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Bioactivity of Essential Oil of *Artemisia argyi* Lévl. et Van. and Its Main Compounds Against *Lasioderma serricorne*

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Abstract: ***Artemisia argyi*** **Lévl. et Van., a perennial herb with a strong volatile odor, is widely distrbuted in the world. Essential oil obtained from** ***Artemisia argyi*** **was analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 32 components representing 91.74% of the total oil were identified and the main compounds in the oil were found to be eucalyptol (22.03%), β-pinene (14.53%), β-caryophyllene (9.24%) and (-)-camphor (5.45%). With a further isolation, four active constituents were obtained from the essential oil and identified as eucalyptol, β-pinene, β-caryophyllene and camphor. The essential oil and the four isolated compounds exhibited potential bioactivity against** ***Lasioderma serricorne*** **adults. In the progress of assay, it showed that the essential oil, camphor, eucalyptol, β-caryophyllene and β-pinene exhibited strong contact toxicity against** ***L. serricorne*** **adults with LD_{50} values of 6.42, 11.30, 15.58, 35.52, and 65.55 μg/adult, respectively. During the fumigant toxicity test, the essential oil, eucalyptol and camphor showed stronger fumigant toxicity against** ***L. serricorne*** **adults than β-pinene (LC_{50} = 29.03 mg/L air) with LC_{50} values of 8.04, 5.18 and 2.91 mg/L air. Moreover, the essential oil, eucalyptol, β-pinene and camphor also exhibited the strong repellency against** ***L. serricorne*** **adults, while, β-caryophyllene exhibited attracting activity relative to the positive control, DEET. The study revealed that the bioactivity properties of the essential oil can be attributed to the synergistic effects of its diverse major and minor components. The results indicate that the essential oil of** ***A. argyi*** **and the isolated compounds have potential to be developed into natural insecticides, fumigants or repellents in controlling insects in stored grains and traditional Chinese medicinal materials.**

Key words: *L. serricorne*, *A. argyi*, antagonistic storage, bioactivity, essential compound

1 INTRODUCTION

Antagonistic storage has been used as one of traditional Chinese medicinal materials conservation methods. It mainly utilizes some traditional Chinese medicinal materials having special volatile odor to store with medicinal materials vulnerable to insects, so as to prevent the insects. With the improvement of the 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 grain insects, we took *Artemisia argyi* as research object and *Lasioderma serricorne* adults as the target insects. It was expected that this research work would provide some theoretical basis for the conception of antagonistic storage.

Abbreviations: ***A. argyi***: *Artemisia argyi*; ***L. serricorne***: *Lasioderma serricorne*; **RI**: Retention Index; **MS**: mass spectrum; **DEET**: N,N-diethyl-3-methyl-benzamide.

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The cigarette beetle *Lasioderma serricorne* (Frbricius) (Coleoptera: Anobiidae) is widely distributed and is of considerable economic importance in tropical to temperate climates[1]. This beetle is destructive primary insect pests of stored cereals, tobacco, oilseeds, dried fruits and traditional Chinese medicinal materials[2]. Development and survival are affected by type of food, temperature and humidity, therefore the life cycle is different[1]. Currently, recommended pest control measures in durable stored products rely heavily on the use of synthetic insecticides or fumigants which pose possible health hazards to warm-blooded animals, risk of environmental pollution, development of resistance by insects and pest resurgence[3]. These problems have necessitated a search 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 activity against stored product insects, they have been proven more effective than traditionally used pesticides in some cases[7–16].

Besides insecticidal and repellent activity, essential oils from different plant sources have exhibited several biological activities, including antimicrobial[17] and antifungal[18], nematicidal[19], larvicidal[20, 21], and acaricidal[22]. As a consequence, this vast arsenal of bioactive compounds has attracted significant and crescent attention of researchers in recent years. During our screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Artemisia argyi aerial* parts was found to possess insecticidal toxicity against *L. serricorne* adults. *A. argyi* is a genus of annual herb in the Lamiaceae family. Artemisia species, widespread throughout the world, are important medicinal plants which are receiving phytochemical attention due to their biological and chemical diversities[23].

A. argyi has been commonly used as a kind of traditional Chinese medicine in China for a very long history[24]. It is one of the most popular plants in Chinese traditional preparations and frequently used for the treatment for the diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria and viruses[25, 26]. The biological activities of essential oils extracted from *A. argyi* leaves have been studied. For example, the essential oil from leaves of *A. argyi* is reported to show anti-histamatic effect and antifungal activity[24]. Recently, the chemical composition of essential oil extracted from leaves or flowers of *A. argyi* has been reported[23]. However, the repellent and insecticidal activity of essential oil extracted from *A. argyi* aerial parts against *L. serricorne* has never been explored. Hence we decided to investigate the chemical constituents, repellent and insecticidal activity of the essential oil against *L. serricorne* and isolated of active constituent compounds from the essential oil for the first time.

2 EXPERIMENTAL PROCEDURES

2.1 Chemicals

Eucalyptol, β-pinene, β-caryophyllene and camphor were isolated from *A. argyi* essential oil on a silica gel column (45 mm × 500 mm) (Qingdao Marine Chemical Plant, Shandong province, China).

2.2 Material

2.2.1 Plants

Dried aerial parts (2.0 kg) of *A. argyi* were collected in October 2013 in Jining City (35.23° N latitude and 116.33° E longitude), Shandong province of 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-Dushuahan-2013-10-22-006) was deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University.

2.2.2 Insects

The cigarette beetle *Lasioderma serricorne* were obtained from laboratory cultures maintained for the last 2 years in dark in incubators at 29 ± 1℃ 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 Isolation of the Essential Oil and Purification of Four Constitunent Compounds

The powder (2.0 kg) of *A. argyi* aerial parts was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil (9.1 g) with a yield of 0.50% (v/w) was stored in airtight containers in a refrigerator at 4℃ for subsequent experiments. The crude essential oil (8 mL) was chromatographed on a silica gel column (45 × 500 mm) (Qingdao Marine Chemical Plant, Shandong province, China) by gradient elution with n-hexane first, then with n-hexane-ethyl acetate, and last with ethyl acetate. Fractions (120 mL) were collected and concentrated at 35℃, and similar fractions according to thin layer chromatography (TLC) profiles were combined to yield 20 fractions. Fractions (4–7, 11–15) that possessed contact toxicity, with similar TLC profiles, were pooled and

further purified by preparative silica gel column chromatography (PTLC) until the pure compounds for determining structure as eucalyptol (**1**, 1.05 g), β-pinene (**2**, 0.65 g), camphor (**4**, 0.26 g), β-caryophyllene (**3**, 0.36 g) were obtained. The isolated compounds were elucidated based on nuclear magnetic resonance. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker Avance DRX 500 instruments using $CDCl_3$ as solvent with TMS as internal standard.

2.4 GC-FID and GC-MS analysis

GC-MS analysis was performed on a Thermo Finnigan Trace DSQ instrument equipped with a flame ionization detector and a HP-5MS (30 m × 0.25 mm × 0.25 μm) capillary column. The column temperature was programmed at 50℃ for 2 min, then increased at 2℃/min to the temperature of 150℃ and held for 2 min, and then increased at 10℃/min until the final temperature of 250℃ was reached, where it was held for 5 min. The injector temperature was maintained at 250℃ 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 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 and Wiley 275 libraries or with mass spectra from literature[28]. Relative percentages of the individual components of the essential oil were obtained by averaging the GC peak area% reports.

2.5 Isolated Constituent Compounds

Eucalyptol (**1**, Fig. 1). Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 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, H-9, 10), 1.07 (3H, s, H-7); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 73.61 (C-8), 69.77 (C-1), 32.94 (C-4), 31.51 (C-3, 5), 28.89 (C-2, 6), 27.58 (C-7), 22.83 (C-9, 10). The ^{1}H and ^{13}C-NMR data were in agreement with the reported data[29].

β-Pinene (**2**, Fig. 1). Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 4.65 (1H, s, H-10β), 4.58 (1H, s, H-10α), 2.57 (1H, m, H-3β), 2.49 (1H, t, J = 5.0 Hz, H-1), 2.33 (1H, m, H-7β), 2.26 (1H, dd, J = 10.0 Hz, H-3α), 2.00 (1H, m, H-5), 1.85 (1H, m, H-4β), 1.85 (1H, m, H-4α), 1.44 (1H, d, J = 10.0 Hz, H-7α), 1.26 (3H, s, Me-8), 0.74 (3H, s, Me-9); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 152.4 (C-2), 105.9 (C-10), 51.8 (C-1), 40.7 (C-6), 40.4 (C-5), 27.0 (C-7), 26.1 (C-8), 23.6 (C-3), 23.6 (C-4), 21.8 (C-9). Its NMR data were identical to the literature data[30].

Camphor (**3**, Fig. 1). Colorless crystal. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 2.37 (1H, m, H-3b), 2.11 (1H, t, J = 4.5 Hz, H-6a), 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, Me-8), 0.93 (3H, s, Me-10), 0.85 (3H, s, Me-10); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 219.83 (C-6), 57.74 (C-1), 46.82 (C-7), 43.33 (C-5), 43.05 (C-4), 29.92 (C-2), 27.06 (C-8), 19.81 (C-3), 19.17 (C-9), 9.28 (C-10). The ^{1}H and ^{13}C-NMR data were in accord with the reported data[31].

β-Caryophyllene (**4**, Fig. 1). Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 5.33 (1H, m, H-5), 4.97 (1H, s, H-12a), 4.85 (1H, s, H-12b), 2.37 (1H, m, H-9), 2.33 (1H, m, H-7b), 2.23 (1H, m, H-7a), 2.11 (1H, m, H-1), 2.02 (1H, m, H-6b), 1.94 (1H, m, H-6a), 1.72 (1H, m, H-2b), 1.69 (1H, m, H-3b), 1.65-1.67 (2H, m, H-10), 1.64 (3H, s, Me-15), 1.60 (1H, m, H-3a), 1.52 (1H, m, H-2a), 1.02 (3H, s, Me-12), 1.00 (3H, s, Me-13); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 154.74 (C-2), 135.58 (C-6), 124.31 (C-5), 111.67 (C-12), 53.54 (C-9), 48.48 (C-1), 40.33 (C-11), 39.96 (C-7), 34.79 (C-3), 33.02 (C-10), 30.09 (C-13), 29.36 (C-8), 28.38 (C-4), 22.66 (C-14), 16.32 (C-15). The ^{1}H and ^{13}C-NMR data were consistent with the literature data[32].

2.6 Bioactivity

2.6.1 Insecticidial activity

2.6.1.1 Contact Toxicity

The contact toxicity of the essential oil/pure compounds against *L. serricorne* adults was tested as described by Liu and Ho[33]. 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. Aliquots of 0.5 μL of the dilutions were

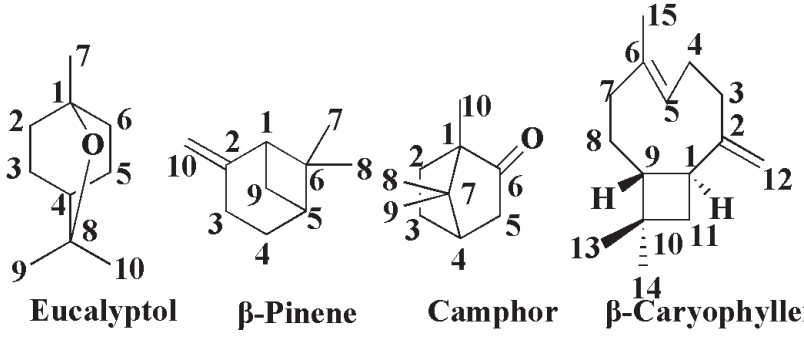

Fig. 1 Constituent compounds isolated from the essential oil of *A. argyi* aerial parts.

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 (diameter 2.5 cm, height 5.5 cm, volume 25 mL) (10 insects/vial) with culture media and kept in incubators (29-30℃ and 70-80% r.h). Mortality of insects was observed after 24 h. The observed mortality data were corrected for control mortality using Abbott's formula. The LD_{50} values were calculated by using Probit analysis[34] (IBM SPSS V20.0).

2.6.1.2 Fumigant Toxicity

The fumigant activity of the essential oil and the pure compounds against *L. serricorne* adults was tested as described by Liu and Ho[33]. 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 μL 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 to form a sealed chamber. Preliminary experiments demonstrated that 20 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 (29-30℃ and 70-80% r.h). The insects were then transferred to clean vials with some culture media and returned to the incubator for 24 h. Mortality of insects was observed and results from all replicates were calculated by using Probit analysis to determine LC_{50} values (IBM SPSS V20.0)[34].

2.6.2 Repellency tests

The repellent activity to *Lasioderma serricorne* adults was tested using the area preference method[14]. 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 (39.3, 7.9, 1.6, 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 μl 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 μl 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. Care was taken so that the attachment did not prevent free movement of insects from the one half to the other, but the distance between the filter paper halves remained sufficient to prevent seepage of test samples from one half to the other. Each remade filter paper after treatment with solid glue was placed in a Petri dish with the seam oriented in one of four randomly selected different directions to avoid any insecticidal stimuli affecting the distribution of insects. 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:

$$PR(\%) = [(Nc - Nt)/(Nc + Nt)] \times 100$$

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. The averages were then assigned to different classes (0 to V) in Table 1[35]. A commercial repellent, DEET (N,N-diethyl-3-methyl- benzamide), was purchased from Dr. Ehrenstorfer, Germany and used as a positive control.

3 RESULTS and DISCUSSION

3.1 Chemical compounds of the essential oil

The *A. argyi* essential oil was dark blue with a density of 0.91 g/mL. The oil sample was analyzed by GC-FID and GC-MS, and the components were identified based on their RI values as well as by comparing their mass spectra with those reported in literature. GC-MS analysis of *A. argyi* essential oil revealed 32 components representing 91.74% of the oil (Table 2). The main composition of the oil was as follows: eucalyptol (22.03%), β-pinene (14.53%), β-caryophyllene (9.24%), (-)-camphor (5.45%), germacrene D (5.32%), thujone (5.14%) and chamazulene (5.06%).

Table 1 The scale to be assign repellency of the essential oil of *A. argyi* aerial parts and its constituents.

Class	Percent repellency	Class	Percent repellency	Class	Percent repellency
0	>0.01 to <0.1	II	20.1–40	IV	60.1–80
I	0.1–20	III	40.1–60	V	80.1–100

Table 2 Chemical components of the essential oil of *A. argyi*.

Peak no.	Components	RI[a]	%RA[b]	Identification Methods[c]
1	o-Xylene	871	0.21	MS, RI
2	α-Phellandrene	1184	0.68	MS, RI, Co
3	1R-α-Pinene	1205	1.13	MS, RI
4	Camphene	1256	0.51	MS, RI
5	β-Pinene	1357	14.53	MS, RI
6	α-Pinene	1438	0.67	MS, RI
7	(+)-4-Carene	1541	0.73	MS, RI
8	2-Isopropyltoluene	1576	0.56	MS, RI
9	Eucalyptol	1600	22.03	MS, RI
10	Crithmene	1739	1.30	MS, RI
11	Fenchene	1676	0.22	MS, RI
12	Terpinolene	1883	0.30	MS, RI
13	Linalool	1451	0.32	MS, RI
14	Thujone	1963	5.14	MS, RI
15	(-)-Camphor	2151	5.45	MS, RI
16	L(-)-Borneol	2268	1.01	MS, RI
17	3-Methylenecyclopentene	2284	0.23	MS, RI
18	Terpineol-4-ol	2333	4.03	MS, RI, Co
19	α-Terpineol	2408	2.80	MS, RI
20	Bornyl acetate	1554	0.23	MS, RI
21	Germacrene D	2095	5.32	MS, RI, Co
22	b-Elemen	2198	3.50	MS, RI
23	β-Caryophyllene	2254	9.24	MS, RI
24	α-Caryophyllene	2332	0.81	MS, RI
25	b-cis-Farnesene	2357	3.01	MS, RI
26	γ-Elemene	2431	0.55	MS, RI
27	d-Cadinene	2496	0.43	MS, RI
28	Ibuprofen	1930	0.37	MS, RI
29	(2E,6E)-3,7,11-Trimethyl-9-(phenylsulfonyl)-2,6,10-dodecatrien-1-ol	2616	1.34	MS, RI
30	α-Bisabolol	2842	0.54	MS, RI
31	Chamazulene	2784	5.06	MS, RI
32	3,3'-Dimethylbiphenyl	2836	0.38	MS, RI
	Total		91.74	

[a] Retention index (RI) relative to the homologous series of n-hydrocarbons on the HP-5 MS capillary column.
[b] Relative area (peak area relative to the total peak area).
[c] MS = mass spectrum, Co = co-injection with standard compound.

The chemical composition of the essential oil of *A. argyi* aerial parts in the present study was not the same as that reported in previous studies. For example, eucalyptol, (-)-camphor and viridiflorol were the main volatile components of *A. argyi* from Zunyi City, Guizhou Province, China, moreover, the content and composition of the volatile oil of *A. argyi* were various with different growing period[36]. However, eucalyptol, camphor, and caryophyllene were common constituents in *A. argyi*[37, 38]. 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 Bioactivity

3.2.1 Insecticidial activity

3.2.1.1 Contact toxicity

The essential oil of *A. argyi* aerial parts exhibited much stronger contact toxicity against *Lasioderma serricorne* adults than the four isolated compounds with LD_{50} value of 6.42 μg/adult. When compared with the positive control, pyrethrins (LD_{50} = 0.24 μg/adult), the essential oil demonstrated 10 times less toxic against *Lasioderma serricorne* adults (Table 3). Four constituent compounds, eucalyptol, β-pinene, β-caryophyllene and camphor exhibited contact toxicity against *Lasioderma serricorne* adults, with LD_{50} values of 15.58, 65.55, 35.52 and 11.30 μg/adult, respectively (Table 3). Moreover, camphor possessed almost 1, 3 and 6 times more toxicity than eucalyptol, β-caryophyllene and β-pinene, respectively.

3.2.1.2 Fumigant toxicity

Camphor (LC_{50} = 2.91 mg/L air) exhibited stronger fumigant toxicity against *Lasioderma serricorne* adults than eucalyptol (LC_{50} = 5.18 mg/L air) and the crude essential oil of *A. argyi* aerial parts (LC_{50} = 8.04 mg/L air), while β-pinene showed a LC_{50} value of 29.03 mg/L air (Table 4). However, β-caryophyllene did not show fumigant toxicity at the tested concentrations. Camphor showed almost 2, 3 and 10 times stronger fumigant toxicity than eucalyptol, the crude essential oil and β-pinene against *Lasioderma serricorne* adults, respectively. Compared with the positive control, phosphine ($LC_{50} = 9.23 \times 10^{-3}$ mg/L air), camphor exhibited weaker fumigant toxicity against *L. serricorne* adults, eucalyptol, the crude essential oil and β-pinene exhibited much less toxicity to *L. serricorne* adults. However, when compared with other essential oils, *A. argyi* essential oil possessed stronger fumigant toxicity against *Lasioderma serricorne* adults, e.g. essential oils of *Elsholtzia stauntonii* (LD_{50} = 10.99 μL/L)[39].

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 non-target organisms, the essential oil of *A. argyi* aerial parts and its isolated constituent compounds show potential to be developed as possible natural fumigants or insecticides for the control of *Lasioderma 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.

3.2.2 Repellent activity

Artemisia argyi essential oil and three isolated constituents exhibited strong repellent activity against *L. serricorne* adults. The results presented in Fig. 2. Data showed that at tested concentration of 39.32 nL/cm^2, the crude essential oil showed strongest (class V) repellency against *Lasioderma serricorne* adults at 2 h and 4 h after expo-

Table 3 Contact toxicity of the essential oil of *A. argyi* aerial parts and its constituents against *Lasioderma serricorne* adults.

Insects	Treatment	LD_{50} (μg/adult)	95% FL *	Slope ± SE	Chisquare (χ^2)
Lasioderma serricorne	*Artemisia argyi*	6.42	5.62- 7.32	2.25 ± 0.30	15.13
	Eucalyptol	15.58	12.88-18.02	3.87 ± 0.55	15.18
	β-Pinene	65.55	58.13-76.09	3.75 ± 0.46	21.62
	Camphor	11.30	7.78-14.07	1.47 ± 0.28	16.13
	β-Caryophyllene	35.52	31.89-39.54	3.07 ± 0.37	15.41
	Pyrethrins **	0.24	0.16- 0.35	1.31 ± 0.20	17.36

* Fiducial limits, ** data from Yang[40].

Table 4 Fumigant toxicity of the essential oil of *A. argyi* aerial parts and its constituents against *Lasioderma serricorne* adults.

Insects	Treatment	LC_{50} (mg/L air)	95% FL *	Slope ± SE	Chisquare (χ^2)
Lasioderma serricorne	Artemisia argyi	8.04	7.00- 9.22	2.22 ± 0.30	8.84
	Eucalyptol	5.18	4.63- 5.70	4.84 ± 0.59	16.79
	β-Pinene	29.03	26.38-31.79	5.41 ± 0.58	17.48
	Camphor	2.91	2.57- 3.26	2.72 ± 0.34	13.11
	β-Caryophyllene	–	–	–	–
	Phosphine **	9.23×10^{-3}	$7.13 \times 10^{-3} - 11.37 \times 10^{-3}$	2.12 ± 0.27	11.96

* Fiducial limits, ** data from Yang[40].

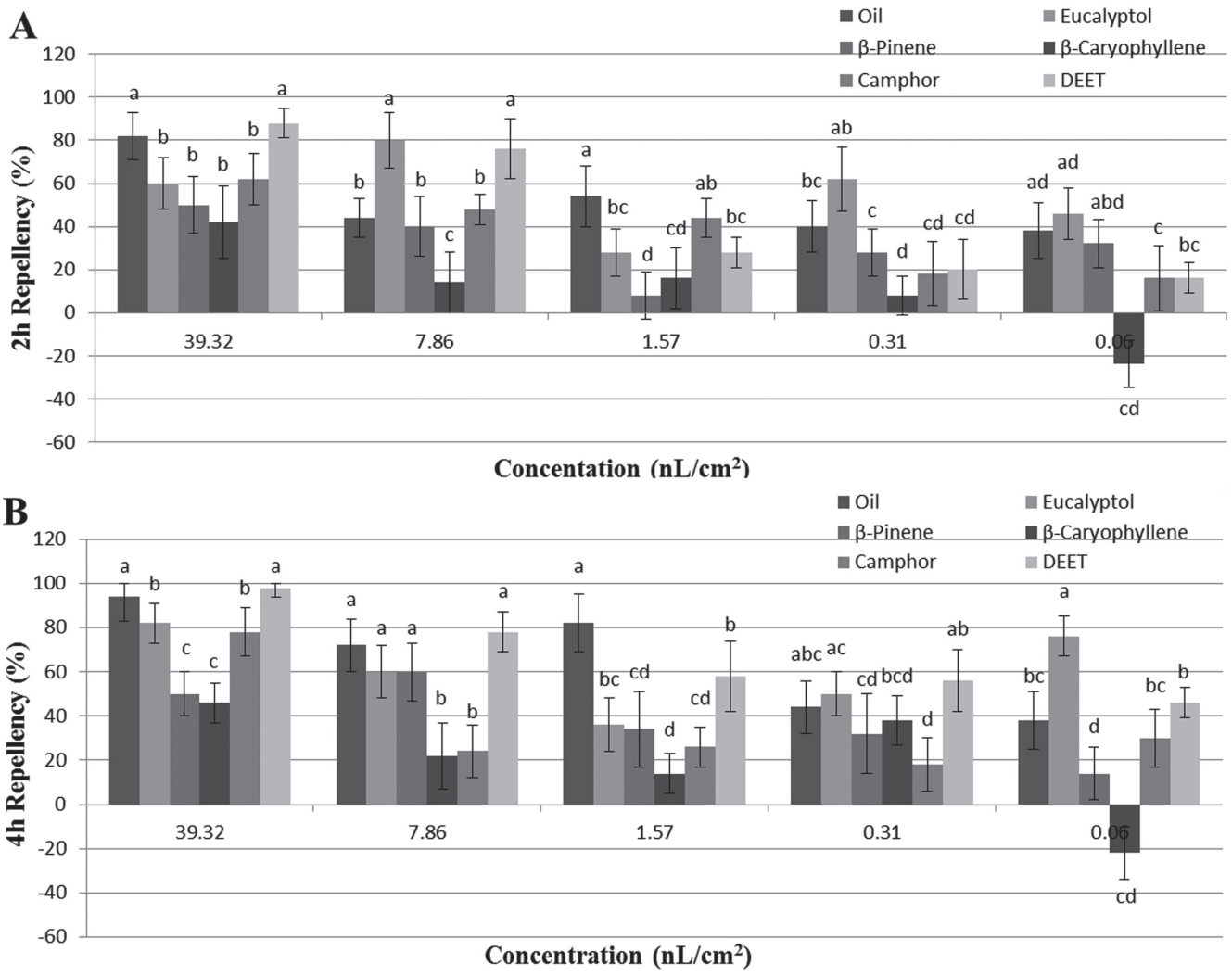

Fig. 2 Percentage repellency (PR) of the essential oil from *A. argyi* aerial parts and its constituents against *Lasioderma serricorne* at 2 h (A) and 4 h (B) after exposure[a].

[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.

sure. At the lowest assayed concentration (0.06 nL/cm^2), eucalyptol showed much stronger (class IV) repellency (76%) than the positive control, DEET (class III, *PR* = 46%) at 4 h after exposure against *Lasioderma serricorne* adults. Compared with the positive control, DEET, the essential oil and eucalyptol exhibited stronger repellency against *Lasioderma serricorne* adults, because at the concentrations of 1.57, 0.31 and 0.06 nL/cm^2, the essential oil and eucalyptol exhibited higher level of repellency against *Lasioderma serricorne* adults at 2 h after exposure, and at the concentrations of 0.06 nL/cm^2, the essential oil, eucalyptol and β-pinene exhibited the same and higher level of repellency than DEET against *Lasioderma serricorne* adults at the concentrations of 0.31 and 0.06 nL/cm^2 at 2 h after exposure. However, β-caryophyllene even exhibited attracting action against *Lasioderma serricorne* adults at the concentrations of 0.06 nL/cm^2 at 2 h and 4 h after exposure. And more remarkably, camphor showed a decrease in repellency against *Lasioderma serricorne* adults at the concentrations of 1.57 and 7.86 nL/cm^2 at 4 h after exposure, compared with the repellent activity at 2 h after exposure. It might be attributed to its non-persistent volatility. However, there's on sufficient reports about it at present. Hence, further studies would be conducted in the future. In this paper, we report to isolate four repellent constituents from the essential oil of *Artemisia argyi* aerial parts against *L. serricorne* adults for the first time.

4 CONCLUSION

To the best of our knowledge, this report is the first one to demonstrate the biocontrol of *A. argyi* essential oil. The chemical composition of this essential oil was described in detail and appreciable bioactivity of the oil and its major constituents were also demonstrated. The results suggested that *A. argyi* essencial oil and its major constituents have potential uses in the study of ecological prevention and control of storage pests. Since the natural resources of *A. argyi* are abundant, further investigations that focus on more detailed biological activity studies should be conducted to elucidate the bioactivity mechanism of tested essential oils for various applications.

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