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Research Article

Contact Toxicity and Repellency of the Essential Oil from *Bupleurum bicaule* Helm against Two Stored Product Insects

Xiao-Meng Wei,[1] Shan-Shan Guo,[2] Hua Yan,[3] Xian-Long Cheng,[3] Feng Wei,[1,3] and Shu-Shan Du[2]

[1] *Faculty of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 102488, China*
[2] *Beijing Key Laboratory of Traditional Chinese Medicine Protection and Utilization, Faculty of Geographical Science, Beijing Normal University, Beijing 100875, China*
[3] *National Institutes for Food and Drug Control, China Food and Drug Administration, Beijing 100050, China*

Correspondence should be addressed to Feng Wei; weifeng@nifdc.org.cn and Shu-Shan Du; dushushan@bnu.edu.cn

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Essential oils obtained from many plants showed various kinds of insecticidal properties; some of them have been considered as alternative insecticides for pest control. The present study was aimed at determining the chemical composition of the essential oil from the roots of *Bupleurum bicaule* Helm, as well as evaluating the contact and repellent activities of the oil and four identified compounds against *Lasioderma serricorne* and *Liposcelis bostrychophila* adults. The essential oil was extracted by hydrodistillation, and its components were analyzed by gas chromatography-mass spectrometry (GC-MS). 26 components were determined and the main compounds included trans-2-isopropylbicyclo[4.3.0]non-3-en-8-one (25.9%), 4,5-dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene (23.5%), and 1,4-dimethoxy-2-tert-butylbenzene (4.3%). It was found that the essential oil exhibited contact toxicity against *L. serricorne* (LD_{50} = 11.91 μg/adult), but the contact toxicity against *L. bostrychophila* could not be observed. The essential oil also showed strong repellent activity against *L. serricorne* with percent repellency of 100% at 78.63 nl/cm^2. Four chemical compounds, 1,4-dimethoxy-2-tert-butylbenzene, bornyl acetate, (2E,4E)-2,4-nonadienal, and β-bisabolene, exhibited various levels of bioactivities. The experimental results indicated that the essential oil of *B. bicaule* and its individual compounds could be used in insecticidal and repellent strategies for stored product insects.

1. Introduction

The economy of agriculture and food industry has been threatened by insects in the process of production, transportation, and storage. It is estimated that about 10–40% of the world's annual stored product loss was caused by the insects [1]. The cigarette beetle (*Lasioderma serricorne* Fabricius) and the booklouse (*Liposcelis bostrychophila* Badonnel) are two major insects in the warehouses of stored foods, grains, and some herbal medicines [2, 3]. Under appropriate environmental conditions, they can reproduce rapidly [4, 5]. The insects not only consume the stored commodities, but also lead to contamination and deterioration of stored products in the short term. A great effort has been focused on the prophylaxis of insects in stored products. Many methods, such as the cold storage, the light traps, and the chemical treatment [5] have been successively used to control the insects. Among them the chemical treatment is the most commonly used method with different chemical synthetic insecticides. However, a series of negative effects such as insecticide residues and environmental damage have been exposed, so that the development of the new environmental friendly, economical, and effective methods [2, 6] is necessary. Antagonistic storage, a traditional method, has been used for Chinese medicinal material conservation for a long history. Our ancestors skillfully used the special odors of some Chinese herbs to repel the insects and to achieve the purpose of protecting another kind of medicinal materials [7]. For instance, Rhizoma Alismatis is easily infected by insects. If it is stored with Cortex Moutan which has a special odor, Rhizoma Alismatis would not be susceptible to insects, and the quality of the two medicinal materials would be

maintained for a long time [8]. The antagonistic storage method is mostly related to the special odor reeked off by the stored materials. Thus, the essential oils of the stored materials could be considered as the effective elements in antagonistic storage method. The research on the essential oils would be helpful to reveal the mechanism of the antagonistic storage method. In practice, this kind of research has become a hot topic in the field of controlling stored product insects [9–11].

Bupleurum bicaule Helm (Chinese name: Zhuiye Chaihu) is widely distributed in northeast region of China, and its roots have been medicinally used for the treatment of fever, cold, hypochondriac pain, hepatitis, biliary tract infection, irregular menstruation, uterine prolapse, and anal prolapse [12–15]. As is well known, essential oils are abundant in Umbelliferae (Apiaceae) plants [16]. Many of these essential oils obtained from Umbelliferae (Apiaceae) plants have been proven to possess insecticidal activities against certain kinds of insects. For examples, the essential oil of *Heracleum persicum* showed fumigant toxicity against *Callosobruchus maculatus* [17], *Pimpinella anisum* essential oil also showed strong fumigant toxicity against *Ephestia kuehniella* and *Sitophilus oryzae* [18], and *Ferula asafetida* essential oil possessed strong repellent activity against *Tribolium castaneum* [19].

The phytochemistry of *Bupleurum* plants has been investigated, and some of the secondary metabolites obtained from *B. salicifolium* showed antifeedant activity against *Spodoptera littoralis* [20]. For *B. bicaule*, the chemical components of the essential oil obtained from this plant have been reported [21]. In this work, the essential oil of *B. bicaule* was further investigated, and its insecticidal and repellent activities against the cigarette beetles and booklice adults were evaluated; meanwhile, the same kinds of biological activity tests were carried out with four representative compounds identified in the sample of the essential oil.

2. Materials and Methods

2.1. Plant Materials and Preparation of the Essential Oil. Roots of *B. bicaule* were collected in Hailar, Inner Mongolia Autonomous Region, China (northern latitude: 9°5′–19°27′; east longitude: 119°30′–120°35′) in August 2016. The species was identified by Dr. Yan H. (National Institutes for Food and Drug Control, China Food and Drug Administration, China). The voucher specimen (NIFDC-20160801-01) was deposited at Herbarium (NIFDC) of National Institutes for Food and Drug Control. After air drying for one week (25–28°C and 13–20% RH), the dried roots (2.4 kg) were ground to powders with a grinding mill. The extraction of essential oil was carried out by hydrodistillation using a modified Clevenger apparatus for 8 h [22]. The distilled oils were dried over anhydrous sodium sulfate and stored in glass vials at 4°C.

2.2. GC-MS and GC-FID Analysis. The GC-MS analysis of *B. bicaule* essential oil was performed by an Agilent 6890N GC/MS instrument equipped with an Agilent 5973N mass selective detector and a HP-5 MS column (30 m × 0.25 mm × 0.25 μm). The carrier gas was helium, and the flow rate was 1.0 mL/min. Analytical conditions are as follows: column temperature was programmed from 50°C (2 min) to 150°C at 2°C/min and kept isothermal for 2 min, then increased to 250°C at 10°C/min, and held for 5 min. The injector temperature was 250°C. The MS spectra were obtained in the electron-impact mode with ionization energy of 70 eV, and mass range was from m/z 50 to 550.

The essential oil was diluted with *n*-hexane to prepare a GC sample solution with content of 1% (v/v), and the sample solution of 0.1 μL was injected into the instrument. Relative percentages of the individual components were determined by GC-FID via percentage peak area calculations. These components were further identified based on their retention index and by comparison with NIST 05 (Standard Reference Data, Gaithersburg, MD) and Wiley 275 GC-MS databases (Wiley, New York, NY).

2.3. Test Insects. The cigarette beetles were originally delivered from Henan University of Technology, China, since 2013, and the species was identified by Dr. Lv J. H. (School of Food Science and Technology, Henan University of Technology, China). The booklice were adopted from China Agricultural University and identified by Dr. Liu Z. L. (China Agricultural University, China). The cigarette beetles were reared on wheat flour mixed with yeast (10 : 1, w/w) at 12-13% moisture content, and the booklice were cultured with the flour mixture, which was prepared with flour, yeast, and milk powder at 10 : 1 : 1 mixed proportion [2]. Insect rearing were carried out in the dark in incubators at 29-30°C and 70–80% RH. All the unsexed insects used in the experiments were one to two weeks old. The escape proof was made of polytetrafluoroethylene (Beijing Sino-Rich Co., Ltd., Beijing, China) coating on all insect containers and experimental Petri dishes.

2.4. Bioactivities

2.4.1. Preparation of the Treatment Solutions. The essential oil and four representative compounds found in the oil were selected for bioactivity assays. The chemical compounds (2E,4E)-2,4-nonadienal and β-bisabolene were purchased from TCI (Shanghai) Development Co., Ltd. (Shanghai, China). 1,4-Dimethoxy-2-tert-butylbenzene was obtained from JW&Y PharmLab (Shanghai), PharmLab Co., Ltd. (Shanghai, China). Bornyl acetate was purchased from Acros Organics Co., Ltd. (Geel, Belgium). N,N-Diethyl-3-methylbenzamide (DEET) was obtained from the National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang, China). Each of the above samples was dissolved in the solvent of *n*-hexane, and their stock solutions with the known concentrations were prepared.

2.4.2. Contact Toxicity. In the evaluation of contact toxicity, two kinds of experimental methods were used based on the size of the insects. The contact toxicity against cigarette beetles was carried out with the methods described in the literature [23]. Before the formal test, the appropriate concentration range was determined by preliminary tests. Ultimately, the essential oil and four selected compounds

were diluted with n-hexane into five concentrations. Drop 0.5 μL dilutions on the dorsal thorax of each insect using a TopPette (0.5–10 μL, Dragon Laboratory Instruments Co., Ltd., Beijing, China). Ten insects were used for each concentration, and the experiment was replicated five times for each concentration. Then the treated and control insects were transferred to glass vials (2.5 cm in diameter, 5.5 cm in height). After being cultured in an incubator (29-30°C and 70–80% R.H) for 24 h, the mortality of tested insects was observed and recorded, and the LD_{50} values were calculated by Probit analysis (SPSS 20.0) [24].

For booklice, the contact toxicity was tested as described [25]. A filter paper of 5.5 cm in diameter was treated with 300 μL dilutions of each test concentration. The treated filter paper was fixed in a 5.5-cm-diameter Petri dish with solid glue, and 10 test insects were placed in each Petri dish. Five concentrations (in n-hexane) and five replicates of each concentration were used. In the above experiments, n-hexane was used as a negative control. The chemical synthetic insecticide pyrethrins was usually served as a positive control; its mortality was taken from Yang et al.'s data with the same experimental methods [26]. The mortality of insects was recorded after these covered-Petri dishes were placed in an incubator at 29-30°C and 70–80% RH for 24 h. The mortality data was corrected by Abbott's formula [27]:

$$\text{corrected mortality}\,(\%) = 100 \times \frac{(M_c - M_t)}{(1 - M_c)}, \qquad (1)$$

where M_t is the observed mortality and M_c is the control mortality. With these data, the LD_{50} values were calculated by Probit analysis (SPSS 20.0).

2.4.3. Repellent Activity. The repellent activity of *B. bicaule* essential oil and four components against cigarette beetles and booklice was investigated with the method proposed by Zhang et al. [28]. The experiments were set with five concentrations (cigarette beetles: 78.63, 15.73, 3.15, 0.63, and 0.13 nL/cm^2; booklice: 63.17, 12.63, 2.53, 0.51, and 0.10 nL/cm^2) and two observation times (2 h and 4 h). For cigarette beetles, the essential oil and four selected compounds were diluted with n-hexane. Filter paper (9 cm in diameter) was symmetrically cut into two pieces, and 500 μL dilution of each concentration was dropped evenly on half of the filter paper, while the other half was treated with 500 μL of n-hexane as a negative control. After being air-dried for 30 s, both two pieces were stuck to the bottom of the Petri dish (9 cm in diameter) with solid glue abreast. For booklice, due to its tiny body, the diameters of the Petri dish and filter paper were reduced to 5.5 cm. Filter paper was cut into two pieces, and one piece was treated with 150 μL of each solution separately. The other half (negative control) was treated with the same volume of n-hexane. For each test, 20 insects were placed at the center of the disk and covered quickly with lids. All the above procedures were repeated five times for each concentration, and each experiment was repeated three times.

As a commercial insect repellant, DEET was served as positive control. Counts of insects on each half of the paper were recorded separately. The percent repellency (PR) of each test was calculated by the following formula [23]:

$$\text{PR}\,(\%) = \left[\frac{(N_c - N_t)}{(N_c + N_t)}\right] \times 100, \qquad (2)$$

where N_c is the number of insects in the negative control half and N_t is the number of insects in the tested half. The percent repellency was transformed to arcsine square root values for analysis of variance (ANOVA), and the effect on the transformed arcsine of the percent repellency of different treatments (the essential oil, the four compounds, and the control) at each concentration and exposure time was analyzed separately. Significant differences in repellence rates among treatments were given by the analysis of Tukey's test (SPSS 20.0 for Windows 2007).

3. Results and Discussion

3.1. Chemical Constituents of the Essential Oil. The yellow essential oil was obtained from *B. bicaule* with a yield of 0.04% *(v/w)*. Its chemical components were analyzed by the GC-MS. 26 compounds were identified and their relative contents in the oil sample were determined. The total amount of all the identified compounds was 85.5% (w/w) of the essential oil. The content of trans-2-isopropylbicyclo[4.3.0]non-3-en-8-one was 25.9%, and the content of 4,5-dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene was 23.5%. These two compounds could be considered as the major components of the essential oil. The results are summarized in Table 1.

With the further analysis of the GS-MS results, it was found that the essential oil contained a plenty of monoterpenes and sesquiterpenes, and their contents accounted for 27.0% and 21.3% of the essential oil, respectively. The other types of chemical components included aldehydes, alkynes, and aromatic compounds. In a published paper [21], Xu and his partners reported that 19 compounds were identified in the root oil of *B. bicaule* collected from Inner Mongolia, China, and all the types of chemical components mentioned above also existed in Xu et al.'s oil sample. The five types of components accounted for about 60% of the essential oils in the two works. However, the significant difference between the results presented in Xu's paper and our results could be figured out. The compound with the highest content predicted in Xu's paper was 4-tridecen-6-yne (42.6%), but only its isomer, 6-tridecen-4-yne, was discovered in our sample and its content only accounted for 2.7% of the essential oil. The highest content compound in our sample was trans-2-isopropylbicyclo[4.3.0] non-3-en-8-one (25.9%), and it was not reported in the literature.

The other differences were showed in Figure 1, the content of aldehydes (12.6%) in Xu's paper was higher than that of terpenoids (6.2%), and the monoterpenes only accounted for a minor proportion (0.8%). However, in our case, the content of terpenoids was 48.3% (27.0% for monoterpenes and 21.3% for sesquiterpenes), which was much higher than that of aldehyde compounds (4.8%). The above differences might be caused by many variables such as the growth environment

Table 1: Chemical composition of the essential oil of *Bupleurum bicaule*.

Compounds	Relative content (%)	[a]RI_{exp}	[b]RI_{lit}	Identification methods[c]
Aldehydes				
(2E,4E)-2,4-Octadienal	0.8	1113	1109	MS; RI
(2E,4E)-2,4-Nonadienal	1.2	1214	1217	MS; RI
(2E,4E)-2,4-Decadienal	2.8	1317	1316	MS; RI
Monoterpenes				
Bornyl acetate	1.1	1280	1281	MS; RI
Trans-2-isopropylbicyclo[4.3.0]non-3-en-8-one	25.9	1765	1765	MS; RI
Sesquiterpenes				
α-Longipinene	0.5	1352	1351	MS; RI
β-Elemene	0.8	1391	1393	MS; RI
β-Cuvebene	1.5	1390	1388	MS; RI
1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	1.9	1456	1454	MS; RI
Calarene	2.1	1467	1463	MS; RI
γ-Gurjunene	2.0	1479	1472	MS; RI
(E,E)-α-Farnesene	0.4	1505	1505	MS; RI
β-Bisabolene	2.7	1510	1511	MS; RI
Cuparene	2.9	1502	1499	MS; RI
(E)-Nerolidol	0.5	1565	1564	MS; RI
Spathulenol	0.9	1582	1578	MS; RI
Caryophyllene oxide	1.4	1583	1580	MS; RI
Humulene oxide II	1.4	1607	1611	MS; RI
α-Cadinol	1.4	1653	1654	MS; RI
τ-Muurolol	0.6	1662	1660	MS; RI
Juniper camphor	0.3	1690	1693	MS; RI
Alkynes				
(6Z)-6-Tridecen-4-yne	2.7	1470	1470	MS; RI
Aromatic compounds				
1,4-Dimethoxy-2-tert-butylbenzene	4.3	1477	1477	MS; RI
Myristicin	1.6	1529	1523	MS; RI
2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	0.3	1772	1773	MS; RI
Others				
4,5-Dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene	23.5	1357	1357	MS; RI
Total	85.5			

[a]Retention index (RI) in this experiment relative to the homologous series of n-alkanes (C_5–C_{36}) on the HP-5 MS capillary column. [b]RI in literature. [c]Mass spectrum.

of plants, the harvest time, and treatment of samples. These differences might further affect the biological activities.

It was reported that terpenoids and aromatic compounds exhibited excellent bioactivities against different stored product insect pests [29–32] and aldehydes showed fair contact toxicity and repellent activities [33]. Here, four representative compounds were selected from 26 components identified in our sample as they belong to the chemical types of monoterpenes, sesquiterpenes, aldehydes, or aromatic compounds, respectively. Their bioactivity tests were evaluated as well. They are bornyl acetate, β-bisabolene, (2E,4E)-2,4-nonadienal, and 1,4-dimethoxy-2-tert-butylbenzene. The molecular structures of the four selected compounds are shown in Figure 2.

3.2. Contact Toxicity. The results of contact toxicity of the essential oil and selected compounds against cigarette beetles and booklice are listed in Table 2. The results showed that the essential oil exhibited fair contact toxicity against cigarette beetles with a LD_{50} value of 11.91 μg/adult but expressed unobservable toxicity against booklice in our measure range.

Among the four selected compounds, (2E,4E)-2,4-nonadienal showed the strongest toxicity against cigarette beetles (LD_{50} = 2.31 μg/adult) and booklice (LD_{50} = 32.23 $\mu g/cm^2$).

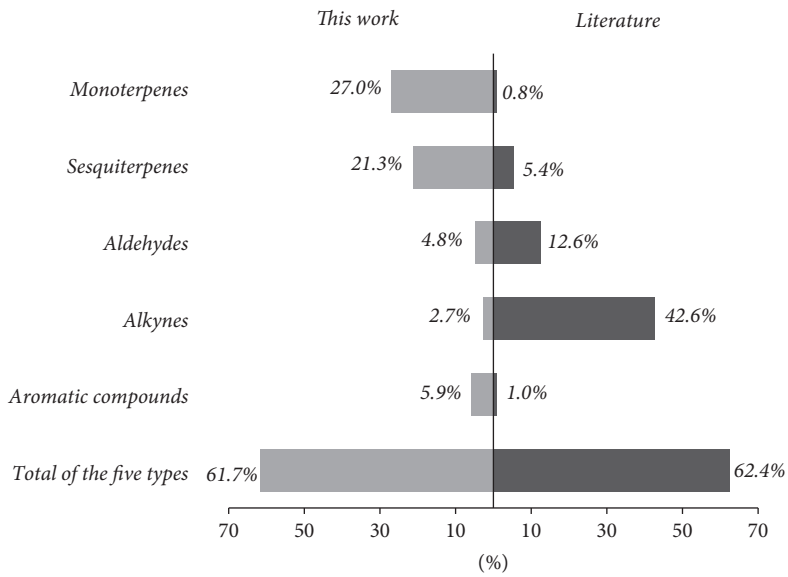

Figure 1: Percentage of different types of compounds in essential oils of this work and literature.

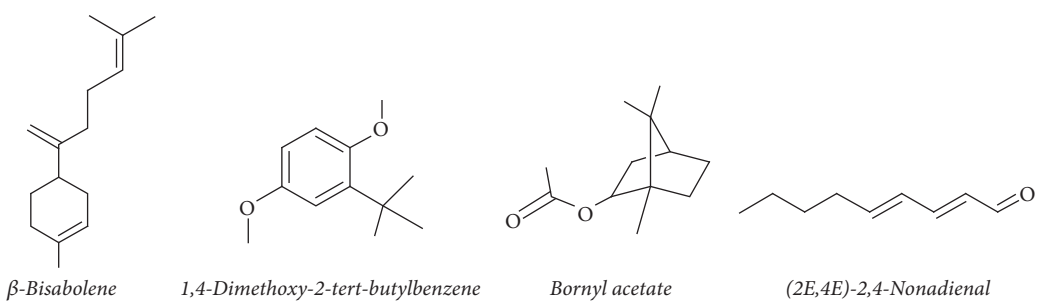

Figure 2: Molecular structures of selected compounds from the essential oil of *Bupleurum bicaule*.

Table 2: Contact toxicity of *Bupleurum bicaule* essential oil and its constituents against *Lasioderma serricorne* (LS) and *Liposcelis bostrychophila* (LB) adults.

Insects	Treatments	Concentrations (%)	LD_{50} (μg/adult)/(μg/cm^2)	95% FL (μg/adult)/(μg/cm^2)	Slope ± SE	Chi square (χ^2)
LS	Essential oil	1.91–10.00	11.91	7.04–15.66	2.01 ± 0.39	17.50
	β-Bisabolene	2.97–15.00	24.99	21.68–29.14	3.86 ± 0.56	3.61
	1,4-Dimethoxy-2-tert-butylbenzene	1.97–10.00	23.56	20.03–27.24	3.18 ± 0.55	2.90
	Bornyl acetate	0.90–4.40	9.42	8.14–10.87	0.98 ± 0.17	7.53
	(2E,4E)-2,4-Nonadienal	0.27–1.30	2.31	2.03–2.61	4.00 ± 0.62	9.44
	Pyrethrins[a]	--	0.24	0.22–0.30	3.34 ± 0.32	13.11
LB	Essential oil	0–5.00	--	--	--	--
	β-Bisabolene	0–50.00	--	--	--	--
	1,4-Dimethoxy-2-tert-butylbenzene	0.12–1.00	62.34	55.97–69.66	6.42 ± 0.87	3.93
	Bornyl acetate	0.20–0.40	36.67	34.67–38.55	16.73 ± 2.28	7.04
	(2E,4E)-2,4-Nonadienal	0.20–0.40	32.23	30.59–33.97	17.08 ± 2.31	9.66
	Pyrethrins[a]	--	18.72	17.60–19.92	2.98 ± 0.40	10.56

[a]Data from Yang et al. [26].

Table 3: Percent repellency (PR) of the essential oil from *Bupleurum bicaule* and its constituents against *Lasioderma serricorne* adults at 2 h and 4 h after exposure. *Comparison of different samples at the same concentration, df = 5.

Times	Treatments	Concentrations (nL/cm^2)				
		78.63	15.73	3.15	0.63	0.13
2 h	Essential oil	100 ± 0^{c}	74 ± 12^{c}	70 ± 12^{c}	66 ± 11^{b}	38 ± 4^{bc}
	β-Bisabolene	90 ± 6^{bc}	12 ± 9^{b}	34 ± 15^{bc}	10 ± 16^{a}	56 ± 12^{c}
	1,4-Dimethoxy-2-tert-butylbenzene	62 ± 11^{a}	40 ± 13^{bc}	34 ± 15^{bc}	6 ± 12^{a}	14 ± 12^{b}
	Bornyl acetate	74 ± 12^{ab}	-28 ± 19^{a}	-4 ± 17^{a}	-4 ± 9^{a}	-36 ± 17^{a}
	(2E,4E)-2,4-Nonadienal	56 ± 12^{a}	66 ± 20^{c}	4 ± 19^{ab}	16 ± 17^{ab}	60 ± 10^{c}
	DEET	88 ± 7^{b}	76 ± 14	28 ± 7^{bc}	20 ± 14^{ab}	16 ± 7^{b}
	F	15.878	20.895	9.823	5.244	39.261
	P	0	0	0	0.002	0
4 h	Essential oil	100 ± 0^{c}	62 ± 15^{bcd}	24 ± 19^{ab}	28 ± 19^{bc}	12 ± 18^{b}
	β-Bisabolene	88 ± 12^{bc}	26 ± 17^{b}	24 ± 11^{ab}	14 ± 17^{abc}	34 ± 18^{bc}
	1,4-Dimethoxy-2-tert-butylbenzene	46 ± 20^{a}	32 ± 9^{bc}	34 ± 17^{ab}	-4 ± 16^{ab}	28 ± 4^{bc}
	Bornyl acetate	68 ± 13^{ab}	-4 ± 12^{a}	14 ± 12^{ab}	-8 ± 18^{ab}	-18 ± 11^{a}
	(2E,4E)-2,4-Nonadienal	50 ± 19^{a}	72 ± 9^{cd}	12 ± 18^{a}	-14 ± 16^{a}	32 ± 19^{bc}
	DEET	98 ± 2^{c}	78 ± 9^{d}	58 ± 16^{b}	56 ± 14^{c}	46 ± 7^{c}
	F	15.428	17.091	2.713	5.753	13.079
	P	0	0	0.044	0.001	0

*Different letters in the same column (2 h and 4 h, resp.) mean significant differences ($P < 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before analysis.

Bornyl acetate also showed certain toxicity against the two insects (LD_{50} = 9.42 μg/adult and 36.67 μg/cm^2, resp.), while the toxicity of 1,4-dimethoxy-2-tert-butylbenzene and β-bisabolene was relatively weak. No toxicity against booklice was observed for β-bisabolene under the testing concentrations.

From the results, it could be calculated that the contact toxicity of (2E,4E)-2,4-nonadienal against booklice was only 1.7 times less than that of pyrethrins (LD_{50} = 18.72 μg/cm^2). In You et al.'s work [34], it was reported that the perilla aldehyde isolated from the essential oil of Purple *Perilla* aerial parts also showed significant contact toxicity against cigarette beetles (LD_{50} = 3.82 μg/adult). By the structural analysis, it was found that both of (2E,4E)-2,4-nonadienal and perilla aldehyde have aldehyde fragment. The toxic characteristic of these compounds against insects might be related to the presence of an aldehyde group in these compounds. Therefore, the content of (2E,4E)-2,4-nonadienal could be considered as one of the key elements which would affect the contact toxicity of the essential oil against certain kinds of insects.

3.3. Repellency. The results of the ANOVAs indicated that the repellent rate against both insects significantly differ with the different treatments. These results are given in Tables 3 and 4.

The essential oil showed strong repellency against cigarette beetles. After 2 h of exposure, the percent repellency (PR) of the essential oil reached 100% at the highest concentration (78.63 nL/cm^2). The PR values continuously declined with the decrease of the tested concentrations. The repellent characteristics of the four compounds against the cigarette beetles were variable. At the dose of 78.63 nL/cm^2, all the four compounds showed definite repellent activities against cigarette beetles, although their individual efficiency of repellency was not as good as that of the essential oil treatment. The PR values of β-bisabolene, 1,4-dimethoxy-2-tert-butylbenzene, bornyl acetate, and (2E,4E)-2,4-nonadienal were 90%, 62%, 74%, and 56%, respectively, after 2 h of exposure. However, the repellent characteristics of the four compounds were obviously different at lower concentrations. The experimental data were further analyzed by Tukey' test, and the statistical results (see Table 3) could be used to exam the repellency level of each sample. Testing results of 0.13 nL/cm^2 showed that β-bisabolene and (2E,4E)-2,4-nonadienal possessed a higher level of repellency than DEET (P = 0.019 and 0.009, resp.) after 2 h of exposure. The repellency of 1,4-dimethoxy-2-tert-butylbenzene was comparable to DEET (P = 0.966), while the bornyl acetate showed an insect attractant property.

As the results show in Table 4, the *B. bicaule* essential oil showed relatively weak repellency against booklice; the highest repellent rate of the essential oil was only 56% relative to DEET (PR = 98%) at the dose of 12.63 nL/cm^2 after 2 h of exposure. However, some of individual compounds showed certain repellent activities. At low concentration (0.10 nL/cm^2), the repellency of the four compounds was at the same level as that of DEET (P = 0.952, 0.855, 1.000, and 1.000, resp.) after 2 h of exposure. At the highest concentration (63.17 nL/cm^2), only β-bisabolene showed an obvious repellent activity with PR value of 76% after 2 h of exposure.

The experimental results and data analysis showed that the repellent rates of the essential oil were almost comparable to those of the positive control at all testing concentrations against cigarette beetles. Each of the four compounds exhibited a different level of the repellent activity against

Table 4: Percent repellency (PR) of the essential oil from *Bupleurum bicaule* and its constituents against *Liposcelis bostrychophila* adults at 2 h and 4 h after exposure. *Comparison of different samples at the same concentration, df = 5.

Times	Treatments	Concentrations (nL/cm^2)				
		63.17	12.63	2.53	0.51	0.10
2 h	Essential oil	50 ± 12^{ab}	56 ± 12^{bc}	4 ± 17^{a}	24 ± 19^{a}	-6 ± 20^{a}
	β-Bisabolene	76 ± 15^{bc}	74 ± 15^{cd}	42 ± 19^{ab}	12 ± 18^{a}	18 ± 16^{a}
	1,4-Dimethoxy-2-tert-butylbenzene	52 ± 19^{ab}	8 ± 19^{a}	36 ± 13^{ab}	22 ± 18^{a}	16 ± 13^{a}
	Bornyl acetate	12 ± 14^{a}	20 ± 10^{ab}	32 ± 9^{ab}	10 ± 18^{a}	34 ± 16^{a}
	(2E,4E)-2,4-Nonadienal	18 ± 20^{a}	74 ± 8^{cd}	24 ± 18^{a}	20 ± 10^{a}	28 ± 14^{a}
	DEET	100 ± 0^{c}	98 ± 2^{d}	78 ± 10^{b}	66 ± 8^{a}	8 ± 3^{a}
	F	8.980	12.170	5.299	1.998	2.173
	P	0	0	0.002	0.115	0.091
4 h	Essential oil	44 ± 13^{abc}	48 ± 15^{a}	16 ± 10^{b}	36 ± 10^{a}	$-10 \pm 16a$
	β-Bisabolene	74 ± 22^{bc}	64 ± 20^{a}	-16 ± 15^{a}	48 ± 20^{a}	16 ± 18^{ab}
	1,4-Dimethoxy-2-tert-butylbenzene	20 ± 15^{ab}	28 ± 20^{a}	18 ± 16^{b}	30 ± 14^{a}	18 ± 18^{ab}
	Bornyl acetate	10 ± 19^{a}	24 ± 10^{a}	38 ± 12^{bc}	22 ± 18^{a}	44 ± 18
	(2E,4E)-2,4-Nonadienal	28 ± 8^{ab}	36 ± 13^{a}	46 ± 7^{bc}	14 ± 3^{a}	8 ± 20^{ab}
	DEET	96 ± 3^{c}	82 ± 5^{a}	68 ± 3^{c}	54 ± 6^{a}	22 ± 5^{ab}
	F	7.308	2.695	10.321	2.014	2.564
	P	0	0.045	0	0.113	0.054

*Different letters in the same column (2 h and 4 h, resp.) mean significant differences ($P < 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before analysis.

cigarette beetles. Accordingly, it could be considered that these compounds would make various degrees of contribution to the strong repellency of the essential oil. Among them, β-bisabolene (a sesquiterpene) might be the most important one which majorly affected the repellent activity against cigarette beetles. It has been reported that the sesquiterpenes showed repellent activities against several insects. In Khani and Heydarian's work, the essential oil of *Teucrium polium* subsp. *capitatum* (L.), which was rich in sesquiterpene, showed 60% and 52% repellency against *Teucrium castaneum* and *Callosobruchus maculatus* adults [35]. Based on the above findings, it was believed that the essential oil of *B. bicaule* and the types of sesquiterpenes compounds would be the exploration direction of natural repellents.

4. Conclusions

In this work, the chemical composition of *B. bicaule* essential oil was determined, and its contact and repellent activities against two stored product insects were investigated along with four individual components. A compound (2E,4E)-2,4-nonadienal with striking contact toxicity was found, and the essential oil showed considerable contact and repellent activities, which could be attributed to the existence of different chemical compounds in essential oil. Some of those components might be the material basis of active effect. This work would provide scientific basis for further development and utilization of *B. bicaule* in stored product insects control. There must be a certain relationship between the bioactivity and structure of the compounds, which deserves further study and discussion.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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