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Research Article

Chemical Constituents and Insecticidal Activities of the Essential Oil of Cinnamomum camphora Leaves against Lasioderma serricorne

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During our screening program for agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Cinnamomum camphora* leaves was found to possess strong fumigant and contact toxicity against *Lasioderma serricorne* adults with LC_{50}/LD_{50} values of 2.5 mg/L air and 21.25 μ g/adult, respectively. The essential oil obtained by hydrodistillation was investigated by GC and GC-MS. The main components of the essential oil were identified to be *D*-camphor (40.54%), linalool (22.92%), cineole (11.26%), and 3,7,11-trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate (4.50%). Bioactivity-directed chromatographic separation on repeated silica gel columns led to the isolation of *D*-camphor and linalool. *D*-camphor and linalool showed strong fumigant toxicity (LC_{50} = 2.36 and 18.04 mg/L air, resp.) and contact toxicity (LD_{50} = 13.44 and 12.74 μ g/adult, resp.) against *L. serricorne*. The results indicate that the essential oil of *C. camphora* and its active compounds had the potential to be developed as natural fumigants and insecticides for control of *L. serricorne*.

1. Introduction

The cigarette beetle, *Lasioderma serricorne* Fabricius, is one of the most widespread and destructive primary insect pests of stored cereals [1]. The infestations of stored product insects currently not only cause significant losses due to the consumption of grains but also result in the rise of temperature and moisture which lead to an accelerated growth of molds, including toxigenic species [2]. Control of stored product insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides, and lethal effects on nontarget organisms in addition to direct toxicity to users [3]. These problems have necessitated a search for alternative

ecofriendly insect pest control methods [4]. Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecules [5, 6]. The use of essential oils or their constituents with low mammalian toxicity can effectively prevent and/or suppress insect pest especially in storage [5]. During our screening program for new agrochemicals from local wild plants and Chinese medicinal herbs, the essential oil from *C. camphora* leaves has been found to possess contact and fumigant activities towards *L. serricorne*.

Cinnamomum camphora (L.) Presl (family: Lauraceae) is a Chinese medicinal plant widespread in China and has long been prescribed in traditional medicine for treatment of inflammation-related diseases, such as rheumatism, sprains,

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bronchitis, and muscle pains [7]. Pharmacological studies have revealed that the plant possesses a wide range of therapeutic activities, including anthelmintic [8], sperm viability effect [9], antibacterial [10], antiallergic [11], and antiinflammatory [12]. *C. camphora* is a well-known chemotype; on distillation, the wood from different groups of trees may yield camphor, linalool, safrole, or cineole as the major chemical. The use of *C. camphora* as a source of leaf oil has expanded in recent years, and it is now an important source of natural linalool (which is still preferred over the synthetic form for some fragrant applications) [13]. A literature survey has shown that there is no report on insecticidal activity of C. camphora essential oil against L. serricorne adults; thus, we decided to investigate the chemical constituents and contact/fumigant activity of the essential oil of C. camphora against L. serricorne for the first time and to isolate any biologically active compounds from its essential oil.

2. Experimental

- 2.1. Tested Insect Species. L. serricorne were obtained from laboratory cultures maintained for the last 2 years in the dark in incubators at $29 \pm 1^{\circ}$ C and 70-80% relative humidity. The insects were reared in glass containers (0.5 L) containing wheat flour at 12-13% moisture content mixed with yeast (wheat feed/yeast, 10:1, w/w). Adults used in all the experiments were about 7 ± 2 days old regardless of gender.
- 2.2. Plant Materials. Leaves (3.5 kg) of *C. camphora* were collected in May 2013 from Suzhou City (31.97°N latitude and 120.49°E longitude), Jiangsu Province, China. The leaves were air-dried for one week and ground to powder. The species was identified according to the voucher specimen (BNU-CMH-Dushuahan-2013-05-25-006) deposited at the Herbarium of College of Resources Science and Technology, Beijing Normal University (BNU).
- 2.3. Extraction and Analysis of Essential Oil. The ground powder of C. camphora leaves was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with n-hexane. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in air-tight container in a refrigerator at $4^{\circ}C$.

GC-MS analysis was performed on a Thermo Finnigan Trace DSQ instrument equipped with a flame ionization detector and an HP-5MS (30 m \times 0.25 mm \times 0.25 μ m) capillary column. The column temperature was programmed at 50°C for 2 min and then increased at 2°C/min to the temperature of 150°C and held for 2 min and then increased at 10°C/min until the final temperature of 250°C was reached, where it was held for 5 min. The injector temperature was maintained at 250°C and the volume injected was 0.1 mL of 1% solution (diluted in n-hexane). The carrier gas was helium at flow rate of 1.0 mL/min. Spectra were scanned from 50 to 550 m/z. Most constituents were identified by comparison of their retention indices with those reported in the literature. The retention indices were determined in relation to a homologous series of n-alkanes ($C_{10} - C_{36}$) under the same operating conditions. Further identification was

made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from the literature [14]. Relative percentages of the individual components of the essential oil were obtained by averaging the GC peak area % reports.

- 2.4. Isolation and Characterization of Two Constituent Compounds. The crude essential oil (5 mL) was chromatographed on a silica gel (Qingdao Marine Chemical Plant, Shandong Province, China) column (30 mm i.d., 500 mm length) by gradient elution with *n*-hexane first, then with *n*-hexane-ethyl acetate, and last with ethyl acetate to obtain 19 fractions. Based on contact toxicity, fractions 3 and 12 were chosen for further fractionation. With PTLC, two purified compounds were obtained. The isolated compounds were elucidated with NMR spectra. NMR experiments were performed on Bruker Avance DRX 500 instrument using CDCl₃ as solvent with TMS as internal standard.
- 2.5. Fumigant Toxicity Bioassay. The fumigant activity of the essential oil/pure compounds against *L. serricorne* adults was tested as described by Liu and Ho [1]. A serial dilution of the essential oil (five concentrations) was prepared in nhexane. Whatman filter papers (diameter 2.0 cm) were each impregnated with $10 \,\mu L$ dilution and then placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, and volume 25 mL). The solvent was allowed to evaporate for 20 s before the cap was placed tightly on the glass vial, each of which contained 10 insects inside to form a sealed chamber. Preliminary experiments demonstrated that 30 s was sufficient for the evaporation of solvents. *n*-Hexane was used as a control. Five replicates were carried out for all treatments and controls, and they were incubated for 24 h. The insects were considered dead if their appendages did not move when probed with a camel brush. The LC₅₀ values were calculated by using Probit analysis [15].
- 2.6. Contact Toxicity. The contact toxicity of the essential oil/pure compounds against L. serricorne adults was measured as described by Liu and Ho [1]. Range-finding studies were run to determine the appropriate testing concentrations. Serial dilutions of the essential oil/compounds (five concentrations) were prepared in *n*-hexane. Aliquots of $0.5 \mu L$ of the dilutions were applied topically to the dorsal thorax of the insects. Controls were determined using n-hexane. Five replicates were carried out for all treatments and controls. Both treated and control insects were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators. Mortality was recorded after 24 h and the LD₅₀ values were calculated using Probit analysis [15]. Positive control pyrethrins (pyrethrin 1:24%; pyrethrin 2:13%; cinnerin 1:2%; cinnerin 2:2%; jasmolin 1:1%; jasmolin 2:1%) were purchased from Dr. Ehrenstorfer GmbH.

3. Results and Discussion

3.1. Chemical Constituent of Essential Oil. The yield of *C. camphora* leaves essential oil was 1.83% (v/w) and the density

TABLE 1: Chemical composition of the essential oil of Cinnamomum camphora leaves.

Compounds	RI^*	Content (%)
α-Pinene	939	2.05
Camphene	952	1.00
2-Thujene	967	1.97
Sabinene	977	1.80
α -Phellandrene	1005	0.40
<i>p</i> -Mentha-2,4(8)-diene	1011	0.44
<i>m</i> -Cymene	1025	0.44
Cineole	1032	11.26
<i>α-trans</i> -Ocimene	1051	0.05
2,2-Dimethylheptane	1055	0.07
2,2,5-Trimethylhexane-3,4-dione	1057	0.03
4,7-Dimethyl-4,4a,5,6-tetrahydrocyclopenta[c]pyran-1,3-dione	1061	0.31
2,5,9-Trimethyldecane	1067	0.08
Linalool	1094	22.92
7,7-Dimethyl-2-methylene-norbornane	1130	0.05
D-Camphor	1146	40.54
endo-Borneol	1182	0.23
(R)-(-)-p-Menth-1-en-4-ol	1197	1.02
<i>p</i> -Menth-1-en-8-ol	1214	2.30
Elixene	1356	0.33
Dihydro-cis-α-copaene-8-ol	1379	0.61
α -Bourbonene	1382	0.03
(S,1Z,5E)-1,5-Dimethyl-8-isopropenyl-1,5-cyclodecadiene	1401	0.22
Caryophyllene	1420	2.16
γ-Elemene	1458	0.98
Germacrene D	1474	0.94
α -Caryophyllene	1478	0.24
3,5-Dimethyl-4-octanone	1578	0.05
Cadina-1(10),4-diene	1596	0.07
3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	1634	4.50
Oxalic acid, di(1-methyl) ester	1672	0.43
1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	1691	0.10
Total		97.62

 $^{^*}$ RI: retention index as determined on a HP-5MS column using the homologous series of n-hydrocarbons.

of the essential oil was determined to be 0.92 g/mL. GC-MS analysis of the essential oil of *C. camphora* leaves led to the identification and quantification of a total of 32 major components, accounting for 97.62% of the total components present (Table 1).

The main constituents of *C. camphora* leaves essential oil were *D*-camphor (40.54%), linalool (22.92%), cineole (11.26%), and 3,7,11-trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate (4.50%). There are five chemotypes existing in *C. camphora* according to different primary component in the essential oil. *C. camphora* could be divided as the following chemical types (physiological types) by the major compounds of their leaf oils: camphor-type; linalool-type; cineol-type; isonerolidol-type; and borneol-type [16]. The results indicated that the *C. camphora* collected from Suzhou was camphor-type. With further isolation, two purified compounds were obtained and analysed by various NMR

techniques including ¹H NMR and ¹³C NMR. Combining all the NMR spectra data, the two isolated compounds were finally recognized as *D*-camphor (1, 1.56 g) and linalool (2, 0.87 g). The spectral data of *D*-camphor matched with the previous report [17]. The data of linalool matched with the previous report [18].

3.2. Fumigant and Contact Toxicity. The essential oil of *C. camphora* leaves showed strong fumigant toxicity against *L. serricorne* adults with LC_{50} value of 2.50 mg/L (Table 2). The isolated compounds *D*-camphor and linalool also exhibited strong fumigant toxicity against *L. serricorne* adults with LC_{50} values of 2.36 and 18.04 mg/L, respectively (Table 2).

The essential oil of *C. camphora* leaves also showed strong contact toxicity against *L. serricorne* adults with LD₅₀ value of 21.25 μ g/adult (Table 2). Compared with the positive control pyrethrins, the crude essential oil demonstrated 89 times less

Treatment	Fumigant toxicity				Contact toxicity		
	LC_{50} (μ g/mL air)	95% FL	χ^2	LD_{50} (μ g/adult)	95% FL	χ^2	
C. camphora	2.50	2.20-2.91	14.43	21.25	19.16-23.64	11.25	
D-Camphor	2.36	1.91-2.71	14.29	13.44	10.39-16.07	15.38	
Linalool	18.04	12.28-22.72	16.33	12.74	11.25-14.16	13.11	
Phosphine	9.23×10^{-3}	$7.13 \times 10^{-3} - 11.37 \times 10^{-3}$	11.96	_	_	_	
Dyrethrine	_	<u>_</u>	_	0.24	0.16_0.35	1736	

Table 2: Fumigant and contact toxicity of essential oil of Cinnamomum camphora leaves and its main components against Lasio-derma serricorne adults.

toxicity because the pyrethrins had acute contact toxicity to L. serricorne adult with LD₅₀ value of 0.24 μ g/adult. The isolated compounds, D-camphor and linalool also exhibited strong contact toxicity against L. serricorne adults with LD₅₀ values of 13.44 and 12.74 μ g/adult, respectively (Table 2).

In previous research, insecticidal activity of essential oil of C. camphora was proven in the pest control of the stored grain Trogoderma granarium, Tribolium confusum, and Coptotermes curvignathus [19, 20]. However, this is the first report regarding insecticidal action of the essential oil of C. camphora leaves against L. serricorne. There is also no report on fumigant and contact toxicity of D-camphor and linalool against *L. serricorne*. Compared with the commercial fumigant phosphine (LC₅₀ = 9.23×10^{-3} mg/L), the essential oil and D-camphor exhibited almost 271 and 256 times less fumigant toxicity against *L. serricorne* adults. However, compared with the other essential oils in the literature, the crude essential oil suggested stronger level of fumigant toxicity towards L. serricorne adults, for example, essential oils of Cinnamomum cassia bark (LC₅₀ = $11.03 \mu L/L$) [21] and Lavandula stoechas (LC₅₀ = $3.84 \mu L/L$) [22]. There are some reports on insecticidal activity of *D*-camphor. Suthisut et al. tested contact toxicity of camphor toward Tribolium castaneum (LD₅₀ = 70 μ g/mg) and Sitophilus zeamais (LD₅₀ = $137 \,\mu\text{g/mg}$) [23]. In contact toxicity assays, (+)-camphor had weak activity against Tribolium castaneum and Sitophilus oryzae (LC₅₀ values above 500 μ g/cm²) [24]. The component was also effective as a fumigant; LC50 value was found to be 21.64 mg/L air against Sitophilus zeamais [25]. Camphor also exhibited strong contact toxicity (LD₅₀ = 207.26 μ g/cm²) and fumigant toxicity ($LC_{50} = 1.03 \text{ mg/L air}$) against *Liposcelis* bostrychophila [26]. So the toxic effects of C. camphora leaves oil could be attributed to *D*-camphor and other components.

Currently, the use of synthetic chemicals has led to unintended side effects such as ozone depletion, pest resistance, environmental pollution, and toxicity on nontarget organisms [27, 28]. An alternative is to use natural products that possess good efficacy and are environmentally friendly. Essential oils from many plants have been extensively tested to assess their insecticidal and repellent properties as a valuable natural resource. Based on our results, fumigant activity of the crude essential oil and *D*-camphor are quite promising and they show potential to be developed as possible natural fumigants for control of stored product insects. Among the oil and compounds assayed against *L. serricorne*, the essential oil is the most promising as possible natural fumigants. Although *D*-camphor is more toxic than the oil,

the difference is quite small. Therefore, there is no need for further isolation. However, for the practical application of the essential oil/compounds as novel fumigant and insecticides, further studies on the safety of the essential oil/compounds to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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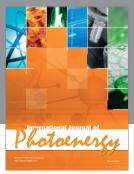
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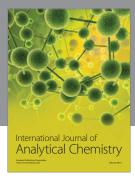
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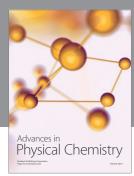
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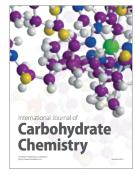
















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