Behavioral Response of *Lasioderma serricorne* to Materials from *Cinnamomum camphora*

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Abstract: The attractiveness of *Cinnamomum camphora* (Laurales: Lauraceae) blocks and essential oil to *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) adults was investigated. The results showed that the behavioral response values of *L. serricorne* adults to *C. camphora* blocks significantly increased with increasing doses above the dose of 0.8 g. The highest behavioral response values of *L. serricorne* adults were 93.33% to *C. camphora* blocks and 81.67% to *C. camphora* essential oil, respectively. *C. camphora* blocks had stronger attractiveness to *L. serricorne* adults than *C. camphora* oil. These results demonstrate that the effectiveness to control *L. serricorne* in practice of *C. camphora* blocks and *C. camphora* oil deserve to be further investigated.

Keywords: Attractive potential; *Lasioderma serricorne*; *Cinnamomum camphora*; Volatiles

1 Introduction

Insects often prefer to some special substances that can be used to manipulate insect behavior, particularly for insect monitoring and detecting programs. Many researches showed that the stored product insects could be apparently attracted by the volatiles from stored products or processed foods. For example, *Tribolium castaneum* (Coleoptera: Tenebrionidae)[1], *Sitophilus granarius* (Coleoptera: Dryophthoridae)[2], *T. confusum* (Coleoptera: Tenebrionidae)[3], *Sitophilus zeamais* (Coleoptera: Curculionidae)[4], *Ahasverus advena* (Coleoptera: Cucujidae)[5], *Sitophilus oryzae* (Coleoptera: Curculionidae)[6], and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)[7] preferentially respond to the volatiles from cereal grains, and processed products.

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) ranks as one of the most destructive pest insects of stored products worldwide[8]. Repeated and intensive use of phosphine for decades has resulted in serious negative issues including insecticide resistance, insect resurgence, insecticide residue, environment contamination, and lethal effects on non-target organisms[9]. Development and application of environment-friendly and sustainable control strategies has been considered to be the only effective method to cope with the increasing *Cinnamomum camphora* (Laurales: Lauraceae), commonly known as camphor tree, camphor laurel or camphorwood, originates in China, Japan, Korea, and Vietnam, and has been introduced to many other countries. The components of volatiles from *C. camphora* essential oil mainly include camphor, nerolidol, linalool, 1,8-cineole, safrole or borneol. Camphor has been used for many centuries as a kind of culinary spice, a component of incense, and medicine. The essential oil could be prepared from *C. camphora* wood and leaves by simultaneous distillation extraction method, which is often used by field workers to avoid insect biting in China during harvesting period[10]. However, little is known about the behavioral response of *L. serricorne* adults to the volatiles from *C. camphora* so far. Therefore, this study aims to evaluate the behavioral response of *L. serricorne* adults to *C. camphora* blocks and *C. camphora* essential oil in laboratory.

2 Materials and methods

2.1 Insects

Cultures of the cigarette beetle, *L. serricorne*, were maintained in the laboratory without exposure to any insecticide in the Institute of Stored Product Insects of Henan University of Technology, China. They were reared on sterilized diet (wheat feed/yeast, 95:5, W:W) at (27 ± 2) °C, 75% ± 5% relative humidity, and a 12:12 light:dark photoperiod. Healthy, unsexed 3-d-old adults were randomly chosen for bioassays.

2.2 *C. camphora* Blocks and *C. camphora* Essential Oil

C. camphora blocks and *C. camphora* essential oil (purity 98%) was purchased from Jishui Natural Essential Oil Industry Ltd., China. The specification of one *C. camphora* block (Wuyuan, China) is 2.5 cm (length) × 2 cm (width) × 2 mm (thickness). *C. camphora* blocks and *C. camphora* essential oil were respectively prepared form fresh branches and leaves.

2.3 Analysis of *C. camphora* Essential Oil

The *C. camphora* essential oil was analyzed using a Shimadzu GC-MS QP2010 (Shimadzu, Kyoto, Japan) fitted with a fused silica DB-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness) capillary column with helium as carrier gas. Temperature was programmed initially at 60 ℃ for 2 min, and then increased at a rate of 10 ℃/min to 280 ℃/min, then

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maintained for 5 min. The interface temperature was 250 ℃. Injection was in unsplit mode. The MS was operated in the EI mode at 70 eV. The chromatograph was coupled to a Shimadzu QP5000 mass selective detector – EIMS (Shimadzu, Kyoto, Japan) at 70 eV. Identification of components was made by comparing their relative retention indices and mass spectra with those of authentic samples or those found in the literature, and the amount of the component was determined by the percentage of its peak area of total peak area[11]. NIST 11 and NIST 11s Library of Mass Spectra were used for identifying the compounds.

2.4 Bioassay Procedure

The behavioral response of *L. serricorne* adults to *C. camphora* blocks and *C. camphora* essential oil evaluated by using a two-choice pitfall bioassay was similar to that described by Germinara et al.[12].

The test arena was a white round sheet iron barrel (22 cm in diameter by 25 cm high) with two diametrically opposed holes (3 cm in diameter) 2.5 cm from the side wall. Two petri dishes (6 cm in diameter by 1.2 cm high) were positioned under each hole to collect responding insects. The petri dish wall and upside brim of the arena were coated with polyfluortetraethylene to avoid insects escaping. The tested *C. camphora* blocks and *C. camphora* essential oil were laid at the center of petri dish. 20 *L. serricorne* adults were kept in a petri dish (6 cm in diameter by 1.2 cm high) at the center of the test arena and allowed 20 min to acclimatize the test environment prior to release. The test arena was covered with a piece of black cotton cloth tightly tied with a band at the upper edge to prevent insects from escaping. The bioassays were carried out in dark at (27 ± 2) ℃, and 75% ± 5% relative humidity. *C. camphora* blocks were tested with doses of 0.4, 0.6, 0.8, 1.0, 2.0, and 3.0 g, and *C. camphora* essential oil was tested with doses of 0.05, 0.2, 0.4, 0.6, 0.8, and 1.0 mL. Each collecting petri dish was covered by a piece of Whatman No.1 filter paper (6 cm in diameter) on the inside bottom. A specific tested dose of *C. camphora* blocks was put on the filter paper in one petri dish, or a specific tested dose of *C. camphora* essential oil was evenly sprayed on the filter paper as a treatment. The filter paper in the other petri dish was treated nothing as a control. Each experiment was replicated four times. The number of insects present in the control (N_c) and treated (N_t) dishes was counted after 24, 48, and 72 h of exposure, respectively. The behavioral response values (BRV) of *L. serricorne* adults to *C. camphora* blocks or *C. camphora* essential oil were calculated as follows[13]:

$$\mathrm{BRV} = [\,(N_t - N_c\,)\,/\,(\,N_c + N_t\,)\,] \times 100\%$$

2.5 Statistical Analysis

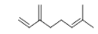
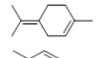
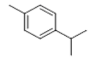
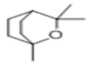
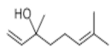
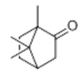
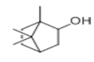
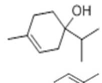
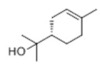
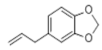
The behavioral response values of *L. serricorne* adults to *C. camphora* blocks or *C. camphora* essential oil were determined and transformed to arcsine square-root values before subjecting to two-way analysis of variance (ANOVA) with BRVs as response variable, and dose, and exposure time as fixed effects. The mean BRVs were compared and separated by Scheffe's test at $P = 0.05$. These analysis were performed using SPSS version 16.0 software.

3 Results

3.1 Components of *C. camphora* Essential Oil

The components of *C. camphora* essential oil are listed in Table 1. The main components of *C. camphora* essential oil included myrcene, terpinolene, *p*-cymene, 1,8-cinede, linalool, isoborned, (1R,4R)-(+)-camphor, terpinen-4-ol, α-terpineol, safrole.

Table 1 Composition from *C. camphora* essential oil

No.	Component	Structural formula	Amount (%)
1	myrcene		1.01
2	terpinolene		0.34
3	*p*-cymene		0.89
4	1,8-cinede		28.19
5	linalool		0.56
6	(1R,4R)-(+)-camphor		52.91
7	D-Isoborneol		0.58
8	terpinen-4-ol		2.33
9	α-terpineol		6.06
10	safrole		7.13

3.2 Behavioral Response of *L. serricorne* Adults to *C. camphora* Blocks

L. serricorne adults had significantly different behavioral responses to *C. camphora* blocks ($F = 13.25$, d$f = 5$, 36, $P < 0.001$) and *C. camphora* essential oil at different tested doses ($F = 2.71$, d$f = 5$, 36, $P < 0.001$). *C. camphora* blocks showed strong attractive potential during the whole test period (Table 2). The behavioral response values of *L. serricorne* adults to *C. camphora* blocks significantly increased with increasing doses when tested at above 0.8 g of blocks, and the highest behavioral response values were 93.33, 86.67, and 86.67% after 24, 48, and 72 h of exposure, respectively.

3.3 Behavioral Response of *L. serricorne* Adults to *C. camphora* Essential Oil

C. camphora essential oil exhibited the strongest attractive potential to *L. serricorne* adults when tested at 0.6 mL. The highest behavioral response values of *L. serricorne* adults to *C. camphora* essential oil were respectively 78.33%, 80.00%, and 81.67% after 24, 48, and 72 h of exposure to 0.6 mL of *C. camphora* essential oil, respectively (Table 3). *C. camphora* blocks had stronger attractive potential to *L. serricorne* adults than *C. camphora* essential oil.

4 Discussion

The results indicate that both *C. camphora* essential oil and blocks have stronger attractiveness to *L. serricorne* adults. The

Table 2 The behavioral response values of *L. serricorne* adults to *C. camphora* blocks %

Dose (g)	Observation time		
	24 h	48 h	72 h
0.4	73.33±1.67^{Abcd}	71.67±3.33^{Aab}	46.67±3.33^{Bb}
0.6	70.00±0.00^{Acd}	68.33±10.14^{Aab}	71.67±9.28^{Aa}
0.8	56.67±4.41^{Ad}	56.67±4.41^{Ab}	65.00±7.64^{Aab}
1.0	88.30±4.41^{Aab}	78.33±4.41^{Aab}	83.33±3.33^{Aa}
2.0	80.00±10.41^{Aabc}	76.67±11.67^{Aab}	86.67±8.34^{Aa}
3.0	93.33±3.33^{Aa}	86.67±6.01^{Aa}	86.67±8.33^{Aa}

Note: Data is Means ± SD of four replicates. Different lowercases indicate significant differences in the same column, and different capital letters indicate significant difference in the same row (P < 0. 05). The same as Table 3.

Table 3 The behavioral response values of *L. serricorne* adults to *C. camphora* essential oil %

Dose (mL)	Exposure time		
	24 h	48 h	72 h
0.05	38.33±6.01^{Ac}	43.33±6.01^{Abc}	40.00±7.64^{Acd}
0.2	61.67±8.33^{Aab}	61.67±3.33^{Aab}	71.67±1.67^{Aab}
0.4	50.00±2.89^{Abc}	58.33±4.41^{Aab}	56.67±4.41^{Abc}
0.6	78.33±3.33^{Aa}	80.00±7.64^{Aa}	81.67±6.01^{Aa}
0.8	33.33±4.41^{Ac}	35.00±5.00^{Ac}	31.67±6.01^{Ad}
1.0	65.00±11.55^{Aab}	65.00±11.55^{Aab}	63.33±13.02^{Aabc}

behavior response of insects to volatiles depends on various factors, including insect species, developmental stage, dose, compound formulation, application method and special environmental condition, and so forth. Some compounds that strongly attract insects, specially sex pheromones, have been developed as attractants for detecting and monitoring programs [14-15], or as attracticides[16].

The *C. camphora* essential oil, prepared by steam distilled method from *C. camphora* wood and leaves, is often used by field workers during harvesting seasons to avoid insects (mostly the mosquitoes) biting in China[10]. Camphor, a white crystalline substance, obtained from *C. camphora* tree, has been used for many centuries as a common culinary spice, a component of incense, and as a traditional medicine. Meanwhile, camphor is also a common insect repellent and a flea-killing substance. However, in the present research, *C. camphora* blocks and *C. camphora* essential oil could apparently attract *L. serricorne* adults. These demonstrate that different insect species differently response to the same chemical substance. The response mechanism of *L. serricorne* adults to *C. camphora* blocks and *C. camphora* essential oil needs to be further investigated, and the response of different developmental stages of *L. serricorne* to *C. camphora* blocks and *C. camphora* essential oil also should be studied.

This research results show that *C. camphora* blocks and *C. camphora* essential oil had potent potential as an attractant for controlling *L. serricorne*. The proper formulation, main components, dose, reasonable application strategy and environmental effect deserve to be researched, so that they can be exploited for effectively preventing disinfestation by *L. Serricorne* in practice in the future.

Conflict of Interest

The authors declare that there is no conflict of interest.

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