Acute toxicity and repellent activity of essential oil from Atalantia guillauminii Swingle fruits and its main monoterpenes against two stored product insects

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ABSTRACT
The objective of this work was to analyze the essential oil from Atalantia guillauminii fruits in China and evaluate their chemical compositions, insecticidal and repellent activities against Tribolium castaneum and Lasioderma serricorne adults. The essential oil was obtained by hydro-distillation and its yield was 0.12% (V/W). All together 25 chemical compositions were identified by GC-FID and GC-MS, and the main chemical compositions were D-limonene (41.8%), β-pinene (10.9%) and selina-3,7(11)-diene (6.8%). The bioactivity of the obtained essential oil and its two main compositions (D-limonene and β-pinene) was investigated by fumigation, contact and repellent activity bioassay. The essential oil and its two main compositions all showed significant fumigant toxicity, contact toxicity and repellent activity against two target insects. These results suggest that the essential oil and its two main compositions have potential to be developed as natural insecticides and repellents for the control of stored product insect pests.

Introduction
In the past few decades, human population is growing at an alarming rate and is expected to exceed 2.4 billion by 2050.[1] Although agriculture industry has increased production with the advent of the Green Revolution,[2] the postharvest losses caused by stored product insects are still severe.[3] In a report by Hashemi, the loss of stored products from stored products insects was estimated to be about 5–10% in temperate regions and about 20–30% in the tropical zone.[4] At present, Tribolium castaneum (Coleoptera: Tenebrionidae) and Lasioderma serricorne (Coleoptera: Anobiidae) adults are considered as two of the most destructive and widespread insect pests of stored products throughout the world, because of their rapid breeding speed, strong adaptability and wide distribution.[7] Generally, the standard practices to control those insects mainly rely on the use of synthetic insecticides, including organochlorines, pyrethroids, organophosphates and carbamates.[8,9] Unfortunately, there is growing evidence that the extensive and indiscriminate use of those pesticides can cause serious harm to humans, animals and the environment.[10–13] Therefore, there is a considerable interest in developing new structure types as safer alternatives.

With in-depth research, many plant secondary metabolites have been shown to have significant insecticidal[14,15] and repellent[16,17] effects on stored product insect pests. Among them, essential oils isolated from aromatic plants have received more attention, owing to their high efficacy, minimal residual, low risk to the environment and innocuous toxicity to fishes, mammals, and birds.[18–20] In the United States, commercial development of insecticides and repellents based on edible plant essential oils has been greatly facilitated by exemption from registration.[21] The lemon, citronella and eucalyptus
essential oils have been registered by US Environmental Protection Agency as repellent materials because of their good efficacy and low toxicity. These advantages, considered together, prompt us to systematically survey the bioactivity of essential oils from wild plants on stored product insect pests.

During the screening program for new agrochemicals from wild plants and folk herbs, plants from Atalantia Corr., family Rutaceae, were found to possess significant acute toxicity and repellent activity against stored product insects. In previous studies, Yang et al. reported the effect of Atalantia guillauminii leaves essential oil on the fumigation toxicity, contact toxicity and repellent activity of the T. castaneum, L. serricorne, and Liposcelis bostrychophila adults. Pang et al. evaluated the contact toxicity and repellent effects of Atalantia buxiolium (Poir).Oliv. leaves essential oil and its main compositions, β-caryophyllene and caryophyllene oxide, on the adults of T. castaneum, L. serricorne, and L. bostrychophila. Wu et al. investigated the antifeeding activity of three chemical compositions, severinolide, atalantin and cyclopiatalantin, isolated from A. buxiolium root bark against Plutella xylostella. These findings prompt us to systematically investigate the bioactivity of this genus on stored product insects.

A. guillauminii is one of the representative plants in Atalantia Corr., family Rutaceae. However, there is less report on practical application of A. guillauminii. In this work, chemical compositions of the essential oil from A. guillauminii fruits were determined by GC-FID and GC-MS. The bioactivity of the essential oil against T. castaneum and L. serricorne adults was evaluated by fumigation, contact and repellent activity bioassay. Meanwhile, to the best of our knowledge, negligible published studies so far have evaluated the fumigation, contact and repellent activities of A. guillauminii fruits essential oil on stored product insects. Herein, it is reasonable to suppose that the A. guillauminii fruits essential oil have potential to be developed into new, efficient, environmentally friendly and natural insecticides and insect repellents. In the past, the fruits of A. guillauminii are discarded as useless part. The development of these useless parts can make full use of wasted plant resources.

Materials and methods

Experimental samples and the extraction of essential oils

The fruits of Atalantia guillauminii Swingle were harvested from Puer Country of Yunnan Province, China (Latitude 22°02′–24°50′ N, Longitude 99°09′–102°19′ E) in October 2019. The sample was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing, China) and voucher specimens (BNU-dushushan-20191013-1) were deposited at the Herbarium (BNU) of the College of Resources Science and Technology, Faculty of Geographical Science, Beijing Normal University. The fruits were air-dried for a week. The essential oil of A. guillauminii fruits was obtained by hydrodistillation using a modified Clevenger-type apparatus for 4 h. Anhydrous sodium sulfate was used to remove excess water after extraction. The distilled essential oil was stored in dark airtight containers in a refrigerator at 4°C until being used in the treatments.

Solvents and chemicals

D-Limonene (97% pure) and β-pinene (95% pure) were purchased from Sigma-Aldrich (St. Louis, MO, USA). n-Hexane was purchased from Beijing Chemical Works (Beijing, China). Anhydrous sodium sulfate was purchased from Ziyi reagent (Shanghai, China). DEET (N, N-diethyl-3-methyl-benzamid, 98% pure) was purchased from the National Center of Pesticide Standards (Shenyang, China). Polytetrafluoroethylene was purchased from Beijing Sino-rich Tech Co., Ltd (Xuanwu District, Beijing, China).

Insect culture

Two stored product insects, the adults of Tribolium castaneum (Coleoptera: Tenebrionidae) and Lasioderma serricorne (Coleoptera: Anobiidae) were selected in this experiment and were identified
by Dr. Liu, Z.L. (College of Plant Protection, China Agricultural University, Beijing, China). The two target insects were obtained from laboratory cultures in a permanent dark incubator at 28–30°C and 70–80% relative humidity and reared in glass containers (0.5 L), containing wheat flour mixed with yeast (10:1, w/w) at 12–14% moisture content. All containers housing insects and the petri dishes used in experiments were made escape proof with a coating of polyterafluoroethylene. The unsexed insects used in all of the experiments were approximately 7–14 days old post-emergence.

Analysis of volatile compositions

Chemical compositions of the essential oil were identified by gas chromatographic-mass spectrometry (GC-MS) on an Agilent 6890 N apparatus equipped with a flame ionization detector (FID) and an Agilent 5973 N mass spectrometer (70 eV). A capillary column of HP-5 MS (30 m × 0.25 mm × 0.25 μm) was used. Detector and injector temperature were set at 250°C. The GC settings were as follows: the column temperature was initially held at 50°C for 2 min, and then ramped at a rate of 2°C/min to 150°C held for 2 min, and then ramped at a rate of 10°C/min to 250°C held for 5 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The sample (1 μL) was diluted to 1% with n-hexane and injected into the GC with a 1:10 split ratio. Spectra were scanned from 20 to 550 m/z at 2 scans s⁻¹.

The retention indices were determined in relation to a homologous series of n-alkanes (C₅–C₃₀) under the same operating conditions. Chemical compositions were identified by comparison of their retention indices (RI cal. a) with those reported in the literatures (RI lit. b). The further identification of the compositions was based on 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. Quantification of the essential oil was performed by using gas chromatograph with a flame ionization detector (GC-FID). The relative percentage of the compositions was calculated from the GC peak areas by the normalization method without using correction factors. For these two main compositions, the identities were confirmed by comparison (co-injection) with standard samples (D-Limonene and β-Pinene).

Fumigation toxicity bioassay

The fumigant toxicity of the essential oil and its two main compositions against T. castaneum and L. serricorne adults was evaluated according to a previous protocol. Range-finding studies were run to determine the appropriate testing concentration of the essential oil and compositions (maximum concentration, 50% V/V). Each sample was serially diluted with n-hexane to obtain five testing concentrations, and the selected testing concentrations were shown in Table 2. Ten insect adults were introduced into a glass vial (diameter 2.5 cm, height 5.5 cm), which was equipped with a screw cap. A filter paper (diameter 2.5 cm) was placed into a screw cap and impregnated with 10 μL of a testing solution with the micropipette as well as the n-hexane which was used as the blank control. After the blank solvent evaporating for 20 s, the screw cap was closed. Each sample and blank treatment was replicated five times. Then, all the glass vials were transferred and kept in the aforementioned incubator. Finally, samples and controls were monitored at 24 h and the number of dead insects were recorded. The LC₅₀ values were calculated using SPSS V17.0 Probit analysis.

Contact toxicity bioassay

The contact toxicity of the essential oil and its two main compositions against T. castaneum and L. serricorne adults was tested as described by Liu and Ho. Range-finding studies were run to determine the appropriate testing concentration of the tested essential oil and compositions (maximum concentration, 50% V/V). All samples were, respectively, diluted in n-hexane to obtain five different testing concentrations. The selected testing concentrations of each sample were shown in
Table 4. Aliquots of 0.5 μL of the dilutions were topically applied on the dorsal thorax of insects. Ten insect adults treated with a testing solution were transferred to a glass vial (diameter 2.5 cm, height 5.5 cm) and then the glass vial was sealed with a screw cap.

Insects treated with n-hexane alone were used as negative controls. Each treatment was replicated five times. Both the treated and the control insects were subsequently transferred and kept in an incubator under the same laboratory conditions. Insect mortality was recorded after 24 h, and the LD$_{50}$ values were calculated using SPSS V17.0 Probit analysis.[28]

Repellent activity bioassay

The repellent activity of the essential oil and its two main compositions against *T. castaneum* and *L. serricorne* adults was tested using the area preference method.[29] The tested essential oil and compositions were prepared in n-hexane to obtain five testing concentrations. The testing concentrations of the three samples were 78.63, 15.73, 3.15, 0.63, and 0.13 nL/cm$^2$. A filter paper disk with a 9 cm diameter was cut into two halves. One half was uniformly treated with 500 μL of each concentration of each sample. The other half (control) was treated with 500 μL of n-hexane as a negative control. Both the treated half and the control half disks were air-dried for 30 s to absolutely evaporate the blank solvent and then fixed to the bottom of the Petri dish (diameter 9 cm) with solid glue. Twenty insect adults were released at the center of each filter paper disk, and a lid was placed over the filter paper disk. Five replicates were used for each concentration of each sample. Counts of the insects present on the negative control were recorded after 2 h and 4 h of exposure, respectively. In addition, the value of percent repellency (PR) was calculated by the following formula:

PR (%) = [(Nc – Nt)/(Nc + Nt)] ×100

where Nc was the number of insects present in the negative control half and Nt was the number of insects present in the treated half. Analysis of variance (One-Way ANOVA) and Tukey’s test were conducted by using SPSS 17.0 (IBM Crop., Armonk, NY, USA) for Windows 7. PR values were subjected to an arcsine square-root transformation before Analysis of variance (One-Way ANOVA) and Tukey’s test. The results showed significant differences at $P < .05$ levels. The mean values were assigned to different classes (0 to V) using the scales described by Liu and Ho.[27] PR < 0.1, Class 0; PR = 0.1–20.0, Class I; PR = 20.1–40.0, Class II; PR = 40.1–60.0, Class III; PR = 60.1–80.0, Class IV; PR = 80.1–100.0, Class V.

Results

Chemical compositions of the essential oil

The light yellow Atalantia guillauminii fruits essential oil emits a fresh fragrance. The yield of the essential oil from the fruits of *A. guillauminii* was 0.12% (V/W) and its density was 0.87 g/mL. Using GC-MS, a total of 25 aromatic compositions were identified, representing 97.1% of the oil. The chemical compositions and relative content of the total oil were presented in Table 1. The essential oil of *A. guillauminii* is dominated by monoterpenes hydrocarbons 52.2% and sesquiterpene hydrocarbons 27.7%. The main compositions of the essential oil were D-limonene 41.8%, β-pinene 10.9% and selina-3,7(11)-diene 6.8%, the compounds elemicin 5.5%, elixene 4.9% and β-cocimene 3.2% also present in the essential oil in considerable amounts. The abundance of low-content compounds ranges from 0.2% to 2.6%, accounting for 20.9% of the total oil.

Fumigant toxicity of the essential oil and main compositions

Mortality of the essential oil and its two main compositions (D-limonene and β-pinene) against *T. castaneum* and *L. serricorne* adults are shown in Table 2. The results showed the mortality rate of insects depended on the concentration of the essential oil and compositions, and increased with increasing the essential oil and compositions concentrations. The essential oil exhibited significant
Table 1. Chemical profile of the essential oil from A. guillauminii fruits.

| No. | Compound Name          | RI cal. | RI lit. | Identification method | Relative content (%) |
|-----|------------------------|---------|---------|-----------------------|---------------------|
| 1   | β-Pinene               | 980     | 978     | RI, MS, Co            | 10.9                |
| 2   | α-Phellandrene         | 1004    | 1007    | RI, MS                | 2.3                 |
| 3   | D-Limonene             | 1030    | 1032    | RI, MS, Co            | 41.8                |
| 4   | β-Ocimene              | 1040    | 1044    | RI, MS                | 3.2                 |
| 5   | 4-terpinol             | 1176    | 1175    | RI, MS                | 1.8                 |
| 6   | Phellandral            | 1281    | 1280    | RI, MS                | 0.2                 |
| 7   | Geranyl acetate        | 1386    | 1392    | RI, MS                | 1.8                 |
| 8   | β-Elemene              | 1391    | 1394    | RI, MS                | 0.4                 |
| 9   | Methyleneugenol        | 1405    | 1410    | RI, MS                | 1.1                 |
| 10  | Caryophyllene          | 1418    | 1417    | RI, MS                | 1.0                 |
| 11  | α-Bergamotene          | 1430    | 1428    | RI, MS                | 2.6                 |
| 12  | Aromandendrene         | 1436    | 1440    | RI, MS                | 0.7                 |
| 13  | Elixene                | 1445    | -       | MS                    | 4.9                 |
| 14  | γ-Muurolene            | 1475    | 1474    | RI, MS                | 1.0                 |
| 15  | Acoradien              | 1483    | 1483    | RI, MS                | 1.9                 |
| 16  | Germacrene D           | 1485    | 1480    | RI, MS                | 1.2                 |
| 17  | Ledene                 | 1495    | 1497    | RI, MS                | 0.4                 |
| 18  | δ-Cadinene             | 1530    | 1528    | RI, MS                | 0.8                 |
| 19  | Selim-5,7(11)-diene    | 1545    | 1542    | RI, MS                | 6.8                 |
| 20  | Elemicin               | 1560    | 1558    | RI, MS                | 5.5                 |
| 21  | Globulol               | 1578    | 1575    | RI, MS                | 1.9                 |
| 22  | β-Spathuleno           | 1583    | 1582    | RI, MS                | 0.4                 |
| 23  | Viridiflorol           | 1585    | 1584    | RI, MS                | 0.2                 |
| 24  | γ-Eudesmol             | 1630    | 1635    | RI, MS                | 1.2                 |
| 25  | t-Muurolol             | 1645    | 1645    | RI, MS                | 3.1                 |

|                  | Monoterpenes hydrocarbons | Oxygenated monoterpenes | Sesquiterpene hydrocarbons | Oxygenated sesquiterpenes | Other | Total |
|------------------|---------------------------|-------------------------|----------------------------|---------------------------|-------|-------|
|                  | 52.2                      | 3.8                     | 27.7                       | 6.8                       | 6.6   | 97.1  |

|                  | Relative area (peak area relative to the total peak area). |

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Table 2. Mortality of two target insects in fumigation tests at 24 h.

| Insects | Essential Oil C (%) (V/V) | M (% ± SE) | D-Limonene C (%) (V/V) | M (% ± SE) | β-Pinene C (%) (V/V) | M (% ± SE) | n-Hexane C (%) (V/V) | M (% ± SE) |
|---------|---------------------------|------------|------------------------|------------|----------------------|------------|----------------------|------------|
| TC      | 5.0                       | 92.0 ± 4.9 | 5.0                    | 92.0 ± 3.7 | 10.0                 | 100.0 ± 0.0 | 100.0                | 0.0 ± 0.0  |
|         | 3.3                       | 84.0 ± 4.0 | 3.3                    | 80.0 ± 3.2 | 6.7                  | 86.0 ± 6.0  | -                    | -          |
|         | 2.2                       | 62.0 ± 3.7 | 2.2                    | 62.0 ± 3.7 | 4.4                  | 62.0 ± 3.7  | -                    | -          |
|         | 1.5                       | 42.0 ± 3.7 | 1.5                    | 38.0 ± 5.8 | 3.0                  | 28.0 ± 4.9  | -                    | -          |
|         | 1.0                       | 12.0 ± 3.7 | 1.0                    | 16.0 ± 5.1 | 2.0                  | 6.0 ± 4.0   | -                    | -          |
| LS      | 50.0                      | 100.0 ± 0.0 | 10.0                   | 98.0 ± 2.0 | 20.0                 | 98.0 ± 2.0  | 100.0                | 0.0 ± 0.0  |
|         | 33.3                      | 96.0 ± 2.4 | 6.7                    | 80.0 ± 3.2 | 13.3                 | 90.0 ± 0.0  | -                    | -          |
|         | 22.2                      | 74.0 ± 2.4 | 4.4                    | 52.0 ± 3.7 | 8.9                  | 56.0 ± 4.0  | -                    | -          |
|         | 14.8                      | 54.0 ± 2.4 | 3.0                    | 24.0 ± 5.1 | 5.9                  | 38.0 ± 3.7  | -                    | -          |
|         | 9.9                       | 30.0 ± 4.5 | 2.0                    | 14.0 ± 4.0 | 4.0                  | 6.0 ± 4.0   | -                    | -          |

C (%): concentration (% V/V); M: mortality of treated insects; SE: standard error

Fumigant toxicity against *T. castaneum* and *L. serricorne* adults with LC50 values of 6.4 and 48.4 mg/L air, respectively. The two tested compositions also exhibited significant fumigant toxicity against two target insect adults. D-Limonene exhibited fumigant toxicity against *T. castaneum* and *L. serricorne* adults with LC50 values of 6.3 and 13.7 mg/L air, respectively, while β-pinene exhibited fumigant toxicity against *T. castaneum* and *L. serricorne* adults with LC50 values of 14.5 and 28.3 mg/L air, respectively. The specific experimental results are shown in Table 3.
### Table 3. Fumigant toxicity of *A. guillauminii* fruits essential oil and its major compositions against two target insects at 24 h.

| Insects | Samples | $LC_{50}$ (95% Fiducial Limits) (mg/L air) | $LC_{50}$ (95% Fiducial Limits) (mg/L air) | Slope ± SE | Chi-Square ($\chi^2$) | $P$-value |
|---------|---------|------------------------------------------|------------------------------------------|------------|-----------------------|-----------|
| TC      | Essential Oil | 6.4 (5.7–7.2) | 14.5 (12.2–18.4) | 3.6 ± 0.4 | 17.4 | 0.792 |
|         | D-Limonene | 6.3 (5.5–7.1) | 15.0 (12.3–19.6) | 3.4 ± 0.4 | 13.3 | 0.945 |
|         | β-Pinene | 14.5 (13.3–15.9) | 25.4 (22.5–30.1) | 5.3 ± 0.5 | 18.0 | 0.758 |
|         | MeBr a | 1.8 | - | - | - | - |
| LS      | Essential Oil | 48.4 (42.3–54.0) | 98.5 (85.3–121.4) | 4.2 ± 0.5 | 7.7 | 0.999 |
|         | D-Limonene | 13.7 (12.3–15.2) | 27.8 (23.9–34.4) | 4.2 ± 0.4 | 13.5 | 0.942 |
|         | β-Pinene | 28.3 (25.7–31.1) | 52.0 (45.7–62.2) | 4.9 ± 0.5 | 14.0 | 0.928 |
|         | Phosphine b | 9.2 × 10^{-3} (7.1 × 10^{-3} – 11.4 × 10^{-3}) | - | 2.1 ± 0.3 | 12.0 | 0.971 |

The mortality of the negative control was 0 for the three insects;
a) Data from Liu & Ho 22-23;
b) Data from Yang et al. 290

### Contact toxicity of the essential oil and main compositions

As it is shown in Table 4, the essential oil at the highest testing concentrations caused over 95% mortality to two target insect adults. The essential oil exhibited significant contact toxicity against *T. castaneum* and *L. serricorne* adults with LD_{50} values of 15.8 μg/adult and 12.2 μg/adult, respectively. Among the two tested compositions, the results showed that the two target insect adults were all sensitive to D-limonene and β-pinene. D-Limonene exhibited significant contact toxicity against *T. castaneum* and *L. serricorne* adults with LD_{50} values of 14.2 μg/adult and 13.1 μg/adult, respectively. β-Pinene exhibited significant contact toxicity against *T. castaneum* and *L. serricorne* adults with LD_{50} values of 21.6 μg/adult and 61.5 μg/adult, respectively. The specific experimental results are shown in Table 5.

### Repellent activity of the essential oil and main compositions

The results of the repellent activity for the essential oil, D-limonene and β-pinene against the two target insect adults are presented in Figure 1. Data indicated that the essential oil of *A. guillauminii* fruits and the two tested compositions possessed approximate repellent activity with positive control DEET against *T. castaneum* adults at the testing concentrations of 78.63 nL/cm² and 15.73 nL/cm² after 2 h and 4 h of exposure. However, the situation seems to be different for *L. serricorne* adults. At the highest testing concentrations (78.63 nL/cm²), the essential oil and β-pinene showed weak repellent activity at 2 h and 4 h after exposure, with repellent rates ranging from Class III to Class I. Fortunately, the tested compound, D-limonene showed strong repellent activity (80% and 74%, respectively) against *L. serricorne* adults at the highest testing concentrations (78.63 nL/cm²) after 2 h and 4 h of exposure; meanwhile, D-limonene possessed approximate repellent activity with DEET at the highest testing

### Table 4. Mortality of two target insects in contact tests at 24 h.

| Insects | Essential Oil | D-Limonene | β-Pinene | n-Hexane |
|---------|--------------|------------|----------|----------|
| TC      | C (%) (V/V) | M (%) ± SE | C (%) (V/V) | M (%) ± SE | C (%) (V/V) | M (%) ± SE |
| 10.0    | 98.0 ± 2.0  | 10.0       | 94.0 ± 4.0 | 20.0     | 98.0 ± 2.0 | 100.0     | 0.0 ± 0.0 |
| 6.7     | 76.0 ± 2.4  | 6.7        | 80.0 ± 4.5 | 13.3     | 84.0 ± 2.4 | -         | -         |
| 4.4     | 58.0 ± 3.7  | 4.4        | 66.0 ± 5.1 | 8.9      | 76.0 ± 2.4 | -         | -         |
| 3.0     | 40.0 ± 3.2  | 3.0        | 50.0 ± 4.5 | 5.9      | 68.0 ± 3.7 | -         | -         |
| 2.0     | 20.0 ± 3.2  | 2.0        | 18.0 ± 3.7 | 4.0      | 40.0 ± 3.2 | -         | -         |
| LS      | C (%) (V/V) | M (%) ± SE | C (%) (V/V) | M (%) ± SE | C (%) (V/V) | M (%) ± SE |
| 10.0    | 100.0 ± 0.0 | 5.0       | 78.0 ± 2.0 | 20.0     | 80.0 ± 4.5 | 100.0     | 0.0 ± 0.0 |
| 6.7     | 92.0 ± 3.7  | 3.3        | 50.0 ± 4.5 | 13.3     | 46.0 ± 5.1 | -         | -         |
| 4.4     | 70.0 ± 3.2  | 2.2        | 32.0 ± 2.0 | 8.9      | 24.0 ± 5.1 | -         | -         |
| 3.0     | 48.0 ± 3.7  | 1.5        | 16.0 ± 2.4 | 5.9      | 8.0 ± 3.7  | -         | -         |
| 2.0     | 36.0 ± 2.4  | 1.0        | 2.0 ± 2.0  | 4.0      | 4.0 ± 2.4  | -         | -         |

C (%): concentration (%, V/V); M: mortality of treated insects; SE: standard error
At the other assayed concentrations, the essential oil and two tested compositions provided varying degrees of repellent activity against *T. castaneum* and *L. serricorne* adults at 2 h and 4 h after exposure. And their repellent activity ranged from Class IV to Class 0.

**Discussions**

**Chemical compositions of the essential oil**

As it is shown in Table 1, monoterpenes were the main fraction in *Atalantia guillauminii* fruits, accounting for 56.0% of the total oil. Among them, D-limonene (41.8%), β-pinene (10.9%) and β-ocimene (3.2%) were the three compositions with the highest content. However, the results were not identical with those that were reported in previous studies. In 2016, Nguyễn et al. reported that the main compositions of *A. guillauminii* (fruits, harvested from Pu Mat National Park, Nghe An province) essential oil were sabinene (36.4%), β-phellandrene (19.5%) and α-phellandrene (8.0%).

| Insects | Samples | $\text{LD}_{50}$ (95% Fiducial Limits) (μg/adult) | $\text{LD}_{90}$ (95% Fiducial Limits) (μg/adult) | Slope ± SE | Chi-Square ($\chi^2$) | $P$-value |
|---------|---------|---------------------------------------------|---------------------------------------------|-----------|---------------------|-----------|
| TC      | Essential Oil | 15.8 (13.9–17.8) | 37.5 (31.3–49.1) | 3.4 ± 0.4 | 8.7 | 0.997 |
|         | D-Limonene   | 14.2 (12.2–16.1) | 35.2 (29.3–46.6) | 3.2 ± 0.4 | 19.9 | 0.860 |
|         | β-Pinene     | 21.6 (16.4–25.8) | 65.5 (53.3–91.9) | 2.7 ± 0.4 | 8.9 | 0.996 |
|         | Pyrethrins a | 0.3 (0.2–0.3) | – | 3.3 ± 0.3 | 13.1 | 0.950 |
| LS      | Essential Oil | 12.2 (10.5–13.8) | 27.9 (23.7–35.6) | 3.6 ± 0.5 | 10.2 | 0.990 |
|         | D-Limonene   | 13.1 (11.6–15.0) | 30.1 (24.1–42.5) | 3.5 ± 0.4 | 7.1 | 0.999 |
|         | β-Pinene     | 61.5 (54.9–70.5) | 132.1 (107.3–181.5) | 3.9 ± 0.5 | 17.9 | 0.760 |
|         | Pyrethrins a | 0.2 (0.2–0.4) | – | 1.3 ± 0.2 | 17.4 | 0.791 |

The mortality of the negative control was 0 for the three insects; a Data from Yang et al. [30]

![Figure 1](image-url)
And, the essential oil of *A. guillauminii* fruits is dominated by monoterpenes 77.6% and sesquiterpene 11.7%. The same thing also happened to the *A. guillauminii* leaves essential oil. In 2015, Yang et al. reported that the main compositions of the essential oil from *A. guillauminii* leaves (harvested from Xishuangbanna, China) were β-thujene (27.2%), elemicin (15.0%) and eudesma-3,7(11)-diene (9.6%), while Nguyễn et al. analyzed the chemical compositions of *A. guillauminii* leaves essential oil, and β-phellandrene (33.4%), α-phellandrene (10.6%) and o-cymene (5.8%) were determined to be the main compositions. The above findings suggest that there are great variations in the chemical compositions of the essential oil from same plant. The large climate and geographical location differences between the two areas may be the two main reasons for the difference in essential oil composition. Therefore, if the *A. guillauminii* fruits essential oil is developed as a novel insecticide and repellent for practical application, further research on plant cultivation and essential oil standardization is needed because the chemical composition of the essential oil varies greatly depending on the plant population.

**Fumigant and contact toxicity**

The essential oil of *A. guillauminii* fruits showed significant fumigant and contact toxicity against *T. castaneum* and *L. serricorne* adults. The results exhibited that *T. castaneum* adults were more susceptible to the fumigation toxicity of the essential oil of *A. guillauminii* fruits than *L. serricorne* adults. However, contact toxicity of the essential oil of *A. guillauminii* fruits against two target insects is the opposite. Compared to the positive control, although *A. guillauminii* fruits essential oil exhibited moderate fumigant and contact toxicity to the two target insects, it was significantly stronger than other plants reported in the published literature. For example, in the same method of experiment, Cao et al. evaluated the fumigation toxicity of the essential oil isolated from *Haplophyllum dauricum* (L.) G. Don fruits against *T. castaneum* adults with the LC₅₀ value of 14.6 mg/L air. Wu et al. reported that the essential oil of *Platycladus orientalis* (L.) Franco possessed fumigant toxicity to *L. serricorne* adults with the LC₅₀ value of 145.4 mg/L air. Qi et al. showed the contact toxicity of *Ligusticum pteridophyllum* Franch. rhizomes essential oil against *T. castaneum* adults with the LD₅₀ value of 88.0 µg/adult. Wu et al. confirmed the contact toxicity of the essential oil extracted from *Zingiber zerumbet* rhizomes against *L. serricorne* adults with the LD₅₀ value of 48.3 µg/adult. Compared with their results, the essential oil of *A. guillauminii* fruits was 2.3, 3.0, 5.6, and 4.0 times more toxic to adults of *L. serricorne* and *T. castaneum*, respectively.

Among the two tested compositions (D-limonene and β-pinene), it was believed that D-limonene could be one of the key compositions which would affect the fumigant and contact toxicity of *A. guillauminii* fruits essential oil. When testing the fumigation toxicity of *L. serricorne* adults, D-limonene showed the strongest fumigation activity, which was 3.5-fold higher than the effect of the EO and 2.1-fold higher than that of β-pinene. Moreover, D-limonene also showed significant fumigation toxicity against other insects, including *Rhizophyta dominica* (F.), *Sitophilus oryzae* (L.), *Lycoriella ingenua* Dufour and *Sitophilus zeamais* Motsch. According to the previous literature, monoterpenoids and sesquiterpenoid compositions are fast-acting neurotoxins in insects, possibly interacting with multiple types of receptors. However, β-pinene showed weaker contact toxicity against two target insects. This may be due to the difference in the three-dimensional structure of the compound. The astonishing thing is that the total oil containing only 41.8% of D-limonene can produce insecticidal toxicity similar to that of D-limonene. The results indicated that synergy among various compositions increases the penetration of toxic substances through the skin of insects rather than through inhibition of detoxicative enzymes.

During the three tested samples (*A. guillauminii* fruits essential oil, D-limonene and β-pinene) acted on the dorsal thorax of target insects, we also observed the typical neurotoxic symptoms of target insects, such as tremors, wing movements, gradual loss of direction, erratic leg and even death. Currently, the mechanism of neurotoxicity action of the essential oil from *A. guillauminii* fruits and its
two main compositions is still unclear and needs further elucidation. Additional studies on the safety of the essential oil/compositions to human are also necessary.

**Repellent activity**

In the present research, the essential oil from *A. guillauminii* fruits showed strong repellent effect on adults of *T. castaneum*. Data showed that at all testing concentrations, the essential oil showed almost the same level of repellent activity as the positive control (DEET) at 2 h and 4 h after exposure. Even at the lowest assayed concentration (0.13 nL/cm$^2$), the repellent activity of the essential oil (66% and 76%, respectively) is stronger than the positive control (8% and 22%, respectively). The PR value peaked at 78.63 nL/cm$^2$ (90%, Class V) at 4 h of exposure. For *L. serricorne* adults, the essential oil showed weaker repellent activity than that of *T. castaneum* adults. It showed 30% (Class II) and 50% (Class III) repellency at the highest testing concentrations (78.63 nL/cm$^2$) at 2 h and 4 h of exposure, respectively. Fortunately, as the exposure duration prolonged and the testing concentration of the sample decreased, the repellent activity of the essential oil increased significantly. At the dose of 3.15 and 0.63nL/cm$^2$, the essential oil showed the same repellent activity level as DEET at 2 h and 4 h after exposure. Besides, the PR value peaked at 15.73 nL/cm$^2$ at 4 h of exposure and its calculated percentage repellency (PR) value was 74% (Class IV).

The two tested compositions, D-limonene and β-pinene showed strong repellent activity against *T. castaneum* adults. The PR value of D-limonene peaked at 15.73 nL/cm$^2$ (94%, Class V) at 4 h of exposure, while β-pinene peaked at 78.63 nL/cm$^2$ (94%, Class V) at 4 h of exposure. For *L. serricorne* adults, D-limonene and β-pinene showed different levels of repellent activity. At the highest testing concentration (78.63 nL/cm$^2$), D-limonene showed strong repellent activity (80% and 74%, respectively) against *L. serricorne* adults at 2 h and 4 h after exposure, while β-pinene showed weak repellent activity (18% and 30%, respectively) against *L. serricorne* adults at 2 h and 4 h after exposure. However, at the lowest assayed concentration (0.13 nL/cm$^2$), the repellent activity of β-pinene (66% and 76%, respectively) is higher than that of D-limonene (8% and 22%, respectively). Most notably, at the dose of 3.15 nL/cm$^2$, β-pinene showed insect attractant properties (PR = −10%) at 2 h after exposure, while D-limonene showed insect repellent properties (PR = 78%) at 2 h after exposure.

In general, the variety of the percentage repellency values was obviously affected by the tested sample, testing concentrations of the samples, the exposure duration of experimental test and the sensitivity of insects. The ability of the essential oil from *A. guillauminii* fruits to repel or attract the two target insects may be also related to the synergism and antagonism of their various main and minor compositions. Consulting the past literature, many compositions have been reported their repellent or attractant effects on the two target insects. For example, Liang et al.[46] evaluated the repellent effects of selina-3,7(11)-diene on the adults of *T. castaneum* and *Liposcelis bostrychophila*. The results showed that at the highest testing concentration, selina-3,7(11)-diene possessed the same repellent level (class V) as DEET against *T. castaneum* and *L. bostrychophila* adults. You et al.[47] investigated the repellent activity of 4-terpineol against *L. serricorne* adults. The results identified that, at the testing concentration of 39.32 nL/cm$^2$ at 2 h after exposure, 4-terpineol exhibited strong repellency (88%, Class V) to *L. serricorne* adults. In addition, some of the other relatively high compositions such as elemicin (5.5%), elixene (4.9%) and β-oicmene (3.2%) may also have potential repellent or attractant effects on insect pests, and further investigation is needed.

**Conclusion**

This work demonstrated that the essential oil isolated from *Atalantia guillauminii* fruits and its two main compositions D-limonene and β-pinene possessed significant fumigant, contact and repellent activities against *Tribolium castaneum* and *Lasioderma serricorne* adults. Considering the currently synthetic insecticides are highly toxic to humans, other non-target organisms and environment, the essential oil of *A. guillauminii* fruits and its two main compositions are quite promising, and they show potential to be developed into natural insecticides and repellents to control stored product insects.
Conflicts of Interest

The authors declare no conflict of interest.

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