Insecticidal and Repellent Activities of Volatile Constituents from

*Litsea dilleniifolia* P. Y. Pai et P. H. Huang

Against Stored-Product Insects

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Abstract: The volatile oil from leaves of *Litsea dilleniifolia* P. Y. Pai et P. H. Huang was obtained by hydrodistillation. The qualitative and quantitative analysis of volatile constituents were performed by GC/MS and GC/FID. The volatile oil along with two major components was evaluated for their fumigant, contact toxicity and repellency against stored-product insects, namely *Tribolium castaneum* and *Liposcelis bostrychophila*. The volatile oil mainly contained decanal (48.4%), eremanthin (10.4%), zerumbone (10.1%) and estragole (9.2%). In bioassays, *L. dilleniifolia* volatile oil showed toxicity and repellent activities against *T. castaneum* and *L. bostrychophila*. The volatile oil, decanal and estragole at 78.63 nL/cm² could cause 82-92% repellency against *T. castaneum* at 4h post-exposure. Among them, decanal (LC₅₀ = 3.90 mg/L air) and estragole (LC₃₀ = 5.33 mg/L air) had significant fumigant toxicity against *T. castaneum*, and estragole was strongly toxic to *L. bostrychophila* (LC₃₀ = 0.61 mg/L air). The two components displayed a similar level of toxicity against *T. castaneum* in contact assays (LD₅₀ = 21.91 and 20.41 µg/adult, respectively). This work indicates that *L. dilleniifolia* is promising to be developed as botanical insecticides and repellents to control pest damage in warehouses and storage rooms.

Keywords: Botanical insecticides; *Liposcelis bostrychophila*; repellency; *Tribolium castaneum*; the volatile oil; toxicity. © 2021 ACG Publications. All rights reserved.

1. Plant Source

Leaves of *L. dilleniifolia* (530g) were collected in May 2019. The collection site is Chongzuo in Guangxi Province of China. The species was identified by Dr. Q. R., Liu (College of Life Sciences,

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Beijing Normal University) and voucher specimens (BNU-CMH-Dushushan-20190522-01) were deposited in the Herbarium of Beijing Normal University (Herbarium Code: BNU).

2. Previous Studies

The genus *Litsea* is primarily distributed in the tropics and subtropics [1]. It is reported that natural extracts from *Litsea* plants have bioactivities against stored-product insects. The chloroform extract and the essential oil of *L. cubeba* (Lour.) Pers. fruit could be remarkable toxicants and repellents to prevent *L. bostrychophila*, *Sitophilus zeamais* and *Lasioderma serricorne* [2-3]. The essential oil of *L. salicifolia* Roxb. ex Wall. was toxic to *S. zeamais* in fumigation and exerted contact, repellent and antifeedant effects on *T. castaneum* and *S. zeamais* [4]. In our previous work, the essential oil of *L. pungens* Hemsl. showed potency in fumigant, contact and repellent assays against *T. castaneum* and *L. bostrychophila* [5]. *L. dilleniifolia* P. Y. Pai et P. H. Huang from the genus *Litsea*, is predominant in the southwest of China. It is commonly used for wood or for synthetic fragrance [6]. There are no reports available about its bioactivities against storage pests at present.

3. Present Study

In this work, qualitative and quantitative analysis of the volatile oil were carried out by GC/MS and GC-FID. *T. castaneum* and *L. bostrychophila* were adopted as target insects. Fumigant, contact toxicity and repellent activities of the volatile oil from *L. dilleniifolia* were evaluated for the first time. To further know its active ingredients, two major components in the oil were also assessed.

**Table 1.** Chemical composition of the volatile oil from *L. dilleniifolia* P. Y. Pai et P. H. Huang

| No. | RI exp. a | RRI lit. b | Compound | Area (%) | Identified Method f |
|-----|-----------|------------|----------|---------|---------------------|
| 1   | 1102      | 1069-1112  | Nonanal<sup>c</sup> | 1.4     | MS; RI              |
| 2   | 1200      | 1185-1218  | Decanal<sup>c</sup> | 48.4    | MS; RI; Co          |
| 3   | 1214      | 1188-1203  | Estragole<sup>d</sup> | 9.2     | MS; RI; Co          |
| 4   | 1541      | 1536-1583  | (E)-Nerolidol<sup>e</sup> | 1.5     | MS; RI              |
| 5   | 1574      | 1545-1620  | Spathulenol<sup>e</sup> | 2.1     | MS; RI              |
| 6   | 1638      | 1607-1654  | Cubenol<sup>e</sup> | 2.6     | MS; RI              |
| 7   | 1733      | -          | Zerumbone<sup>e</sup> 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one<sup>e</sup> | 10.1     | MS                  |
| 8   | 1736      | -          | Eremanthin<sup>e</sup> | 3.4     | MS                  |
| 9   | 2018      | 2018       | Phyto<sup>e</sup> | 10.4    | MS; RI              |
| 10  | 2128      | 2087-2148  | Alkane ketones | 49.8    | MS; RI              |
|     |           |            | Phenylpropanoids | 9.2     | Oxygenated sesquiterpenes |
|     |           |            |                 | 30.1    | (E)-Nerolidol<sup>e</sup> |
|     |           |            | Total           | 91.0    |                     |

* a RI exp.: calculated retention index of individual constituents (HP-5MS column). b RRI lit.: range of retention index from literature values in the NIST library. c alkane ketones; d phenylpropanoids; e oxygenated sesquiterpenes; f MS: mass spectra; Co: co-injection with correspondingly standards.

The yield of the volatile oil was about 0.08% (w/w, g/g). Results of GC/MS and GC/FID analysis were shown in Table 1. The volatile oil was mainly composed of alkane ketones (49.8%), oxygenated
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sesquiterpenes (30.1%) and phenylpropanoids (9.2%). Major components included decanal (48.4%), eremanthin (10.4%), zerumbone (10.1%) and estragole (9.2%).

Investigations on the chemical profiles of L. dilleniifolia volatile oil were rarely available. Literature retrieval suggested that the essential oils of L. glaucescens leaf, L. guatemalensis leaf and L. cubea fruit, L. salicifolia fruit collected from China, Thailand, France, Mexico and Guatemala had a high percentage of monoterpenoids [3-4, 9-11]. The largest proportion of essential oils from Litsea species are identified as monoterpenes, which could be widely divided into two major types namely menthane and cineole. It is reported that menthane-type monoterpenoids present in most species of Litsea [1]. It is inconsistent with our findings and certainly there are other studies that contradict this. The essential oil of L. cubea distributed in North-east India revealed methylheptenone (30.9%) as the most abundant constituent in fruit [12]. In the essential oil of L. kostermansii leaf, sesquiterpenes took up the highest proportion at 99% [13]. Genes are the determinants of chemical disparities among species. The genotype of L. dilleniifolia collected here might differ greatly from that of some other Litsea plants. Besides gene factors, those arose from physiological and environmental factors, including plant organ, age, seasonality, soil and climate conditions, also influence the oil composition in quality and quantity [14-15]. It is significant to research the regulation of essential oil production in plants through these factors.

The results of fumigant toxicity were presented in Table 2. The volatile oil at the concentration of 50% resulted in 0% mortality of T. castaneum, so it was considered inactive here. Notably, decanal and estragole showed potent toxicity against T. castaneum in fumigation (LC50 = 3.90 and 5.33 mg/L air, respectively). On the other hand, the volatile oil and decanal were believed ineffective at the concentration of 10%, while estragole was strongly toxic to L. bostrychophila (LC50 = 0.61 mg/L air).

Table 2. Fumigant toxicity of the volatile oil from L. dilleniifolia against target insets

| Target insets * | Treatments | LC50 (mg/L air) b | 95% LCL-UCL c | Slope ± SE | Chi square χ² (df) | P-value |
|-----------------|------------|------------------|---------------|-----------|-------------------|---------|
| TC              | L. dilleniifolia | 0% mortality at the maximum concentration of 50% used | | | | |
|                 | Decanal | 3.90 | 3.40-4.45 | 4.41 ± 0.61 | 11.14 | 0.599 |
|                 | Estragole | 5.33 | 4.51-6.07 | 3.43 ± 0.45 | 17.31 | 0.794 |
|                 | MeBr d | 1.75 | - | - | - | - |
| LB              | L. dilleniifolia | 7 % ± 6 % mortality at the maximum concentration of 10 % used | | | | |
|                 | Decanal | 0.61 | 0.55-0.67 | 6.06 ± 0.76 | 15.95 | 0.596 |
|                 | Estragole | 1.35×10⁻³ | (1.08-1.62)×10⁻³ | 8.71 ± 0.65 | 9.78 | 0.926 |

Table 3. Contact toxicity of the volatile oil from L. dilleniifolia against target insets

| Target insets * | Treatments | LD50 (µg/adult)/ LC50 (µg/cm²) b | 95% LCL-UCL c | Slope ± SE | Chi square χ² (df) | P-value |
|-----------------|------------|---------------------------------|---------------|-----------|-------------------|---------|
| TC              | L. dilleniifolia | 44.03 | 21.48-63.80 | 1.30 ± 0.36 | 7.76 | 0.859 |
|                 | Decanal | 21.91 | 17.63-26.94 | 2.59 ± 0.39 | 9.86 | 0.705 |
|                 | Estragole | 20.41 | 18.95-22.36 | 5.18 ± 0.53 | 15.64 | 0.870 |
|                 | Pyrethrins d | 0.26 | 0.22-0.30 | 3.34 ± 0.32 | 13.11 | - |
| LB              | L. dilleniifolia | 265.14 | 234.71-301.73 | 4.80 ± 0.64 | 10.53 | 0.651 |
|                 | Decanal | 67.93 | 61.43-75.16 | 5.23 ± 0.60 | 16.86 | 0.533 |
|                 | Estragole | 30.22 | 28.13-32.49 | 8.04 ± 0.96 | 6.25 | 0.995 |
|                 | Pyrethrins e | 18.72 | 17.60-19.92 | 2.98 ± 0.40 | 10.56 | 0.987 |

* TC: T. castaneum; LB: L. bostrychophila. b LC50/ LD50: median lethal concentration/dose. c LCL-UCL: lower-upper confidence limit. d data from You et al (2014) [18]. e data from Yang et al (2014) [3].
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The results of contact toxicity were shown in Table 3. The volatile oil was effective against *T. castaneum* and *L. bostrychophila*, and its toxicity was weaker than the two individual components. Decanal and estragole had a similar level of potency against *T. castaneum* (LD$_{50} = 21.91$ and 20.41 µg/adult, respectively). Furthermore, they were much more toxic to *L. bostrychophila* with LC$_{50}$ values of 67.93 and 30.22 µg/cm$^2$ respectively, which were about 3.9 and 8.8 times stronger than the volatile oil.

| Table 4. Repellent activities of the volatile oil from *L. dilleniifolia* against target insets |
|-----------------------------------------------|
| **Target inset** | **Times** | **Treatments** | **Concentration (nL/cm$^2$), PR % (Mean ± SE)** |
| L. dilleniifolia | 2 h | Decanal | 72 ± 10 a | 52 ± 11 a | 42 ± 20 a | 28 ± 26 a | 18 ± 13 a | 78.63 |
| L. dilleniifolia | 2 h | Estragole | 66 ± 11 a | 70 ± 8 a | 78 ± 4 a | 84 ± 8 b | 52 ± 26 a | 65.73 |
| L. dilleniifolia | 2 h | DEET | 90 ± 3 ab | 94 ± 4 b | 56 ± 34 a | 36 ± 33 a | 32 ± 25 a | 8.63 |
| L. dilleniifolia | 2 h | F, P | 100 ± 0 b | 98 ± 3 b | 78 ± 14 a | 66 ± 10 a | 8 ± 5 a | 0.63 |
| L. dilleniifolia | 2 h | df | 5.035, 0.012 | 12.014, 0 | 0.716, 0.557 | 1.138, 0.364 | 0.653, 0.592 | 0.13 |
| L. dilleniifolia | 4 h | Decanal | 28 ± 14 a | 66 ± 10 a | 12 ± 19 ab | 36 ± 10 ab | 2 ± 12 a | 78.63 |
| L. dilleniifolia | 4 h | Estragole | 44 ± 16 ab | 86 ± 8 c | 70 ± 12 b | 54 ± 8 ab | 22 ± 8 a | 65.73 |
| L. dilleniifolia | 4 h | DEET | 12 ± 19 ab | 82 ± 8 a | 68 ± 5 a | 54 ± 8 ab | 22 ± 8 a | 8.63 |
| L. dilleniifolia | 4 h | F, P | 12 ± 19 ab | 38 ± 10 ab | 12 ± 19 ab | 36 ± 10 ab | 2 ± 12 a | 0.63 |
| L. dilleniifolia | 4 h | df | 3.16, 19 | 3.16, 19 | 3.16, 19 | 3.16, 19 | 3.16, 19 | 0.13 |
| L. bostrychophila | 2 h | Decanal | 92 ± 4 a | 54 ± 15 a | 32 ± 15 a | 28 ± 24 ab | 14 ± 20 a | 63.17 |
| L. bostrychophila | 2 h | Estragole | 82 ± 9 a | 64 ± 22 a | 52 ± 14 a | 64 ± 24 b | 36 ± 24 a | 54.15 |
| L. bostrychophila | 2 h | DEET | 96 ± 3 a | 82 ± 8 a | 68 ± 11 a | 44 ± 29 a | -12 ± 29 a | 36 ± 24 a |
| L. bostrychophila | 2 h | F, P | 1998, 0.155 | 0.421, 0.740 | 0.607, 0.620 | 2.117, 0.138 | 0.301, 0.824 | 0.10 |
| L. bostrychophila | 2 h | df | 3.16, 19 | 3.16, 19 | 3.16, 19 | 3.16, 19 | 3.16, 19 | 0.13 |
| L. bostrychophila | 4 h | Decanal | 26 ± 12 a | 14 ± 19 a | -30 ± 19 a | 12 ± 19 ab | 18 ± 15 a | 63.17 |
| L. bostrychophila | 4 h | Estragole | 26 ± 1 a | 54 ± 9 b | 44 ± 16 bc | 38 ± 10 ab | 20 ± 23 a | 54.15 |
| L. bostrychophila | 4 h | DEET | 92 ± 5 b | 84 ± 3 b | 82 ± 8 c | 54 ± 17 b | 28 ± 14 a | 36 ± 24 a |
| L. bostrychophila | 4 h | F, P | 7.717, 0.002 | 5.856, 0.007 | 8.797, 0.001 | 3.567, 0.038 | 1.728, 0.201 | 0.10 |
| L. bostrychophila | 4 h | df | 3.16, 19 | 3.16, 19 | 3.16, 19 | 3.16, 19 | 3.16, 19 | 0.13 |

*TC: T. castaneum; LB: L. bostrychophila. PR: values of percentage repellency were subjected to an arcsine square-root transformation. Means in the same concentration condition and post-exposure followed by the same letters mean having no significant difference (P>0.05, Tukey’s HSD tests).*

The results of repellent activity were listed in Table 4. The volatile oil, decanal and estragole were repellent towards *T. castaneum*. The volatile oil and two components at the maximum concentration of 78.63 nL/cm$^2$ could result in 82-92% (Class V) repellency against *T. castaneum* at 4h post-exposure. Among them, estragole at 78.63 nL/cm$^2$ showed ≥90% (Class V) PR values at 2h post-exposure. At 15.73 nL/cm$^2$, it could be comparable to DEET and there was no significant difference between them (P>0.05, Tukey’s HSD test) at 2h post-exposure. According to the total PR values, the volatile oil was obviously repellent to *L. bostrychophila* at the highest concentration of 63.17 nL/cm$^2$. Decanal and estragole had weak repellency within the concentration range of 63.17-0.10 nL/cm$^2$.

Here, decanal was proved to have contact activity against *T. castaneum* with the LD$_{50}$ value of 21.91 µg/adult, which was about 4 times weaker than 2-dodecanone (LD$_{50} = 5.21$ µg/adult) and 2-tridecanone (LD$_{50} = 5.35$ µg/adult) [19-20]. The three components share the same chemical skeleton.
Among them, 2-dodecanone and 2-tridecanone possess a carbonyl group individually, while decanal has an aldehyde group. It was speculated that the contribution of carbonyl group to the contact toxicity could be much stronger than that of aldehyde group in this class of alkane ketones. Some researchers believe that the presence of a carbonyl group in ketone compounds is important and the double bond of the carbonyl group is likely to enhance the inhibition on the enzyme acetylcholinesterase (AChE). It has been suggested that competitive inhibition of AChE could be the mechanism of action for essential oil components [21]. Furthermore, decanal had strong toxicity against *T. castaneum* but was inactive against *L. bostrychophila* in fumigant bioassays. The sensitivity to the same substance varies among insect species. Researchers also proposed that a variety of insects differ in their sensitivity to insect repellents and differences have loose relation to the taxonomic distance between groups [22].

Estragole exists in many aromatic plants and it has been extensively applied in foodstuffs as flavoring agents [23]. There are many research articles published about its bioactivities against stored-product insects including *Sitophilus zeamais*, *S. oryzae*, *Callosobruchus chinensis*, *Lasioderma serricorne* and *Blattella germanica*. This compound has the potential to serve as a good toxicant to control a wide range of storage pests. Volatile constituents from natural plants have been extensively investigated for developing eco-friendly biopesticides, for they have obvious bioactivities against pests, diverse mechanisms of action, relatively mild toxicity to non-target organisms and potential to be used as reducing and stabilizing agents for the synthesis of nanopesticides [24]. This work provides some evidence for *L. dilleniifolia* in the development of botanical insecticides and repellents.

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Supporting Information

Supporting information accompanies this paper on [http://www.acgpubs.org/journal/records-of-natural-products](http://www.acgpubs.org/journal/records-of-natural-products)

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