Chemical Composition and Bioactivity of Essential Oil of *Atalantia guillauminii* against Three Species Stored Product Insects

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Abstract: The toxic and repellent activities of the essential oil extracted from the leaves of *Atalantia guillauminii* Swingle were evaluated against three stored product insects, red flour beetles (*Tribolium castaneum*), cigarette beetles (*Lasioderma serricorne*) and booklice (*Liposcelis bostrychophila*). The essential oil obtained by hydrodistillation was investigated by GC-MS. The main constituents of the essential oil were β -thujene (27.18%), elemicin (15.03%), eudesma-3, 7(11)-diene (9.64%), followed by (-)-4-terpeniol (6.70%) and spathulenol (5.25%). The crude oil showed remarkable contact toxicity against *T. castaneum*, *L. serricorne* adults and *L. bostrychophila* with LD₅₀ values of 17.11, 24.07 μ g/adult and 55.83 μ g/cm² respectively and it also displayed strong fumigant toxicity against *T. castaneum*, *L. serricorne* adults with LC₅₀ values of 17.60 and 12.06 mg/L respectively, while weak fumigant toxicity against *L. bostrychophila* with a LC₅₀ value of 16.75 mg/L. Moreover, the essential oil also exhibited the same level repellency against the three stored product insects, relative to the positive control, DEET. At the same concentrations, the essential oil was more repellent to *T. castaneum* than to *L. serricorne*. Thus, the essential oil of *A. guillauminii* may be potential to be developed as a new natural fumigant/repellent in the control of stored product insects.

Key words: *A. guillauminii*, *T. castaneum*, *L. serricorne*, *L. bostrychophila*, bioactivity, essential oil composition

1 INTRODUCTION

Coleopterans have global importance in grain storage and industrial food production and can cause damage to the appearance of products as well as weight loss and reduced nutrient levels, rendering products unfit for human consumption¹. The red flour beetle (*Tribolium castaneum* Herbst) (Coleoptera: Tenebrionidae) is world-widely distributed and among the most economically important stored product pests². Infestations can also result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species³. *Lasioderma serricorne* Fabricius (Fabricius, 1792) (Coleoptera: Anobiidae), variously known as the cigarette beetle or the tobacco beetle, is cosmopolitan and

occurs frequently in tropical and subtropical areas. This insect is usually found in perishable food products such as cereals, legumes, tobacco, tobacco products and traditional Chinese medicinal materials in warehouses⁴. The booklouse (*Liposcelis bostrychophila* Badonnel) (Psocoptera) is an important emerging pest of stored products. It feed on a wide variety of foods, but they are particularly associated with amylaceous products, such as grain and flour^{5, 6}. The three stored product insects were also widespread in traditional Chinese medicinal materials.

Abbreviations: *A. guillauminii*: *Atalantia guillauminii*; *T. castaneum*: *Tribolium castaneum*; *L. serricorne*: *Lasioderma serricorne*; *L. bostrychophila*: *Liposcelis bostrychophila*; **RI**: Retention Index; **MS**: mass spectrum

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Currently, control of stored product insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbance of the environment, increasing application costs, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to the users^{7,8)}. Thus, there is a considerable interest in developing natural products which are relatively less damaging to mammalian health and the environment than existing conventional pesticides, and expected to be used as alternatives to non-selective synthetic pesticides to control the pests in the fields of medical and food security. In recent years, the researchers working in different fields have been concentrating their efforts on the search for natural products as alternatives to conventional insecticides and fumigants, as well as the re-evaluation of traditional botanical pest control agents^{9,10)}. Essential oils or their constituents may be ideal alternatives. Investigations have confirmed that some plant essential oils not only repel insects, but possess contact and fumigant toxicity against stored product pests as well as exhibited feeding inhibition or harmful effects on the reproductive system of insects¹¹⁾. In addition, antibacterial and nematocidal activities of essential oils have been reported^{12–18)}.

During the screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Atalantia guillauminii* leaves was found to possess strong insecticidal toxicity against the stored product insects, *T. castaneum*, *L. serricorne* and *L. bostrychophila*. *Atalantia* is a genus of about 17 species of flowering plants in the family Rutaceae, native to tropics and subtropics regions of Asia¹⁹⁾. Many species of *Atalantia*, such as *A. buxifolia*, *A. monophylla*, *A. racemosa* and *A. wightii*, were used in traditional medicine. For example, *A. buxifolia* have long been used in traditional Chinese medicine for the treatment of cough, stomachache, hernia, rheumatism, hemiplegia, snakebite and traumatic injury²⁰⁾. *A. monophylla* possesses the same effect as *A. buxifolia* and also used for the treatment of antispasmodic, itch and other skin complaints²¹⁾. Moreover, they all have antifungal, antioxidant activities, anti-allergic, antihepatotoxic, anti-inflammatory, antimalarial, antimicrobial, antiviral, cytotoxic and insecticidal activities against *Helicoverpa armigera*, *Spodoptera litura* and so on^{22–27)}. However, there is no report on medicinal use of *A. guillauminii*. *A. guillauminii* is a dungarunga distributed in Yunnan Province of China¹⁹⁾. In addition, a literature survey has shown that there is no report on the volatile constituents and insecticidal activity of *A. guillauminii*. Here, the chemical constituents and insecticidal activities of the essential oil of *A. guillauminii* leaves were investigated against stored product insects for the first time.

2 EXPERIMENTAL PROCEDURES

2.1 Material

2.1.1 Insects

Red flour beetles (*T. castaneum*), cigarette beetles (*L. serricorne*) and booklouse (*L. bostrychophila*) were obtained from laboratory cultures maintained in the dark in incubators at 29–30°C and 70–80% relative humidity (RH). The red flour beetles and cigarette beetles were reared on wheat flour mixed with yeast (10:1, w/w) at 12–13% moisture content while the booklouse were reared on a 1:1:1 mixture, by mass, of milk powder, active yeast and flour. The unsexed adults beetles/booklouse used in all the experiments were about 1–2 weeks old. All containers housing insects and the petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon).

2.1.2 Plant

Fresh leaves (1.3 kg) of *Atalantia guillauminii* were harvested in 2011 from Xishuangbanna (northern latitude: 21° 08' ~ 22° 36'; east longitude: 99° 56' ~ 101° 50') (Yunnan Province, China). The leaves were air dried for 1 week and ground to a powder. The plant species was identified and voucher specimens (BNU-dushushan-2011-06-12-24) were deposited at the Herbarium of College of Resources Science and Technology, Beijing Normal University (Beijing, China).

2.2 Extraction of Essential oil

The ground powder of *A. guillauminii* (2.0 kg) was subjected to hydrodistillation using a modified Clevenger-type apparatus for 10 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in an airtight container in a refrigerator at 4°C.

2.3 Analysis of Essential Oil

Components of the essential oil of *A. guillauminii* were separated and identified by using Bruker-320 gas chromatography/mass spectrometry (GC/MS) and equipped with a flame ionization detector and a capillary column with VF-5 (30 m × 0.25 mm × 0.25 μm). The GC settings were as follows: The initial oven temperature was held at 50°C for 4 min and ramped at 10°C/min to 290°C for 17 min. The injector temperature was maintained at 250°C. The samples (1 μL, dilute to 1% with hexane) were injected neat, with a split ratio of 1:60. The carrier gas was helium at flow rate of 1 mL/min. Spectra were scanned from 45 to 650 m/z at 2 scans/s. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C₈–C₂₄) 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 literature²⁸⁻³⁰. Component relative percentages were calculated based on GC peak areas, without using correction factors.

2.4 Contact Toxicity

The contact toxicity of the essential oil against *T. castaneum* and *L. serricorne* adults was measured by using topical application as described by Liu and Ho⁵. Serial dilutions of essential oil (10-1.97% (v/v) for *T. castaneum* and *L. serricorne*; five concentrations) were prepared in *n*-hexane. Preliminary experiments were conducted to determine appropriate ranges of test concentrations. Aliquots (0.5 µL) of the dilutions were applied topically to the dorsal thorax of the insects. Controls were established using *n*-hexane. Both treated and control insects were then transferred to glass vials (ten insects per vial) with culture media and kept in incubators at 29-30°C and 70-80% RH. Mortality of insects was observed daily until end-point mortality was reached 24 h after treatment. The experiments were repeated three times. While the contact toxicity of the essential oil against *L. bostrychophila* was also tested as described⁵. A 5.5 cm diameter filter paper was treated with 300 µL of the solution of the essential oil. The filter paper after treated with solid glue was placed in a 5.5 cm diameter petri dish and 10 booklice were put on the filter paper. A cover was put and all the petri dishes were kept in incubators. *n*-Hexane was used as a negative control. Five concentrations (in *n*-hexane) and five replicates of each concentration were used. Mortality of insects was observed after 24 h. The LD₅₀ values were calculated by using Probit analysis³¹.

2.5 Fumigant Toxicity

Serial dilution of the essential oil were prepared in *n*-hexane. Filter papers (Whatman Cat. No. 1001020, diameter 2 cm) were impregnated with 10 µL of an appropriate concentration (10-1.97% (v/v) for *T. castaneum* and *L. serricorne*; five concentrations) of essential oil and each was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 25 mL). Preliminary experiments were conducted to determine appropriate ranges of test concentrations. Fluon was used inside each glass vial to prevent insect contact with the treated filter paper. The solvent was allowed to evaporate for 20 s was before the cap was screwed tightly on each glass vial containing ten insects. Preliminary experiments demonstrated that 20 s was sufficient for solvent evaporation. *n*-Hexane was used as a control. Five replicates were carried out for all treatments and controls, which were incubated for 24 h. The experiments were repeated three times. While the fumigant activity of the essential oil against *L. bostrychophi-*

la was tested as described⁵. A filter paper strip (3.5 × 1.5 cm) treated with 10 µL of an appropriate concentration of the test essential oil. The impregnated filter paper was then placed in the bottom cover of a 250 mL volume of glass bottle. Ten unsexed adults of the booklouse in a small glass bottle (8 mL) were put into the glass bottle and exposed for 24 h. Five concentrations of the oil were used in the experiments and each concentration with five replicates. *n*-Hexane was used as a negative control. The LC₅₀ values were calculated by using Probit analysis³¹.

2.6 Repellency Tests

The repellent effects of the essential oil against *T. castaneum*, *L. serricorne* and *L. bostrychophila* were assessed by using assays on petri dishes³². Petri dishes 9 cm in diameter were used to confine beetles during the experiment for *T. castaneum* and *L. serricorne*. The essential oil of *A. guillauminii* were prepared in *n*-hexane (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm²), and absolute *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 absolute *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 reassembled 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 was then calculated using the formula

$$PR(\%) = [(N_c - N_t) / (N_c + N_t)] \times 100$$

Where N_c is the number of insects present in the negative control half and N_t is the number of insects present in the treated half. The averages were then assigned to different classes (0 to V) using the following scale (percentage repellency)³³. Class, % repellency: 0, >0.01 to <0.1; I, 0.1-20.0; II, 20.1-40.0; III, 40.1-60.0; IV, 60.1-80.0; and V, 80.1-100.

As for the booklouse, petri dishes and filter papers were changed to 6 cm in diameter and the concentration of the oil used in the experiments were 31.58, 6.32, 1.26, 0.25, 0.05 nL/cm². The half filter paper was treated with 150 µL of the solution. As a positive control, a commercial repellent DEET (N, N-diethyl-3-methyl-benzamide), was used under the conditions as the oil. Analysis of variance

(ANOVA) and Tukey's test were conducted by using SPSS statistics 20 for Windows 2007. Percentage was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

3 RESULTS AND DISCUSSION

3.1 Chemical Constituents of Essential Oil

The essential oil yield of *A. guillauminii* was 0.15% (v/w) and the density of the concentrated essential oil was determined as 0.90 g/mL. The results of GC/MS of *A. guillauminii* essential oil are presented in Table 1. 49 components which were accounted for 99.51% of the total oil were identified (Table 1). The main components in the essential oil of *A. guillauminii* were β -thujene (27.18%), elemicin (15.03%), eudesma-3, 7(11)-diene (9.64%), followed by (-)-4-terpeniol (6.70%), spathulenol (5.25%) and elixene (3.43%). Among the 49 compounds, 18 components belonged to monoterpenoids, corresponding to 45.81% of the whole oil, while 27 constituents were sesquiterpenoids (36.53% of the crude essential oil) and others were accounted for 17.17% of the total oil. A literature survey has shown that the chemical composition of *A. guillauminii* essential oil was calculated for the first time. However, the constituents of essential oils derived from other species of *Atalantia* have been reported. Das and Swamy revealed that the major compounds of *A. monophylla* essential oil collected from Nagamalai hills of Madurai district, Tamil Nadu were α -asarone (28.82%), sabinene (13.19%), eugenol methyl ether (12.71%), 1,2-di-

methoxy-4-(2-methoxyethenyl)benzene (11.63%) and β -pinene (5.3%)³⁴. Ten compounds were identified by Manimaran *et al.* on essential oil from *A. monophylla* leaves collected from Narrtha hills, Pudukkottai and the major compounds identified in that study were methyl eugenol (36.46%), sabinene (24.89%) and elemicin (24.61%)³⁵. It has been proved that there was variation in the chemical composition of essential oils collected in different areas. The difference in chemical constitution may be probably due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants as well as other factors. Thus for the practical application of the essential oil as a novel fumigant/repellent, further studies on plant cultivation and essential oil standardization are needed because chemical composition of the essential oil varies greatly with the plant population.

3.2 Contact Toxicity

The crude essential oil of *A. guillauminii* leaves showed pronounced contact toxicity against *T. castaneum*, *L. serricornis* and *L. bostrychophila* with LD₅₀ values of 17.11, 24.07 μ g/adult and 55.83 μ g/cm² respectively (Table 2). When compared with the positive control, pyrethrins, the essential oil demonstrated 65.8, 100 and 3.0 times less toxic against *T. castaneum*, *L. serricornis* adults and *L. bostrychophila* respectively. It showed that *L. bostrychophila* was the most susceptible to the contact toxicity of the essential oil of *A. guillauminii* and *L. serricornis* adults were more tolerant than *T. castaneum* adults. However, compared with the other essential oils in the literature, the essential oil of *A. guillauminii* leaves pos-

Table 1 Chemical constituents of essential oil derived from leaves of *A. guillauminii*.

Peak no.	Compounds	RI ^a	%RA ^b	Identification Methods ^c
1	α -Thujene	927	0.51	MS, RI
2	1R- α -Pinene	931	1.94	MS, RI, Co
3	β -Thujene	967	27.18	MS, RI
4	β -pinene	981	0.85	MS, RI
5	α -Phellandrene	1005	0.21	MS, RI
6	o-Cymene	1012	2.95	MS, RI
7	D-Limonene	1014	0.63	MS, RI
8	α -Terpinene	1017	0.42	MS, RI
9	β -Phellandrene	1030	2.04	MS, RI
10	γ -Terpinene	1057	0.92	MS, RI
11	cis- β -Terpineol	1060	0.19	MS, RI
12	Terpinolene	1080	0.29	MS, RI
13	Linalool	1094	0.17	MS, RI
14	cis-p-Menth-2-en-1-ol	1126	0.38	MS, RI
15	trans-p-Menth-2-en-1-ol	1140	0.14	MS, RI

Table 1 Continued.

Peak no.	Compounds	RI ^a	%RA ^b	Identification Methods ^c
16	Sabina ketone	1160	0.27	MS, RI
17	(-)-4-Terpeniol	1179	6.70	MS, RI
18	α -Terpieol	1191	0.23	MS, RI
19	cis-Piperitol	1196	0.06	MS, RI
20	Elixene	1313	3.43	MS, RI
21	Copaene	1374	0.24	MS, RI
22	β -Cubebene	1392	0.40	MS, RI
23	β -Elemene	1393	0.88	MS, RI
24	Methyleugenol	1401	1.57	MS, RI, Co
25	Viridiflorene	1403	0.78	MS, RI
26	Caryophyllene	1420	1.28	MS, RI, Co
27	α -Bergamotene	1433	0.46	MS, RI
28	α -Guaiene	1442	0.21	MS, RI
29	(+)-Aromadendrene	1448	0.15	MS, RI
30	α -Caryophyllene	1454	0.40	MS, RI
31	(-)-Alloaromadendrene	1458	0.65	MS, RI
32	α -Bulnesene	1501	0.74	MS, RI
33	β -Guaiene	1506	0.27	MS, RI
34	Calamenene	1511	0.24	MS, RI
35	Cadinene	1519	0.67	MS, RI
36	Eudesma-3,7(11)-diene	1536	9.64	MS, RI
37	Hedycaryol	1550	0.46	MS, RI
38	Elemicin	1554	15.03	MS, RI
39	α -Cedrene epoxide	1562	0.74	MS, RI
40	Spathulenol	1578	5.25	MS, RI
41	Caryophyllene oxide	1583	1.12	MS, RI, Co
42	Diepicedrene-1-oxide	1588	0.19	MS, RI
43	Viridiflorol	1590	1.96	MS, RI
44	Guaiol	1602	2.14	MS, RI
45	γ -Eudesmol	1621	2.78	MS, RI
46	β -Eudesmol	1648	1.13	MS, RI
47	τ -Muurolol	1662	0.11	MS, RI
48	2,3,4,5-tetramethyl-Tricyclo[3.2.1.02,7]oct-3-ene	1680	0.30	MS, RI
49	Epiglobulol	1723	0.21	MS, RI
Monoterpenoids			45.81	
Sesquiterpenoids			36.53	
Others			17.17	
Total			99.51	

^a Retention index (RI) as determined on an VF-5 MS column using a homologous series of *n*-hydrocarbons.^b Relative area (peak area relative to the total peak area).^c MS = mass spectrum, Co = co-injection with standard compound.

Table 2 Toxicity of *A. guillauminii* essential oil against *T. castaneum* (TC), *L. serricorne* (LS) and *L. bostrychophila* (LB) adults.

Insect	Compound	Fumigant toxicity		Contact toxicity	
		LC ₅₀ (mg/L air)	95% confidence limits	LD ₅₀ (µg/adult)/(µg/cm ²)	95% confidence limits
TC	Crude oil	17.60	15.83 – 19.63	17.11	14.57 – 19.56
	Pyrethrinsa	–	–	0.26	0.22 – 0.30
	MeBrb	1.75	–	–	–
LS	Crude oil	12.06	9.97 – 14.06	24.07	19.97 – 27.93
	Pyrethrinsa	–	–	0.24	0.16 – 0.35
	Phosphinea	9.23×10^{-3}	$7.13 - 11.37 \times 10^{-3}$	–	–
LB	Crude oil	>16.75	–	55.83	51.05 – 61.20
	Pyrethrinsc	–	–	18.72	17.60 – 19.92
	Dichlorvosc	1.35×10^{-3}	–	–	–

^b Data from Liu and Ho³³⁾, ^a Data from You *et al.*⁵⁰⁾, ^c Data from Yang *et al.*⁵¹⁾.

sessed stronger contact toxicity against *T. castaneum* adults, e.g. essential oils of *Dracocephalum moldavica* (LD₅₀ = 18.28 µg/adult)³⁶⁾, *Murraya exotica* (LD₅₀ = 20.94 µg/adult)³⁷⁾, *Evodia lepta* (LD₅₀ = 166.94 µg/adult)³⁸⁾. While the essential oil of *A. guillauminii* leaves also exhibited stronger contact toxicity against *L. bostrychophila* than the other reported oils, for example essential oils of *Lonicera japonica* (LD₅₀ = 64.04 µg/cm²)⁵⁾, *Foeniculum vulgare* (LD₅₀ = 90.36 µg/cm²)³⁹⁾, *Acorus calamus* (LD₅₀ = 100.21 µg/cm²)⁴⁰⁾, *Curcuma wenyujin* (LD₅₀ = 208.85 µg/cm²)⁴¹⁾.

3.3 Fumigant toxicity

The crude essential oil also displayed strong fumigant toxicity against *T. castaneum*, *L. serricorne* with LC₅₀ values of 17.60, 12.06 mg/L air respectively while exhibited weak fumigant toxicity against *L. bostrychophila* (LC₅₀ > 16.75 mg/L air (Table 2). Fumigation plays a very important role in insect pest elimination in stored products. The fumigant activity (LC₅₀) of one of the currently used grain fumigants, methyl bromide, against *T. castaneum* adults is reported to be 1.75 mg/mL air. Compared with methyl bromide, the essential oil of *A. guillauminii* was 10 times less toxic to *T. castaneum* adults. Nevertheless, compared with the other essential oils in the literature, the essential oil of *A. guillauminii* leaves possessed stronger fumigant toxicity against *T. castaneum* adults, e.g. essential oils of *Ostericum sieboldii* (LC₅₀ = 20.92 mg/mL air)⁴²⁾, *Evodia rutaecarpa* (LC₅₀ = 24.57 mg/mL air)⁴³⁾, *Artemisia vulgaris* (LC₅₀ = 279.86 µL/L air)⁴⁴⁾. Compared with phosphine (LC₅₀ = 9.23 µg/L air), the essential oil of *A. guillauminii* was 1.3×10^3 times less toxic to *L. serricorne* adults (Table 2). However the essential oil also showed stronger fumigant toxicity against *L. serricorne* adults, e.g. the essential oil of *Agastache foeniculum* (LC₅₀ = 21.565 µL/L

air)⁴⁵⁾. Moreover, considering that the currently used fumigants are synthetic insecticides, the fumigant activity of the essential oil of *A. guillauminii* is quite promising, showing its potential to be developed as possible natural fumigants for the control of stored product insects. Further studies are needed to assess the fumigant activity of the crude essential oil against other stored product insects at different life stages.

3.4 Repellency

In addition to contact and fumigant activities, the repellent effect of the essential oil of *A. guillauminii* against the three stored product insects was also investigated. The results are presented in Table 3 and 4. This essential oil strongly repelled all species of stored product insects, but the repellent effect was more marked on *T. castaneum* than on *L. serricorne*. Data showed that at the tested concentration of 0.63 nL/cm², the crude oil still showed moderate repellency (76 % and 60 %) (class IV and class III) against the red flour beetle, *T. castaneum* adults at 2 and 4 h after exposure (Table 3). While no repellency of the positive control, DEET, but some insect attractant properties were observed at 0.63 and 0.13 nL/cm² at 4 h after exposure. What's more, at the other assayed concentrations, the essential oil of *A. guillauminii* exhibited strong repellency (class V) at 2 and 4 h after exposure (Table 3).

Compared with the positive control, DEET, the crude oil of *A. guillauminii* possessed the same level repellency against the cigarette beetle, *L. serricorne* at tested concentrations. At the assayed concentration of 78.63 nL/cm², the crude oil was strongly repellent (class V) at 2 and 4 h after exposure (Table 3). With the decreasing of the sample concentration, the repellent activity of the essential oil of *A. guillauminii* was decreased. When at 0.13 nL/cm², compared with the positive control, DEET (46 %, class III),

Table 3 Percentage repellency (PR) after two exposure times for the essential oil against *T. castaneum* (TC) and *L. serricorne* (LS) adults^a.

Insect	Compound	2h					4h				
		78.63*	15.73*	3.15*	0.63*	0.13*	78.63*	15.73*	3.15*	0.63*	0.13*
TC	Crude oil	98 ± 3a V	96 ± 3a V	82 ± 10a V	76 ± 15a IV	40 ± 10a II	100 ± 0a V	94 ± 6a V	84 ± 9a V	60 ± 11a III	30 ± 12a II
	DEET	100 ± 0a V	96 ± 3a V	74 ± 16a IV	66 ± 9a IV	18 ± 16b I	94 ± 6a V	80 ± 10a IV	46 ± 17b III	2 ± 20b -	8 ± 22b -
LS	Crude oil	84 ± 7a V	60 ± 8a III	42 ± 11a III	24 ± 7a II	10 ± 12a I	82 ± 9a V	64 ± 13a IV	62 ± 8a IV	60 ± 8a III	16 ± 17a I
	DEET	88 ± 7a V	76 ± 14a IV	28 ± 7b II	20 ± 14a I	16 ± 7a I	98 ± 4a V	78 ± 9a IV	58 ± 16a III	56 ± 14a III	46 ± 7b III

* Concentration (nL/cm²); ^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.

Table 4 Percentage repellency (PR) after two exposure times for the essential oil against *L. bostrychophila* (LB)^a.

Insect	Compound	2h					4h				
		31.58*	6.32*	1.26*	0.25*	0.05*	31.58*	6.32*	1.26*	0.25*	0.05*
LB	Crude oil	90 ± 9a V	92 ± 5a V	88 ± 7a V	40 ± 22a II	22 ± 15a II	92 ± 5a V	88 ± 8a V	82 ± 5a V	54 ± 14a III	38 ± 15a II
	DEET	100 ± 0a V	92 ± 7a V	92 ± 7a V	74 ± 9b IV	30 ± 10a II	96 ± 7a V	90 ± 10a V	90 ± 6a V	72 ± 19b IV	32 ± 7a II

* Concentration (nL/cm²); ^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.

the essential oil of *A. guillauminii* showed lesser repellency against the cigarette beetles (16%, class I) at 4 h after exposure (Table 3).

At tested concentrations, compared with the positive control, the crude oil of *A. guillauminii* also showed the same level repellency against the booklice, *L. bostrychophila* at 2 and 4 h after exposure (Table 4). Moreover, at the assayed concentrations of 31.58, 6.32, 1.26 nL/cm², the oil exhibited strongly repellent (class V) against the booklice at 2 and 4 h after exposure, while at the lowest tested concentration of 0.05 nL/cm², class II repellency against the booklice still was observed at 2 and 4 h after exposure (Table 4). Many essential oils were evaluated for repellency against insects⁴⁶⁾. In China, the essential oils derived from spice and Chinese medicinal herbs were also evaluated for insecticidal activity and repellency against insects^{40, 41, 47–49)}. In this article, we report the insecticidal and repellent activities of the essential oil of *A. guillauminii* for the first time. Above all, the essential oil of *A. guillauminii* possessed strong repellency against the three stored product insects. These findings, considered together, suggest that the essential oil of *A. guillauminii* show potential for development as natural repellents for stored product.

4 CONCLUSION

Based on mass screening, essential oil of *A. guillauminii* leaves was examined for their bioactivities against red flour beetles *T. castaneum*, cigarette beetles *L. serricorne* and booklice *L. bostrychophila*. The essential oil possessed strong contact and fumigant toxicity against the three stored product insects. Furthermore, the essential oil also exhibited strongly repellence against the three stored product insects. These findings suggest that the essential oil show potential for development as a natural fumigant and repellent for stored products.

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