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*Research Article*

# Identification of Repellent and Insecticidal Constituents from *Artemisia mongolica* Essential Oil against *Lasioderma serricorne*

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The aims of this research were to determine the chemical composition and insecticidal and repellent activities of the *Artemisia mongolica* essential oil against *Lasioderma serricorne* and to isolate active constituents from the essential oil. The essential oil of *A. mongolica* was obtained by hydrodistillation and 36 components were identified with GC-MS. Eucalyptol (39.88%), (S)-cis-verbenol (14.93%), 4-terpineol (7.20%), (−)-camphor (6.02%), and $\alpha$-terpineol (4.20%) were found to be major components. With a further isolation process, five constituents obtained from the essential oil were identified as eucalyptol, verbenol, 4-terpineol, camphor, and $\alpha$-terpineol. In the progress of assay, it showed that *L. serricorne* adults had different sensitivities to the crude essential oil and isolated constituents. 4-Terpineol exhibited strongest contact activity against *L. serricorne*, showing the $LD_{50}$ value of 8.62 $\mu$g/adult. Moreover, camphor and $\alpha$-terpineol showed stronger fumigant activity ($LC_{50}$ = 2.91 and 3.27 mg/L air, resp.) against *L. serricorne* than crude essential oil and other constituents. In addition, the essential oil, eucalyptol, verbenol, and $\alpha$-terpineol showed comparable repellency against *L. serricorne* adults. The results indicate that the essential oil and isolated compounds have potential to provide more efficient and safer natural insecticides or repellents for control of insects in food and Chinese medicinal materials preservation.

## 1. Introduction

Antagonistic storage has been used as a kind of traditional Chinese medicine conservation methods. It mainly utilizes some traditional Chinese medicinal materials having special odor to store with medicinal materials vulnerable to insects to deter the insects. With improvement of sense of environmental protection and medication security, it is believed that this method would have broad prospects of application in the future. In order to inherit and develop the traditional method of prevention and control of stored insects, we took *Artemisia mongolica* as research object and *Lasioderma serricorne* adults as the target insects. It was expected that this research work would provide some of the theoretical basis for the conception of antagonistic storage.

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), is a kind of serious pest species of stored botanicals worldwide. The cigarette beetle occurs frequently in tropical and subtropical areas. They cause significant damage during storage of perishable food products such as cereals, legumes, tobacco, and traditional Chinese medicinal materials in warehouses [1, 2]. Currently, recommended pest control measures in durable stored products are mainly the use of synthetic insecticides or fumigants

which pose possible health risks to warm-blooded animals, environmental pollution, resistance by insects, and pest resurgence [3]. These problems have necessitated some studies for alternative ecologically safe insect pest control methods [4].

The use of essential oils or their constituents with low mammalian toxicity can effectively prevent insect pest especially in storage [5]. Investigations in several countries confirm that some plant essential oils not only repel insects, but also possess contact and fumigant toxicity against stored product pests as well as exhibiting feeding inhibition or harmful effects on the reproductive system of insects [6]. Essential oils and their constituents of many plants including medicinal herbs, spices, and fruits have been evaluated successfully for insecticidal or repellent activities against stored product insects, and they have been proven more effective than traditionally used pesticides in some cases [7–9]. As a consequence, this vast arsenal of bioactive compounds has attracted significant and crescent attention of researchers in recent years [10].

During our screening program for new botanical pesticides from Chinese medicinal herbs and wild plants, the essential oil of *A. mongolica* aerial parts was found to possess insecticidal and repellent activities against *L. serricorne* adults. *Artemisia mongolica* (Fisch. ex Bess.) Nakai is a perennial herb of *Artemisia* genus in the Compositae family. And it is used in Inner Mongolia as a substitute of the traditional medicinal herb Folium *Artemisiae Argyi* [11]. This plant was found to have strongly resistant to insects and pathogens [12]. A literature survey has shown that there are no reports on repellent and insecticidal activities of the essential oil derived from *A. mongolica* aerial parts against *L. serricorne*. There is only one report on insecticidal activity of the *A. mongolica* essential oil against *Sitophilus zeamais* [13]. However, the insect is different from us and the repellent activity of the essential oil is not investigated. Moreover, no active compound is isolated from the essential oil. Hence, the aim of the present study was to investigate the chemical composition and repellent and insecticidal activities of essential oil against *L. serricorne* and to further isolate active compounds from the essential oil for the first time.

## 2. Experimental

*2.1. Plant Materials and Essential Oil Extraction.* Dried aerial parts (whole grass, 8.0 kg) of *A. mongolica* were harvested in September 2013 from Qianshan Mountain Scenic Spot (41.0°N latitude and 123.1°E longitude), Anshan City, Liaoning Province, China. The aerial parts were air-dried for one week and ground to a powder. The plant was identified by Dr. Liu, Q. R. (College of Life Sciences, Beijing Normal University, Beijing, China) and a voucher specimen (BNU-CMH-Dushushan-2013-09-017-007) was deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University. The powder was submitted to hydrodistillation using a modified Clevenger-type apparatus for 6 h. The oil was dried by anhydrous sodium sulfate. The essential oil was stored in a refrigerator at 4°C for further use.

*2.2. Insects.* The cigarette beetle, *L. serricorne,* was obtained from laboratory cultures maintained for the last 2 years in the dark in incubators at 28–30°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 (10 : 1, w/w). Adults used in all the experiments were about 7 ± 2 days old.

*2.3. GC-FID and GC-MS Analysis.* The volatile components of the *A. mongolica* essential oil were analyzed by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) using an Agilent 6890N gas chromatograph hooked to an Agilent 5973N mass selective detector. The same column and analysis conditions were used for both GC-FID and GC-MS. They were equipped with a HP-5MS (30 m × 0.25 mm × 0.25 $\mu$m) capillary column. The GC settings were as follows: the column temperature was held at 50°C for 2 min, then increased at 2°C/min to 250°C and held there for 2 min, and then increased at 10°C/min until the final temperature reached 250°C, where it was held for 5 min. The injector temperature was maintained at 250°C and the volume injected was 1 $\mu$L of 1% solution (diluted in *n*-hexane). The helium gas used as the carrier gas at a 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 literatures. The retention indices were determined in relation to a homologous series of *n*-alkanes ($C_5$–$C_{36}$) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 (Standard Reference Data, Gaithersburg, MD, USA) and Wiley 275 libraries (Wiley, New York, NY, USA) or with mass spectra from literature [14]. Relative percentages of the individual components of the essential oil were obtained by averaging the GC-FID peak area % reports.

*2.4. Purification and Characterization of Five Compounds.* The crude essential oil (7 mL) was chromatographed on a silica gel (Qingdao Marine Chemical Plant, Shandong province, China) column (500 mm × 45 mm) by gradient elution with *n*-hexane first, then with *n*-hexane-ethyl acetate, and last with ethyl acetate. Fractions (200 mL) were collected and concentrated at 35°C, and similar fractions according to thin layer chromatography (TLC) profiles were combined to yield 25 fractions. According to similar TLC profiles, fractions (5–8, 13–17) were pooled and further purified by preparative silica gel column chromatography (PTLC) until obtaining the pure compounds. The pure compounds were identified as eucalyptol (**1**, 1.39 g), verbenol (**2**, 0.61 g), 4-terpineol (**3**, 0.26 g), camphor (**4**, 0.23 g), and $\alpha$-terpineol (**5**, 0.19 g). The isolated compounds were elucidated based on nuclear magnetic resonance. About 10 mg or 10 $\mu$L samples were dissolved into 500 $\mu$L $CDCl_3$ containing TMS as internal standard. $^1$H and $^{13}$C-NMR spectra were recorded on Bruker Avance DRX 500 instruments with the magnetic field of 11.74 Tesla. All NMR spectra were phased and baseline corrected with MestReNova software (version 8.1.2).

*2.5. Contact Toxicity.* The contact toxicity of the essential oil against *L. serricorne* adults was measured as described by Liu and Ho [15]. A serial dilution of the essential oil/compounds (five concentrations) was 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. Ten insects were used for each concentration and control, and the experiment was replicated five times. Both treated and control insects were then transferred to glass vials with culture media and kept in incubators. Mortality was recorded after 24 h and the $LD_{50}$ values were calculated using Probit analysis (IBM SPSS V20.0) [16]. We used Probit analysis in regression analysis. We chose mortality as Response Frequency, and we chose totality as Total Observed, and we chose concentration as Covariate. Then we chose Natural log Transform and Logit Model. We can get the $LD_{50}$ values from the output. The observed mortality data were corrected for control mortality using Abbott's formula. The positive control pyrethrins (pyrethrin I, 24%; pyrethrin II, 13%; cinnerin I, 2%; cinnerin II, 2%; jasmolin I, 1%; jasmolin II, 1%) were purchased from Dr. Ehrenstorfer, Augsburg, Germany.

*2.6. Fumigant Toxicity.* The fumigant activity of the essential oil against *L. serricorne* adults was tested as described by Liu and Ho [15]. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil/compounds (five concentrations) was prepared in *n*-hexane. A Whatman filter paper (diameter 2.0 cm) was 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, 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. *n*-Hexane was used as a control. Five replicates were carried out for all treatments and controls, and they were incubated under the same conditions as rearing. Mortality was determined after 24 h of treatment, and the $LC_{50}$ values were calculated using Probit analysis (IBM SPSS V20.0) [16].

*2.7. Repellency Tests.* The repellent activity to *L. serricorne* adults was tested using the area preference method [8]. Petri dishes (9 cm in diameter) were used to confine red flour beetles and cigarette beetles during the experiment. The crude essential oil and the isolated compounds were diluted in *n*-hexane to five concentrations (39.32, 7.86, 1.57, 0.31, and 0.06 nL/cm$^2$), and *n*-hexane was used as the control. Filter paper (9 cm in diameter) was cut in half and 500 $\mu$L of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 500 $\mu$L of *n*-hexane. Both the treated half and the control half were then air-dried to evaporate the solvent completely (30 s). A full disk was carefully remade by attaching the tested half to the negative control half with tape. Each remade filter paper after treatment with solid glue was placed in a Petri dish. Twenty insects were released in the center of each filter paper disk, and a cover was placed over the Petri dish. Five replicates were used and the experiment was repeated three times. Counts of the insects present on each strip were made after 2 and 4 h. The percent repellency (PR) of each volatile oil/compound was then calculated using the formula:

$$\mathrm{PR}\,(\%) = \left[\frac{(Nc - Nt)}{(Nc + Nt)}\right] \times 100. \quad (1)$$

*Nc* is the number of insects present in the negative control half while *Nt* is the number of insects present in the treated half. Analysis of variance (One-Way ANOVA and GLM Univariate) and Tukey's test were conducted by using SPSS 20.0 for Windows 2007. Percentage was subjected to an arcsine square-root transformation before variance and Tukey's tests. A commercial repellent, DEET (N,N-diethyl-3-methylbenzamide), was purchased from Dr. Ehrenstorfer, Augsburg, Germany, and used as a positive control.

## 3. Results and Discussion

*3.1. Chemical Composition of the Essential Oil.* The *A. mongolica* essential oil was green with a yield of 0.12% (v/w) and density of 0.91 g/mL. A total of 36 components of the essential oil of *Artemisia mongolica* were identified, accounting for 94.19% of the total oil (Table 1). The main compounds in the essential oil were eucalyptol (39.88%), verbenol (14.93%), 4-terpineol (7.20%), camphor (6.02%), and $\alpha$-terpineol (4.20%), followed by $\gamma$-terpinene (2.71%) and cis-sabinol (2.71%).

The chemical composition of the essential oil of *A. mongolica* aerial parts in the present study was not same as that reported in previous study. For example, eucalyptol, germacrene D, camphor, artemisia ketone, and calarene were the main volatile components of *A. mongolica* harvested in August from Xiaolongmeng National Forest Park, Mentougou District, Beijing [13]. These differences of chemical content and composition of the essential oils might have been due to harvest time and local, climatic, and seasonal factors as well as storage duration of medicinal herbs, and these differences may result in different biological activities.

*3.2. Isolated Compounds*

*3.2.1. Eucalyptol (**1**, Figure 1).* Colorless oil, $C_{10}H_{18}O$. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ ppm: 2.03 (2H, t, H-2), 1.68 (2H, t, H-6), 1.52 (4H, m, H-3, 5), 1.42 (1H, m, H-4), 1.25 (6H, s, 9, 10-$CH_3$), 1.07 (3H, s, 7-$CH_3$); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ ppm: 73.6 (C-8), 69.8 (C-1), 32.9 (C-4), 31.5 (C-3, 5), 28.9 (C-2, 6), 27.6 (C-7), 22.8 (C-9, 10). The $^1$H and $^{13}$C-NMR data were in agreement with the reported data [17].

*3.2.2. $\alpha$-Terpineol (**2**, Figure 1).* Colorless oil, $C_{10}H_{18}O$. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ ppm: 5.41 (1H, s, H-6), 2.06 (1H, m, H-2a), 2.00 (1H, m, H-2b), 1.90 (1H, m, H-4), 1.82 (1H, m, H-3a), 1.78 (3H, s, 10-$CH_3$), 1.52 (1H, s, H-3b), 1.30 (1H, m, H-5a), 1.25 (1H, m, H-5b), 1.21 (3H, s, 8-$CH_3$), 1.19 (3H, s, 9-$CH_3$); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ ppm: 134.0 (C-1), 120.6 (C-6), 72.8 (C-7), 45.0 (C-4), 31.0 (C-2), 27.5 (C-8), 26.9 (C-3),

Table 1: Chemical composition of the essential oil of *Artemisia mongolica*.

| Number | Compound | RI[a] | Relative content (%) |
|---|---|---|---|
| 1 | $\alpha$-Pinene | 931 | 0.60 |
| 2 | Camphene | 941 | 0.32 |
| 3 | $\alpha$-Phellandrene | 1010 | 0.59 |
| 4 | Eucalyptol | 1031 | 39.88 |
| 5 | $\beta$-Ocimene | 1050 | 0.25 |
| 6 | $\gamma$-Terpinene | 1061 | 2.71 |
| 7 | Fenchene | 1076 | 0.54 |
| 8 | Thujone | 1112 | 0.27 |
| 9 | (−)-Camphor | 1150 | 6.02 |
| 10 | Sabenene | 1153 | 1.11 |
| 11 | 4-Terpineol | 1175 | 7.20 |
| 12 | $\alpha$-Terpineol | 1188 | 4.50 |
| 13 | b-Terpinene | 1195 | 0.31 |
| 14 | Isoterpinolone | 1233 | 1.30 |
| 15 | o-Cymene | 1250 | 1.63 |
| 16 | 2-Thujene | 1303 | 0.13 |
| 17 | 2,2-Dimethylheptane | 1307 | 0.16 |
| 18 | $\gamma$-Elemene | 1340 | 0.47 |
| 19 | $\beta$-Caryophyllene | 1420 | 1.98 |
| 20 | (−)-Zingiberene | 1452 | 0.16 |
| 21 | Germacrene D | 1458 | 0.39 |
| 22 | cis-p-Menth-2-en-1-ol | 1465 | 0.38 |
| 23 | Helminthogermacrene | 1484 | 0.87 |
| 24 | Elixene | 1496 | 0.22 |
| 25 | Butylbenzene | 1499 | 0.38 |
| 26 | cis-Sabinol | 1505 | 2.71 |
| 27 | $\gamma$-Cadinene | 1512 | 0.12 |
| 28 | (S)-cis-Verbenol | 1560 | 14.93 |
| 29 | Ibuprofen | 1597 | 2.30 |
| 30 | 3,8-p-Menthadiene | 1634 | 0.16 |
| 31 | cis-Piperitol | 1666 | 0.21 |
| 32 | trans-Carveol | 1694 | 0.19 |
| 33 | n-Butylbenzene | 1721 | 0.25 |
| 34 | Orientin | 1772 | 0.38 |
| 35 | Thuja-2,4(10)-diene | 1867 | 0.46 |
| 36 | Undecane | 2058 | 0.11 |
| | Total | | **94.19** |

[a]RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons.

26.7 (C-9), 24.0 (C-5), 23.4 (C-10). The $^1$H and $^{13}$C-NMR data were in accord with the reported data [18].

*3.2.3. 4-Terpineol (**3**, Figure 1).* Colorless oil, $C_{10}H_{18}O$. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ ppm: 5.32 (1H, m, H-5), 2.17 (2H, m, H-6), 1.94 (2H, m, H-3), 1.71 (3H, s, 10-$CH_3$), 1.67 (1H, m, H-7), 1.58 (2H, m, H-2), 0.97 (3H, d, $J$ = 7.0 Hz, 8-$CH_3$), 0.94 (3H, d, $J$ = 7.0 Hz, 9-$CH_3$); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ ppm: 133.9 (C-4), 118.5 (C-5), 71.8 (C-1), 36.8 (C-7), 34.6 (C-2), 30.8 (C-6), 27.1 (C-10), 23.3 (C-3), 16.8 (C-8, 9). The $^1$H-NMR data referred to the published data [19].

*3.2.4. Camphor (**4**, Figure 1).* Colorless crystal, $C_{10}H_{16}O$. Melting point 177°C. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ ppm: 2.37 (1H, m, H-3b), 2.11 (1H, t, $J$ = 4.5 Hz, H-6b), 1.96 (1H, m, H-4), 1.87 (1H, d, $J$ = 18.0 Hz, H-3a), 1.70 (1H, m, H-6a), 1.39 (2H, m, H-5), 0.98 (3H, s, 8-$CH_3$), 0.93 (3H, s, 9-$CH_3$), 0.85 (3H, s, 10-$CH_3$); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ ppm: 219.8 (C-6), 57.7 (C-1), 46.8 (C-7), 43.3 (C-5), 43.1 (C-4), 29.9 (C-2), 27.1 (C-8), 19.8 (C-3), 19.2 (C-9), 9.3 (C-10). Its NMR data were consistent with the literature data [20].

*3.2.5. Verbenol (**5**, Figure 1).* White needle crystal, $C_{10}H_{16}O$. Melting point 65°C. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ ppm: 5.39 (1H, s, H-2), 4.48 (1H, s, H-1), 2.47 (1H, m, H-6), 2.31 (1H, m, H-5a), 1.99 (1H, m, H-4), 1.75 (3H, s, 10-$CH_3$), 1.37 (3H, s, 9-$CH_3$), 1.33 (1H, m, H-5b), 1.10 (1H, m, H-8); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ ppm: 147.4 (C-3), 119.3 (C-2), 73.6 (C-1), 48.2 (C-4), 47.7 (C-7), 39.0 (C-6), 35.6 (C-5), 26.9 (C-10), 22.7 (C-9), 22.6 (C-8). The $^1$H and $^{13}$C-NMR data referred to the literature data [21].

*3.3. Insecticidal Toxicity.* The essential oil of *A. mongolica* aerial parts exhibited contact toxicity against *L. serricorne* adults with a $LD_{50}$ value of 22.32 $\mu$g/adult. Moreover, 4-terpineol possessed almost 1.3, 1.4, and 2 times more toxicity than camphor, $\alpha$-terpineol, and eucalyptol, respectively. It is suggested that 4-terpineol exhibited the strongest contact activity against *L. serricorne*. However, compared with pyrethrins (positive control), the essential oil showed 93 times less toxicity and 4-terpineol showed 36 times less toxicity (Table 2).

Camphor and $\alpha$-terpineol showed stronger fumigant toxicity ($LC_{50}$ = 2.91 and 3.27 mg/L air, resp.) against *L. serricorne* than eucalyptol ($LC_{50}$ = 5.47 mg/L air), verbenol ($LC_{50}$ = 5.32 mg/L air) and 4-terpineol ($LC_{50}$ = 6.90 mg/L air). However the crude essential oil of *A. mongolica* aerial parts showed a $LC_{50}$ value of 6.08 mg/L air (Table 3). Camphor and $\alpha$-terpineol showed almost 2 times stronger fumigant toxicity than eucalyptol, verbenol, 4-terpineol, and the crude essential oil against *L. serricorne* adults. However, compared with the other essential oils in the literature, the essential of *A. mongolica* aerial parts possessed stronger fumigant toxicity against *L. serricorne* adults, for example, the essential oil of *Agastache foeniculum* ($LC_{50}$ = 21.57 $\mu$L/L air) [22].

The currently used fumigants are synthetic insecticides and the most effective fumigants (e.g., phosphine and methyl bromide) are also highly toxic to humans and other nontarget organisms; the essential oil of *A. mongolica* aerial parts and its five compounds show potential to be developed as possible natural fumigants or insecticides for the control of *L. serricorne* adults. However, for the practical application of the essential oil and the isolated constituents as novel insecticides or fumigants, further studies on the safety of the essential oil to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce costs.

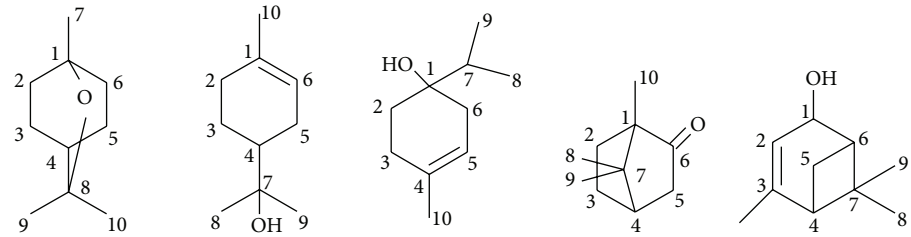


Figure 1: Compounds isolated from the essential oil of *Artemisia mongolica*.

Table 2: Contact toxicity of *Artemisia mongolica* essential oil and its constituents against *Lasioderma serricorne*.

| Treatment | $LD_{50}$ ($\mu$g/adult) | 95% FL[a] | Slope ± SE | Chi square ($\chi^2$) |
|---|---|---|---|---|
| *A. mongolica* | 22.32 | 20.05–24.93 | 3.05 ± 0.37 | 19.27 |
| Eucalyptol | 15.58 | 12.88–18.02 | 3.87 ± 0.55 | 15.18 |
| Verbenol | — | — | — | — |
| 4-Terpineol | 8.62 | 7.38–9.85 | 3.63 ± 0.47 | 12.65 |
| Camphor | 11.30 | 7.78–14.07 | 1.47 ± 0.28 | 16.13 |
| $\alpha$-Terpineol | 11.99 | 10.42–13.42 | 3.12 ± 0.43 | 18.96 |
| Pyrethrins | 0.24 | 0.16–0.35 | 1.31 ± 0.20 | 17.36 |

[a]Fiducial limits.

Table 3: Fumigant toxicity of *Artemisia mongolica* essential oil and its constituents against *Lasioderma serricorne*.

| Treatment | $LC_{50}$ (mg/L air) | 95% FL[a] | Slope ± SE | Chi square ($\chi^2$) |
|---|---|---|---|---|
| *A. mongolica* | 6.08 | 4.58–7.38 | 1.79 ± 0.27 | 17.62 |
| Eucalyptol | 5.47 | 4.73–6.17 | 3.97 ± 0.47 | 25.30 |
| Verbenol | 5.32 | 4.84–5.83 | 3.98 ± 0.47 | 18.62 |
| 4-Terpineol | 6.90 | 6.04–7.84 | 3.58 ± 0.40 | 24.84 |
| Camphor | 2.91 | 2.57–3.26 | 2.72 ± 0.34 | 13.11 |
| $\alpha$-Terpineol | 3.27 | 3.17–3.38 | 12.12 ± 1.51 | 19.09 |
| Phosphine | $9.23 \times 10^{-3}$ | $7.13 \times 10^{-3}$–$11.37 \times 10^{-3}$ | 2.12 ± 0.27 | 11.96 |

[a]Fiducial limits.

*3.4. Repellent Activity. A. mongolica* essential oil and its isolated constituents exhibited comparable repellent activity against *L. serricorne* adults. The results are presented in Figure 2. Data showed that at tested concentration of 39.32 nL/cm$^2$ verbenol and 4-terpineol showed strong repellency (80% and 88%, resp.) against *L. serricorne* adults at 2 h after exposure, and eucalyptol and $\alpha$-terpineol exhibited strong repellency (82% and 80%, resp.) against *L. serricorne* adults at 4 h after exposure. At the lowest assayed concentration (0.06 nL/cm$^2$), the crude essential oil and eucalyptol showed 60% and 46% repellency against *L. serricorne* adults at 2 h after exposure, whereas the crude essential oil and eucalyptol showed 40% repellency and 76% repellency against *L. serricorne* adults at 4 h after exposure. However, the positive control, DEET (N,N-diethyl-3-methylbenzamide), showed strong repellency (88%, 76% and 98%, 78%) against *L. serricorne* adults at 2 and 4 h after exposure. When compared with the positive control, DEET, $\alpha$-terpineol and verbenol exhibited stronger repellency than DEET against *L. serricorne* adults at 2 h after exposure. It is due to the fact that at the concentrations of 1.57, 0.31, and 0.06 nL/cm$^2$ crude essential oil exhibited stronger repellency than DEET ($P$ = 0.003, 0.000, and 0.003) against *L. serricorne* adults at 2 h after exposure. At the concentrations of 1.57 and 0.31 nL/cm$^2$, $\alpha$-terpineol exhibited stronger repellency than DEET ($P$ = 0.000 and 0.012) against *L. serricorne* adults at 2 h after exposure. At the concentration of 0.31 nL/cm$^2$, verbenol exhibited stronger repellency than DEET ($P$ = 0.023) against *L. serricorne* adults at 2 h after exposure. In addition, at the concentrations of 0.31 and 0.06 nL/cm$^2$, eucalyptol exhibited stronger repellency than DEET ($P$ = 0.001 and 0.029) against *L. serricorne* adults at 2 h after exposure. At the concentration of 0.06 nL/cm$^2$, eucalyptol exhibited stronger repellency than DEET ($P$ = 0.018) against *L. serricorne* adults at 4 h after exposure. Many essential oils and their constituents have been also evaluated for repellency against insects [23, 24].

The insecticidal and repellent activities of the crude essential oil and the isolated compounds were different. The results indicated that the bioactivity properties of the essential oil are related to the synergistic effects of its diverse major and

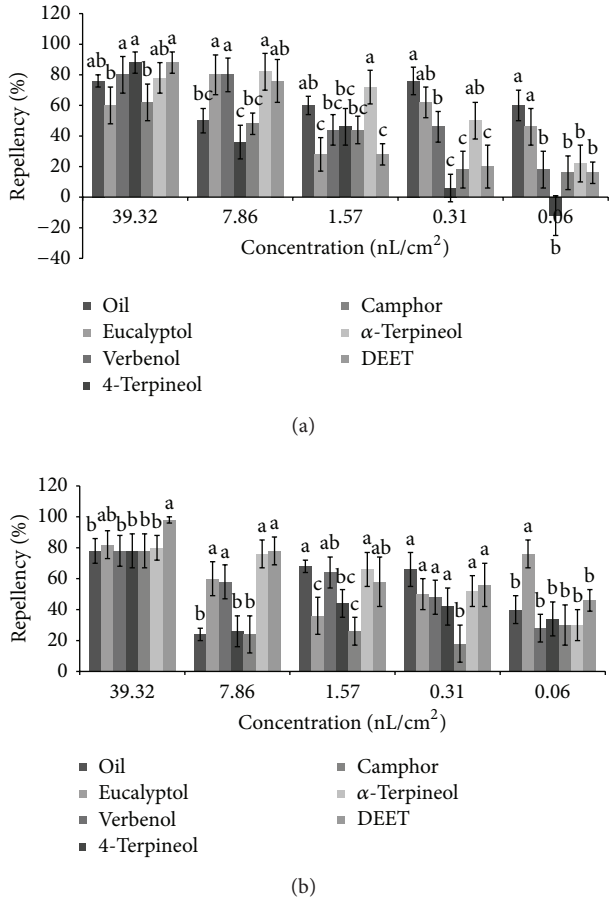


Figure 2: Percentage repellency (PR) of *Artemisia mongolica* essential oil and its constituents against *Lasioderma serricorne* at 2 h (a) and 4 h (b) after exposure. [a]means in the same column followed by the same letters do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

minor components. Hence, it is significant to isolate chemical constituents in both high and low percentages for their appreciable bioactivity. In addition, further investigations that focus on efficiency and safety of the pure compounds should be conducted, while structure modification is a considerable method.

## 4. Conclusions

This report is the first one to investigate insecticidal and repellent activities of the essential oil of *A. mongolica* aerial parts against *L. serricorne* adults and to isolate five compounds from the essential oil. The work indicates that the essential oil of *A. mongolica* aerial parts and its isolated constituents possess significant insecticidal and repellent activities against *L. serricorne* adults. As rich in natural resources, *A. mongolica* has a very good perspective of comprehensive utilization in foods, as well as traditional Chinese medicinal materials, and can thus substitute for more toxic synthetic insecticides, fumigants, and repellents. Further investigations that involve the insecticidal and repellent mechanism of *A. mongolica* essential oil should be conducted.

## Conflict of Interests

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

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