

## Investigation of Pesticidal Effects of *Peucedanum terebinthaceum* Essential Oil on Three Stored-Product Insects

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**Abstract:** The aim of this work was to evaluate the bioactivities of *Peucedanum terebinthaceum* (Fisch.) Fisch. ex Turcz. essential oil and its three second rich constituents against *Tribolium castaneum* Herbst, *Lasioderma serricornis* Fabricius and *Liposcelis bostrychophila* Badonnel. The essential oil from aerial part of *P. terebinthaceum* was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry. Thirty-three constituents were confirmed by GC-MS, which accounting for 91.5% of the total oil. The principal constituents included  $\beta$ -thujene (21.4%),  $\beta$ -terpinene (11.8%), germacrene D (9.4%) and dihydro-cis- $\alpha$ -copaene-8-ol (8.0%). Besides,  $\beta$ -myrcene (6.2%), Linalyl isovalerate (4.3%),  $\alpha$ -pinene (4.0%), caryophyllene (3.6%), (Z)- $\alpha$ -farnesene (3.6%) and  $\beta$ -elemene (3.0%) were also detected in relatively lower content. The essential oil possessed promising potential in pest control, as it showed strong contact toxicity and repellent effects on *T. castaneum* and *L. serricornis*. Three major constituents  $\alpha$ -pinene, caryophyllene and  $\beta$ -myrcene were toxic to three insect species in contact assays and showed repellent effects on *T. castaneum* and *L. bostrychophila*. This work revealed the insecticidal capacity of *P. terebinthaceum* and would provide some information for the development of new strategies in the control of insect pests.

**Keywords:** Fumigant toxicity; Contact toxicity; *Tribolium castaneum* Herbst; *Lasioderma serricornis* Fabricius; *Liposcelis bostrychophila* Badonnel. © 2019 ACG Publications. All rights reserved.

### 1. Introduction

*Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *Lasioderma serricornis* Fabricius (Coleoptera: Anobiidae) and *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae) are important stored-product insects with cosmopolitan habitat [1]. As secondary pests, they do great harm to the processed products such as flour [2]. Except damaging stored food commodities directly, these insects are responsible for the product contamination, which are caused by fungal infections [2] and insect excreta [3].

For its high efficiency, synthetic chemical insecticide has been served as a powerful insect controlling management. Regarding to consumers' safety and healthy, synthetic insecticides are not allowed to have direct contact with stored products such as grain and tobacco. Thus fumigation is a

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much proper approach to control stored-products insect pest. Nowadays, phosphine is widely considered as a suitable fumigation and commercially available [4]. However, continuous and discriminate use of synthetic pesticides has resulted in serious problems. Apart from the negative effects on the environment [5] and non-target organisms [6], the development of insect resistance is one of the most concerns [4, 7-9]. Above mentioned problems have necessitated the search for alternatives to synthetic pesticides and it has promoted the emergence of several ecological approaches [10]. Among those existing alternative strategies, plant-derived insecticides are considered a promising one.

To protect themselves from pathogens and insect pests, plants have evolved a natural defensive capacity, which would synthesize low-molecule-weighted substances such as monoterpenoids and sesquiterpenoids [11-13]. These substances could be easily extracted by hydrodistillation and are generally known as essential oils [14]. Traditionally, essential oils are of great industrial and therapeutic values [15]. Moreover, essential oils have found their applications in botanical insecticidal management. Currently, quite a lot studies have indicated bioactive properties of essential oils, including insecticidal [16], repellent [17, 18] and antimicrobial [19, 20]. Additionally, the complex constituents of essential oils provide various insecticidal mechanisms thus effectively reduce the development of resistance [12, 16].

Umbelliferae covers a series of aromatic plants with hollow stems. Plants in this family usually possess unique flavour and have been used as seasonings in food, such as coriander, carrot and fennel. Umbelliferae also plays an important role in traditional Chinese medicine, as several typical medicinal plants including *Angelica sinensis* (Oliv.) Diels, *Ligusticum sinense* Oliv. and *Radix bupleuri* chinese belong to it. And there are some researches about the antioxidant activity of Umbelliferae plants [21]. Apart from their medical values, *A. sinensis* [22] and *L. sinense* [23] have been reported to possess repellent activity against mosquito vectors. As traditional Chinese medicines, the repellent potency showed by *A. sinensis* and *L. sinense* means a great deal to safe and green management control on mosquito vectors. Moreover, there are some published papers recording the insecticidal potential of Umbelliferae plants against stored insect pests [24-27], house flies [28] and fire ants [29].

However, the reports on *Peucedanum terebinthaceum* are quite insufficient, let alone researches about the insecticidal activity of *P. terebinthaceum*. As a second rich compound of *P. terebinthaceum* essential oil,  $\alpha$ -pinene is found in the oils of many species of many coniferous trees, notably the pine. According to already published literatures, the fumigant toxicity of  $\alpha$ -pinene has been confirmed against mosquito vectors, such as *Lycoriella mali* [30], *Culex pipiens molestus* [31, 32] and *Aedes aegypti* [33]. Moreover, the contact and fumigant toxicity of  $\alpha$ -pinene against *T. castaneum* [34, 35], *L. serricornis* and *L. bostrychophila* [36] has been reported. Another compound caryophyllene is a natural bicyclic sesquiterpene, constituting many essential oils. It has been reported to repel *Diaphorina citri* [37] and be toxic to the malaria vector *Anopheles subpictus*, the dengue vector *Aedes albopictus* and the Japanese encephalitis vector *Culex tritaeniorhynchus* [38]. And  $\beta$ -myrcene has been found to possess insecticidal activity against mosquito vectors [39]. In present work, we qualitatively and quantitatively analyzed the chemical constituents of the essential oil extracted from *P. terebinthaceum* by GC-FID and GC-MS. Based on GC-MS results, we aimed at evaluating the insecticidal and repellent activities of the essential oil isolated from *P. terebinthaceum* and its three constituents against three species of stored insect pests.

## 2. Materials and Methods

### 2.1. Plant Material

The aerial parts of *P. terebinthaceum* were collected at Anshan City, Liaoning Province (122°10' E - 123°41' E, 40°27' N - 41°34' N). The collections were identified by Dr. Q.R. Liu (College of Life Science, Beijing Normal University, Beijing, China). Voucher specimen (BNU-CMH-Dushushan-2013-09-01-013) were deposited in the Herbarium (BNU) of the College of Resources Science and Technology, Faculty of Geographical Science, Beijing Normal University.

## 2.2. Insects Culture

*T. castaneum* was introduced from He'nan University of Technology. *L. serricorne* and *L. bostrychophila* were from China Agriculture University. These three target insects were identified by Dr. Z.L. Liu (College of Plant Protection, China Agriculture University, Beijing, China). *T. castaneum* and *L. serricorne* were cultured on a mixture of yeast and flour (1:10, w/w). *L. bostrychophila* was cultured on a mixture of milk powder, yeast and flour (1:2.5:10, w/w/w). Testing insects were maintained in dark incubator at  $30 \pm 1$  °C and 70-80% relative humidity.

## 2.3. Essential Oil

The aerial parts of *P. terbinthaceum* (400 g) were grounded into powder and subjected to hydrodistillation for 6 h to obtain the essential oil. The extracted essential oil was dehydrated by anhydrous sodium sulfate. The final volume (0.9 mL) was measured for yield calculation. The processed oil was stored in a refrigerator at 4 °C until further bioassay use.

## 2.4. Chemicals

$\alpha$ -Pinene (95%), caryophyllene (>90%) and  $\beta$ -myrcene (>70%) were purchased from Tokyo Chemical Industry (Toshima, Kita-Ku, Tokyo, Japan). The solvent *n*-hexane was purchased from Beijing Chemical Works (Beijing, China). Fluon was served to prevent the escape of insect pests and purchased from Beijing Sino-Rich Material Science (Beijing, China).

## 2.5. GC-MS and GC-FID analysis

An Agilent 6890N gas chromatograph equipped with an Agilent 5973N mass selective detector (70 eV) were served for the performance of gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The carrier gas was helium, with a flow rate of 1000  $\mu$ L/min. One  $\mu$ L (1% solution) of sample was injected. The scanning frequency was from 50 to 550 m/z. A HP-5MS (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) capillary column was served. The column temperature was set at 50°C for 2 min at the beginning, then increased to 150°C at 2°C/min and held for 2 min, finally increased to 250°C at 10°C/min and held for 5 min. Retention index (RI) was calculated referring to a homologous series of *n*-alkanes (C<sub>8</sub>-C<sub>40</sub>). Mass spectra and calculated RIs were compared with those recorded in NIST 05 (Standard Reference Data, Gaithersburg, MD, USA) and Wiley 275 libraries (Wiley, New York, NY, USA), to confirm the constituents. Relative percentages of individual constituents were determined by averaging the GC-FID peak area%.

## 2.6. Bioassays

In present work, three second rich compounds *i.e.*  $\alpha$ -pinene, caryophyllene and  $\beta$ -myrcene of *P. terbinthaceum* essential oil were selected and evaluated the fumigant, contact and repellent activity against *T. castaneum*, *L. serricorne* and *L. bostrychophila*. According to our previous works,  $\alpha$ -pinene and caryophyllene showed great insecticidal activity against various insect species. But the insecticidal and repellent activity of  $\beta$ -myrcene was not evaluated. Thus here the insecticidal and repellent activity of three second rich compounds were compared. On the other aspect, the insecticidal and repellent activities of four most abundant compounds *i.e.*  $\beta$ -thujene,  $\beta$ -terpinene, germacrene D and dihydro-cis- $\alpha$ -copaene-8-ol were not evaluated, as they are neither commercially available nor chemically separation suitable. It is truly a deficiency and requires further researches.

### 2.6.1. Fumigant Toxicity

Pre-experiments (three sample concentrations set as 50%, 10% and 2% for *T. castaneum* and *L. serricorne*, 5%, 1% and 0.2% for *L. bostrychophila*) were performed to determine appropriate concentration ranges for formal bioassays. Generally, 5-7 sample concentrations were used for LC<sub>50</sub> calculation. And the negative control was solvent *n*-hexane.

Fumigant toxicity of the essential oil and its three components against *T. castaneum* and *L. serricorne* were described by Liu and Ho [40]. Ten testing insects were introduced into a glass vial (height 5.5 cm, diameter 2.5 cm) and a cap with a filter paper which containing 10 µL dilutions was screwed tightly. Parafilm was used to ensure the air tightness.

Regard of the fumigant toxicity against *L. bostrychophila*, we adopted the method described by Zhao et al [41]. A small glass bottle (8 mL) containing 10 insects was put into a large glass bottle (250 mL). A cap with a filter paper strip (3.5 cm × 1.5 cm) was covered on the large bottle and parafilm was used to prevent gas leakage as well.

Each concentration as well as negative control were repeated five times. Treated devices were kept under the same condition as that of insect culture. Mortalities were recorded after 24 h and served for LC<sub>50</sub> calculation.

### 2.6.2. Contact Toxicity

Pre-experiments were performed in advance for range-finding. In formal bioassays, 5-7 concentrations were prepared for LD<sub>50</sub> calculation. And the solvent *n*-hexane was used as a negative control.

The method described by Liu and Ho [40] was adopted to evaluate the contact toxicity against *T. castaneum* and *L. serricorne*. Each insect was treated with 0.5 µL dilutions or solvent on their dorsal thorax. Every 10 insects treated with same concentration of a sample were introduced into a glass bottle.

The contact toxicity against *L. bostrychophila* was performed with the method described by Zhao et al. [41]. A filter paper (diameter 5.5 cm) was treated with 300 µL dilutions. After solvent evaporation, the filter paper was stuck to the bottom of a fluon-covered petri dish (diameter 5.5 cm). Ten testing insects were released at the center, then a cover with two holes was applied on it.

Each concentration of experiments was repeated five times. These devices were kept under the same condition as that of insect culture. Mortalities were counted after 24 hours for LD<sub>50</sub> calculation.

### 2.6.3. Repellent Activity

The repellent activities against *T. castaneum* and *L. serricorne* were adopted from Liu and Ho [40]. Five concentrations (78.63, 15.83, 3.15, 0.63 and 0.13 nL/cm<sup>2</sup>) of samples were prepared in *n*-hexane. A filter paper (diameter 9 cm) were cut into two. One half was treated with 500 µL dilutions and the other half was with 500 µL solvent as control. After solvent evaporation, two halves of the filter paper were stuck on a Petri dish (diameter 9 cm) and twenty testing insects were released at the center of the dish.

Regarding *L. bostrychophila*, these five concentrations were set as 63.17, 12.63, 2.53, 0.51 and 0.10 nL/cm<sup>2</sup>. Besides, the diameter of filter paper and Petri dish was 5.5 cm. The volume of dilutions or solvent was 150 µL for each half. Similarly, twenty insects were released at the center of a dish.

The numbers of insects settling at the control half were recorded after 2 and 4 h. Each concentration of these bioassays were repeated five times. And the devices were kept under the same condition as that of insect culture.

### 2.7. Statistical Analysis

In fumigant and contact toxicities, LC<sub>50</sub> and LD<sub>50</sub> values were determined by Probit analysis [42] using SPSS V20.0 (IBM, New York, NY, USA). Moreover, 95% Confidence intervals, related parameters and chi-square values were estimated as well.

In repellent activity, the percent repellency (PR) was determined by the following equation:

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

where the N<sub>t</sub> is the number of insects on the treated half and the N<sub>c</sub> is that on the control half. The PR values were transformed into arcsine and square root values to perform analysis of variance (ANOVA) and Tukey's HSD test. Differences between means were considered significant when  $p < 0.05$ .

### 3. Results and Discussion

#### 3.1. Chemical Components of the Essential Oil

The yield of *P. terebinthaceum* essential oil was 0.23% (v/w). The essential oil was rich in monoterpenoids (51.7%), followed by sesquiterpenoids (38.6%). Out of 33 constituents identified from the essential oil,  $\beta$ -thujene (21.4%) was the major compound followed by  $\beta$ -terpinene (11.8%), germacrene D (9.4%) and dihydro-cis- $\alpha$ -copaene-8-ol (8.0%). Besides,  $\beta$ -myrcene (6.2%), linalyl isovalerate (4.3%),  $\alpha$ -pinene (4.0%), caryophyllene (3.6%), (Z)- $\alpha$ -farnesene (3.6%) and  $\beta$ -elemene (3.0%) were also detected in our sample.

It has been reported that *Peucedanum* species were usually rich in essential oils and coumarins [43, 44], which were basic substances for most reported pharmacological activities [45]. The yield of essential oils was various, as it could be dependent on several factors such as season [46, 47], harvested stage [48] and plant part [49, 50].

The chemical profile of the essential oil was shown in Table 1. Totally, 33 constituents were detected by GC-MS and accounted for 91.5%. In our sample, monoterpenoids (51.7%) and sesquiterpenoids (38.6%) were the dominated constituents. Usually, essential oils extracted by hydrodistillation or steam-distillation were primarily dominated by monoterpenoids and sesquiterpenoids [45]. According to previous reports,  $\alpha$ -pinene was the common and major constituent (range from 4.0% to 38.7%) detected from the essential oils of *Peucedanum* species. Moreover, germacrene D,  $\beta$ -myrcene and caryophyllene were also reported with various relative contents by previous literatures. In the same genus, chemical constituent differentiations in quality and quantity often occur. As secondary metabolites, the quality and quantity of essential oil components were influenced by environmental (climatic and soil) factors and plant organs [45]. Such a variation in chemical constituents and amount would be a great challenge for commercial development and standardization of essential oils as insecticides. Variations of essential oils in quality and quantity would make a difference to insecticidal effects. There are some papers reporting the influence of different mixture ratios on its insecticidal potential [7, 51, 52]. Thus, it highlighted the importance of insecticidal mechanism researches.

#### 3.2. Fumigant and Contact toxicities

The results of fumigant toxicity were presented in Table 2. One of its compounds  $\alpha$ -pinene showed promising fumigant toxicity to three testing insect species *T. castaneum*, *L. serricornis* and *L. bostrychophila*, with  $LC_{50}$  values of 12.15, 38.07 and 1.43 mg/L air, respectively. And  $\beta$ -myrcene was only fumigant toxic to *T. castaneum* with  $LC_{50}$  of 21.91 mg/L air. However, *P. terebinthaceum* essential oil and caryophyllene showed no fumigant toxicity to any of three testing insect species.

Generally,  $\alpha$ -pinene and  $\beta$ -myrcene exhibited the different level of fumigant toxicity to three testing insect species.  $\alpha$ -Pinene exhibited great fumigant toxicity to three insect species, with  $LC_{50}$  (the concentration lethal to half of given insect species over a certain time) values of 12.15, 38.07 and 1.43 mg/L air for *T. castaneum*, *L. serricornis* and *L. bostrychophila* respectively. The fumigant toxicity of  $\alpha$ -pinene to insect pests has been confirmed, such as *Hypothenemus hampei* [53], *Sitophilus granarius* [54] and *Callosobruchus chinensis* [55]. And it has been reported that essential oils of 10 species of Myrtaceae were toxic to *T. castaneum* in the fumigation, all of which contained  $\alpha$ -pinene within a range of 0.2% - 32.5% [56]. Besides,  $\beta$ -myrcene was toxic to *T. castaneum* ( $LC_{50}$  = 21.91 mg/L air) but failed to cause mortality of *L. serricornis* and *L. bostrychophila* at the maximum testing concentration used. Our results were in accordance with that of Abdelgaleil *et al.* [57]. It is worth noting that *T. castaneum* was much more sensitive to monoterpenoids  $\alpha$ -pinene and  $\beta$ -myrcene than sesquiterpenoid caryophyllene. However, further researches are required to verify if monoterpenoids were much more efficient than sesquiterpenoids to *T. castaneum*.

The essential oil and caryophyllene showed no fumigant toxicity to any of three testing insect species at all. As the essential oil showed no fumigant toxicity, an assumption had been proposed that antagonistic effects existed between some constituents in the essential oil. Especially to *T. castaneum*,  $\alpha$ -pinene and  $\beta$ -myrcene showed fumigant toxicity ( $LC_{50}$  = 12.15 and 21.91 mg/L air, respectively)

while the essential oil was inactive at all. Pavela [12] assessed the acute toxicity of 30 compounds and their mutual binary combinations against *Culex quinquefasciatus* Say larvae and found that the mixture of  $\alpha$ -pinene and  $\beta$ -myrcene showed an antagonistic action. It could partially explain the toxic discrepancy between the essential oil and two individual constituents  $\alpha$ -pinene and  $\beta$ -myrcene.

**Table 1.** Chemical constituents identified in the essential oil extracted from *P. terebinthaceum*

Peak No.	RI <sup>a</sup>	RI <sup>b</sup>	Compounds	Molecular Formula	Relative Content (%)	Identified Method
1	922	920	1,3,6-Heptatriene, 2,5,5-trimethyl-	C <sub>10</sub> H <sub>16</sub>	0.1	MS; RI
2	932	933	$\alpha$ -Pinene	C <sub>10</sub> H <sub>16</sub>	4.0	MS; RI
3	950	954	Fenchene	C <sub>10</sub> H <sub>16</sub>	0.6	MS; RI
4	966	963	$\beta$ -Thujene	C <sub>10</sub> H <sub>16</sub>	21.4	MS; RI
5	988	988	$\beta$ -Terpinene	C <sub>10</sub> H <sub>16</sub>	11.8	MS; RI
6	992	992	$\beta$ -Myrcene	C <sub>10</sub> H <sub>16</sub>	6.2	MS; RI
7	1007	1009	$\alpha$ -Phellandrene	C <sub>10</sub> H <sub>16</sub>	0.7	MS; RI
8	1007	1007	3-Carene	C <sub>10</sub> H <sub>16</sub>	0.6	MS; RI
9	1026	1029	o-Cymene	C <sub>10</sub> H <sub>14</sub>	1.4	MS; RI
10	1050	1052	(E)-Ocimene	C <sub>10</sub> H <sub>16</sub>	0.4	MS; RI
11	1083	1985	Isoterpinolene	C <sub>10</sub> H <sub>16</sub>	0.7	MS; RI
12	1130	1130	Allo-Ocimene	C <sub>10</sub> H <sub>16</sub>	0.1	MS; RI
13	1164	1166	2-Methylundecane	C <sub>12</sub> H <sub>26</sub>	0.2	MS; RI
14	1175	1176	4-Terpineol	C <sub>10</sub> H <sub>18</sub> O	1.5	MS; RI
15	1187	1185	Crypton	C <sub>9</sub> H <sub>14</sub> O	1.2	MS; RI
16	1222	1221	3,7-Dimethylundecane	C <sub>13</sub> H <sub>28</sub>	0.3	MS; RI
17	1235	1237	Cumal	C <sub>10</sub> H <sub>12</sub> O	0.7	MS; RI
18	1280	1278	Bornyl acetate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	0.5	MS; RI
19	1281	1283	Phellandral	C <sub>10</sub> H <sub>16</sub> O	0.5	MS; RI
20	1354	1355	$\alpha$ -Cubebene	C <sub>15</sub> H <sub>24</sub>	0.4	MS; RI
21	1394	1397	$\beta$ -Elemene	C <sub>15</sub> H <sub>24</sub>	3.0	MS; RI
22	1417	1420	Caryophyllene	C <sub>15</sub> H <sub>24</sub>	3.6	MS; RI
23	1442	1443	(Z)- $\alpha$ -Farnesene	C <sub>15</sub> H <sub>24</sub>	3.6	MS; RI
24	1454	1456	$\alpha$ -Caryophyllene	C <sub>15</sub> H <sub>24</sub>	1.2	MS; RI
25	1464	1467	$\beta$ -Selinene	C <sub>15</sub> H <sub>24</sub>	2.0	MS; RI
26	1473	1474	Linalyl isovalerate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	4.3	MS; RI
27	1484	1484	Germacrene D	C <sub>15</sub> H <sub>24</sub>	9.4	MS; RI
28	1497	1499	(E,Z)- $\alpha$ -Farnesene	C <sub>15</sub> H <sub>24</sub>	0.9	MS; RI
29	1574	1574	15-Copaenol	C <sub>15</sub> H <sub>24</sub> O	0.2	MS; RI
30	1620	1608	Dihydro-cis- $\alpha$ -copaene-8-ol	C <sub>15</sub> H <sub>26</sub> O	8.0	MS; RI
31	1624	1625	cis- $\alpha$ -Copaene-8-ol	C <sub>15</sub> H <sub>24</sub> O	0.5	MS; RI
32	1644	1644	Caryophylla-4(12),8(13)-dien-5.beta.-ol	C <sub>15</sub> H <sub>24</sub> O	0.2	MS; RI
33	1645	1645	$\delta$ -Cadinol	C <sub>15</sub> H <sub>26</sub> O	1.4	MS; RI
			Monoterpenoids		51.7	
			Sesquiterpenoids		38.6	
			Total		91.5	

<sup>a</sup> Retention index relative to *n*-alkanes (C<sub>8</sub>-C<sub>40</sub>) on HP-5MS column; <sup>b</sup> Retention index taken from the NIST 05 library; MS: based on comparison of mass spectra with those listed in the NIST 05 and Wiley 275 libraries or with those reported in the literatures.

The results of contact toxicity were presented in Table 3. Totally, *P. terebinthaceum* had a promising potential for insect managing, as the essential oil and its three second rich constituents showed contact toxicity to three insect species. Regarding these two Coleoptera species *T. castaneum* and *L. serricorne*, the contact toxicity of the essential oil ( $LD_{50} = 13.48$  and  $17.93 \mu\text{g}/\text{adult}$ , respectively) could mainly attribute to the existence of  $\beta$ -myrcene ( $LD_{50} = 19.93$  and  $16.61 \mu\text{g}/\text{adult}$ , respectively). And caryophyllene ( $LD_{50} = 52.52 \mu\text{g}/\text{cm}^2$ ) would greatly play an important role in the overall contact effect of the essential oil ( $LD_{50} = 165.07 \mu\text{g}/\text{cm}^2$ ) on *L. bostrychophila*.

Based on Table 3, we found that the  $LD_{50}$  values of  $\alpha$ -pinene and  $\beta$ -myrcene were much higher than that of the essential oil. It indicated that complex interactions would exist between these compounds in *P. terebinthaceum* essential oil. Comparing with the fumigant results, it seems that the interaction in the essential oil would vary with different mode of actions, which need additional researches to verify. If this phenomenon does exist, careful considerations about the applying method should be given.

**Table 2.** Fumigant toxicity of the essential oil from *P. terebinthaceum* on some storage pests

Insects <sup>a</sup>	Samples	LC <sub>50</sub> (mg/L air)	95% Confidence Intervals	Slope $\pm$ SE	$\chi^2$	P-value
TC	<i>P. terebinthaceum</i>	-	-	-	-	-
	$\alpha$ -Pinene <sup>b</sup>	12.15	10.85-13.50	$3.13 \pm 0.38$	16.51	-
	Caryophyllene	-	-	-	-	-
	$\beta$ -Myrcene	21.91	19.72-24.37	$6.36 \pm 0.86$	4.05	0.991
	MeBr <sup>c</sup>	1.75	-	-	-	-
LS	<i>P. terebinthaceum</i>	-	-	-	-	-
	$\alpha$ -Pinene <sup>d</sup>	38.07	34.49-41.72	$5.83 \pm 0.72$	20.01	0.641
	Caryophyllene	-	-	-	-	-
	$\beta$ -Myrcene	-	-	-	-	-
	Phosphine <sup>d</sup>	$9.23 \times 10^{-3}$	$7.13-11.37 \times 10^{-3}$	$2.12 \pm 0.27$	11.96	0.971
LB	<i>P. terebinthaceum</i>	-	-	-	-	-
	$\alpha$ -Pinene <sup>d</sup>	1.43	1.37-1.51	$9.31 \pm 1.03$	14.49	0.912
	Caryophyllene	-	-	-	-	-
	$\beta$ -Myrcene	-	-	-	-	-
	Dichlorvos <sup>e</sup>	$1.35 \times 10^{-3}$	-	-	-	-

<sup>a</sup> TC: *T. castaneum*, LS: *L. serricorne*, LB: *L. bostrychophila* <sup>b</sup> data from Wu et al. [35]; <sup>c</sup> data from Liu et al. [40]; <sup>d</sup> data from Yang [36]; <sup>e</sup> data from Liu et al. [58].

### 3.3. Repellent Activities

The repellent effects were presented in Table 4. The essential oil showed promising repellent potential to three testing insects. The PR values against *T. castaneum* and *L. serricorne* reached 90% at  $78.63 \text{ nL}/\text{cm}^2$  after 2 and 4 h exposure and were more than 85% against *L. bostrychophila*. Regarding to *T. castaneum* and *L. serricorne*, the essential oil showed outstanding repellent effects at all testing concentrations after 2 and 4 h exposure, as its PR values were comparable to that of the positive control DEET, which is commercially available in insect management. The duration of *P. terebinthaceum* was considerable, as it exerted significant repellent effect after 4 h. As for *L. bostrychophila*, the essential oil exhibited significant repellent effects at  $63.17$  and  $12.63 \text{ nL}/\text{cm}^2$  and moderate at  $2.53$ ,  $0.51$  and  $0.10 \text{ nL}/\text{cm}^2$ . Our results confirmed the repellent efficacy of *P. terebinthaceum* in the control of stored-product pests.

**Table 3.** Contact toxicity of the essential oil from *P. terebinthaceum* on some storage pests

Insects <sup>a</sup>	Samples	LD <sub>50</sub> (µg/adult; µg/cm <sup>2</sup> )	95% Confidence Intervals	Slope ± SE	χ <sup>2</sup>	P- value
TC	<i>P. terebinthaceum</i>	13.48	11.03 -15.79	3.40 ± 0.55	4.34	0.987
	α-Pinene <sup>b</sup>	22.47	17.61-26.63	1.82 ± 0.30	17.12	-
	Caryophyllene <sup>c</sup>	25.86	22.61-30.24	2.97 ± 0.39	13.13	-
	β-Myrcene	19.93	17.56-22.77	4.55 ± 0.63	6.32	0.934
	Pyrethrins <sup>b</sup>	0.26	0.22-0.30	3.34 ± 0.32	13.11	0.950
LS	<i>P. terebinthaceum</i>	17.93	16.17-19.87	6.73 ± 0.93	1.34	1.000
	α-Pinene <sup>d</sup>	76.82	68.69-86.82	5.41 ± 0.58	17.48	0.785
	Caryophyllene <sup>c</sup>	43.79	39.16-48.93	3.82 ± 0.43	11.82	-
	β-Myrcene	16.61	14.63-18.81	4.73 ± 0.64	6.14	0.941
	Pyrethrins <sup>d</sup>	0.24	0.16-0.35	1.31 ± 0.20	17.36	0.791
LB	<i>P. terebinthaceum</i>	165.07	155.38-174.23	11.97 ± 1.88	6.43	0.778
	α-Pinene <sup>d</sup>	873.73	830.73-921.29	8.27 ± 0.93	18.63	0.772
	Caryophyllene <sup>c</sup>	52.52	43.52-60.83	2.77 ± 0.39	9.62	-
	β-Myrcene	320.49	301.81-339.97	10.45 ± 1.40	7.44	0.878
	Pyrethrins <sup>c</sup>	18.72	17.60-19.92	2.98 ± 0.40	10.56	0.987

<sup>a</sup>TC: *T. castaneum*, LS: *L. serricorne*, LB: *L. bostrychophila* <sup>b</sup> data from Wu et al. [35]; <sup>c</sup> data from Cao et al. [59]; <sup>d</sup> data from Yang et al. [36].

Regarding to the repellent effects of these three major constituents, they showed different profiles of three stored insect pests. As for *T. castaneum*, β-myrcene showed weak repellent or even attractive effect (at 15.83, 3.15, 0.63 and 0.13 nL/cm<sup>2</sup>), while α-pinene and caryophyllene would mainly explain the repellent effect of the essential oil, as they showed repellent effect. As for *L. serricorne*, all of three major constituents exhibited weak repellency. The different repellent potentials of essential oils and chemical constituents to different insect species were often occur. It has been observed from β-pinene, caryophyllene oxide [59], terpinen-4-ol, g-terpinene [60] and p-cymene-8-ol [61] in previous papers. Moreover, Hori [62] reported the attractive effect of linalool and β-caryophyllene on female *L. serricorne* at 0.1 and 1 µL. Comparing with strong repellent ability of the essential oil to *L. serricorne*, the weak repellency of three second rich constituents indicated the major role that other constituents possibly played and necessitated further researches for repellent activity of individual constituents in the essential oil.

Regarding to *L. bostrychophila*, at the highest concentration (63.17 nL/cm<sup>2</sup>), α-pinene, caryophyllene and β-myrcene all would contribute to the repellent effect of the essential oil after 2 and 4 h. But at other four concentrations (12.63, 2.53, 0.51 and 0.01 nL/cm<sup>2</sup>) this trend was different between 2 and 4 h. At 2 h after exposure, β-myrcene would mainly explain the repellency and the PR value increased with the concentration decreasing from 12.63 to 0.51 nL/cm<sup>2</sup>. At 4 h after exposure, β-myrcene would be the major contribution at 12.63 nL/cm<sup>2</sup>, while β-myrcene and caryophyllene together were the contribution at 2.53, 0.51 and 0.01 nL/cm<sup>2</sup>.

The essential oil exerted strong repellent effects on *T. castaneum* and *L. serricorne* at all five testing concentrations. The results indicated the promising potential of the essential oil to repel *T. castaneum* and *L. serricorne*. As for *L. bostrychophila*, the essential oil exerted notable repellent effect at 63.17 and 12.63 nL/cm<sup>2</sup>. And the major constituent β-myrcene would deserve a deep research for its repellent ability.



**Table 4.** Repellency of *P. terebinthaceum* essential oil and its three major compounds against *T. castaneum*, *L. serricorne* and *L. bostrychophila*

Treatment	2 h (% ± SE <sup>c</sup> )					4 h (% ± SE <sup>c</sup> )				
TC <sup>a</sup>	78.63 <sup>b</sup>	15.83 <sup>b</sup>	3.15 <sup>b</sup>	0.63 <sup>b</sup>	0.13 <sup>b</sup>	78.63 <sup>b</sup>	15.83 <sup>b</sup>	3.15 <sup>b</sup>	0.63 <sup>b</sup>	0.13 <sup>b</sup>
<i>P. terebinthaceum</i>	94 ± 2bc	98 ± 2c	70 ± 5bc	64 ± 8bc	28 ± 10bc	96 ± 4b	76 ± 10cd	54 ± 6bc	38 ± 7c	36 ± 10b
α-Pinene	48 ± 5a	34 ± 7b	8 ± 4a	88 ± 7c	70 ± 9c	56 ± 2a	38 ± 6bc	28 ± 9b	94 ± 4d	18 ± 7b
Caryophyllene	82 ± 6b	38 ± 7b	30 ± 7ab	16 ± 8a	22 ± 7b	98 ± 2b	24 ± 7b	28 ± 7b	-46 ± 5a	-16 ± 7a
β-Myrcene	36 ± 4a	-12 ± 7a	14 ± 6a	24 ± 9ab	-14 ± 8a	46 ± 7a	-8 ± 9a	-10 ± 4a	-6 ± 6b	28 ± 7b
DEET	100 ± 0c	98 ± 3c	78 ± 14c	66 ± 10c	8 ± 5ab	96 ± 3b	82 ± 8d	68 ± 5c	54 ± 8c	22 ± 8b
LS <sup>a</sup>	78.63 <sup>b</sup>	15.83 <sup>b</sup>	3.15 <sup>b</sup>	0.63 <sup>b</sup>	0.13 <sup>b</sup>	78.63 <sup>b</sup>	15.83 <sup>b</sup>	3.15 <sup>b</sup>	0.63 <sup>b</sup>	0.13 <sup>b</sup>
<i>P. terebinthaceum</i>	90 ± 5c	72 ± 4bc	64 ± 7c	60 ± 3c	36 ± 10b	96 ± 2c	72 ± 4c	46 ± 5c	66 ± 9b	46 ± 7c
α-Pinene	-22 ± 2a	30 ± 7b	42 ± 10c	-4 ± 5a	22 ± 5b	-8 ± 5a	-16 ± 8ab	28 ± 6bc	-10 ± 10a	10 ± 8b
Caryophyllene	42 ± 9b	-16 ± 7a	6 ± 4b	28 ± 8bc	-48 ± 9a	28 ± 8b	-2 ± 7b	6 ± 5ab	56 ± 6b	-46 ± 8a
β-Myrcene	14 ± 9b	-10 ± 7a	-22 ± 6a	4 ± 7ab	10 ± 6b	8 ± 7ab	-34 ± 6a	-10 ± 6a	-8 ± 7a	4 ± 5b
DEET	88 ± 7c	76 ± 14c	28 ± 7c	20 ± 14abc	16 ± 7b	98 ± 4c	78 ± 9c	58 ± 15c	56 ± 14b	46 ± 7c
LB <sup>a</sup>	63.17 <sup>b</sup>	12.63 <sup>b</sup>	2.53 <sup>b</sup>	0.51 <sup>b</sup>	0.10 <sup>b</sup>	63.17 <sup>b</sup>	12.63 <sup>b</sup>	2.53 <sup>b</sup>	0.51 <sup>b</sup>	0.10 <sup>b</sup>
<i>P. terebinthaceum</i>	86 ± 5c	82 ± 8b	14 ± 6bc	16 ± 7ab	32 ± 7bc	90 ± 3b	68 ± 9b	20 ± 7b	38 ± 5b	22 ± 5b
α-Pinene	82 ± 4bc	10 ± 6a	-6 ± 7ab	-6 ± 6a	-20 ± 9a	62 ± 7a	10 ± 7a	-22 ± 10a	10 ± 4a	-2 ± 9a
Caryophyllene	52 ± 6a	16 ± 9a	-24 ± 6a	28 ± 7bc	10 ± 4b	80 ± 9ab	6 ± 8a	44 ± 9bc	54 ± 7b	30 ± 7b
β-Myrcene	58 ± 2ab	22 ± 5a	40 ± 4c	58 ± 7c	50 ± 8c	62 ± 4a	54 ± 5b	48 ± 8bc	46 ± 5b	14 ± 7ab
DEET	94 ± 6c	82 ± 5b	86 ± 8d	70 ± 12c	56 ± 3c	92 ± 5b	84 ± 3b	82 ± 8c	54 ± 17b	28 ± 14b

<sup>a</sup> TC: *T. castaneum*, LS: *L. serricorne*, LB: *L. bostrychophila*; <sup>b</sup> Concentration (nL/cm<sup>2</sup>); <sup>c</sup> The values are expressed as the means ± error of five independent experiments; means followed by the same lower-case are not significantly different according to Tukey's HSD test. ANOVA was applied to the data and the differences between the mean values were given at the 5% significance level according to Tukey's HSD test.

#### 4. Conclusion

In this work, we analyzed the chemical constituents of *P. terebinthaceum* essential oil in quality and quantity by GC-MS and GC-FID. The analysis showed that 33 constituents were totally confirmed by GC-MS and accounted for 91.5%, among which were dominated by monoterpenoids (51.7%) and sesquiterpenoids (38.6%). The principle constituents of the essential oil were  $\beta$ -thujene (21.4%),  $\beta$ -terpinene (11.8%), germacrene D (9.4%) and dihydro-cis- $\alpha$ -copaene-8-ol (8.0%).

Besides, we evaluated the insecticidal and repellent activities of the essential oil and its three second rich constituents against three stored insect pests. The essential oil exhibited great contact toxicity on three testing insect species ( $LC_{50} = 13.48$  and  $17.93 \mu\text{g}/\text{adult}$ ,  $165.07 \mu\text{g}/\text{cm}^2$  against *T. castaneum*, *L. serricornis* and *L. bostrychophila*, respectively). The essential oil showed great repellent effect on three insect species, as PR values against *T. castaneum* and *L. serricornis* reached 90% at  $78.63 \text{ nL}/\text{cm}^2$  after 2 and 4 h exposure and were more than 85% against *L. bostrychophila*. Three major constituents  $\alpha$ -pinene, caryophyllene and  $\beta$ -myrcene all exhibited great contact toxicity to three stored insects. The  $LC_{50}$  values of  $\alpha$ -pinene were 22.47, 76.82  $\mu\text{g}/\text{adult}$  and 873.73  $\mu\text{g}/\text{cm}^2$  against *T. castaneum*, *L. serricornis* and *L. bostrychophila*, respectively, while  $LC_{50}$  values of caryophyllene were 25.86, 43.79 and 52.52 and of  $\beta$ -myrcene were 19.93, 16.61 and 320.49. These three major compounds showed different trends in repellent effect on three insect species. Our results suggest that *P. terebinthaceum* essential oil might have potential to be used as a natural insecticide.

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