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Locomotory and physiological responses induced by clove and cinnamon essential oils in the maize weevil *Sitophilus zeamais*

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ABSTRACT

Plant essential oils have been suggested as a suitable alternative for controlling stored pests worldwide. However, very little is known about the physiological or behavioral responses induced by these compounds in insect populations that are resistant to traditional insecticides. Thus, this investigation evaluated the toxicity (including the impacts on population growth) as well as the locomotory and respiratory responses induced by clove, *Syzygium aromaticum* L., and cinnamon, *Cinnamomum zeylanicum* L., essential oils in Brazilian populations of the maize weevil *Sitophilus zeamais*. We used populations that are resistant to phosphine and pyrethroids (PyPhR), only resistant to pyrethroids (PyR1 and PyR2) or susceptible to both insecticide types (SUS). The PyPhR population was more tolerant to cinnamon essential oil, and its population growth rate was less affected by both oil types. Insects from this population reduced their respiratory rates (i.e., CO_2 production) after being exposed to both oil types and avoided (in free choice-experiments) or reduced their mobility on essential oil-treated surfaces. The PyR1 and PyR2 populations reduced their respiratory rates, avoided (without changing their locomotory behavior in no-choice experiments) essential oil-treated surfaces and their population growth rates were severely affected by both oil types. Individuals from SUS population increased their mobility on surfaces that were treated with both oil types and showed the highest levels of susceptibility to these oils. Our findings indicate that *S. zeamais* populations that are resistant to traditional insecticides might have distinct but possibly overlapping mechanisms to mitigate the actions of essential oils and traditional insecticides.

1. Introduction

Essential oils are complex mixtures of volatile organic compounds that can be extracted from seeds, stems, leaves and flowers and have great potential for use as fungicides, bactericides and insecticides [1–4]. Although it is not completely clear how such products will operationally be applied, essential oils have been suggested as suitable alternatives for controlling stored pests worldwide [5–8] because these plant products apparently do not pose the same risks (e.g., pest resistance, hazards to human health and environmental contamination) as traditional insecticides. Essential oils' modes of action include the disruption of the physiological functions of the GABAergic [5–7] and aminergic [8–11] systems as well as the inhibition of acetylcholinesterase actions [12–14] in the insect nervous system.

The plant species that produce essential oils with potential insecticidal activities for pest control include clove, *Syzygium aromaticum*, and cinnamon, *Cinnamomum zeylanicum*. The insecticidal activity of clove and cinnamon oils is well documented [15–24], but relatively little attention has been paid to the respiratory and locomotory responses induced by these compounds in insects, although these physiological and behavioral traits indicate the adaptability of insect populations to the environmental conditions to which they are exposed [25–32].

The control efficacy of clove and cinnamon oils might be reduced in insect populations that have evolved physiological and behavioral mechanisms to resist traditional insecticides. Indeed, such resistance mechanisms have been reported for traditional insecticides in some stored pests [28–31,33–35], which reinforces that need of taking this information in consideration before simply replace the use of traditional insecticides by essential oils.

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a serious cosmopolitan pest of stored grains throughout tropical regions, including Brazil [31,36–38]. It is one of the most destructive and widespread key pests of stored maize [38,39]. The management of this pest in storage facilities relies heavily on the use of chemical insecticides, but many cases of resistance and control failure have been reported because of the inadequate application of this

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strategy [34,40,41, 1995, 42,43]. The insecticidal actions of clove and cinnamon oils and their major constituents on *S. zeamais* have been reported elsewhere [44–47], but the potential involvement of the respiratory and locomotory strategies used by these insects to mitigate these oil actions have been completely neglected. Here, we evaluated the toxicity (including the effects on the population growth rates) of clove and cinnamon essential oils as well as the effects of exposure to these oils on the behavioral (locomotory) and respiratory rates of four Brazilian populations of *S. zeamais* with distinct susceptibilities to traditional insecticides (phosphine and pyrethroids).

2. Materials and methods

2.1. Insect populations

We used four Brazilian populations of *S. zeamais* (originally obtained between 2004 and 2007) with documented and frequently checked levels of insecticide resistance [35,48,49]: the population of Guarapuava-PR, which is resistant to phosphine and pyrethroids (PyPhR); the populations of Coimbra-MG (PyR1) and Cristalina-GO (PyR2), which are only resistant to pyrethroids; and the population of Guaxupé-MG, which is susceptible to both types of insecticide (SUS). All of the insect populations have been maintained in glass containers in rearing facilities under controlled conditions (27 ± 2 °C, 75 ± 5% relative humidity, 14 h: 10 h lighting regime [D:L]) and reared on insecticide-free maize grains. The same controlled environmental conditions were used in all of the bioassays.

2.2. Clove and cinnamon essential oils

The essential oils of clove, *S. aromaticum*, and cinnamon, *C. zeylanicum*, were kindly provided by the Clínica de Doenças de Plantas do Departamento de Fitopatologia of the Federal University of Viçosa (Viçosa, Minas Gerais, Brazil). Chromatographic analyses of these oils revealed that eugenol (>90%) and sesquiterpene β-caryophyllene (>7%) are their major constituents [65]. When concentration-response and behavioral bioassays were conducted, analytical-grade acetone (99.8%; Vetec Fine Chemicals, Duque de Caxias, Rio de Janeiro — RJ, Brazil) was used as the solvent.

2.3. Concentration and survival bioassays

Concentration-mortality bioassays were carried out in a completely randomized experimental design with four replicates. At least six different concentrations of either clove oil or cinnamon oil (applied as 1 mL of solution and ranging from 0.35 to 2.10 μL of essential oil/cm^{-2}) were used to estimate their lethal toxicity to each *S. zeamais* population. The control was treated with acetone only. The essential oils and acetone were applied in filter papers (60 mm diameter) in a non-fumigant way (i.e. by using a Hamilton microsyringe — Hamilton Company, Reno, NV, USA). The filter papers were allowed to completely dry (the drying process was complete after 1 h) before being used in the experiments. Each replicate consisted of 20 adult insects placed in Petri dishes (60 mm diameter × 10 mm height) that were covered on the bottom with essential oil-impregnated filter paper. After adding the insects, the Petri dishes were covered with a piece of organza veil and a rubber band to prevent the insects from escaping. After 24 h of exposure, the mortality was recorded. Insects were considered dead if unable to walk when prodded with a fine hair brush.

For the survival bioassays, we used the same exposure procedures as in the concentration-mortality bioassays. However, only four essential oil concentrations were applied (0.35; 0.70; 1.40 and 2.10 μL of essential oil/cm^2) and the number of dead insects was recorded every three hours for a period of 120 h.

2.4. Population growth rate bioassays

The developmental rate (instantaneous rate of increase — r_i) was used to estimate the essential oil effects on the biological development of each population. Experiments were performed using 0.8-L capacity glass jars containing 250 g of insecticide-free maize grains. These grain masses were treated with four doses (60, 120, 250 and 500 μL/Kg of grains) of clove or cinnamon essential oils. For the control, the grains were treated with acetone alone. Twenty adult insects were released in each jar and left to colonize the grain mass for 60 days under controlled conditions (27 ± 2 °C, 75 ± 5% relative humidity, 14 h h:10 h lighting regime [D:L]). After this period, the total number of (adult) live insects and the grain weight in each experimental unit were recorded. Five 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 [50].

2.5. Behavioral (locomotory) responses

Two behavioral bioassays were carried out in arenas that were either fully treated or half-treated with essential oils using methodologies that were previously described for synthetic insecticides [33,34,40,42,51]. The control treatments consisted of acetone only. In the bioassays with fully treated arenas, the filter papers were impregnated with 3 mL of essential oil solution (Clove: 0.64 μL of essential oil/cm^2; *Cinnamon*: 1.23 μL of essential oil/cm^2) at the concentration corresponding to the determined LC_{50} of SUS population (i.e. Guaxupé-MG), which was the most susceptible to the actions of each oil type. After drying for 20 min, the filter papers were placed in Petri dishes (135 × 20 mm). The inner walls of each Petri dish were coated with Teflon® PTFE (DuPont, Wilmington, DE, USA) to prevent the insects from escaping. The movement of each insect within the arena was recorded for 10 min using an automated video tracking system equipped with a CCD camera (ViewPoint Life Sciences Inc., Montreal, CA). The parameters that were recorded for the fully treated arenas included the walked distance

Table 1
Toxicity of clove and cinnamon essential oils to Brazilian populations of *Sitophilus zeamais*.

Oil type	Population	Number of insects	Slope ± S.E.	LC_{50} (95% FI) μL of oil/cm^{-2}	LC_{95} (95% FI) μL of oil/cm^{-2}	TR* (LC_{50})	χ^2	*P*
Clove	SUS	480	11.0 ± 1.13	0.45 (0.43–0.48)	0.64 (0.60–0.71)	–	2.76	0.25
	PyR1	560	3.1 ± 0.48	0.66 (0.41–1.00)	2.33 (1.37–7.33)	1.47 (0.95–2.08)	7.70	0.05
	PyR2	560	3.2 ± 0.32	0.65 (0.57–0.75)	2.16 (1.69–3.05)	1.44 (1.33–1.56)	3.28	0.19
	PyPhR	640	3.9 ± 0.37	0.92 (0.82–1.03)	2.43 (2.02–3.14)	2.04 (1.91–2.15)	4.55	0.21
Cinnamon	SUS	480	6.7 ± 1.21	0.69 (0.44–1.06)	1.23 (0.88–9.58)	–	4.76	0.09
	PyR1	640	5.1 ± 0.57	0.95 (0.87–1.05)	1.99 (1.69–2.55)	1.38 (1.98–0.99)	2.05	0.36
	PyR2	640	4.1 ± 0.47	1.47 (1.32–1.65)	3.74 (3.03–5.14)	2.13 (3.0–1.56)	4.03	0.26
	PyPhR	480	2.8 ± 0.37	1.74 (1.74–2.16)	6.83 (4.67–12.84)	2.52 (3.95–2.04)	4.29	0.23

* Toxicity ratio = LC_{50} of the determined population/LC_{50} of the most susceptible (SUS) population. PyPhR: phosphine- and pyrethroids-resistant population; PyR1 and PyR2: pyrethroid-resistant populations; SUS: phosphine- and pyrethroids-susceptible population.