Chemical composition and biological activities of essential oils of different plants of *Ligusticum* genus against three stored insects

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**ABSTRACT**

Apiaceae L. contains many potential plants as repellent and insecticidal resources. However, *Ligusticum* applied in the stored insect control is still under-evaluated. Hence, the chemical composition and bioactivities of five *Ligusticum* plants with abundant resources were investigated in this study against *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Lasioderma serricorne* (Coleoptera: Anobiidae), and *Liposcelis bostrychophila* (Psocoptera: Liposcelididae). The contact toxicity was found 24 h after treatment with *Ligusticum pteridophyllum*, *Ligusticum ajanense*, *Ligusticum chuanxiong*, *Ligusticum thomsonii*, and *Ligusticum jeholense* essential oils (LD\(_{50}\) = 87.99, 44.01, 21.53, 125.37, and >50% µg/adult; 89.82, 93.51, 28.42, 105.55, and 50.16 µg/adult; 7.87, 308.62, 67.82, and 195.06 µg/cm\(^2\) respectively). The five plants’ essential oils possessed poor fumigant toxicity against three target insects at the highest concentration. Moreover, all analyzed oils have good repellent activity in a dose-dependent manner. In conclusion, the tested oils have the potential to be developed as contact and repellent agents in protecting the stored products.

**Introduction**

*Tribolium castaneum* (Coleoptera: Tenebrionidae), *Lasioderma serricorne* (Coleoptera: Anobiidae), and *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) are the most destructive storage insects of stored products, which could cause huge economic losses by eating and contaminating grains.\(^1\) In order to reduce the economic losses, synthetic products are widely used. While along this way, it could have various side effects, such as pest resistance and toxicity to non-target organism.\(^1, 2\) Therefore, essential oils, which applied in cosmetics and food industry,\(^3\) are developed as a substitute to synthetic products for their superior properties, such as easy degradation,\(^4-6\) friendly environment,\(^7\) abundant resources,\(^8\) low residue,\(^9\) and safety.\(^10\) Accumulated evidence has demonstrated that Apiaceae essential oils possessed insecticidal and repellent properties against many kinds of stored insects.\(^11-13\)

*Ligusticum* L. is an important genus of Apiaceae family, widely distributed in Europe, Asia, and North America.\(^14\) Found in China, *Ligusticum pteridophyllum*, *Ligusticum ajanense*, *Ligusticum chuanxiong*, *Ligusticum thomsonii*, and *Ligusticum jeholense* are the five abundant and representative plants of *Ligusticum* L. genus.\(^15\) *L. pteridophyllum* and *L. chuanxiong* are documented for their antifungal and insecticidal ability of their essential oils.\(^16, 17\) However, there are fewer reports on the insecticidal and repellent activities of *Ligusticum* L against *T. castaneum*, *L. serricorne*, and *L. bostrychophila*. Therefore, the objective of this study was to comparatively analyze the chemical
composition and target biological activities of the essential oils of these five species for developing promising eco-friendly pesticide agents.

**Materials and methods**

**Plant material and essential oil extraction**

*L. pteridophyllum*, *L. ajanense*, *L. chuanxiong*, *L. thomsonii*, and *L. jeholense* fresh roots were collected from a wild population. The collected specimen was air dried for half a month and then ground to powder at the laboratory of traditional Chinese medicine protection and utilization of Beijing Normal University. Essential oils of plant materials were extracted by hydrodistillation with Clevenger-type apparatus for 4 hours. The residual distilled water in the extractions was removed with anhydrous sodium sulfate. The extracted essential oils were sealed in a glass container and then kept within a refrigerator at 4°C for subsequent applications.

**Insect culture**

*T. castaneum* and *L. serricorne* were cultured in a glass container with dried wheat flour and 5% yeast. 20–30 adults of *T. castaneum* and *L. serricorne* were put into the container covered with gauze. This container was kept within an incubator. The temperature was set to 28–30°C with relative humidity 70–80%. Two days later, these adults were taken out; then, the container was put in the incubator again. In each experiment, the adults (7–10 days old) of the same generation were selected.

*L. bostrychophila* was reared on wheat flour mixed with yeast and milk powder (1:1:1, w/w) in a triangle bottle applied polytetrafluoroethylene. Some folded small pieces of paper were put into the triangle bottle. 20–40 adults of *L. bostrychophila* were put into the bottle covered with gauze. The bottle was kept in an incubator. The temperature was set to 28–30°C with relative humidity 70–80%. The remaining steps were the same as those of *T. castaneum* and *L. serricorne*.

**GC-MS and GC-FID analysis**

Quartz capillary column was HP-5 MS (30 m × 0.25 mm × 0.25 μm). The initial temperature of the column was 50°C, and it was maintained for 3 minutes, and the temperature rise rate was 10°C/min until 290°C; the temperature of the vaporization chamber was 250°C; carrier gas was high-purity helium with flow rate of 1.0 mL/min; Ionization mode was EI; electronic energy was 70 ev; ion source temperature was set to 200°C; injection volume was 1 μL; mass scanning range ranged from 45 to 650 m/Z. The retention indices (RI) of the components were analyzed using a series of n-alkanes (C5–C36). Further identification was done by NIST 05 (Standard Reference Data, Gaithersburg, MD) and Wiley 275 libraries (Wiley, New York, NY) or with mass spectra from the literature.[18]

**Contact toxicity bioassay**

Contact toxicity of essential oils against *T. castaneum* and *L. serricorne* was tested according to Liu and Ho’s report.[19] Five concentrations of essential oils and isolated components were prepared with *n*-hexane. Aliquots of 0.5 μL of the test solution was treated to the thorax of the test insect. *n*-hexane was acted as control. Ten adult insects were treated with each concentration, and then, they were immediately transferred to a glass bottle and cultured in the incubator. Each treatment was replicated five times. Mortality should be recorded after 24 h, and probit analysis was applied to calculate the LD₅₀ values.

The procedures of *L. bostrychophila* was conducted as Zhao et al. reported.[20] Five concentrations of essential oils and isolated components were diluted with *n*-hexane. *n*-hexane was acted as control. The bottom of the Petri dish was covered with a filter paper disk (5.5 cm in diameter) and socked with
300 μL of the tested solution. The inner wall of the dish was coated with polytetrafluoroethylene to prevent the escape of *L. serricorne*. Ten treated *L. bostrychophila* were put into the filter paper. The glass dish was covered with a lid and kept in the incubator. Each treatment was replicated five times. Mortality should be recorded after 24 h, and probit analysis was applied to calculate the LD₅₀ values.

**Fumigant toxicity bioassay**

The fumigant activity of two essential oils and isolated components against *T. castaneum* and *L. serricorne* was tested according to Liu and Ho’s report.[^19] Five concentrations of essential oils and isolated components diluted with *n*-hexane were determined by preliminary test. *n*-hexane was set as control. Ten adult insects were put into a glass bottle with a diameter of 2.5 cm and a height of 5.5 cm. A filter paper with 2 cm in diameter was put in the bottle cap. Ten microliters of the diluted test solution was dropped to the filter paper. After solvent evaporation for 2 s, the glass bottle was covered with the cap. In addition, for the glass bottle of *L. serricorne*, the bottleneck was coated with polytetrafluoroethylene to prevent the escape of *L. serricorne*. All treatments and controls were conducted for five times. After 24 h, putting the glass bottle down, and observing for 5 minutes, if the insects did not move, they were considered dead. The mortality data was recorded.

The fumigant activity against *L. bostrychophila* was conducted according to Zhao et al.’s report.[^20] Ten test *L. bostrychophila* were picked up with a brush and put into a small glass bottle with a diameter of 0.5 cm and a height of 1.6 cm. The inner wall of the bottleneck was coated with polytetrafluoroethylene to prevent the escape of the test insects. The small glass bottle was placed in a large grinded bottle (250 mL) with a height of 12 cm and a diameter of 4 cm. The *n*-hexane was used as control and diluent solvent. Five concentrations were determined according to the preliminary test. Ten test insects were put into the small glass bottle. The test solution was dropped on the filter paper strip (3 cm in length, 2 cm in width, Whatman) with a pipette. The filter paper strip was stuck in the cap with solid glue. Then, the grinded bottle was quickly covered with the cap. Twenty-four hours later, the mortality of the test insect was observed and recorded. If the insect body does not move, it will be dead.

**Repellent activity bioassay**

The repellency of the essential oils and isolated components against *T. castaneum* and *L. serricorne* was evaluated by the method of area preference.[^19] Five concentrations of treatment solution were determined by preliminary tests, and *n*-hexane acted as controls and diluent. The filter paper with a diameter of 9 cm was cut into two equal parts from the middle. One-half part was treated with 500 μL of the test solution and another with equal *n*-hexane with a pipette. After the solvent was evaporated, the two parts were pasted on the bottom of the dish with solid glue (the wall of the dish is coated with polytetrafluoroethylene for *L. serricorne*). The number of test insects on the treatment (Nt) and control (Nc) filter papers at 2 and 4 h exposure was observed and recorded. The repellency rate (%) was calculated according to the formula. The repellent procedure for *L. bostrychophila* was same to the *T. castaneum* and *L. serricorne* except the diameter of filter paper was 6 cm and aliquot for one-half part was 150 μL. The value of percent repellency (PR) was calculated as follows:

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

**Statistical analysis**

All data was calculated by SPSS version 20.0 (IBM Corp., Armonk, NY, USA). PR values were subjected to arcsine square-root transformation before analysis of variance (ANOVA) and Tukey’s test. Probit analysis was used to calculate the value of LD₅₀.[^21]
Results

Essential oil chemical composition

The essential oils of air-dried root of five different Ligusticum species – L. pteridophyllum, L. ajanense, L. chuanxiong, L. thomsonii, and L. jeholense – were obtained by hydrodistillation. 0.15%, 0.65%, 0.43%, 0.20%, and 0.34% of yellow essential oils were yielded, respectively.

The results of chemical analysis of essential oils extracted from these five different Ligusticum species are listed in Table 1. As a result, a total of 12 compounds were identified from L. pteridophyllum, 13 from L. ajanense, 24 from L. chuanxiong, 21 from L. thomsonii, and 25 from L. jeholense, representing 98.3%, 72.3%, 96.5%, 80.8%, and 78.4% of the oils. The dominant compounds of these five different Ligusticum species were myristicin, apiol, ligustilide, and sylvestrene, respectively.

Contact toxicity

The results of contact ability of five Ligusticum essential oils against T. castaneum, L. serricorne, and L. bostrychophila are summarized in Table 2. Among the tested essential oils, L. chuanxiong (LD50 = 21.53 μg/adult) was more toxic than L. ajanense (LD50 = 21.53 μg/adult) to T. castaneum, followed by L. pteridophyllum (LD50 = 44.01 μg/adult) and L. thomsonii (LD50 = 87.99 μg/adult). L. chuanxiong (LD50 = 28.42 μg/adult) possessed the strongest contact ability to L. serricorne. However, the L. bostrychophila adult was more sensitive to L. pteridophyllum (LD50 = 7.87 μg/adult). The L. jeholense essential oil could not lead to 100% mortality at a concentration of 50 μg against T. castaneum and L. bostrychophila.

Fumigant toxicity

The fumigant toxicity of five Ligusticum essential oils against T. castaneum, L. serricorne, and L. bostrychophila is listed in Table 3. Overall, the essential oils extracted from five different Ligusticum species showed weak fumigant toxicity to the target stored insects. L. chuanxiong exhibited fumigant toxicity to L. serricorne with LD50 of 119.30 μg/cm2.

Repellent activity

The repellent activity of the five Ligusticum essential oils posed to T. castaneum, L. serricorne, and L. bostrychophila is listed in Figure 1. The results of this study demonstrated that the tested essential oils showed a good repellent activity against T. castaneum and L. serricorne at the concentrations of 78.63, 15.83, and 3.15 nL/cm2 at 2 h. Besides, compared to other tested samples, L. ajanense and L. chuanxiong essential oils possessed the strongest repellent activity against L. serricorne adults at the concentration of 78.63 nL/cm2 at 2 h. For L. bostrychophila, the tested essential oils exhibited pronounced repellent activity at concentrations of 63.17, 12.63, and 2.53 nL/cm2.

Discussion

This study revealed that the chemical composition of the five essential oils was totally different. The dominant compounds present in L. pteridophyllum, L. ajanense, L. chuanxiong, L. thomsonii, and L. jeholense were myristicin, apiol, ligustilide, D-limonene, and sylvestrene with the percentage of 90.5%, 43.6%, 46.2%, 40.2%, and 13.7%, respectively.

Previous research has reported that the bioactivity of essential oils is correlated with their bioactive compounds.[24] Therefore, we conducted a comprehensive study to compare the bioactivities of these five economic Ligusticum essential oils. The study revealed that the tested essential oils possessed strong contact toxicity on T. castaneum, L. serricorne, and L. bostrychophila. The results of contact
Table 1. Chemical components of five essential oils.

| No. | Compound name                        | RI exp. | RI lit. | Relative content (%) | Identified method |
|-----|--------------------------------------|---------|---------|----------------------|-------------------|
|     |                                      |         |         | L. pteridophyllum L. ajanense L. chuanxiong L. thomsonii L. jeholense |                   |
| 1   | β-Thujene                            | 966     | 966     | 1.3                  | 7.5               | MS;RI             |
| 2   | 3-Carene                             | 1010    | 1010    | 0.1                  |                   | MS;RI             |
| 3   | 1-methyl-3-(1-methylethyl)-Benzene    | 1023    | 1024    | 0.1                  |                   | MS;RI             |
| 4   | α-Terpineol                          | 1190    | 1191    | 1.3                  | 1.4               | 2.6               | MS;RI             |
| 5   | Phellandral                           | 1273    | 1273    | 0.1                  |                   | MS;RI             |
| 6   | 2-Methoxy-4-vinylphenol              | 1315    | 1316    | 0.4                  |                   | MS;RI             |
| 7   | α-Terpinylacetate                    | 1351    | 1353    | 0.1                  |                   | MS;RI             |
| 8   | 1,2-dimethoxy-4-(2-propenyl)-Benzene | 1384    | 1384    | 0.1                  |                   | MS;RI             |
| 9   | Myristicin                           | 1528    | 1526    | 90.5                 | 2.4               | 14.7              | 10.1              | MS;RI             |
| 10  | Elemicin                             | 1562    | 1561    | 1.3                  | 2.6               |                   | 1.8               | MS;RI             |
| 11  | Spathulonol                          | 1582    | 1582    | 0.2                  |                   |                   | 0.2               | MS;RI             |
| 12  | Butylidenephthalide                  | 1672    | 1673    | 0.3                  |                   |                   | 3.0               | MS;RI             |
| 13  | β-Pinene                             | 979     | 979     | 0.3                  | 0.3               | 0.7               |                   | MS;RI             |
| 14  | Caryophyllene oxide                  | 1578    | 1578    | 0.6                  |                   |                   | 5.4               | MS;RI             |
| 15  | Carotol                              | 1594    | 1594    | 0.2                  |                   |                   | 5.6               | MS;RI             |
| 16  | Apiol                                | 1682    | 1682    | 43.6                 |                   |                   |                   | MS;RI             |
| 17  | Selina-3,7(11)-diene                 | 1533    | 1532    | 0.4                  |                   |                   |                   | MS;RI             |
| 18  | D-Limonene                           | 975     | 975     | 18.5                 |                   | 0.1               | 40.2              | MS;RI             |
| 19  | B-cubebene                           | 1388    | 1388    | 2.7                  |                   |                   |                   | MS;RI             |
| 20  | ε-Muurolene                          | 1458    | 1458    | 0.2                  |                   |                   |                   | MS;RI             |
| 21  | β-Selinene                           | 1508    | 1509    | 0.3                  | 2.2               |                   | 6.8               | MS;RI             |
| 22  | γ-Gurjunene                          | 1479    | 1479    | 0.2                  |                   |                   |                   | MS;RI             |
| 23  | Ligustilide                          | 1682    | 1683    | 46.2                 |                   |                   |                   | MS;RI             |
| 24  | Senkyunalolide A                     | 1675    | 1675    | 26.9                 |                   |                   |                   | MS;RI             |
| 25  | Neocnidilide                         | 1735    | 1735    | 6.2                  |                   |                   |                   | MS;RI             |
| 26  | 3-n-butylphthalide                   | 1640    | 1640    | 4.9                  |                   |                   |                   | MS;RI             |
| 27  | 1,3,5-undecatriene                   | 1155    | 1157    | 1.8                  |                   |                   |                   | MS;RI             |
| 28  | 2-Methyl nonane                      | 1222    | 1220    | 0.1                  |                   |                   |                   | MS;RI             |
| 29  | O-Cymene                             | 1224    | 1224    | 0.1                  | 0.4               | 1.6               |                   | MS;RI             |
| 30  | β-Myrcene                            | 1256    | 1256    | 0.1                  | 0.9               |                   |                   | MS;RI             |
| 31  | Heneicosane                          | 1261    | 1260    | 0.1                  |                   |                   |                   | MS;RI             |
| 32  | Ferulic acid                         | 1266    | 1266    | 4.0                  |                   |                   |                   | MS;RI             |
| 33  | Caryophyllene oxide                  | 1423    | 1423    | 0.3                  | 1.0               | 7.1               |                   | MS;RI             |
| 34  | Cryptone                             | 1692    | 1692    | 0.1                  |                   |                   | 1.1               | MS;RI             |
| 35  | α-Pinene                             | 938     | 938     | 6.5                  |                   |                   |                   | MS;RI             |
| 36  | β-Terpinen                           | 1056    | 1056    | 0.3                  |                   |                   |                   | MS;RI             |
| 37  | n-Caprylaldehyde                     | 1007    | 1007    | 0.3                  |                   |                   |                   | MS;RI             |
| 38  | α-Thujene                            | 930     | 930     | 1.7                  |                   |                   |                   | MS;RI             |
| 39  | Limonene oxide                       | 1137    | 1137    | 0.6                  |                   |                   |                   | MS;RI             |

(Continued)
Table 1. (Continued).

| No. | Compound name | RI exp. | RI lit. | L. pteridophyllum | L. ajanense | L. chuanxiong | L. thomsonii | L. jeholense | Identified method |
|-----|---------------|---------|---------|-------------------|-------------|---------------|-------------|-------------|------------------|
| 40  | Carveol       | 1215    | 1215    | 2.4               |             |               |             |             | MS;RI            |
| 41  | β-Elemene     | 1410    | 1410    | 1.1               | 2.3         |               |             |             | MS;RI            |
| 42  | α-Bisabolene  | 1505    | 1505    | 0.6               | 2.5         |               |             |             | MS;RI            |
| 43  | β-Cyclogermacrane | 1495  | 1495    | 0.5               |             |               |             |             | MS;RI            |
| 44  | γ-Asarone     | 1578    | 1578    | 6.4               |             |               |             |             | MS;RI            |
| 45  | Ligustilide   | 1809    | 1809    | 0.6               |             |               |             |             | MS;RI            |
| 46  | Zerumbene     | 1733    | 1732    | 1.2               |             |               |             |             | MS;RI            |
| 47  | Sylvestrene   | 1009    | 1009    | 13.7              |             |               |             |             | MS;RI            |
| 48  | γ-Terpinene   | 1066    | 1064    | 0.8               |             |               |             |             | MS;RI            |
| 49  | Verbenol      | 1146    | 1148    | 0.7               |             |               |             |             | MS;RI            |
| 50  | Phellandral   | 1273    | 1273    | 0.3               |             |               |             |             | MS;RI            |
| 51  | α-Cubebeene   | 1351    | 1351    | 0.2               |             |               |             |             | MS;RI            |
| 52  | α-Copaene     | 1376    | 1376    | 0.2               |             |               |             |             | MS;RI            |
| 53  | γ-Muurolene   | 1477    | 1477    | 0.4               |             |               |             |             | MS;RI            |
| 54  | β-Farnesene   | 1440    | 1441    | 1.7               |             |               |             |             | MS;RI            |
| 55  | Germacrene D  | 1480    | 1481    | 1.1               |             |               |             |             | MS;RI            |
| 56  | Cuparene      | 1500    | 1501    | 0.1               |             |               |             |             | MS;RI            |
| 57  | Ambrettolid   | 1952    | 1952    | 3.8               |             |               |             |             | MS;RI            |
|     | **Total**     | **98.3**| **72.3**| **96.5**          | **80.8**    | **78.4**      |             |             |                  |

aRI exp., retention index as determined on an HP-5 MS column using the homologous series of n-alkanes
bRI lit., retention index taken from the NIST 05 library and the literatures.
cMS, based on comparison of mass spectra with those listed in the NIST 05 and Wiley 275 libraries and with published data.
Table 2. Contact toxicity of five essential oils against T. castaneum (TC), L. serricorne (LS), and L. bostrychophila (LB) adults at 24 h.

|        | LD₅₀ (µg/adult; µg/cm²) | Slope ± SE | Chi-square (χ²) | p-Value |
|--------|--------------------------|------------|-----------------|---------|
| TC     | L. pteridophyllum        | 87.99 (77.19–99.00) | 3.48 ± 0.41 | 12.89 | .954  |
|        | L. ajanense              | 44.01 (39.73–48.68) | 0.37 ± 0.43 | 14.60 | .963  |
|        | L. chuanxiong            | 21.53 (19.12–23.92) | 0.23 ± 0.28 | 17.90 | .909  |
|        | L. thomsonii             | 125.37 (102.80–219.32) | 0.24 ± 0.35 | 13.27 | .933  |
|        | L. jeholense             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | Pyrethrins a              | 0.26 (0.22–0.30) | 3.34 ± 0.32 | 13.11 | .950  |
| LS     | L. pteridophyllum        | 89.82 (80.59–99.44) | 4.26 ± 0.47 | 10.20 | .990  |
|        | L. ajanense              | 93.51 (86.15–101.82) | 0.43 ± 0.48 | 9.51 | .994  |
|        | L. chuanxiong            | 28.42 (26.24–31.06) | 0.14 ± 0.15 | 9.52 | .908  |
|        | L. thomsonii             | 105.55 (86.21–123.04) | 0.13 ± 0.18 | 13.80 | .944  |
|        | L. jeholense             | 50.16 (44.68–56.19) | 0.02 ± 0.84 | 11.25 | .981  |
|        | Pyrethrins a              | 0.24 (0.16–0.35) | 1.31 ± 0.20 | 17.36 | .791  |
| LB     | L. pteridophyllum        | 7.87 (6.89–8.85) | 3.60 ± 0.42 | 9.11 | .996  |
|        | L. ajanense              | 308.62 (281.34–339.16) | 0.83 ± 0.09 | 10.22 | .990  |
|        | L. chuanxiong            | 67.82 (61.86–74.57) | 4.16 ± 0.47 | 10.48 | .988  |
|        | L. thomsonii             | 195.06 (179.62–212.83) | 5.06 ± 0.57 | 11.93 | .971  |
|        | L. jeholense             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | Pyrethrins a              | 18.72 (17.60–19.92) | 2.98 ± 0.40 | 10.56 | .981  |

aData from Yang et al. [22]

Table 3. Fumigant toxicity of Ligusticum essential oils against T. castaneum (TC), L. serricorne (LS), and L. bostrychophila (LB) adults at 24 h.

|        | LD₅₀ (µg/adult; µg/cm²) | Slope ± SE | Chi-square (χ²) | p-Value |
|--------|--------------------------|------------|-----------------|---------|
| TC     | L. pteridophyllum        | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. ajanense              | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. chuanxiong            | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. thomsonii             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. jeholense             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | MeBr a                    | 1.7 | - | - | - |
| LS     | L. pteridophyllum        | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. ajanense              | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. chuanxiong            | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. thomsonii             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. jeholense             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | Phosphine b              | 119.3 (110.6–130.2) | 0.08 ± 0.01 | 9.52 | .908  |
|        | L. thomsonii             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. jeholense             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | Dichlorvos c              | 9.2 × 10⁻³ (7.1 × 10⁻³ – 11.4 × 10⁻³) | 2.1 ± 0.3 | 12.0 | .971  |
| LB     | L. pteridophyllum        | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. ajanense              | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. chuanxiong            | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. thomsonii             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | L. jeholense             | > 50.0 (mortality 0% ± 0%) | - | - | - |
|        | Dichlorvos c              | 1.4 × 10⁻¹ (1.1 × 10⁻¹ – 1.6 × 10⁻¹) | 8.7 ± 0.7 | 9.8 | .926  |

Data from Liu and Ho [21] b Data from Yang et al. [22] c Data from Liu et al. [23]

The toxicity test also indicated that the increasing concentration of essential oils could promote the toxic potential over the target stored insects. Overall, the contact toxicity of L. chuanxiong (LD₅₀ = 21.53, 28.42 µg/adult, respectively) against T. castaneum and L. serricorne was stronger than the other tested essential oils, in some cases, even greater than those reported essential oils of other plants over the same stored insects, such as Valeriana officinalis, Juniperus formosana, and Zanthoxylum dissitum. However, it was less toxic to L. bostrychophila compared to L. pteridophyllum essential oil. L. jeholense essential oil showed weak contact toxicity against the target stored insects. The above finding indicated that the contact toxicity of L. chuanxiong essential oil has the potential to be developed into a contact agent for inhibiting the damage caused by the stored insects. The
L. pteridophyllum, L. ajanense, L. chuanxiong, and L. jeholense essential oils and isolated compounds were presented with poor fumigant toxicity against the tested stored insects. We supposed that this may result from the interactions between various compounds. One important thing need to further study is the fumigant toxicity of isolated myristicin, apiol, ligustilide, and sylvestrene.

The behavioral responses of T. castaneum, L. serricorne, and L. bostrychophila adults to the L. pteridophyllum, L. ajanense, L. chuanxiong, L. thomsonii, and L. jeholense essential oils were evaluated. It suggested that the tested essential oils were presented with positive repellent effects on the target stored insects at high concentrations, which indicated that this behavioral response was in a dose-dependent manner by olfactory stimulus. [26] Among different stimuli, L. chuanxiong may be the proper repellent to T. castaneum when compared to the other essential oils.

To protect the stored products from damage by the stored insects, L. pteridophyllum, L. ajanense, L. chuanxiong, and L. thomsonii essential oils could be developed as one of the promising and eco-friendly repellents and contact agents in stored insect control strategies. However, in the present study,
we did not conduct the bioactivities of predominant compounds against *T. castaneum*, *L. serricorne*, and *L. bostrychophila*, which needs to be further studied.

**Conclusion**

The present study revealed that *L. pteridophyllum*, *L. ajanense*, *L. chuanxiong*, and *L. thomsonii* essential oils possessed good repellent activity and contact toxicity against *T. castaneum*, *L. serricorne*, and *L. bostrychophila*. High contact toxicity and good repellent activity made these five plants’ excellent affordable resources for the agents of eco-friendly alternatives to synthetic insecticides.

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**Conflicts of interest**

The authors declare no conflict of interest.

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