

RESEARCH ARTICLE

Toxicity to, oviposition and population growth impairments of *Callosobruchus maculatus* exposed to clove and cinnamon essential oils

Luis Oswaldo Viteri Jumbo^{1,2}, Khalid Haddi^{1*}, Lêda Rita D. Faroni³, Fernanda F. Heleno³, Frederico G. Pinto⁴, Eugênio E. Oliveira^{1*}

1 Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil, **2** Embrapa Tabuleiros Costeiros, Aracaju, SE, Brazil, **3** Departamento de Engenharia Agrícola, Universidade Federal de Viçosa, Viçosa, MG, Brazil, **4** Departamento de Química, Universidade Federal de Viçosa, Campus Rio Paranaíba, MG, Brazil

* khalid.haddi@ufv.br (KH); eugenio@ufv.br (EEO)



OPEN ACCESS

Citation: Viteri Jumbo LO, Haddi K, Faroni LRD, Heleno FF, Pinto FG, Oliveira EE (2018) Toxicity to, oviposition and population growth impairments of *Callosobruchus maculatus* exposed to clove and cinnamon essential oils. PLoS ONE 13(11): e0207618. <https://doi.org/10.1371/journal.pone.0207618>

Editor: Nicolas Desneux, Institut Sophia Agrobiotech, FRANCE

Received: May 4, 2018

Accepted: November 2, 2018

Published: November 16, 2018

Copyright: © 2018 Viteri Jumbo et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Grants from the CAPES Foundation to KH, from the National Council of Scientific and Technological Development (CNPq) to LRDF and EEO and from the Secretaria Nacional de Educación Superior Ciencia y Tecnología of Ecuador (SENESCYT-Ecuador) to LOVJ supported this work. The funders had no role in study design,

Abstract

The use of plant essential oils has been shown to efficiently control insect pests of stored beans, significantly reducing the threats associated with synthetic insecticides. Here, we evaluated the potential of applications of essential oils of clove, *Syzygium aromaticum* L., and cinnamon, *Cinnamomum zeylanicum* L., to control *Callosobruchus maculatus*, considered as one of the most cosmopolitan pests of stored beans. Using four combinations of couples (i.e., unexposed couples, exposed females, exposed males, and exposed couples), we also evaluated how sublethal exposure to these essential oils impacted *C. maculatus* oviposition. Bioassays results revealed that both essential oils exhibited insecticidal activities similar to the synthetic pyrethroid insecticide deltamethrin. Furthermore, oil dosage increments proportionately decreased the growth rate and reduced the losses in bean weight caused by cowpea weevils, and offspring emergence was almost abolished when parents were exposed to the LD₂₀ of each essential oil. Finally, significant oviposition impairments were perceived only in couples where females were exposed (i.e., females exposed and exposed couples) to the LD₂₀ of cinnamon and clove essential oils. Thus, by exhibiting similar insecticidal activities as synthetic insecticides and by significantly affecting the oviposition of sublethally exposed *C. maculatus* females, the cinnamon and clove essential oils represent valuable tools with potential of integration into the management of *C. maculatus* infestations.

Introduction

Plant essential oils have gained a reputation as being potentially bioactive compounds against many insect species, which has portrayed them as safer tools in terms of the environment and human health [1–6]. Despite the potential of essential oils to control pests of stored products, few studies have addressed the physiological and biological responses of stored product pests when the exposure occurred at sublethal levels.

data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

It has been well recognized that stored product pests when sublethally exposed to synthetic insecticides can exhibit not only detrimental (what is somehow expected), but under certain circumstances also positive responses on their physiology and behavior [7–11]. Although the mechanisms explaining such sublethal responses (i.e., positive or detrimental) are still not well understood, it has been described that individuals sublethally exposed to synthetic insecticides show alterations in relevant life traits (e.g., the development time, longevity, fertility, fecundity, immune capacities, locomotion, navigation, sexual communication, oviposition and feeding) [7]. It is worthy to note, however, that similar life trait alterations seem to be elicited by sublethal exposure to botanical insecticides, which in their turn can trigger insect responses that either increase [12–16] or compromise the efficacy of these alternative control tools [9, 17, 18].

Among the essential oils that have been shown to adequately control insect pests, the oils extracted from clove, *Syzygium aromaticum* (L.), and cinnamon, *Cinnamomum zeylanicum* (L.), plants have drawn particular interest because of their promising insecticidal activities against various pests of stored products such as the maize weevil *Sitophilus zeamais* and the red flour beetle *Tribolium castaneum* [2, 12–16]. In insects, these essential oils have neurotoxic action both as fumigants and or contact insecticides and their metabolites can act upon variety of molecular targets including inhibition of acetylcholinesterase or disturbing the functions of GABAergic and aminergic systems [19].

The cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae), is a cosmopolitan pest of legume seeds and is among the most serious pests of stored products in tropical countries [20–22]. The insect larvae represent the most destructive stage, as adult cowpea bruchid do not feed [23, 24]. However, as the availability of a specific host is highly discontinuous and because these adult insects have to live in hosts that are normally treated with insecticides [25–27], these insects might have to face insecticidal sublethal exposures prior to deciding where they are going to lay eggs.

Most of the cowpea, *Vigna unguiculata* (L.) Walp, production occurs in tropical countries with high contribution of small farmers. In this context, the control of *C. maculatus*, when accomplished, is mainly achieved by the application of a small group of synthetic molecules (e.g., phosphine and pyrethroid insecticides, such as deltamethrin). Dependence on a small group of synthetic molecules raises the risk of selecting resistant populations as well as increases the hazard risks to human health and to the environment [2, 28].

Thus, it is urgently needed to develop alternatives to the chemical control of *C. maculatus* that not only can reduce the concerns outlined above but also can be prone of embracing the actual trend in developing new botanical-derived insecticides based on the inclusion of the active ingredient (i.e. EOs) in stable delivery systems (nanoparticles, nanoemulsion, etc) [29, 30]. Therefore, we investigated the chemical composition of clove and cinnamon essential oils and evaluated whether these oils would adequately control *C. maculatus*. We also characterized the biological responses (e.g., oviposition, offspring emergence and population growth) of *C. maculatus* exposed to sublethal amounts of each type of essential oil.

Materials and methods

Insect rearing

The original population of *C. maculatus* was field-collected from small farms in the Viçosa region (Minas Gerais State, Brazil) during the year 2015, and the population was maintained on pest- and insecticide-free cowpea beans under laboratory conditions ($27 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH, 12 h scotophase). The bean grains had a water content of 12% and were offered *ad libitum*. The farm-owners gave permission to collect samples of *C. maculatus* from their fields and since *C. maculatus* is not an endangered or protected species in Brazil, no specific permissions were required for such collection.

Extraction and chemical characterization of essential oils

Locally purchased cinnamon bark and dried flower buds of clove were used for essential oil extraction, as described by [31]. Briefly, the primary material was ground and sieved to obtain a fine powder (less than 1 mm) that was extracted at room temperature by constant percolation with hexane, followed by hydrodistillation for 6 h. Then, the distillate was extracted twice with dichloromethane and dried over anhydrous sodium sulfate. The distilled oils were stored in airtight screw-capped vials at -10°C until use.

The components of the cinnamon and clove essential oils were determined by gas chromatography-mass spectrometry (GCMS-QP2010, Shimadzu). The separation was done on a capillary column of fused silica ($30\text{ m} \times 0.22\text{ mm}$) with stationary phase RTX5 (0.25- μm -thick film). The initial column temperature was 60°C for 2 min, followed by increase of $3^{\circ}\text{C min}^{-1}$ up to 240°C , and this temperature was maintained for 15 min. The temperatures of the injector and detector were maintained at 220°C and 240°C , respectively.

The carrier gas was helium with a flow of 1.8 mL min^{-1} . Samples were diluted in dichloromethane and injected in a $1.0\text{ }\mu\text{L}$ split ratio of 1:20. Data acquisition was made in full-scan mode, with a scanning range between 29 and 400 m/z . Experimental mass spectra were compared with known mass spectra (The National Institute of Standards and Technology (NIST 14) Mass Spectra Library, 2017). The arithmetic index (AI) was calculated according to [32], using the retention times of the essential oil compounds and a homologous series of $\text{C}_8\text{-C}_{26}$ n-alkane standards following the formula: $\text{AI}(x) = 100 P_z + 100[(t(x) - t(P_z))/t(P_{z+1}) - t(P_z)]$; where x : compound at time t ; P_z : alkane before x ; and P_{z+1} : alkane after x . The relative percentage of each compound was calculated by the integral ratio of its respective peak area and the total area of all the compounds of the sample. The calculated AI for each compound was compared with values reported in the literature [32].

Insecticidal activity

We conducted dose-mortality bioassays to determine the lethal doses of the cinnamon and clove essential oils to adult *C. maculatus*. These bioassays followed procedures previously described elsewhere [33]. Briefly, each essential oil was pure (i.e., without diluents) and was applied using a $25\text{-}\mu\text{L}$ microsyringe (Hamilton, Reno, NV, USA) to 50 g of beans that were placed in 0.8-L glass jars. After the application, the jars were manually shaken for 60 s to ensure a complete distribution of the essential oils. Twenty unsexed 1-2-day-old *C. maculatus* adults were placed in each jar, and the jars were sealed with a fine porous cloth to allow ventilation; jars were kept under controlled conditions ($27 \pm 2^{\circ}\text{C}$, $75 \pm 5\%$ relative humidity, 12 h scotophase). The insect mortality was recorded after a 24-h exposure period. Insects were considered dead if they did not respond to fine paintbrush stimuli (i.e., two subsequent touches in 2 min intervals). Five doses of each essential oil were tested in the bioassays (e.g., *Cinnamon*: 20, 60, 120, 160 and $200\text{ }\mu\text{L kg}^{-1}$. *Clove*: 20, 40, 80, 120, $160\text{ }\mu\text{L kg}^{-1}$). Five replications were used per dose, and the control treatment did not receive any oil application. As a positive control, we used the pyrethroid insecticide deltamethrin (25 g L^{-1} ; EC; Bayer Crop Science, SP, Brazil) diluted in distilled water to obtain the desired doses (e.g., 64, 72, 80, 88, 96, $104\text{ }\mu\text{L a.i kg}^{-1}$). The application and the conditions of bioassays were similar to those described for the essential oils.

Effects of essential oils on the biological development and bean-mass losses

Effects on instantaneous rate of population growth (r_i) and bean-mass losses. The instantaneous rate of increase (r_i) test was carried out in 0.8-L glass jars, where 20 unsexed 1-2-day-old adults of *C. maculatus* were allowed to colonize 50 g of beans treated with an essential oil based on the concentration-mortality results previously obtained (see Results section).

We measured the instantaneous rate of increase (r_i) of groups of *C. maculatus* that were subjected to different sublethal exposures to cinnamon ($LD_{20} = 106.2$, $LD_{40} = 123.0$, $LD_{60} = 139.4$, $LD_{80} = 161.4 \mu\text{L kg}^{-1}$ of bean) and clove ($LD_{20} = 48.6$, $LD_{40} = 67.6$, $LD_{60} = 90.2$, $LD_{80} = 125.8 \mu\text{L kg}^{-1}$ of bean) essential oils. Five replicates were used for each combination of concentration and essential oil. All the glass jars were maintained at $27 \pm 2^\circ\text{C}$, $75 \pm 5\%$ relative humidity and 12 h scotophase. The control treatment did not receive any essential oil application. The number of F_1 insects was counted after 45 days, and the instantaneous rate of increase for each population was calculated as follows: $r_i = \ln(N_f/N_i)/\Delta t$, where N_f is the final number of observed adults, N_i is the initial number of *C. maculatus*, and Δt is the duration of the experiment [34].

The grain masses provided for insect colonization were weighed at the start (day 0) and at the end (day 45) of the bioassays to calculate the percentage of grain loss.

Effects on average and cumulative emergence. The bioassays for the average emergence were conducted using the same experimental procedures described for the instantaneous rate of increase (r_i). The progeny formed by the adult *C. maculatus* emerging from the beans were assessed in two days intervals starting from the 20th day after treatments began until no adult emergence was observed (i.e., 20 days after emergence of the 1st adults). After each assessment, the emerged adults were removed.

Effects of sublethal exposure to essential oils on the *C. maculatus* oviposition

Newly emerged (< 48 h old) groups of *C. maculatus* adult males and females were exposed separately to clove- and cinnamon-essential-oil-treated beans at the LD_{20} values for clove (i.e., $48.6 \mu\text{L kg}^{-1}$ of beans) and for cinnamon (i.e., $106.2 \mu\text{L kg}^{-1}$ of beans) essential oils. After a 24-h exposure period, we paired *C. maculatus* couples in four combinations (i.e., unexposed couple, exposed female, exposed male, and exposed couple) and allowed each couple to oviposit in 20 g of untreated beans. At 3-day intervals, the couples were moved to new 20-g bean masses, and this process was repeated for a total period of 9 days. The number of oviposited eggs was assessed under microscope after 3, 6 and 9 days. Twenty couples were used for each treatment combination.

Statistical analyses

Dose-mortality data were subjected to probit analysis [35], and 95% confidence intervals for toxicity ratios were estimated following [36]; the values were considered significant if the range did not include the value 1. Regression analyses were performed to detect trends in cumulative and average emergence that resulted in each treatment over time. Regression analysis was performed using the curve-fitting procedure of Sigma Plot 12.0. The regression model was chosen based on parsimony, lower standard errors, and steep increases in R^2 with increases in model complexity. The regression models for each treatment were considered different from each other if the confidence limits of their parameters did not overlap. We also conducted linear regression to assess the effects of increasing lethal exposure to essential oils on the r_i and grain-mass losses of *C. maculatus*. The data on the number of eggs used in each treatment combination were submitted to repeated measures ANOVA. The assumptions of normality and homogeneity of variance were tested for all parameters, and no data transformations were necessary (PROC UNIVARIATE, SAS Institute Inc., Cary, NC, USA).

Results

Chemical composition of the essential oils

The chemical analyses showed that the two main components of cinnamon and clove essential oils were eugenol and β -caryophyllene (Table 1). However, the cinnamon essential oil

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

Constituents	Arithmetic index				Concentration	
	<i>S. aromaticum</i>		<i>C. zeylanicum</i>		<i>S. aromaticum</i>	<i>C. zeylanicum</i>
	a	b	a	b	(%)	
eugenol	1363	1356	1364	1356	87.4	73.1
β -caryophyllene	1415	1417	1414	1417	11.5	7.7
α -humulene	1447	1452	1447	1452	1.1	0.4
α -pinene	-	-	931	932	-	0.7
α -phellandrene	-	-	1004	1002	-	0.3
p-cymene	-	-	1022	1020	-	1.0
limonene	-	-	1026	1024	-	0.5
eucalyptol	-	-	1028	1026	-	0.7
linalool	-	-	1100	1095	-	2.6
E-cinnamaldehyde	-	-	1268	1267	-	2.3
methyleugenol	-	-	1405	1403	-	0.6
cinnamyl acetate	-	-	1444	1443	-	2.5
acetyeugenol	-	-	1528	1521	-	3.6
caryophyllene oxide	-	-	1576	1582	-	0.5
benzyl benzoate	-	-	1760	1759	-	3.4

^a calculated

^b tabulated.

<https://doi.org/10.1371/journal.pone.0207618.t001>

additionally contained a wide range of other compounds in smaller amounts, including acetyeugenol, benzyl benzoate, linalool, cinnamyl acetate and cinnamaldehyde.

Insecticidal activity

The mortality levels obtained in the dose-mortality bioassays were satisfactorily described by the probit model [goodness-of-fit tests exhibiting low χ^2 -values (<10) and high *P*-values (>0.05)]. The toxicity ratios (TR) were estimated relative to the LD₅₀ for deltamethrin. The toxicities of the clove and cinnamon essential oils were similar to the pyrethroid-based insecticide deltamethrin (Table 2).

Effects of essential oils on the biological development and bean-mass losses

Effect on instantaneous rate of population increase (r_i) and bean weight loss. The instantaneous rate of population increase (r_i) decreased in a dose-dependent manner for the two essential oils used (Fig 1). The extinction stage (negative r_i) was reached when the *C. maculatus* insects were in contact with concentrations equal to or higher than LD₆₀ for clove

Table 2. Toxicity of clove and cinnamon essential oils and deltamethrin on adults of *Callosobruchus maculatus*.

Insecticide	Slope \pm SD	LD ₂₀ (95% FL)	LD ₄₀ (95% FL)	LD ₅₀ (95% FL)	LD ₆₀ (95% FL)	χ^2	<i>P</i>	TR ₅₀ (95% CL)
Clove	4.0 \pm 0.32	48.6 (42.0–54.0)	68.0 (62.0–74.0)	78.2 (71.6–84.8)	90.0 (84.0–98.0)	5.25	0.15	0.94 (0.9–1.0)
Cinnamon	9.3 \pm 1.02	106.4 (96.0–114.0)	124.0 (116.0–130.0)	131.0 (124.0–137.0)	138.0 (132.0–146.0)	3.90	0.14	1.56 (1.5–1.6)
Deltamethrin	13.9 \pm 1.25	72.8 (68.8–76.0)	80.0 (76.8–83.2)	83.7 (80.6–86.6)	87.2 (84.0–90.3)	9.91	0.08	1.00 (0.9–1.0)

SD standard deviation; LD: Lethal dose ($\mu\text{L kg}^{-1}$); FL = Fiducial limits; χ^2 = Chi-square for lack-of-fit to the probit model, and *P* = Probability associated with the chi-square statistic; TR₅₀ = Toxicity ratio determined by LD₅₀ of each the essential oil /LD₅₀ of deltamethrin; CL = Confidence limits of TR₅₀.

<https://doi.org/10.1371/journal.pone.0207618.t002>

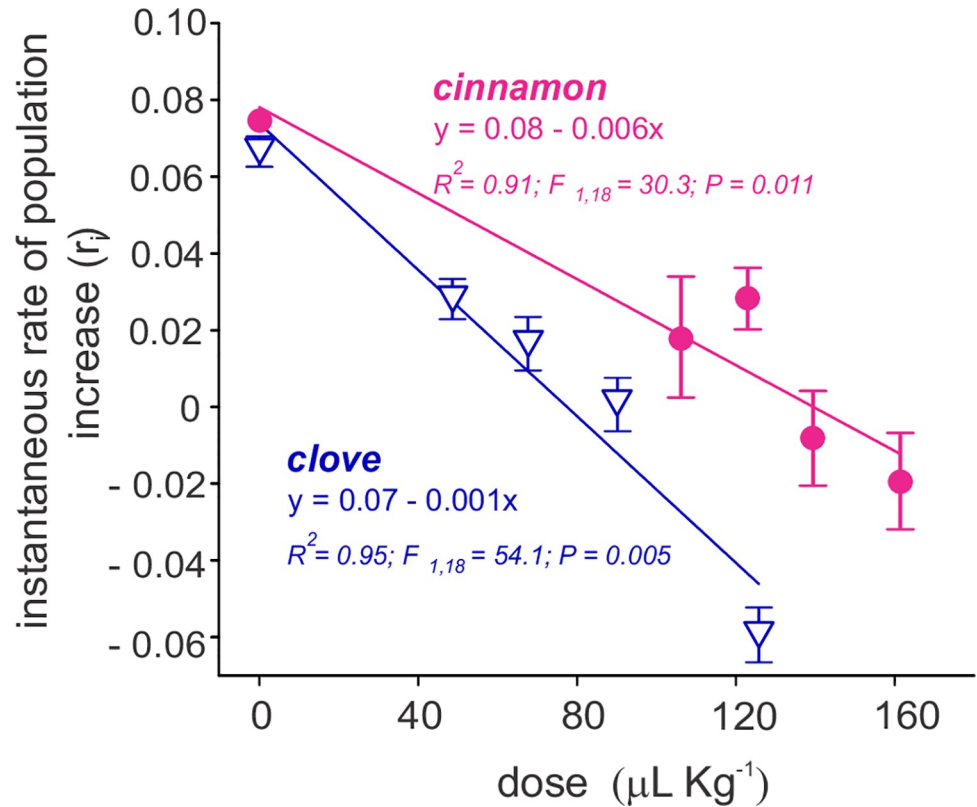


Fig 1. Instantaneous rate of population increase of *C. maculatus* exposed to clove and cinnamon essential oils. The symbols represent the means of five replicates of the LD₀ (control), LD₂₀, LD₄₀, LD₆₀ and LD₈₀ for each oil. The doses are expressed in µL of essential oil/kg beans. The vertical bars represent the SD.

<https://doi.org/10.1371/journal.pone.0207618.g001>

(i.e., 67.6 µL kg⁻¹ of bean) and cinnamon (i.e., 139.4 µL kg⁻¹ of bean) essential oils. Similar trends were observed for the bean mass losses, where the LD₂₀ concentrations of clove ($F = 21.3; P < 0.001$) and cinnamon ($F = 69.8; P < 0.001$) essential oils significantly reduced grain loss when applied as a contact treatment (Fig 2). These losses were reduced from approximately 15%, when the beans were incubated with *C. maculatus* insects in the absence of essential oils, to less than 6%, when the bean was treated with clove and cinnamon essential oils.

Positive correlations were observed between the instantaneous rate of increase of *C. maculatus* and the mass losses in grain masses treated with clove ($R^2 = 0.72; P < 0.001$) and cinnamon ($R^2 = 0.83; P < 0.001$) essential oils, between the instantaneous rate of increase and the total number of emerged adult of *C. maculatus* for clove ($R^2 = 0.75; P < 0.001$) and cinnamon ($R^2 = 0.85; P < 0.001$) essential oils, and between the total number of emerged adult of *C. maculatus* and the grain mass losses for clove ($R^2 = 0.98; P = 0.03$) and cinnamon ($R^2 = 0.99; P < 0.001$) essential oils. All of the fitting parameters for the curves in Figs 1 and 2 are presented in supplementary S1 Table.

Effects on average and cumulative emergence. The average emergence of new *C. maculatus* insects was negatively and severely impacted after treatment by either clove or cinnamon essential oil, as observed in the differences between the emergence curves (Fig 3). Treatments with concentrations starting at the LD₂₀ for both essential oils resulted in a near abolition of emergence. Moreover, the total emergence of *C. maculatus* was significantly delayed by treatments of almost all concentrations of the two essential oils when compared to the control, as shown by the lack of overlap between the cumulative emergence curves (Fig 4). All of the

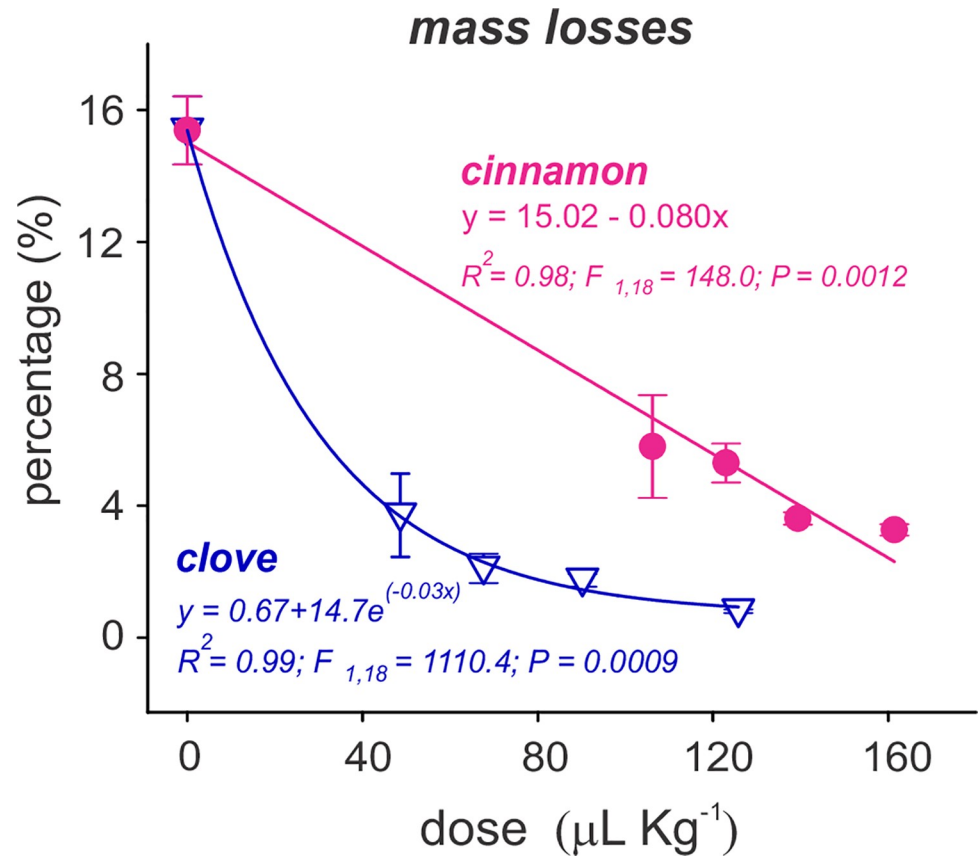


Fig 2. Bean weight losses caused by *C. maculatus* exposed to clove and cinnamon essential oils. The symbols represent the means of five replicates of the LD₀ (control), LD₂₀, LD₄₀, LD₆₀ and LD₈₀ for each oil. The doses are expressed in μL of essential oil/kg beans. The vertical bars represent the SD.

<https://doi.org/10.1371/journal.pone.0207618.g002>

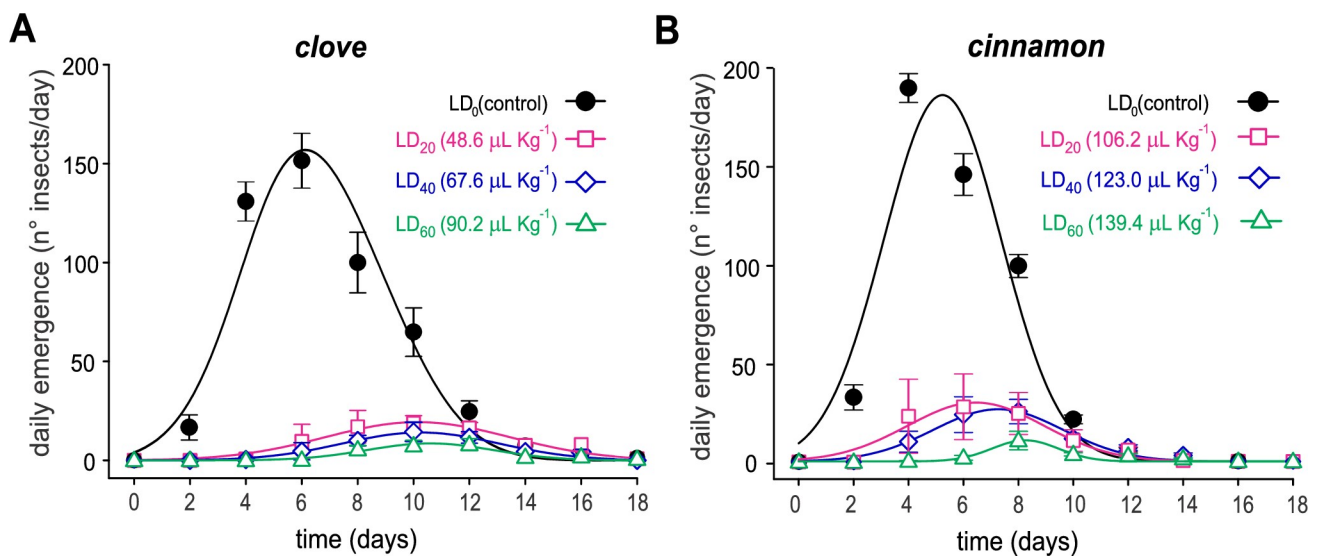


Fig 3. Average emergence of *C. maculatus* exposed to clove (A) and cinnamon (B) essential oils. The symbols represent the means of four replicates of the LD₀ (control), LD₂₀, LD₄₀, and LD₆₀ for each oil. The doses are expressed in μL of essential oil/kg beans. The vertical bars represent the SD.

<https://doi.org/10.1371/journal.pone.0207618.g003>

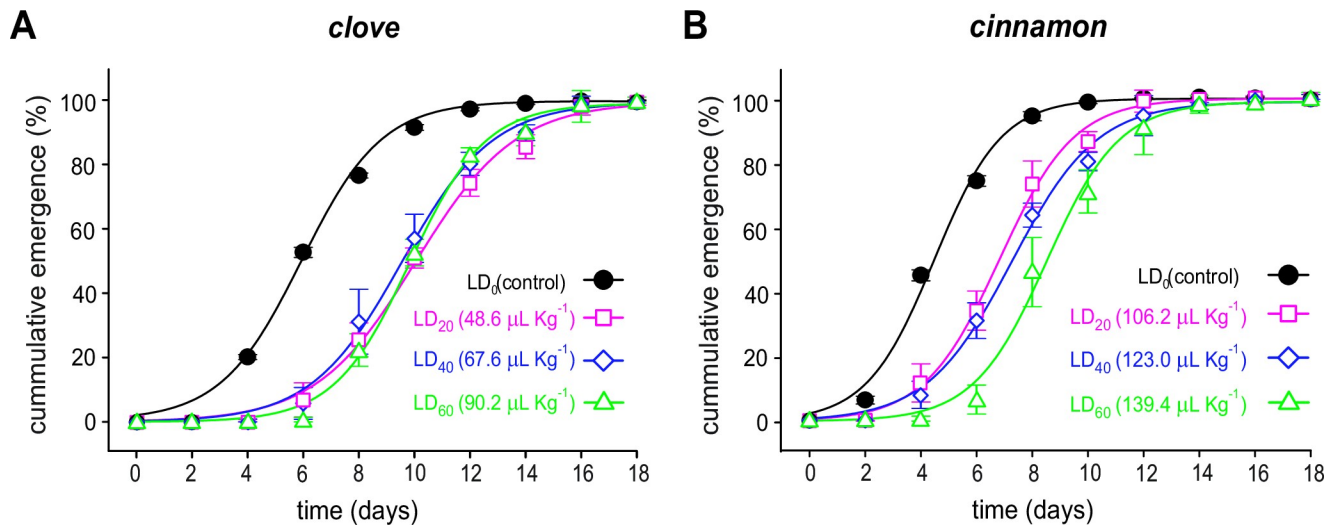


Fig 4. Normalized cumulative emergence of *C. maculatus* exposed to clove (A) and cinnamon (B) essential oils. The symbols represent the means of four replicates of the LD₀ (control), LD₂₀, LD₄₀ and LD₆₀ for each oil. The doses are expressed in µL of essential oil/kg beans. The vertical bars represent the SD.

<https://doi.org/10.1371/journal.pone.0207618.g004>

fitting parameters for the curves in Figs 3 and 4 are presented in supplementary S2 and S3 Tables.

Effects of sublethal concentrations of essential oils on *C. maculatus* oviposition

The results of repeated measures ANOVA showed that there was a significant interaction (Wilks' lambda = 11.77; *df* = 6; *P* < 0.001) between oil types, couple combinations and evaluation times (Fig 5). The sublethal exposure to clove and cinnamon essential oils mediated the effects on *C. maculatus* oviposition, resulting in significant (*F* = 25.1; *df* = 3; *P* < 0.001) differences in the total number of eggs among the four combinations of treatments. When only females were exposed (i.e., exposed females or exposed couples) or when both females and males were exposed (i.e., exposed couples), the total number of laid eggs decreased dramatically in comparison with both the untreated couples and the couples where only males were treated. The two essential oils showed significantly (*F* = 4.19; *df* = 1; *P* = 0.04) different inhibiting effects on oviposition, and this difference was more evident in the oviposition of couples where only females were treated, as the decrease was more important for cinnamon compared to clove and untreated couples. Moreover, the differences between the effects of sublethal exposure to both essential oils were significantly (*F* = 136.2; *df* = 2; *P* < 0.001) higher during the first 3 days of the oviposition period.

Discussion

Plant essential oils are among the most interesting options for cheaper, safer and eco-friendly replacements (or to be used as adjuvants) for synthetic insecticides [2–4, 6]. Here, we demonstrated that applications of clove and cinnamon essential oils not only adequately controlled *C. maculatus* on stored cowpea beans but also were capable of reducing the oviposition and population growth of *C. maculatus* even at sublethal dosages.

Essential oils, such as clove and cinnamon oils, are very complex natural mixtures and can contain various compounds at different concentrations with two or three major components

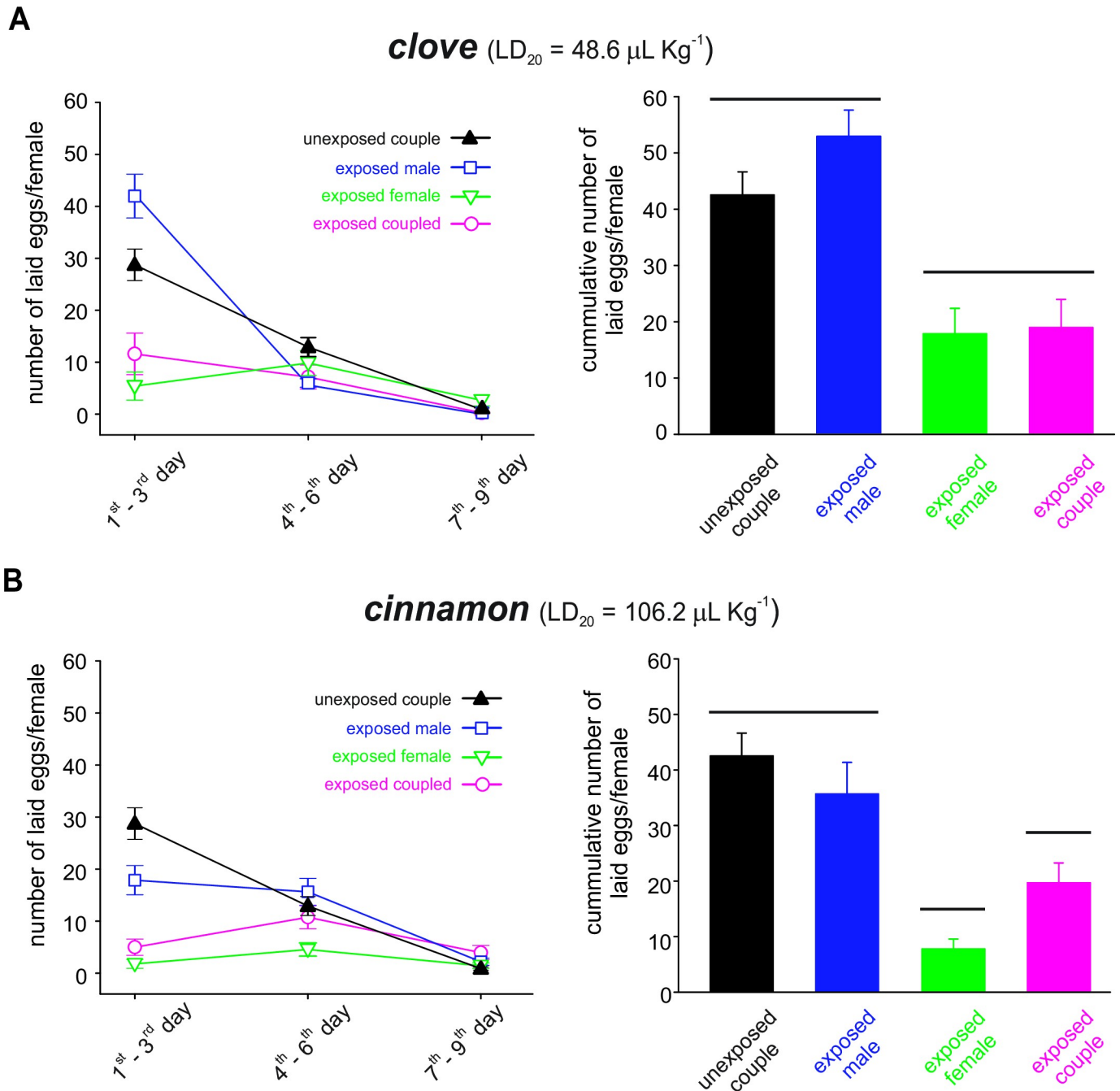


Fig 5. Effects of sublethal exposure to essential oils on *C. maculatus* oviposition. Number of eggs of *C. maculatus* females that were sublethally exposed to clove (A) or cinnamon (B) essential oils and coupled with essential oil-treated or essential oil-untreated partners. The symbols represent the means of 20 replicates (\pm SD) for the number of eggs laid by females of *C. maculatus* at three-day intervals (left panels) and the cumulative number of eggs (right panels) laid on cowpea bean masses during the first 9 days of adulthood. On the right panels, the treatments grouped by the same horizontal line did not differ according to a Tukey HSD test ($P < 0.05$).

<https://doi.org/10.1371/journal.pone.0207618.g005>

that will determine the biological properties of the essential oil [37]. However, synergistic effects between the components of essential oils have been frequently reported in previous studies [38–43]. Our chemical analyses of cinnamon and clove essential oils revealed that their primarily components were eugenol (>70.0%), followed by the sesquiterpene β -caryophyllene

(between 7.0 and 12%). These results are in concordance with previous studies that reported similar compositions [33, 37, 44–48]. It is worth noting that cinnamon essential oil, despite its major components, also contained a range of other compounds, including acetyleugenol, benzyl benzoate, linalool, cinnamyl acetate and cinnamaldehyde (between 2 and 4%), lending more evidence to the hypothesis that essential oil biological activities may be shaped by the potential synergistic and antagonistic interactions among all these molecules and not only by major essential oil compounds [37, 49].

Several studies have reported the insecticidal toxicity of clove and cinnamon essential oils and their primary compounds that successfully control stored product pests [9, 18, 26, 33, 50–52] and other insects [48, 53–56]. The vast majority of these investigations have attributed these essential oil insecticidal activities to their major constituents (i.e., eugenol and β -caryophyllene), as these compounds are known to act on the insects' nervous system by disturbing the functions of GABAergic [57, 58] and aminergic [59–61] systems and by inhibiting the actions of acetylcholinesterase [62–64].

Negative effects on developmental traits, such as rates of growth and progeny emergence of bruchid insects such as *C. maculatus*, have been reported for various essential oils and their components [22, 23, 26, 52, 65–69]. The present investigation demonstrated that treating cowpea bean masses with sublethal dosages (i.e., as lower as their LD_{20}) of these essential oils leads to significant decreases in the *C. maculatus* instantaneous rates of population growth and the bean mass losses, and the application of the essential oils almost abolished *C. maculatus* offspring emergence. Such biological impairments caused by clove and cinnamon essential oils on bruchids can be the result of the direct mortality of adults, repellency, oviposition deterrence or progeny and growth inhibition [51]. However, our oviposition results (i.e., females sublethally exposed to these essential oils decreased their ability to lay eggs even when they were offered to mate with untreated sexual partners in untreated bean masses) revealed an even more complex scenario and potential effects on sexual fitness (e.g., locomotory activities, mating behavior) or on the physiological basis of oviposition (e.g., respiratory activities, oogenesis or hormonal disruption) may also contribute to the reduced performance of essential oil sublethally exposed insects. For instance, similar physiological impairments (e.g., repellence, emergence inhibition, altered respiratory activities and transgenerational behavior changes) have been reported in stored product pests (e.g., *S. zeamais*) exposed to essential oils of cinnamon and clove [9, 13, 17, 18].

Furthermore, as biosynthesis and release of mating signals as well as the production of eggs may be influenced by atmospheric volatiles and gases [70, 71], plant extracts, such as terpenes, can influence the site-choice of egg-laying female insects [70]. Although future experiments are required to isolate the effects of the exposure to essential oils on the physiological basis of oviposition and on the mating behavior, a potential energy trade-off between the detoxification process and oogenesis might be an explanation for the inhibited oviposition observed here [72–74].

In our study we have used drops of pure essential oils on bean masses and although such technique showed good biological activities on *C. maculatus* under laboratory conditions and may have potential applications at small farmer's level, this delivery system may suffer from drawbacks inherent to the volatile nature of essential oils in larger storage facilities. In fact, rapid biodegradation of these compounds due to their poor physicochemical stability, high volatility, and thermal decomposition will require some controlled-release system such as nanotechnological formulations to optimize the action of their active ingredients [75, 76].

Thus, our findings revealed adequate insecticidal activities of clove and cinnamon essential oils against *C. maculatus* and demonstrated that, even at sublethal doses, these botanical compounds impaired the ability of *C. maculatus* to damage cowpea bean masses, which make

them suitable tools that can be integrated into management programs of *C. maculatus*, especially for storage facilities. Further work is also needed to test the applicability and efficacy of nanofomulations of these essential oils under broader stored products conditions.

Supporting information

S1 Data. Raw data used in all the statistical analysis.

(PDF)

S1 Table. Summary of the non-linear regression analyses (Increase rate r_i and bean mass losses) of the curves shown in the Figs 1 and 2.

(PDF)

S2 Table. Summary of the non-linear regression analyses of average emergence curves of the curves shown in the Fig 3.

(PDF)

S3 Table. Summary of the non-linear regression analyses of the normalized cumulative emergence curves shown in the Fig 4.

(PDF)

Author Contributions

Conceptualization: Luis Oswaldo Viteri Jumbo, Khalid Haddi, Fernanda F. Heleno, Eugênio E. Oliveira.

Data curation: Luis Oswaldo Viteri Jumbo, Khalid Haddi.

Formal analysis: Khalid Haddi, Fernanda F. Heleno, Eugênio E. Oliveira.

Investigation: Luis Oswaldo Viteri Jumbo, Fernanda F. Heleno, Frederico G. Pinto.

Methodology: Frederico G. Pinto.

Resources: Luis Oswaldo Viteri Jumbo, Lêda Rita D. Faroni, Eugênio E. Oliveira.

Writing – original draft: Luis Oswaldo Viteri Jumbo, Khalid Haddi, Eugênio E. Oliveira.

Writing – review & editing: Khalid Haddi, Eugênio E. Oliveira.

References

1. Isman MB, Grieneisen ML. Botanical insecticide research: many publications, limited useful data. *Trends in Plant Science*. 2014; 19(3):140–5. <http://dx.doi.org/10.1016/j.tplants.2013.11.005>. PMID: 24332226
2. Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: Low-risk products in a high-stakes world. *Annual Review of Entomology*. 2012; 57(1):405–24. <https://doi.org/10.1146/annurev-ento-120710-100554> PMID: 21942843.
3. Stevenson PC, Isman MB, Belmain SR. Pesticidal plants in Africa: a global vision of new biological control products from local uses. *Industrial Crops and Products*. 2017.
4. Isman MB. Pesticides Based on Plant Essential Oils: Phytochemical and Practical Considerations. *Medicinal and Aromatic Crops: Production, Phytochemistry, and Utilization*: ACS Publications; 2016. p. 13–26.
5. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: a review. *Bioresource Technology*. 2010; 101(1):372–8. <https://doi.org/10.1016/j.biortech.2009.07.048> PMID: 19729299
6. Pavela R. Essential oils for the development of eco-friendly mosquito larvicides: a review. *Industrial Crops and Products*. 2015; 76:174–87.

7. Desneux N, Decourtye A, Delpuech J-M. The sublethal effects of pesticides on beneficial arthropods. *Annu Rev Entomol.* 2007; 52:81–106. <https://doi.org/10.1146/annurev.ento.52.110405.091440> PMID: 16842032
8. Haddi K, Mendes MV, Barcellos MS, Lino-Neto J, Freitas HL, Guedes RNC, et al. Sexual success after stress? Imidacloprid-induced hormesis in males of the neotropical stink bug *Euschistus heros*. *PloS one.* 2016; 11(6):e0156616. <https://doi.org/10.1371/journal.pone.0156616> PMID: 27284906
9. Gonzales Correa YDCG, Faroni LR, Haddi K, Oliveira EE, Pereira E.JG. Locomotory and physiological responses induced by clove and cinnamon essential oils in the maize weevil *Sitophilus zeamais*. *Pesticide Biochemistry and Physiology.* 2015; 125:31–7. <https://doi.org/10.1016/j.pestbp.2015.06.005> PMID: 26615148
10. Guedes R, Smagghe G, Stark J, Desneux N. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annual review of entomology.* 2016; 61:43–62. <https://doi.org/10.1146/annurev-ento-010715-023646> PMID: 26473315
11. Cutler GC, Guedes RN. Occurrence and significance of insecticide-induced hormesis in insects. *Pesticide dose: effects on the environment and target and non-target organisms ACS, Washington.* 2017:101–19.
12. Teodoro AV, Silva MDJDS, Filho JGDS, Oliveira EED, Galvão AS Silva SS. Bioactivity of cottonseed oil against the coconut mite *Aceria guerreronis* (Acari: Eriophyidae) and side effects on *Typhlodromus ornatus* (Acari: Phytoseiidae). *Systematic and Applied Acarology.* 2017; 22(7):1037–47.
13. Freitas RCP, Faroni LRDA, Haddi K, Jumbo LOV, Oliveira EE. Allyl isothiocyanate actions on populations of *Sitophilus zeamais* resistant to phosphine: Toxicity, emergence inhibition and repellency. *Journal of Stored Products Research.* 2016; 69:257–64.
14. Haddi K, Faroni LR, Oliveira EE. Cinnamon Oil. In: Nollet LM, and Hamir Singh Rathore, editor. *Green Pesticides Handbook: Essential Oils for Pest Control: CRC Press; 2017.* p. 117–50.
15. Oliveira NN, Galvão AS, Amaral EA, Santos AW, Sena-Filho JG, Oliveira EE, et al. Toxicity of vegetable oils to the coconut mite *Aceria guerreronis* and selectivity against the predator *Neoseiulus baraki*. *Experimental and Applied Acarology.* 2017:1–12.
16. de Araújo AMN, Faroni LRDA, de Oliveira JV, Navarro DMdAF, Breda MO, de França SM. Lethal and sublethal responses of *Sitophilus zeamais* populations to essential oils. *Journal of Pest Science.* 2017; 90(2):589–600.
17. Haddi K, Oliveira EE, Faroni LR, Guedes DC, Miranda NN. Sublethal exposure to clove and cinnamon essential oils induces hormetic-like responses and disturbs behavioral and respiratory responses in *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Economic Entomology.* 2015; 108(6):2815–22. <https://doi.org/10.1093/jee/fov255> PMID: 26318008
18. Silva S, Haddi K, Viteri Jumbo L, Oliveira E. Progeny of the maize weevil, *Sitophilus zeamais*, is affected by parental exposure to clove and cinnamon essential oils. *Entomologia Experimentalis et Applicata.* 2017; 163(2):220–8.
19. Jankowska M, Rogalska J, Wyszowska J, Stankiewicz M. Molecular Targets for Components of Essential Oils in the Insect Nervous System—A Review. *Molecules.* 2017; 23(1):34.
20. Kang JK, Pittendrigh BR, Onstad DW. Insect resistance management for stored product pests: a case study of cowpea weevil (Coleoptera: Bruchidae). *Journal of Economic Entomology.* 2013; 106(6):2473–90. PMID: 24498750
21. Brisibe EA, Adugbo SE, Ekanem U, Brisibe F, Figueira GM. Controlling bruchid pests of stored cowpea seeds with dried leaves of *Artemisia annua* and two other common botanicals. *African Journal of Biotechnology.* 2011; 10(47):9593–9.
22. Massango H, Faroni L, Haddi K, Heleno F, Jumbo LV, Oliveira E. Toxicity and metabolic mechanisms underlying the insecticidal activity of parsley essential oil on bean weevil, *Callosobruchus maculatus*. *Journal of Pest Science.* 2017; 90(2):723–33.
23. Ileke KD, Bulus DS, Aladegoroye AY. Effects of three medicinal plant products on survival, oviposition and progeny development of cowpea bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae] infesting cowpea seeds in storage. *Jordan Journal of Biological Science.* 2013; 6(1):61–6.
24. Lima MPd, Oliveira JV, Barros R, Torres JB. Identification of cowpea *Vigna unguiculata* (L.) Walp. genotypes resistant to *Callosobruchus maculatus* (Fabr.) (Coleoptera: Bruchidae). *Neotropical Entomology.* 2001; 30(2):289–95.
25. Gbaye OA, Millard JC, Holloway GJ. Legume type and temperature effects on the toxicity of insecticide to the genus *Callosobruchus* (Coleoptera: Bruchidae). *Journal of Stored Products Research.* 2011; 47(1):8–12.
26. Haddi K, Jumbo LV, Costa M, Santos M, Faroni L, Serrão J, et al. Changes in the insecticide susceptibility and physiological trade-offs associated with a host change in the bean weevil *Acanthoscelides obtectus*. *Journal of Pest Science.* 2017:1–10.

27. Gbaye O, Millard J, Holloway G. Synergistic effects of geographical strain, temperature and larval food on insecticide tolerance in *Callosobruchus maculatus* (F.). *Journal of Applied Entomology*. 2012; 136(4):282–91.
28. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*. 2006; 51:45–66. <https://doi.org/10.1146/annurev.ento.51.110104.151146> PMID: 16332203
29. Kah M, Kookana RS, Gogos A, Bucheli TD. A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nature nanotechnology*. 2018;1.
30. Campolo O, Cherif A, Ricupero M, Siscaro G, Grissa-Lebdi K, Russo A, et al. Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: chemical properties and biological activity. *Scientific Reports*. 2017; 7(1):13036. <https://doi.org/10.1038/s41598-017-13413-0> PMID: 29026207
31. Jham GN, Dhingra OD, Jardim CM, Valente VM. Identification of the major fungitoxic component of cinnamon bark oil. *Fitopatologia Brasileira*. 2005; 30(4):404–8.
32. Adams RP. Identification of essential oil components by gas chromatography/mass spectroscopy. *Journal of the American Society for Mass Spectrometry*. 1997; 6(8):671–2.
33. Viteri Jumbo LO, Faroni LRA, Oliveira EE, Pimentel MA, Silva GN. Potential use of clove and cinnamon essential oils to control the bean weevil, *Acanthoscelides obtectus* Say, in small storage units. *Industrial Crops and Products*. 2014; 56(0):27–34. <http://dx.doi.org/10.1016/j.indcrop.2014.02.038>.
34. Walthall WK, Stark JD. Comparison of two population-level ecotoxicological endpoints: The intrinsic (rm) and instantaneous (ri) rates of increase. *Environmental Toxicology and Chemistry*. 1997; 16(5):1068–73.
35. SAS Institute. SAS/STAT User's Guide. SAS, editor. Cary, NC, USA.2008.
36. Robertson JL, Preisler HK. *Pesticide Bioassays with Arthropods*. PCRC, editor. Boca Raton, FL, USA.1992.
37. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—a review. *Food and Chemical Toxicology*. 2008; 46(2):446–75. <https://doi.org/10.1016/j.fct.2007.09.106> PMID: 17996351
38. Fornari T, Vicente G, Vázquez E, García-Risco MR, Reglero G. Isolation of essential oil from different plants and herbs by supercritical fluid extraction. *Journal of Chromatography A*. 2012; 1250:34–48. <https://doi.org/10.1016/j.chroma.2012.04.051> PMID: 22595519
39. Joffe T, Gunning RV, Allen GR, Kristensen M, Alptekin S, Field LM, et al. Investigating the potential of selected natural compounds to increase the potency of pyrethrum against houseflies *Musca domestica* (Diptera: Muscidae). *Pest Management Science*. 2012; 68(2):178–84. <https://doi.org/10.1002/ps.2241> PMID: 21770012
40. Koul O, Singh R, Kaur B, Kanda D. Comparative study on the behavioral response and acute toxicity of some essential oil compounds and their binary mixtures to larvae of *Helicoverpa armigera*, *Spodoptera litura* and *Chilo partellus*. *Industrial Crops and Products*. 2013; 49:428–36.
41. Miresmailli S, Bradbury R, Isman MB. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. *Pest Management Science*. 2006; 62(4):366–71. <https://doi.org/10.1002/ps.1157> PMID: 16470541
42. Omolo M, Okinyo D, Ndiege I, Lwande W, Hassanali A. Fumigant toxicity of the essential oils of some African plants against *Anopheles gambiae* sensu stricto. *Phytomedicine*. 2005; 12(3):241–6. <https://doi.org/10.1016/j.phymed.2003.10.004> PMID: 15830848
43. Kanda D, Kaur S, Koul O. A comparative study of monoterpenoids and phenylpropanoids from essential oils against stored grain insects: acute toxins or feeding deterrents. *Journal of Pest Science*. 2017; 90(2):531–45.
44. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, et al. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review. *Phytotherapy Research*. 2007; 21(6):501–6. <https://doi.org/10.1002/ptr.2124> PMID: 17380552
45. Dayan FE, Cantrell CL, Duke SO. Natural products in crop protection. *Bioorganic & Medicinal Chemistry*. 2009; 17(12):4022–34.
46. Fichi G, Flamini G, Giovanelli F, Otranto D, Perrucci S. Efficacy of an essential oil of *Eugenia caryophyllata* against *Psoroptes cuniculi*. *Experimental Parasitology*. 2007; 115(2):168–72. <https://doi.org/10.1016/j.exppara.2006.07.005> PMID: 16973163
47. Fichi G, Flamini G, Zaralli L, Perrucci S. Efficacy of an essential oil of *Cinnamomum zeylanicum* against *Psoroptes cuniculi*. *Phytomedicine*. 2007; 14(2):227–31.
48. Park I-K, Lee H-S, Lee S-G, Park J-D, Ahn Y-J. Insecticidal and fumigant activities of Cinnamomum cassia bark-derived materials against *Mechorhis ursulus* (Coleoptera: Attelabidae). *Journal of Agricultural and Food Chemistry*. 2000; 48(6):2528–31. PMID: 10888580

49. Koul O, Walia S, Dhaliwal G. Essential oils as green pesticides: potential and constraints. *Biopesticide International*. 2008; 4(1):63–84.
50. Brari J, Thakur D. Insecticidal efficacy of essential oil from *Cinnamomum zeylanicum* Blume and its two major constituents against *Callosobruchus maculatus* (F.) and *Sitophilus oryzae* (L.). *Journal of Agricultural Technology*. 2015; 11(6):1323–36.
51. Athanassiou CG, Rani PU, Kavallieratos NG. The use of plant extracts for stored product protection. *Advances in Plant Biopesticides*: Springer; 2014. p. 131–47.
52. Pérez S, Ramos-López M, Zavala-Sánchez M, Cárdenas-Ortega N. Activity of essential oils as a biorational alternative to control coleopteran insects in stored grains. *Journal of Medicinal Plants Research*. 2010; 4(25):2827–35.
53. Chang S-T, Cheng S-S. Antitermitic activity of leaf essential oils and components from *Cinnamomum osmophloeum*. *Journal of Agricultural and Food Chemistry*. 2002; 50(6):1389–92. PMID: [11879008](#)
54. Cheng S-S, Liu J-Y, Huang C-G, Hsui Y-R, Chen W-J, Chang S-T. Insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against three mosquito species. *Bioresource Technology*. 2009; 100(1):457–64. <https://doi.org/10.1016/j.biortech.2008.02.030> PMID: [18396039](#)
55. Khater HF, Ramadan MY, El-Madawy RS. Lousicidal, ovicidal and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt. *Veterinary Parasitology*. 2009; 164(2):257–66.
56. Wang Z, Kim H-K, Tao W, Wang M, Ahn Y-J. Contact and Fumigant Toxicity of Cinnamaldehyde and Cinnamic Acid and Related Compounds to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *Journal of Medical Entomology*. 2011; 48(2):366–71. PMID: [21485375](#)
57. Tong F, Coats JR. Quantitative structure–activity relationships of monoterpenoid binding activities to the housefly GABA receptor. *Pest Management Science*. 2012; 68(8):1122–9. <https://doi.org/10.1002/ps.3280> PMID: [22461383](#)
58. Bloomquist JR, Boina DR, Chow E, Carlier PR, Reina M, Gonzalez-Coloma A. Mode of action of the plant-derived silphinenes on insect and mammalian GABA A receptor/chloride channel complex. *Pesticide Biochemistry and Physiology*. 2008; 91(1):17–23.
59. Enan EE. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. *Archives of Insect Biochemistry and Physiology*. 2005; 59(3):161–71. <https://doi.org/10.1002/arch.20076> PMID: [15986384](#)
60. Enan EE. Molecular response of *Drosophila melanogaster* tyramine receptor cascade to plant essential oils. *Insect Biochemistry and Molecular Biology*. 2005; 35(4):309–21. <https://doi.org/10.1016/j.ibmb.2004.12.007> PMID: [15763467](#)
61. Kostyukovsky M, Rafaeli A, Gileadi C, Demchenko N, Shaaya E. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. *Pest Management Science*. 2002; 58(11):1101–6. <https://doi.org/10.1002/ps.548> PMID: [12449528](#)
62. Keane S, Ryan M. Purification, characterisation, and inhibition by monoterpenes of acetylcholinesterase from the waxmoth, *Galleria mellonella* (L.). *Insect Biochemistry and Molecular Biology*. 1999; 29(12):1097–104.
63. Lopes KV, Silva LB, Reis AP, Oliveira MG, Guedes RN. Modified alpha-amylase activity among insecticide-resistant and -susceptible strains of the maize weevil, *Sitophilus zeamais*. *Journal of insect physiology*. 2010; 56(9):1050–7. <https://doi.org/10.1016/j.jinsphys.2010.02.020> PMID: [20223242](#).
64. Abdelgaleil SA, Mohamed MI, Shawir MS, Abou-Taleb HK. Chemical composition, insecticidal and biochemical effects of essential oils of different plant species from Northern Egypt on the rice weevil, *Sitophilus oryzae* L. *Journal of Pest Science*. 2016; 89(1):219–29.
65. Paranagama P, Gunasekera J. The efficacy of the essential oils of Sri Lankan *Cinnamomum zeylanicum* fruit and *Micromelum minutum* leaf against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Essential Oil Research*. 2011; 23(1):75–82.
66. Jumbo LOV, Faroni LR, Oliveira EE, Pimentel MA, Silva GN. Potential use of clove and cinnamon essential oils to control the bean weevil, *Acanthoscelides obtectus* Say, in small storage units. *Industrial Crops and Products*. 2014; 56:27–34.
67. Elhag EA. Deterrent effects of some botanical products on oviposition of the cowpea bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *International Journal of Pest Management*. 2000; 46(2):109–13.
68. Singh R. Evaluation of some plant products for their oviposition deterrent properties against the *Callosobruchus maculatus* (F.) on chick pea seeds. *Journal of Agricultural Science and Technology*. 2011; 7(5):1363–7.
69. Ekeh FN, Oleru KI, Ivoke N, Nwani CD, Eyo JE. Effects of *Citrus sinensis* peel oil on the oviposition and development of cowpea beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in some legume grains. *Pakistan Journal of Zoology*. 2013; 45(4):967–74.

70. Hilker M, Meiners T. Plants and insect eggs: how do they affect each other? *Phytochemistry*. 2011; 72(13):1612–23. <https://doi.org/10.1016/j.phytochem.2011.02.018> PMID: 21439598
71. Städler E, Hilker M, Meiners T. Plant chemical cues important for egg deposition by herbivorous insects. *Chemoecology of Insect Eggs and Egg Deposition*. 2002:171–204.
72. Hashem MY, Ismail II, Lutfallah AF, El-Rahman SFA. Effects of carbon dioxide on *Sitotroga cerealella* (Olivier) larvae and their enzyme activity. *Journal of Stored Products Research*. 2014; 59:17–23.
73. Kaur AP, Sohal SK, Kaur M, Singh J. Monitoring growth, survival and enzyme system of melon fruit fly *Bactrocera cucurbitae* (Coquillett) under the influence of affinity purified pea lectin. *Entomological Science*. 2013; 16(1):91–9.
74. Oliveira DM, Gomes FM, Carvalho DB, Ramos I, Carneiro AB, Silva-Neto MA, et al. Yolk hydrolases in the eggs of *Anticarsia gemmatilis* hubner (Lepidoptera: Noctuidae): a role for inorganic polyphosphate towards yolk mobilization. *Journal of insect physiology*. 2013; 59(12):1242–9. <https://doi.org/10.1016/j.jinsphys.2013.09.008> PMID: 24140472
75. Pavela R, Benelli G. Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends in plant science*. 2016; 21(12):1000–7. <https://doi.org/10.1016/j.tplants.2016.10.005> PMID: 27789158
76. de Oliveira JL, Campos EVR, Bakshi M, Abhilash P, Fraceto LF. Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: prospects and promises. *Biotechnology advances*. 2014; 32(8):1550–61. <https://doi.org/10.1016/j.biotechadv.2014.10.010> PMID: 25447424

Copyright of PLoS ONE is the property of Public Library of Science and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.