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Toxic effects of two essential oils and their constituents on the mealworm beetle, *Tenebrio molitor*

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Abstract

The study identified insecticidal effects from the cinnamon and clove essential oils in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). The lethal concentrations (LC_{50} and LC_{90}), lethal time, and repellent effect on larvae, pupae, and adults of *T. molitor* after exposure to six concentrations of each essential oil and toxic compounds were evaluated. The chemical composition of the cinnamon oil was also determined and primary compounds were eugenol (10.19%), *trans*-3-caren-2-ol (9.92%), benzyl benzoate (9.68%), caryophyllene (9.05%), eugenyl acetate (7.47%), α-phellandrene (7.18%), and α-pinene (6.92%). In clove essential oil, the primary compounds were eugenol (26.64%), caryophyllene (23.73%), caryophyllene oxide (17.74%), 2-propenoic acid (11.84%), α-humulene (10.48%), γ-cadinene (4.85%), and humulene oxide (4.69%). Cinnamon and clove essential oils were toxic to *T. molitor*. In toxic chemical compounds, eugenol have stronger contact toxicity in larvae, pupae, and adult than caryophyllene oxide, followed by α-pinene, α-phellandrene, and α-humulene. In general, the two essential oils were toxic and repellent to adult *T. molitor*. Cinnamon and clove essential oils and their compounds caused higher mortality and repellency on *T. molitor* and, therefore, have the potential for integrated management programs of this insect.

Keywords: cinnamon, clove, lethal effect, gas chromatography, pest control, repellency index

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Introduction

The mealworm beetle, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) is a pest of stored products such as starches, food for cats and dogs, and pasta. This insect may also infest broken grains of *Zea mays* (L.) (Poales: Poaceae), *Triticum aestivum* (L.) (Poales: Poaceae), and *Glycine max* (L.) (Fabales: Fabaceae) (Punzo & Mutchmor, 1980; Fazolin *et al.*, 2007; Cosimi *et al.*, 2009). The presence of *T. molitor* in stored grain and bran can contaminate food with fragments of the body, feces, and indirectly by saprophytic microorganisms causing loss of food quality (Loudon, 1988; Schroeckenstein *et al.*, 1990; Barnes & Siva-Jothy, 2000). This insect causes losses up to 15% of grains and flour production worldwide (Dunkel, 1992; Flinn *et al.*, 2003; Neethirajan *et al.*, 2007).

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Tenebrio molitor is controlled primarily with chemical insecticides, but this method has restrictions against stored product insects (Shaaya *et al.*, 1997), due to residual toxicity and insect resistance (Isman, 2006), especially in countries with extensive cereal production for export and domestic consumption (Arthur, 1996; Shaaya *et al.*, 1997). Chemical control of this insect can be achieved by phosphine treatment; however, fumigants cannot kill the eggs of storage pests and several issues have been discussed in the employment of insecticides, such as residue, environment impact, and toxicity to humans (Arthur, 1996; Shaaya *et al.*, 1997; Isman, 2006). Economic, social, and environmental concerns have caused a gradual change to reduce chemical control in starches and stored products (Arthur, 1996; Zettler & Arthur, 2000; Isman, 2006). More selective and biodegradable products, including 'green pesticides', can reduce the use of synthetic chemicals in warehouses (Isman, 2000; Martínez *et al.*, 2015; Plata-Rueda *et al.*, 2017).

Plant essential oils have favorable ecotoxicological properties (low toxicity to humans, degradation, and lower environmental impact), making them suitable to managing insects in organic farming (Chermenskaya *et al.*, 2010; Zanuncio *et al.*, 2016). These oils are plants secondary metabolites and include alkaloids, amides, chalcones, phenols, flavones, lignans, neolignans, or kawapirones which are important in insect–plant relationships (Isman, 2000; Martínez *et al.*, 2015). In this sense, essential oils represent an alternative for pest control as repellents, deterrent of oviposition and feeding, growth regulators, and toxicity to insects with low pollution and quick degradation in the environment (Chermenskaya *et al.*, 2010; Zanuncio *et al.*, 2016; Plata-Rueda *et al.*, 2017). Various studies have focused on the possibility of using plant essential oils for application to stored grain to control insect pests (Zapata & Smagghe, 2010; Stefanazzi *et al.*, 2011; Jemâa *et al.*, 2012).

The cinnamon, *Cinnamomum zeylanicum* Blume (Lauraceae) is a tropical evergreen tree and grows wild in Sri Lanka, Madagascar, India, and Indochina. The inner bark of the tree is used in ethno-medicine and flavoring for foods (Bakkali *et al.*, 2008). Different studies showed that extracts and constituents of *C. zeylanicum* have antimicrobial, insecticidal, and acaricidal properties (Yang *et al.*, 2005; Fichi *et al.*, 2007; Shahverdi *et al.*, 2007). Clove, *Syzygium aromaticum* (L.) Merr at Perry (Myrtaceae) is an evergreen tree and native from Indonesia. Owing to its biological activities, clove oil finds extensive use in medicine, food, cosmetic items, and pest control (Ho *et al.*, 1994; Yoo *et al.*, 2005; Jirovetz *et al.*, 2006). Toxic effects of cinnamon and clove essential oils have been evaluated with success to control of agricultural pests (Regnault-Roger, *et al.*, 1993; Ho *et al.*, 1994; Isman, 2000). There are a variety of insecticides that have toxicological properties, deterrents, and repellents used for the control of *T. molitor*; however, essential oil of cinnamon and clove could be an alternative for the control in stored products. Identification of toxic compounds of cinnamon and clove is important in understanding toxicity as it relates to pest control. In this study, we hypothesized that cinnamon and clove essential oils and their constituents have insecticidal activity in *T. molitor*.

In this series of experiments, we evaluated the toxicity and repellency of cinnamon and clove essential oils and their main constituents against *T. molitor*, in order to contribute for the development of strategies for controlling this insect pest affecting an important source of food.

Materials and methods

Insects

Tenebrio molitor were obtained from the Laboratory of Biological Control of the Institute of Applied Biotechnology to Agriculture (BIOAGRO, Universidade Federal de Viçosa) in Viçosa, Minas Gerais State, Brasil. Adults of *T. molitor* were kept in plastic trays (60 cm long × 40 cm wide × 12 cm) and maintained at 25 ± 1°C, 70 ± 10% RH and 12 : 12 h L:D photoperiod. These insects were fed *ad libitum* with wheat bran (12% protein, 2% lipids, 75% carbohydrates, and 11% mineral/sugar), pieces of sugarcane, *Saccharum officinarum* (L.) (Poaceae) and chayote *Sechium edule* (Jacq.) Swartz (Cucurbitaceae). Sheets of paper were placed on the substrate to facilitate oviposition. Healthy larvae (last instar larval), pupae, and adults of *T. molitor* of 48 h old were chosen for the bioassays.

Essential oils

The essential oils of cinnamon, *C. zeylanicum* and clove, *S. aromaticum* were obtained from Ferquima Industry & Commerce Ltda. (Vargem Grande Paulista, São Paulo State, Brazil), produced in industrial scale by hydrodistillation drag of water vapor (Dapkevicius *et al.*, 1998).

Identification of essential oil compounds

Quantitative analyses of the cinnamon and clove essential oils were performed in quadruplicate using a gas chromatograph (GC-17A series instrument, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector. The following chromatographic conditions were used: a fused silica capillary column (30 m × 0.22 mm) with a DB-5 bonded phase (0.25 µm film thickness); carrier gas N_2 at a flow rate of 1.8 ml min^{-1}; injector temperature 220°C; detector temperature 240°C; column temperature programmed to begin at 40°C (remaining isothermal for 2 min) and then to increase at 3°C min^{-1} to 240°C (remaining isothermal at 240°C for 15 min); injection volume 1.0 µl (1%w/v in CH_2Cl_2); split ratio 1 : 10; column pressure 115 kPa.

The compounds were identified using a gas chromatograph coupled with a mass detector GC/MS (GCMS-QP 5050A; Shimadzu, Kyoto, Japan). The injector and detector temperatures were 220 and 300°C, respectively. The initial column temperature was 40°C for 3 min, with a programmed temperature increase of 3°C min^{-1} to 300°C where it was maintained for 25 min. The split mode ratio was 1 : 10. One microliter of each essential oil containing 1% (w/v in dichloromethane) was injected; helium was used as the carrier gas with a flow rate constant of 1.8 ml^{-1} on the Rtx$^{®}$−5MS capillary column (30 m, 0.25 mm × 0.25 µm; Bellefonte, PA, USA) using the Crossbond$^{®}$ stationary phase (35% diphenyl–65% dimethyl polysiloxane). Mass spectrometer was programmed to detect masses in the range of 29–450 Da with 70 eV ionization energy. Compounds were identified by comparisons of the mass spectra with those available in the NIST08, NIST11 library and Wiley Spectroteca Data Base (7th edition), and by the Retention indices.

Toxicity of essential oils

The essential oil efficacy was determined by calculating the lethal concentration (LC_{50} and LC_{90}) values under laboratory conditions and conducted in triplicate. Six concentrations of cinnamon and clove essential oils besides the control (acetone) were adjusted in 1 ml of stock solution (essential oil and acetone): 1, 2, 4, 8, 16, and 32% (w/v). Aliquots were taken from the stock solution and mixed with acetone in 5 ml glass vials. Different concentrations of each essential oil were applied in 1 µl solution on the thorax of larva, pupa, and adult of *T. molitor*, using a micropipette. For each developmental stage, 50 insects were used per concentration and placed individually in Petri dishes (Ø 90 mm × 15 mm) with perforated cap for ventilation and an absorbent paper, fed with chayote and sugarcane *ad libitum* (larvae and adults) and maintained in the dark. The number of dead insects was counted after essential oil exposure for 48 h.

Toxicity of commercial compounds of two essential oils in T. molitor

Major constituents of the cinnamon and clove essential oils including eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene were tested for toxic effects and obtained commercially. Eugenol (purity 99%), caryophillene oxide (purity 99%), α-humulene (purity 96%), α-phellandrene (purity 85%), and α-pinene (purity 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Six different concentrations of the commercial compounds besides the control (acetone) were adjusted in 1 ml of stock solution (treatment and acetone) used to calculate the lethal LC_{50} and LC_{90} concentrations: 2, 4, 8, 16, 32, and 64 µg insect^{-1}. For each treatment, aliquots were taken from the stock solution and mixed with acetone in 5 ml glass vials. Different concentrations of the each treatment were applied in 1 µl solution on the thorax of larva, pupa, and adult of *T. molitor*. Individuals were placed in Petri dishes and fed with natural diet (10 g wheat bran + 10 g sugarcane in the ratio 1 : 1). A total of 50 larvae, 50 pupae, and 50 adults were used for each concentration and mortality was evaluated for 48 h.

Comparison of the LT_{50} values of the essential oils and their major compounds

Toxicity of cinnamon and clove essential oils, and commercially obtained eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene at the calculated LC_{50} concentration was compared. Acetone was used as a control. For the LC_{50} of each compound, 1 µl was applied on the thorax of larvae, pupae, and adults of *T. molitor* using a micropipette. Insects were individualized in Petri dishes with wheat bran and sugarcane. A total of 240 larvae, 240 pupae, and 240 adults were used for each treatment, with a total of four replicates. Mortality was recorded every 6 h for 48 h, and estimated lethal time values for 50% mortality (LT_{50}) were compared.

Repellency effects of the essential oils and their major compounds

Four Petri dishes (12 × 1.5 cm) were used as an arena, connected to a central board with plastic tubes (diameter 2 cm) at an angle of 45°. The other dishes were distributed around them in equidistant distances and two plates were put together symmetrically opposed (fig. 1). Two hundred and forty individuals (120 larvae and 120 adults) of *T. molitor* were used in this experiment. In each assay replicate, 20 insects were released in the central board and the control group received sugarcane and chayote. A total of 5 µl of the estimated LC_{50} lethal concentration of each essential oil and toxic compounds were applied on absorbent filter paper (2 × 2 cm) placed in two opposite plates used as treatment, and two opposite ones with 5 µl of distilled water on absorbent filter paper represented the control. Four replicates per treatment and control were evaluated by the number of individuals per plate after 12 h calculating the repellency index (RI): $RI = 2G/(G + P)$, where G is the percentage of insects in the treatment and P is the percentage of insects in control. Treatments were classified as neutral if the index was equal to one (1); repellent, higher than one (1), and; attractive lower than one (1).

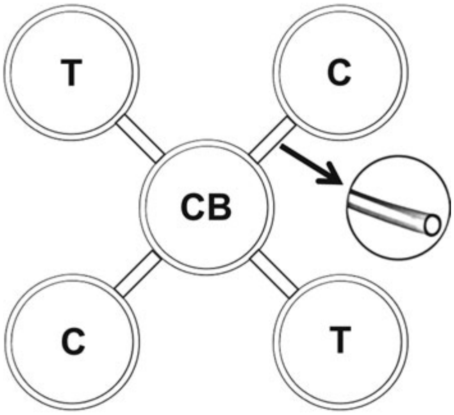

Fig. 1. Schematic drawing of four Petri dishes used as an arena, connected to a central board (CB) with plastic tubes at an angle of 45°. Treatment (T) and control (C) were distributed in equidistant distances and symmetrically opposed. Not drawn to scale.

Statistical analyses

Lethal concentration (LC_{50} and LC_{90}) and their confidence limits for cinnamon and clove essential oils and compounds were determined by logistic regression in dose–response assays based on the concentration Probit-mortality (Finney, 1964) using XLSTAT-PRO (v.7.5) program for Windows (XLSTAT, 2004). Student's t test was used for pairwise comparisons regarding lethal time effects in *T. molitor* using SAS User software (v. 9.0) for Windows (SAS, 2002).

Results

Identification of essential oil compounds

A total of 23 compounds were identified in the two essential oils, which accounted 96–97% of the total composition (fig. 2, table 1). The primary compounds of the essential oil of cinnamon were eugenol (10.19%), *trans*-3-caren-2-ol (9.92%), benzyl benzoate (9.68%), caryophyllene (9.05%), eugenyl acetate (7.47%), α-phellandrene (7.18%), α-pinene (6.92%), cinnamyl acetate (6.91%), safrole (5.51%), nerolidol (5.02%), terpinolene (4.35%), cinnamaldehyde (4.12%), linalol (2.87%), β-pinene (2.43%), terpineol (2.11%), α-copaene (1.96%), myrcene (1.95%), and camphene (1.31%). For clove essential oil, the primary compounds were eugenol (26.64%), caryophyllene (23.73%), caryophyllene oxide (17.74%), 2-propenoic acid (11.84%), α-humulene (10.48%), γ-cadinene (4.85%), and humulene oxide (4.69%).

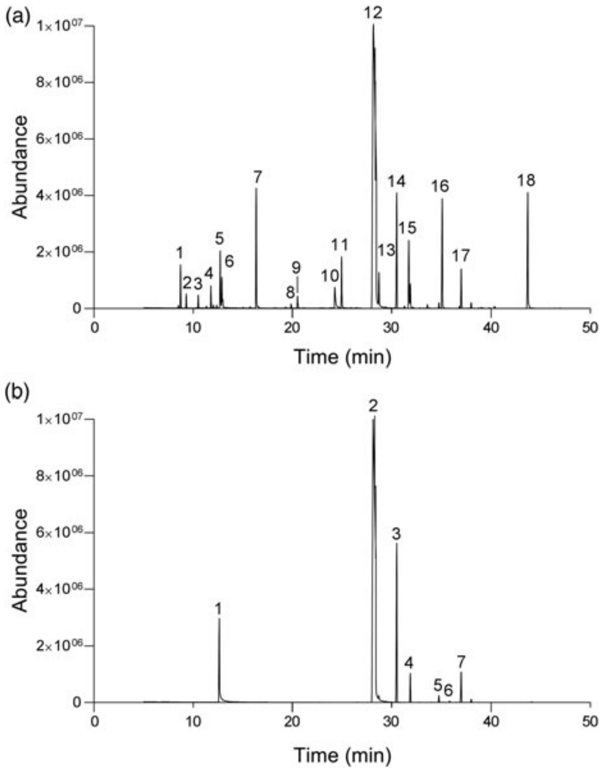

Fig. 2. Gas chromatogram profiles of peak retention of compounds of the cinnamon and clove essential oils. (a) Cinnamon: α-pinene (1), camphene (2), β-pinene (3), myrcene (4), α-phellandrene (5), terpinolene (6), *trans*-3-caren-2-ol (7), linalol (8), α-terpineol (9), cinnamaldehyde (10), safrole (11), eugenol (12), copaene (13), caryophyllene (14), cinnamyl acetate (15), eugenyl acetate (16), nerolidol (17), benzyl benzoate (18). (b) Clove: 2-propenoic acid (1), eugenol (2), caryophyllene (3), α-humulene (4), γ-cadinene (5), caryophyllene oxide (6), humulene oxide (7).

Toxicity of essential oils

Mortality of *T. molitor* was obtained with 16 and 32% (w/v) of each essential oil and two different lethal concentration levels were estimated by Probit (χ^2, $P < 0.0001$) (table 2). The LC_{50} and LC_{90} values indicated that cinnamon essential oil was the most toxic to *T. molitor* larvae ($LC_{50} = 30.4$ μg and $LC_{90} = 55.1$ μg; $\chi^2 = 99.76$, df = 5), followed by clove oil ($LC_{50} = 35.1$ μg and $LC_{90} = 67.2$ μg; $\chi^2 = 60.43$, df = 5). In contrast, clove essential oil was most toxic to *T. molitor* pupae with $LC_{50} = 6.45$ μg and $LC_{90} = 14.6$ μg ($\chi^2 = 60.43$, df = 5) followed by cinnamon oil with $LC_{50} = 10.7$ μg and $LC_{90} = 20.9$ μg ($\chi^2 = 74.74$, df = 5). Clove essential oil was the most toxic to *T. molitor* adults with $LC_{50} = 21.6$ μg and $LC_{90} = 44.5$ μg ($\chi^2 = 12.39$, df = 5) and cinnamon oil with $LC_{50} = 29.8$ μg and $LC_{90} = 56.03$ μg ($\chi^2 = 17.81$, df = 5). Mortality was always <1% in the control.

Toxicity of commercial compounds of two essential oils in T. molitor

The toxicity of commercially obtained eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene in *T. molitor* was estimated by Probit (χ^2, $P < 0.0001$) and evaluated at different concentrations (table 3). To larva stage, dose–response bioassays showed results with eugenol with $LC_{50} = 9.15$ (7.84–10.7) μg and $LC_{90} = 18.9$ μg (16.4–22.6), followed by caryophyllene oxide with $LC_{50} = 9.21$ (7.59–11.1) μg and $LC_{90} = 22.9$ (19.7–27.9) μg, α-pinene with $LC_{50} = 14.02$ (12.1–16.3) μg and $LC_{90} = 27.16$ (23.7–32.0) μg, α-humulene with $LC_{50} = 15.2$ (13.2–17.8) μg and $LC_{90} = 30.9$ (26.8–36.8) μg, and α-phellandrene with $LC_{50} = 17.09$ (14.7–20.1) μg and $LC_{90} = 34.6$ (29.9–41.6) μg. The LC_{50} and LC_{90} values indicated that eugenol was toxic to pupae with $LC_{50} = 13.4$ (11.6–15.5) μg and $LC_{90} = 26.1$ (22.9–30.6) μg, followed by caryophyllene oxide with $CL_{50} = 14.6$ (12.5–17.2) μg and $LC_{90} = 30.9$ (26.7–37.1) μg, α-pinene with $LC_{50} = 17.5$ (15.1–20.8) μg and $LC_{90} = 35.5$ (30.6–43.0) μg, α-phellandrene with $LC_{50} = 18.7$ (16.3–21.8) μg and $LC_{90} = 35.2$ (30.7–41.9) μg, and α-humulene with $LC_{50} = 21.4$ (18.5–25.4) μg and $LC_{90} = 40.4$ (34.6–49.2) μg. To adult stage, increased mortality was observed following application of caryophyllene oxide with $LC_{50} = 25.4$ (21.9–30.4) μg and $LC_{90} = 54.5$ (46.3–67.2) μg, followed by eugenol with $LC_{50} = 26.6$ (23.2–31.4) μg and $LC_{90} = 53.7$ (46.1–65.2) μg, α-phellandrene with $LC_{50} = 27.5$ (24.3–31.9) μg and $LC_{90} = 51.6$ (44.9–61.2) μg, α-pinene with $LC_{50} = 29.9$ (26.1–35.4) μg and $LC_{90} = 56.4$ (48.4–68.5) μg, and α-humulene with $LC_{50} = 31.8$ (27.9–37.4) μg and $LC_{90} = 56.7$ (48.9–68.4) μg. Mortality was always <1% in the control.

Comparison of the LT_{50} values of the essential oils and their major compounds

Larvae, pupae, and adults of *T. molitor* applied with LC_{50} concentration of cinnamon and clove essential oils vs. toxic compounds showed lethal effects at different time points (fig. 3). However, LT_{50} values showed that clove oil took longer to kill insects than the cinnamon essential oil. At a high LC_{50} concentration of cinnamon oil, eugenol took longer to kill the larvae ($t = 5.47$, $P < 0.001$), pupae ($t = 3.98$, $P < 0.001$), and adults ($t = 3.96$, $P < 0.001$) with LT_{50} values of 32.6 ± 0.86, 28.3 ± 0.18, and 44.6 ± 0.85 h, respectively (fig. 3a–c); α-phellandrene took less to kill the larvae ($t = 5.51$, $P < 0.001$), pupae ($t = 4.17$, $P < 0.001$), and adults ($t = 4.88$, $P < 0.001$) with LT_{50} values of 45.6 ± 0.52, 45.6 ± 0.17, and 49.7 ± 0.11 h, respectively (fig. 3d–f); and α-pinene took longer to kill the larvae ($t = 4.45$, $P < 0.001$), pupae ($t = 3.96$, $P < 0.001$), and adults ($t = 5.16$, $P < 0.001$) with LT_{50} values of 47.7 ± 0.27, 48.8 ± 0.43, and 48.9 ± 0.79 h, respectively (fig. 3g–i).

In clove oil, eugenol took longer to kill the larvae ($t = 4.59$, $P < 0.001$), pupae ($t = 3.71$, $P < 0.001$), and adults ($t = 5.08$, $P < 0.001$) with LT_{50} values of 30.1 ± 0.25, 26.7 ± 0.56, and 40.3 ± 0.37 h, respectively; caryophyllene took less to kill the larvae ($t = 3.93$, $P < 0.001$), pupae ($t = 7.31$, $P < 0.001$), and adults ($t = 5.13$, $P < 0.001$) with LT_{50} values of 40.3 ± 0.45, 24.4 ± 0.89, and 36.7 ± 0.34 h, respectively; and α-humulene took longer to kill the larvae ($t = 3.85$, $P < 0.001$), pupae ($t = 3.91$, $P < 0.001$), and adults ($t = 4.16$, $P < 0.001$) with LT_{50} values of 47.5 ± 0.45, 46.1 ± 0.53, and 43.6 ± 0.36 h, respectively.

Repellency effects of the essential oils and their major compounds

The larvae of *T. molitor* RI by cinnamon essential oil and compounds with concentrations estimated for the LC_{50} values differ between them (table 4). Eugenol was the repellent (RI = 1.10 ± 0.05), while α-phellandrene (RI = 0.89 ± 0.08), α-pinene (RI = 0.87 ± 0.09), and cinnamon oil (RI = 0.83 ± 0.04)

Table 1. Chemical composition of cinnamon and clove essential oil.

Compound	R_i	Mean composition (% area)		R_t	MM	m/z
		Cinnamon	Clove			
α-Pinene	943	6.92		8.30	136	121.10
Camphene	943	1.31		9.07	136	121.10
β-Pinene	948	2.43		10.28	136	105.05
Myrcene	958	1.95		11.16	136	83.95
α-Phellandrene	969	7.18		11.49	136	136.05
Terpinolene	919	4.35		12.15	136	136.05
2-Propenoic acid	995		11.84	12.26	130	87.05
trans-3-Caren-2-ol	1052	9.92		12.60	152	134.05
Linalol	1136	2.87		16.11	154	121.10
α-Terpineol	1143	3.11		20.30	154	136.05
Cinnamaldehyde	1189	4.12		24.05	132	132.00
Safrole	1327	5.51		24.70	152	162.00
Eugenol	1392	10.19	26.64	27.90	164	164.00
Copaene	1221	1.96		28.55	204	164.00
Caryophyllene	1494	9.05	23.73	30.32	204	133.05
Cinnamyl acetate	1367	6.91		31.53	176	134.00
α-Humulene	1579		10.48	31.75	204	147.05
γ-Cadinene	1469		4.85	34.50	204	204.05
Eugenyl acetate	1552	7.47		34.83	206	164.00
Caryophyllene oxide	1507		17.74	36.79	220	109.10
Nerolidol	1554	5.02		36.81	220	109.05
Humulene oxide	1590		4.69	37.56	220	109.05
Benzyl benzoate	1733	9.68		46.30	212	211.95

MM, molecular mass; RI, relative intensity; R_i, retention indices; R_t, retention time; m/z, molecular weight.

were attractant. The RI for adults of *T. molitor* differed with the concentration of the cinnamon essential oil and compounds, using the estimated LC_{90} values (table 4). Eugenol was the most repellent (RI = 1.10 ± 0.07), followed by that of the cinnamon oil (RI = 1.06 ± 0.03), while α-phellandrene (RI = 0.90 ± 0.05), and α-pinene (RI = 0.87 ± 0.04) were attractant.

The larvae of *T. molitor* RI by clove essential oil and compounds with concentrations estimated for the LC_{50} values differ between them (table 4). Caryophyllene oxide was repellent (RI = 1.06 ± 0.04), while eugenol (RI = 0.98 ± 0.09), clove oil (RI = 0.84 ± 0.05), and α-humulene were attractant (RI = 0.74 ± 0.04). The RI for adults of *T. molitor* differed with the concentration of the clove essential oil and compounds, using the estimated LC_{90} values (table 4). The clove essential oil (RI = 1.10 ± 0.07) was repellent, while caryophyllene oxide (RI = 1.01 ± 0.06) was neutral. The compounds eugenol (RI = 0.99 ± 0.08) and α-humulene (RI = 0.84 ± 0.05) were attractant.

Discussion

Toxicity and repellency of two essential oils and their compounds against the mealworm beetle, *T. molitor* were determined from the bioassays in the laboratory conditions. Cinnamon and clove essential oils caused substantial mortality and repellency in larva, pupa, and adult stages. The best results were obtained with concentrations of 16 and 32% in *T. molitor* as reported for other stored grain pests according to the concentration of these products (Zettler & Arthur, 2000; Haddi *et al.*, 2015). The susceptibility of stored pest products such as *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) and *Callosobruchus chinensis* Linnaeus (Coleoptera: Chrysomelidae) by cinnamon, and *Sitophilus zeamais* Motschusky (Coleoptera: Curculionidae) and *Tribolium castaneum* Hersbt (Coleoptera: Tenebrionidae) by clove may vary with the method of exposure (contact or fumigation) (Ho *et al.*, 1994; Kim *et al.*, 2003; Correa *et al.*, 2015).

Different concentrations of the cinnamon and clove essential oils showed toxic effects on larva, pupa, and adult of *T. molitor* 48 h after topical application. The dose–response bioassay confirmed toxicity against *T. molitor*, reaching a 90% mortality rate. Increasing concentrations of cinnamon and clove essential oil on different insects have shown immediate toxic responses within 12 h of application (Ho *et al.*, 1994; Choi *et al.*, 2003; Kim *et al.*, 2003; Lee *et al.*, 2008). Comparing the contact toxicity of cinnamon and clove essential oils on developmental stages of *T. molitor*, the pupa was significantly more susceptible followed by adult and larva. The LC_{50} of cinnamon and clove essential oils of pupa (10.7 and 6.45 µg insect^{-1}, respectively), adult (29.8 and 21.6 µg insect^{-1}, respectively), and larva (30.4 and 35.1 µg insect^{-1}, respectively) indicate that small quantities of these essential oils are toxic in all stages of development of this insect.

The chemical composition of cinnamon and clove essential oils revealed 23 compounds detected, identified, and quantified in terms of relative percentages. In particular, eugenol, *trans*-3-caren-2-ol, benzyl benzoate, caryophyllene, eugenyl acetate, α-phellandrene, and α-pinene were the main compounds that were detected in cinnamon oil and eugenol, caryophyllene, caryophyllene oxide, 2-propenoic acid, and α-humulene from clove oil. The results are in accordance with those of previous reports on monoterpenes obtained in essential oils (Simić *et al.*, 2004; Chaieb *et al.*, 2007; Goni *et al.*, 2009). Monoterpenes, with sesquiterpenes, are the main constituents of essential oils extracted from plants, including fruits, vegetables, spices, and herbs (Loza-Tavera, 1999). They are products of the secondary metabolism of plants, although specialized classes occur in some animals and microorganisms (Banthorpe *et al.*, 1972). Monoterpenes are a class of terpenes that consist of two isoprene units, with molecular

Table 2. Lethal concentrations of cinnamon and clove essential oil against different developmental stages of *Tenebrio molitor* after 48 h exposure.

EO	IS	LC	EV (µg insect^{-1})	CI (µg insect^{-1})	χ^2
Cinnamon	Larva	LC_{50}	30.41	26.75–35.58	99.76
		LC_{90}	55.10	47.68–66.08	
	Pupa	LC_{50}	10.77	9.738–11.94	74.74
		LC_{90}	20.96	19.02–23.48	
	Adult	LC_{50}	29.84	26.08–35.22	17.81
		LC_{90}	56.03	48.16–67.87	
Clove	Larva	LC_{50}	35.19	29.76–43.83	60.43
		LC_{90}	67.23	55.70–86.35	
	Pupa	LC_{50}	6.452	5.709–7.302	61.78
		LC_{90}	14.68	13.18–16.66	
	Adult	LC_{50}	21.69	19.22–24.89	12.39
		LC_{90}	44.59	39.15–52.25	

EO, essential oil; IS, insect stage; $LC_{50\text{ and }90}$, lethal concentration causing 50 and 90% mortality; EV, estimated value; CI, confidence interval; χ^2, chi-squared value for the lethal concentrations and fiducial limits based on a log scale with significance level at $P < 0.001$.

Table 3. Lethal concentrations of the cinnamon and clove oil constituents on different developmental stages of *Tenebrio molitor* after 48 h exposure.

Compounds	IS	LC	EV (µg insect^{-1})	CI (µg insect^{-1})	χ^2
Eugenol	Larva	LC_{50}	9.154	7.849–10.74	21.12
		LC_{90}	18.96	16.43–22.68	
	Pupa	LC_{50}	13.41	11.67–15.52	31.75
		LC_{90}	26.13	22.94–30.64	
	Adult	LC_{50}	26.61	23.20–31.43	15.28
		LC_{90}	53.73	46.10–65.25	
Caryophyllene oxide	Larva	LC_{50}	9.217	7.590–11.10	24.24
		LC_{90}	22.99	19.71–27.90	
	Pupa	LC_{50}	14.64	12.54–17.28	33.86
		LC_{90}	30.96	26.73–37.19	
	Adult	LC_{50}	25.46	21.99–30.42	15.12
		LC_{90}	54.59	46.38–67.25	
α-Humulene	Larva	LC_{50}	15.27	13.20–17.87	10.47
		LC_{90}	30.95	26.88–36.82	
	Pupa	LC_{50}	21.41	18.50–25.40	20.08
		LC_{90}	40.45	34.69–49.23	
	Adult	LC_{50}	31.85	27.94–37.45	8.97
		LC_{90}	56.76	48.96–68.47	
α-Phellandrene	Larva	LC_{50}	17.09	14.73–20.11	12.58
		LC_{90}	34.65	29.95–41.64	
	Pupa	LC_{50}	18.75	16.34–21.86	35.61
		LC_{90}	35.27	30.70–41.97	
	Adult	LC_{50}	27.56	24.36–31.94	12.01
		LC_{90}	51.61	44.94–61.28	
α-Pinene	Larva	LC_{50}	14.02	12.19–16.30	5.19
		LC_{90}	27.16	23.73–32.08	
	Pupa	LC_{50}	17.58	15.14–20.80	18.63
		LC_{90}	35.56	30.60–43.02	
	Adult	LC_{50}	29.92	26.10–35.41	11.57
		LC_{90}	56.44	48.41–68.59	

IS, insect stage; $LC_{50\text{ and }90}$, lethal concentration causing 50 and 90% mortality; EV, estimated value; CI, confidence interval; χ^2, chi-squared value for the lethal concentrations and fiducial limits based on a log scale with significance level at $P < 0.001$.

formula of $C_{10}H_{16}$, and may be linear (acyclic) or contain rings. Defensive role in plants of simple monoterpenes have been demonstrated as for more complex compounds (Lerdau *et al.*, 1994). In this study, the main compounds of cinnamon and clove essential oils are monoterpenes and may provide a solution to protect plants or stored products against insect attack.

Chemical compounds of cinnamon and clove essential oils demonstrated toxic activity on different developmental stages of *T. molitor*. Eugenol have stronger contact toxicity in larvae ($LC_{50} = 9.15$ µg), pupae ($LC_{50} = 13.4$ µg), and adult ($LC_{50} = 26.6$ µg), than caryophyllene oxide in larvae ($LC_{50} = 9.21$ µg), pupae ($LC_{50} = 14.6$ µg), and adult ($LC_{50} = 25.4$ µg), followed by α-pinene in larvae ($LC_{50} = 14.0$ µg), pupae ($LC_{50} = 17.5$ µg), and adult ($LC_{50} = 29.9$ µg); α-phellandrene in larvae ($LC_{50} = 17.0$ µg), pupae ($LC_{50} = 18.7$ µg), and adult ($LC_{50} = 27.5$ µg); and α-humulene in larvae ($LC_{50} = 15.2$ µg), pupae ($LC_{50} = 21.4$ µg), and adult ($LC_{50} = 31.8$ µg). Eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene have been highly toxic to *C. chinensis*, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), *S. oryzae*, *S. zeamais* (Coleoptera: Curculionidae), and *T. castaneum* (Ho *et al.*, 1994; Huang *et al.*, 2002) at different developmental stages as well as other insects (Gunasena *et al.*, 1988; Huang *et al.*, 2002; Park *et al.*, 2003). Our results showed that *T. molitor* was more susceptible in the pupal stage followed by larvae and adults exposed to eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene. One possible explanation for the developmental stages difference is that efficacy may be affected by the penetration of the cinnamon and clove compounds into the body and the ability of the insect to metabolize these compounds.

The mechanisms of toxic action of monoterpenoids such as eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene are unknown. However, the insects exposed to the toxic compounds displayed altered locomotion activity, and muscle contractions in legs and abdomen that were observed at high concentrations in LC_{50} test. In some individuals, the paralysis was constant with concentrations near the LC_{50} without recovery signs. Paralysis and muscle contractions in individuals of *T. molitor* at LC_{50} followed by death can be explained by the toxic effect in the nervous system. Different studies have shown that neurotoxic effects of insects exposed to monoterpenoids can cause blockade of octopamine receptor binding sites (Livingstone *et al.*, 1980; Enan, 2001). In this context, octopamine induces hyperextension of the legs and abdomen by increasing the frequency of excitatory postsynaptic potentials received by the appropriate abdominal motor neurons (Livingstone *et al.*, 1980; Enan, 2001). Octopamine has a broad spectrum of biological roles in insects, acting as a neurotransmitter, neurohormone and circulating neurohormone neuromodulator (Orchard, 1982; Kostyukovsky *et al.*, 2002; Farooqui, 2007). In insects, there is some evidence suggesting a role in neuromuscular transmission (Candy, 1978; Whim & Evans, 1988), but is not to say that octopamine is the neuromuscular transmitter, but rather that it may possess a modulating influence on nerve–muscle interaction. The presence of the monoterpenoids in cinnamon and clove essential oils may be responsible for the neurotoxic effect in *T. molitor* and may cause rapid mortality as reported to *S. zeamais* with eugenol, *H. virescens* with caryophyllene oxide, *C. chinensis* with α-humulene, α-phellandrene, and α-pinene causing hyperactivity, hyperextension of the legs and abdomen and rapid knock-down effect or immobilization (Gunasena *et al.*, 1988; Huang *et al.*, 2002; Park *et al.*, 2003).

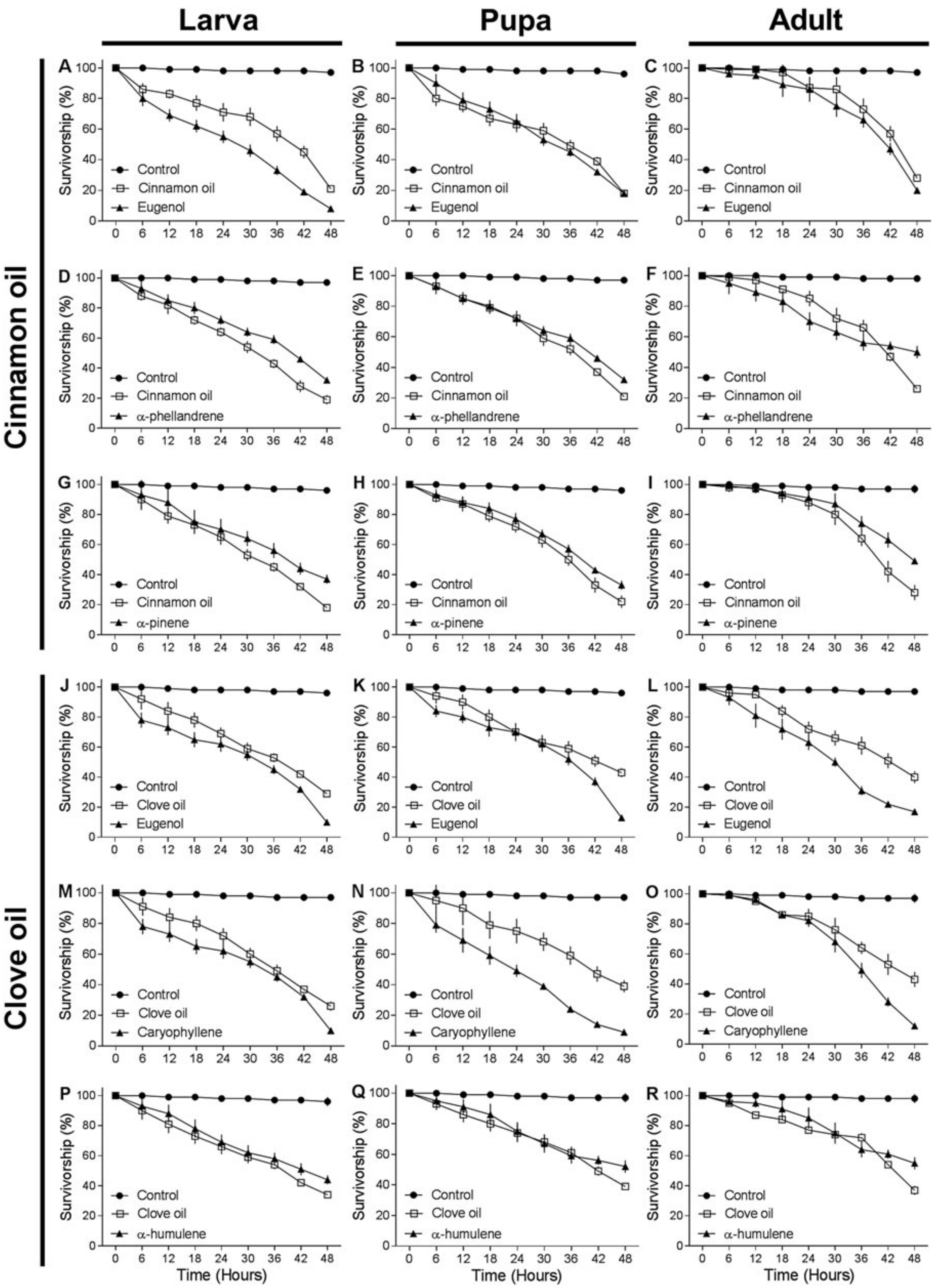

Fig. 3. Lethal time of essential oils and toxic compounds on *Tenebrio molitor* after 48 h topical applied with a LC_{50}: cinnamon oil (a–i) and clove oil (j–r) (control insects were applied with water). Control (●), cinnamon/clove essential oil (□), eugenol, caryophyllene, α-humulene, α-phellandrene, and α-pinene (▲).

Table 4. Repellency index of two essential oils and toxic compounds to level LC_{50} application on larvae and adults of *Tenebrio molitor*.

Stage	Compound	Repellency index	Classification
Cinnamon			
Larva	Cinnamon oil	0.83 ± 0.04	Attractant
	Eugenol	1.10 ± 0.05	Repellent
	α-Phellandrene	0.89 ± 0.08	Attractant
	α-Pinene	0.87 ± 0.09	Attractant
Adult	Cinnamon oil	1.06 ± 0.03	Repellent
	Eugenol	1.10 ± 0.07	Repellent
	α-Phellandrene	0.90 ± 0.05	Attractant
	α-Pinene	0.87 ± 0.04	Attractant
Clove			
Larva	Clove oil	0.84 ± 0.05	Attractant
	Caryophyllene oxide	1.06 ± 0.04	Repellent
	Eugenol	0.98 ± 0.09	Attractant
	α-Humulene	0.74 ± 0.04	Attractant
Adult	Clove oil	1.10 ± 0.07	Repellent
	Caryophyllene oxide	1.01 ± 0.06	Neutral
	Eugenol	0.99 ± 0.08	Attractant
	α-Humulene	0.84 ± 0.05	Attractant

Treatments were classified as neutral if the index was equal to one (1); repellent if higher than one (1); and attractive if lower than one (1).

The compounds of cinnamon and clove essential oils and their constituents induced mortality in larva, pupa, and adult of *T. molitor* within a short period of time. The LT_{50} of *T. molitor* applied with LC_{50} eugenol was approximately 30, 26, and 40 h; caryophyllene oxide was 40, 24, and 36 h; α-humulene was 54, 46, and 43 h; α-phellandrene was 45, 45, and 49 h; and α-pinene was 47, 48, and 48 h for larva, pupa, and adult, respectively. Toxic compounds affect multiple regions of the insect body over a period of time, ranging from 1 to 20–40 h for death. In this period, the necrotic areas were increasing progressively on the insect body. The comparative effects on *T. molitor* between two essential oils and toxic compounds were observed at various time points. An essential oil of quick action should be preferred for protection of products stored to be able to prevent feeding and avoid or reduce damage by insect pests (Isman, 2006; Chermenskaya *et al.*, 2010; Martínez *et al.*, 2015).

The repellency test indicated that eugenol and caryophyllene oxide had a greater effect on *T. molitor* behavior, while cinnamon and clove essential oils had little effect. Odor produced from volatile compounds was repulsive to larvae and adults of *T. molitor* and was observed during early hours after exposition. In exposure to vapors, the volatile substances enter with the air insects inhale through their spiracles as part of their respiratory process (Wasserthal, 1996). The substances are transported to different tissues through the network of tracheas and tracheoles, thus reaching their site of action. Monoterpenes eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene are majority compounds and toxic to insect pests of stored products such as *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae) (Jumbo *et al.*, 2014) and *T. castaneum* (Padin *et al.*, 2000). Our results suggest that cinnamon and clove essential oils and their constituents eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene have high activities of behavioral deterrence against *T. molitor*, as evaluated by the behavioral responses of larvae and adults to different odor sources and the number of insects repelled, indicating their potential to the pest control in stored products.

This study showed the potential of cinnamon and clove essential oil and main compounds to control the *T. molitor* in starches and stored products. In order to prevent or retard the development of insecticide resistance, the toxicity and repellency effects of two essential oils and toxic compounds on *T. molitor* show that they can be used individually or in mixture for the management of populations. The lethalities of eugenol, caryophyllene oxide, α-humulene, α-phellandrene, and α-pinene on *T. molitor* may have advantages by their mode of action on this insect. These findings show that the compounds of two essential oils are a potential source of insecticidal compounds and warrants further investigation.

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