorded. Four replicates of each dose were used. The control treatment did not receive any essential oil application. The instantaneous rate of increase was calculated using the equation $r_i = [\ln(N_f/N_i)]/\Delta T$, where N_f and N_i are the final and initial numbers of live (adult) insects, respectively, and ΔT is the duration of the experiment in days (Walthal and Stark, 1997).

The bioassays for population growth were conducted using the same experimental procedures described above, except the 25 *A. obtectus* adults that were released in each jar were removed 15 days later, following the method described by Trematerra et al. (1996). The progeny of the adult *A. obtectus* obtained from the beans were assessed every other day. Cumulative and daily emergence

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