Research Article

Fumigant Compounds from the Essential Oil of Chinese *Blumea balsamifera* Leaves against the Maize Weevil (*Sitophilus zeamais*)

Sha Sha Chu,¹ Shu Shan Du,² and Zhi Long Liu¹

¹ Department of Entomology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China

² State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University, Beijing 100875, China

Correspondence should be addressed to Zhi Long Liu; zhilongliu@cau.edu.cn

Received 14 December 2011; Revised 5 June 2012; Accepted 13 June 2012

Academic Editor: Mohamed Afzal Pasha

Copyright © 2013 Sha Sha Chu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Essential oil of Chinese medicinal herb, *Blumea balsamifera* leaves, was found to possess fumigant toxicity against the maize weevils, *Sitophilus zeamais*. The main components of the essential oil of *B. balsamifera* were 1,8-cineole (20.98%), borneol (11.99%), β -caryophyllene (10.38%), camphor (8.06%), 4-terpineol (6.49%), α -terpineol (5.91%), and caryophyllene oxide (5.35%). Bioactivity-guided chromatographic separation of the essential oil on repeated silica gel columns led to isolate five constituent compounds, namely, 1,8-cineole, borneol, camphor, α -terpineol, and 4-terpineol. 1,8-Cineole, 4-terpineol, and α -terpineol showed pronounced fumigant toxicity against *S. zeamais* adults (LC_{50} = 2.96 mg/L, 4.79 mg/L, and 7.45 mg/L air, resp.) and were more toxic than camphor (LC_{50} = 21.64 mg/L air) and borneol (LC_{50} = 21.67 mg/L air). The crude essential oil also possessed strong fumigant toxicity against *S. zeamais* adults (LC_{50} = 10.71 mg/L air).

1. Introduction

Fumigants play a very important role in insect pest elimination in stored products not only because of their ability to kill a broad spectrum of pests but also because of their easy penetration into the commodity while leaving minimal residues [1]. Currently, phosphine and methyl bromide (MeBr) are the two common fumigants used for stored-product protection all over the world. Due to insect resistance to phosphine and MeBr as an ozone-depleting compound, there is a global interest in looking for new fumigants [1, 2] and plant essential oils and their components have been shown to possess potential to be developed as new fumigants. Plant essential oils and their components may have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation, and local availability [3, 4]. Essential oils derived from more than 75 plant species have been studied for fumigant toxicity against stored product insects [5].

Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecules [1]. During the screening program

for new agrochemicals from Chinese medicinal herbs, the essential oil of *Blumea balsamifera* DC. (Family: Asteraceae) leaves was found to possess strong fumigant toxicity to the maize weevil, *Sitophilus zeamais* (Motsch). The maize weevil (*S. zeamais*) is one of the most widespread and destructive primary insect pests of stored cereals [6]. Infestations not only cause significant losses due to the consumption of grains; they also result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species. *B. balsamifera* is a perennial evergreen shrub native of Southeast Asia but is distributed throughout tropical Asia. It is a small tree that can grow up to 4 m in height and imparts a strong camphorous odor around it. Use of *B. balsamifera* leaves has been recommended as traditional Chinese medicine in the treatment of various diseases [7]. It has anti-inflammatory, anticatarrhal and expectorant properties which render it useful in the treatment of both upper and lower respiratory tracts like sinusitis, influenza, and asthmatic bronchitis. Several studies on the chemical constituents of *B. balsamifera* have been reported, and a number of flavonoids, sesquiterpenoids, and triterpenoids, have been isolated from this plant [8–17].

FIGURE 1: Constituent compounds isolated from *B. balsamifera* essential oil.

Chemical composition of the essential oil of *B. balsamifera* leaves has been studied [18–22]. However, the essential oil of *B. balsamifera* leaves was not evaluated to have insecticidal activity against grain storage insects and the bioactive (fumigant) constituent compounds of the essential oil have not been isolated and identified from this Chinese medicinal herb. In this paper, we report the isolation and identification of five active compounds contained in the essential oil of *B. balsamifera* leaves against the maize weevil by bioassay-guided fractionation.

2. Experimental

^1H nuclear magnetic resonance (NMR) spectra were recorded on Bruker ACF300 (300 MHz (^1H)) and AMX500 (500 MHz (^1H)) instruments using deuteriochloroform (CDCl_3) as the solvent with tetramethylsilane (TMS) as the internal standard. Electron impact ionone mass spectra (EIMS) were determined on a Micromass VG7035 mass spectrometer at 70 eV (probe). The crude essential oil (20 mL) was chromatographed on a silica gel (Merck 9385, 1,000 g) column (85 mm i.d., 850 mm length) by gradient elution with a mixture of solvents (*n*-hexane, *n*-hexane-ethyl acetate, and acetone). Fractions of 500 mL were collected and concentrated at 40°C, and similar fractions according to TLC profiles were combined to yield 28 fractions. Each fraction was tested with fumigant toxicity bioassay (see the following) to identify the bioactive fractions (fractions 5, 9, 11, 14, and 16). Fractions that possessed fumigant toxicity, with similar TLC profiles, were pooled and further purified by preparative silica gel column chromatography (PTLC) until obtaining three pure compounds for determining structure (Figure 1). The spectral data of 1,8-cineole (1) (2.7 g) matched with the previous reports [23, 24]. The data of camphor (2) (1.6 g) and borneol (3) (1.2 g) matched with the previous reports [23, 25, 26]. The spectral data were identical to the published data of 4-terpineol (4) (1.1 g) and α -terpineol (5) (0.9 g) (Table 3) [23, 27, 28].

2.1. Chinese Medicinal Herb and Hydrodistillation. Fresh leaves of *B. balsamifera* were collected from the suburb of Nanning City (22.8°N, 108.3°E, Guangxi Zhuang Autonomous Region, China) at August 2008. The plant was identified by Dr. QR Liu (College of Life Sciences, Beijing Normal University, China), and a voucher specimen

(CMH-Dafengai-Guangxi-2008-08) was deposited in the Department of Entomology, China Agricultural University. To obtain volatile essential oil, the air-dried samples were first ground to a powder then soaked in water at a ratio of 1 : 4 (w/v) for 1 h, prior to hydrodistillation using a round bottom container over a period of 6 h. The volatile essential oil was collected in a specific receiver, measured, dried over anhydrous sulfate, weighed, and stored in airtight containers.

2.2. Analysis of the Essential Oil. Components of the essential oil of *B. balsamifera* leaves were separated and identified by gas chromatography-mass spectrometry (GC-MS) Agilent 6890N gas chromatography hooked to Agilent 5973N mass selective detector. They equipped with a flame ionization detector and capillary column with HP-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μm). The GC settings were as follows: the initial oven temperature was held at 60°C for 1 min and ramped at 10°C min $^{-1}$ to 180°C for 1 min, and then ramped at 20°C min $^{-1}$ to 280°C for 15 min. The injector temperature was maintained at 270°C. The samples (1 μL) were injected neat, with a split ratio of 1 : 10. The carrier gas was helium at flow rate of 1.0 mL min $^{-1}$. Spectra were scanned from 20 to 550 m/z at 2 scans s $^{-1}$. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature [18–22] or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8 – C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [29]. Component relative percentages were calculated based on GC peak areas without using correction factors.

2.3. Fumigant Toxicity [6]. The maize weevils were obtained from laboratory cultures maintained for the last 15 years in the dark in incubators at 29–30°C and 65–75% r.h. They were reared on whole wheat at 12–13% moisture content. The unsexed adults used in the experiments were 2–4 weeks posteclosion. A Whatman filter paper (CAT number 1001020, diameter 2.0 cm) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 24 mL). Ten microliters of an appropriate concentration of compounds/oil (1.0%–40.0%, 5 concentrations) were

TABLE 1: Chemical constituents of essential oil from *Blumea balsamifera* leaves.

RI*	Compound	Formula	RA** (%)
998	Yomogi alcohol	C ₁₀ H ₁₈ O	2.58
1032	1,8-Cineole	C ₁₀ H ₁₈ O	20.98
1083	Artemisia alcohol	C ₁₀ H ₁₈ O	1.87
1114	β -Thujone	C ₁₀ H ₁₆ O	3.21
1126	ρ -Menth-2-en-1-ol	C ₁₀ H ₁₈ O	1.84
1138	2,2,4-Trimethyl-3-cyclohexene-1-carboxaldehyde	C ₁₀ H ₁₆ O	3.34
1143	Camphor	C ₁₀ H ₁₆ O	8.06
1168	Borneol	C ₁₀ H ₁₈ O	11.99
1179	4-Terpineol	C ₁₀ H ₁₈ O	6.49
1188	α -Terpineol	C ₁₀ H ₁₈ O	5.91
1205	Verbenone	C ₁₀ H ₁₄ O	1.84
1222	<i>cis</i> -Carveol	C ₁₀ H ₁₆ O	2.36
1356	Eugenol	C ₁₀ H ₁₂ O ₂	1.02
1374	Copaene	C ₁₅ H ₂₄	0.28
1387	β -Cubebene	C ₁₅ H ₂₄	0.43
1420	Caryophyllene	C ₁₅ H ₂₄	10.38
1434	β -Gurjunene	C ₁₅ H ₂₄	0.45
1452	<i>cis</i> - β -Farnesene	C ₁₅ H ₂₄	0.37
1455	α -Caryophyllene	C ₁₅ H ₂₄	1.50
1473	γ -Murolene	C ₁₅ H ₂₄	0.35
1480	Germacrene D	C ₁₅ H ₂₄	1.10
1483	(+)- β -Selinene	C ₁₅ H ₂₄	0.95
1523	δ -Cadinene	C ₁₅ H ₂₄	0.53
1578	Spathulenol	C ₁₅ H ₂₄ O	2.64
1583	Caryophyllene oxide	C ₁₅ H ₂₄ O	5.35
1556	Guaia-3,9-diene	C ₁₅ H ₂₄	3.09
2119	Phytol	C ₂₀ H ₄₀ O	0.32
Total			99.23
Monoterpenoids			70.47
Sesquiterpenoids			27.42
Others			1.34

*RI: retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons.

**RA: relative area (peak area relative to total peak area).

added to the filter paper. The solvent was allowed to evaporate for 30 seconds before the cap was placed tightly on the glass vial (with 10 insects). *n*-Hexane was used as controls. The vials were upright and the Fluon (ICI America Inc) coating restricted the insects to the lower portion of the vial to prevent them from the treated filter paper. Six replicates were used in all treatments and controls, and they were incubated at 29–30°C and 65–75% RH for 24 hrs. The insects were then transferred to clean vials with some culture media and kept in an incubator for determination of end-point mortality, which was reached after one week. The insects were considered dead if appendages did not move when probed with a camel brush. The observed mortality data were corrected for control mortality using Abbott's formula. Results from all replicates were subjected to probit analysis using the Probit Program V1.6.3 to determine LC₅₀ and LC₉₀ values [30].

3. Results and Discussion

3.1. Chemical Constituents of the Essential Oil. Hydrodistillation of dried leaves of *B. balsamifera* yielded 0.88% essential oil (v/w), and the density of the essential oil was determined as 0.87%. The results of GC-MS of *B. balsamifera* essential oil are presented in Table 1. A total of 27 components were identified in the essential oil of *B. balsamifera*, accounting for 99.23% of the total oil (Table 1). The main components are 1,8-cineole (20.98%), borneol (11.99%), β -caryophyllene (10.38%), camphor (8.06%), 4-terpineol (6.49%), α -terpineol (5.91%), and caryophyllene oxide (5.35%). Monoterpenoids represented 12 of the 27 compounds, corresponding to 70.47% of the whole oil while 13 of the 27 constituents were sesquiterpenoids (27.42% of the crude essential oil). The chemical composition of *B. balsamifera* essential oil was different from that reported in

TABLE 2: Fumigant toxicity of *Blumea balsamifera* essential oil and its constituent compounds against *Sitophilus zeamais* adults.

Treatments	Conc. (%)	Mortality (% $\pm$ SE)	LC ₅₀ (mg/L air) (95% FL)	LC ₉₀ (mg/L air) (95% FL)	Slope $\pm$ SE	Chi square (χ^2)
Borneol	20.00	98.0 $\pm$ 1.22	29.64 (27.39–31.98)	40.11 (37.67–43.24)	2.37 $\pm$ 0.32	15.34
	14.29	72.0 $\pm$ 3.43				
	10.20	48.0 $\pm$ 3.41				
	7.29	34.0 $\pm$ 2.34				
	5.21	8.0 $\pm$ 1.22				
Camphor	20.00	88.0 $\pm$ 3.43	21.67 (19.74–23.25)	43.54 (39.76–47.32)	2.18 $\pm$ 0.25	8.39
	13.33	65.0 $\pm$ 2.45				
	8.89	47.0 $\pm$ 1.43				
	5.92	36.0 $\pm$ 1.32				
	3.95	10.0 $\pm$ 2.32				
	3.00	95.0 $\pm$ 1.34				
1,8-Cineole	1.50	78.0 $\pm$ 3.43	2.96 (2.76–3.21)	5.46 (4.78–5.93)	1.72 $\pm$ 0.15	9.48
	0.75	56.0 $\pm$ 2.45				
	0.38	35.0 $\pm$ 1.56				
	0.19	12.5 $\pm$ 0.78				
α -Terpineol	10.00	95.0 $\pm$ 2.34	7.45 (6.51–8.32)	12.18 (10.79–13.23)	2.13 $\pm$ 0.21	11.79
	5.00	84.0 $\pm$ 1.68				
	2.50	62.0 $\pm$ 1.87				
	1.25	38.0 $\pm$ 1.54				
	0.66	13.0 $\pm$ 0.89				
4-Terpineol	8.00	94.0 $\pm$ 3.43	4.79 (4.31–5.22)	8.37 (7.67–9.27)	2.07 $\pm$ 0.19	3.14
	4.00	72.0 $\pm$ 2.43				
	2.00	48.0 $\pm$ 1.45				
	1.00	32.0 $\pm$ 1.67				
	0.50	7.0 $\pm$ 0.56				
MeBr*	—	—	0.67	—	—	—
Crude oil	15.0	98.0 $\pm$ 2.54	10.71 (9.76–11.75)	18.82 (17.13–20.67)	1.77 $\pm$ 0.13	5.28
	7.50	86.0 $\pm$ 1.95				
	3.75	65.0 $\pm$ 1.65				
	1.88	42.0 $\pm$ 1.44				
	0.94	16.0 $\pm$ 0.99				

* MeBr: Methyl bromide; values from Liu and Ho [6].

other studies. For example, borneol (33.2%), caryophyllene (8.2%), ledol (7.1%), tetracyclo[6,3,2,0,(2.5).0(1,8) tridecan-9-ol, 4,4-dimethyl (5.2%), phytol (4.6%), caryophyllene oxide (4.1%), guaicol (3.4%), thujopsene-13 (4.4%), dimethoxydurene (3.6%), and γ -eudesmol (3.2%) were the dominant components in the essential oil of *B. balsamifera* leaves collected from Bangladesh (Chittagong, 22.20°N, 91.50°E) [20]. However, borneol (57.7%), caryophyllene (7.6%), and camphor (5.0%) were the three main components of the essential oil of *B. balsamifera* from Guizhou, China (Luodian Country, 25.43°N, 106.75°E) [21], while bornel (52.4%) and camphor (17.7%) were the two components of the essential oil from Yunnan, China (Puer City, 22.48°N, 100.58°E) [22]. There were great geographic variations in chemical composition of essential oils of *B. balsamifera* harvested in three provinces of Vietnam [18]. The major components of the essential oils were borneol (57.8%), caryophyllene (8.3%), δ -cadinol (8.0%), and caryophyllene oxide (3.1%) (Ha Giang, 22.80°N,

104.98°E); borneol (50.6%), camphor (18.7%), caryophyllene (10.1%), δ -cadinol (3.1%), patchoulene (3.0%), and veridiflorol (2.0%) (Hanoi, 21.02°N, 105.51°E); camphor (70.1%), caryophyllene (10.5%), borneol (5.7%), and carvacrol (5.7%) (Dak Lak, 22.80°N, 104.98°E). The above findings suggest that further studies on plant cultivation and essential oil standardization are necessary because chemical composition of the essential oil varies greatly with the plant population.

3.2. Fumigant Toxicity of Isolated Constituent Compounds against the Maize Weevils. 1,8-Cineole (**1**) (LC₅₀ = 2.96 mg/L air), 4-terpineol (**4**) (LC₅₀ = 4.79 mg/L air), and α -terpineol (**5**) (LC₅₀ = 7.45 mg/L air) showed stronger fumigant toxicity than the crude essential oil (LC₅₀ = 10.71 mg/L air) against *S. zeamais* (Table 2). It indicates that fumigant toxicity of the essential oil may be attributed to the three constituent compounds. In the previous studies, 1,8-cineole was found to exhibit fumigant toxicity against

FIGURE 2: Dose response curves of *Sitophilus zeamais* treated with the essential oil and its constituent compounds.

red flour beetles, *Tribolium castaneum* adult with $LC_{50} = 41 \mu\text{L/L}$ air [31], $15.3 \mu\text{L/L}$ air [32], and 1.52 mg/L air [33]. It also possesses fumigant toxicity against several other stored product insects and cockroaches as well as mosquitoes, for example, the rice weevil (*S. oryzae*) ($LC_{50} = 22.8 \mu\text{L/L}$ air [32]), the lesser grain borer (*Rhyzopertha dominica*) ($LC_{50} = 9.5 \mu\text{L/L}$ air [32]). However, compared with the current used fumigant (MeBr, $LC_{50} = 0.67 \text{ mg/L}$ air), the three compounds and the crude essential oil exhibited only 4–16 times less toxic to the maize weevils. The three constituent compounds and the crude essential oil were more toxic to *S. zeamais* than another two isolated compounds, camphor (2) ($LC_{50} = 21.67 \text{ mg/L}$ air) and borneol (3) ($LC_{50} = 29.64 \text{ mg/L}$ air) (Figure 2). Compared with the other essential oils in the

previous studies that were tested using a similar bioassay, *B. balsamifera* essential oil exhibited stronger fumigant toxicity against *S. zeamais* adults, for example, essential oils of *Schizonepeta multifida* [34], *Murraya exotica* [35], and several essential oils from Genus *Artemisia* [36–38]. Moreover, the two other active constituent compounds, 4-terpineol and α-terpineol, have been found to possess strong fumigant toxicity against several grain storage insects, such as *S. granarius*, *S. oryzae*, *T. castaneum*, *T. confusum*, and *R. dominica* [31, 32, 39–43]. The two other active constituents, camphor and borneol, were also demonstrated fumigant toxicity against grain storage insects [31, 41, 44, 45].

Considering the currently used fumigants are synthetic insecticides, fumigant activity of the crude essential oil of

TABLE 3: ^1H and ^{13}C values (δ (ppm)) of the isolated compounds.

Number	1,8-Cineole		Camphor		Borneol		4-Terpineol		α -Terpineol	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	—	72.7	—	220.0	3.15	76.1	—	133.9	—	133.9
2	1.52	37.3	2.14; 1.89	40.4	1.67; 1.42	35.8	5.37	123.3	5.37	123.3
3	1.40	24.2	1.99	34.4	1.42	45.2	2.19; 1.94	40.8	2.04; 1.79	24.1
4	1.74	39.6	1.60; 1.35	28.4	1.52; 1.27	23.6	—	71.7	1.71	46.5
5	1.52; 1.27	24.2	1.84; 1.59	31.5	1.49; 1.24	30.0	1.88; 1.63	30.3	1.74; 1.49	19.1
6	1.65; 1.40	37.3	—	61.6	—	52.7	2.01; 1.91	24.7	2.01; 1.91	31.2
7	1.31	25.4	1.26	15.6	1.16	13.3	1.71	23.1	17.1	23.1
8	—	76.8	—	39.8	—	50.4	1.90	37.6	—	73.3
9	1.26	25.4	1.11	21.8	1.11	19.8	1.01	14.9	1.26	27.7
10	1.26	25.4	1.11	21.8	1.11	19.8	1.01	14.9	1.26	27.7

B. balsamifera leaves and the three isolated active compounds are quite promising and they show potential to be developed as possible natural fumigants for control of stored product insects with low toxicity to humans. Although dried leaves of *B. balsamifera* were used as a common Chinese medicinal herb [7], there are no toxicity data for this herb and the three isolated compounds and available on human consumption. For the practical use of the three compounds and crude essential oil as novel botanical insecticides, further studies are necessary on the safety of these materials to human, on phytotoxicity to crop seeds, and on the development of formulations to improve efficacy and stability, and to cut cost as well. The isolated three active compounds exhibited fumigant toxicity against several grain storage insects [31, 32, 39–43]. However, little has been done on mechanisms of action of these three monoterpenes. In addition, further testing is necessary to evaluate the spectrum of fumigant activity against other stored-product insects (as well as other life stages of these stored-product insects, especially eggs).

Acknowledgments

This work was funded by the Hi-Tech Research and Development of China 2011AA10A202 and 2006AA10A209. The authors are grateful to Dr. SL Liu, Department of Biology, GuangXi Traditional Chinese Medical University, for his collection of the medicinal herb. They are grateful to Dr. QR Liu, College of Life Sciences, Beijing Normal University, Beijing, China, for identification of Chinese medicinal herb.

References

- [1] J. L. Zettler and F. H. Arthur, "Chemical control of stored product insects with fumigants and residual treatments," *Crop Protection*, vol. 19, no. 8-10, pp. 577–582, 2000.
- [2] M. B. Isman, "Plant essential oils for pest and disease management," *Crop Protection*, vol. 19, no. 8-10, pp. 603–608, 2000.
- [3] M. B. Isman, "Plant essential oils as green pesticides for pest and disease management," in *Agricultural Applications in Green Chemistry*, vol. 887 of ACS Symposium, pp. 41–51, 2004.
- [4] M. B. Isman, "Botanical insecticides: for richer, for poorer," *Pest Management Science*, vol. 64, no. 1, pp. 8–11, 2008.
- [5] S. Rajendran and V. Sriranjini, "Plant products as fumigants for stored-product insect control," *Journal of Stored Products Research*, vol. 44, no. 2, pp. 126–135, 2008.
- [6] Z. L. Liu and S. H. Ho, "Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook f. et Thomas against the grain storage insects, *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst)," *Journal of Stored Products Research*, vol. 35, no. 4, pp. 317–328, 1999.
- [7] Jiangsu New Medical College, *Encyclopedia of Chinese Medicinal Substances*, Shanghai People's Publisher, Shanghai, China, 1977.
- [8] N. Ruangrunsi, P. Tappayuthpijarn, P. Tantivatana, R. P. Borris, and G. A. Cordell, "Traditional medicinal plants of Thailand. I. Isolation and structure elucidation of two new flavonoids, (2R,3R)-dihydroquercetin-4/-methyl ether and (2R,3R)-dihydroquercetin-4/-7-dimethyl ether from *Blumea balsamifera*," *Journal of Natural Products*, vol. 44, no. 5, pp. 541–545, 1981.
- [9] Y. Fujimoto, A. Soemartono, and M. Sumatra, "Sesquiterpenelactones from *Blumea balsamifera*," *Phytochemistry*, vol. 27, no. 4, pp. 1109–1111, 1988.
- [10] N. C. Barua and R. P. Sharma, "(2R,3R)-7,5/-dimethoxy-3,5,2/-trihydroxyflavanone from *Blumea balsamifera*," *Phytochemistry*, vol. 31, no. 11, p. 4040, 1992.
- [11] D. M. H. Ali, K. C. Wong, and P. K. Lim, "Flavonoids from *Blumea balsamifera*," *Fitoterapia*, vol. 76, no. 1, pp. 128–130, 2005.
- [12] N. Osaki, T. Koyano, T. Kowithayakorn, M. Hayashi, K. Komiyama, and M. Ishibashi, "Sesquiterpenoids and plasmin-inhibitory flavonoids from *Blumea balsamifera*," *Journal of Natural Products*, vol. 68, no. 3, pp. 447–449, 2005.
- [13] C. Y. Ragasa, A. L. K. C. Co, and J. A. Rideout, "Antifungal metabolites from *Blumea balsamifera*," *Natural Product Research*, vol. 19, no. 3, pp. 231–237, 2005.
- [14] M. Chen, J. J. Qin, J. J. Fu et al., "Repellency of an over-the-counter essential oil product in China against workers of red imported fire ants," *Planta Medica*, vol. 76, pp. 897–902, 2010.
- [15] O. Shiota, J. M. Oribello, S. Sekita, and M. Satake, "Sesquiterpenes from *Blumea balsamifera*," *Journal of Natural Products*, vol. 74, no. 3, pp. 470–476, 2011.
- [16] N. Saewan, S. Koysoomboon, and K. Chantrapromma, "Antityrosinase and anti-cancer activities of flavonoids from *Blumea balsamifera* DC," *Journal of Medicinal Plant Research*, vol. 5, no. 6, pp. 1018–1025, 2011.

- [17] F. Nessa, Z. Ismail, and N. Mohamed, "Xanthine oxidase inhibitory activities of extracts and flavonoids of the leaves of *Blumea balsamifera*," *Pharmaceutical Biology*, vol. 48, no. 12, pp. 1405–1412, 2010.
- [18] U. Sakee, S. Maneerat, T. P. T. Cushnie, and W. De-Eknamkul, "Antimicrobial activity of *Blumea balsamifera* (Lin.) DC. extracts and essential oil," *Natural Product Research*, vol. 25, no. 19, pp. 1849–1856, 2011.
- [19] T. T. Nguyen, N. L. T. Le, V. T. Nguyen, and Q. T. Nguyen, "Study on the chemical composition of essential oil extracted from leaves of *Blumea balsamifera* (L.) DC in Vietnam," *Tap Chi Duoc Hoc*, pp. 12–13, 2004.
- [20] N. I. Bhuiyan, J. U. Chowdhury, and J. Begum, "Chemical components in volatile oil from *Blumea balsamifera* (L.) DC," *Bangladesh Journal of Botany*, vol. 38, no. 1, pp. 107–109, 2009.
- [21] Y. Hao, Z. Yu, and Z. H. Ding, "The study on chemical constituents of volatile oil on *Blumea balsamifera* growing in Guizhou," *Journal of Guiyang Medical College*, vol. 25, pp. 121–122, 2000 (Chinese).
- [22] P. Du, X. J. Zhang, and X. D. Sun, "Chemical constituents of volatile oil from *Blumea balsamifera* (Linn.) DC. in Yunnan," *Chemistry and Industry of Forest Products*, vol. 29, pp. 115–118, 2009 (Chinese).
- [23] F. Bohlmann, R. Zeisberg, and E. Klein, "Naturally occurring terpene derivatives. ¹³C NMR spectra of monoterpenes," *Organic Magnetic Resonance*, vol. 7, pp. 426–432, 1975.
- [24] G. J. Martin, S. Lavoine-Hanneguelle, F. Mabon, and M. L. Martin, "The fellowship of natural abundance ²H-isotopomers of monoterpenes," *Phytochemistry*, vol. 65, no. 20, pp. 2815–2831, 2004.
- [25] E. M. Elgendy and S. A. Khayyat, "Oxidation studies on some natural monoterpenes: citral, pulegone, and camphene," *Russian Journal of Organic Chemistry*, vol. 44, no. 6, pp. 814–822, 2008.
- [26] V. P. Talzi, "A ¹³C and ¹H NMR analysis of perfumes," *Russian Journal of Applied Chemistry*, vol. 79, no. 1, pp. 107–116, 2006.
- [27] K. N. Gurudutt, M. A. Pasha, B. Ravindranath, and P. Srinivas, "Reactions of oxiranes with alkali metals: intermediacy of radical-anions," *Tetrahedron*, vol. 40, no. 9, pp. 1629–1632, 1984.
- [28] U. Krings, B. Hardebusch, D. Albert, R. G. Berger, M. Maróstica, and G. M. Pastore, "Odor-active alcohols from the fungal transformation of α -farnesene," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 24, pp. 9079–9084, 2006.
- [29] X. Zhou, X. S. Yang, and C. Zhao, "Chemical components of volatile oil from *Blumea balsamifera*," *Journal of Instrumental Analysis*, vol. 20, pp. 76–78, 2001 (Chinese).
- [30] M. Sakuma, "Probit analysis of preference data," *Applied Entomology and Zoology*, vol. 33, no. 3, pp. 339–347, 1998.
- [31] D. Suthisut, P. G. Fields, and A. Chandrapatya, "Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids," *Journal of Stored Products Research*, vol. 47, no. 3, pp. 222–230, 2011.
- [32] B. H. Lee, P. C. Annis, F. Tumaalii, and W. S. Choi, "Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects," *Journal of Stored Products Research*, vol. 40, no. 5, pp. 553–564, 2004.
- [33] A. K. Tripathi, V. Prajapati, K. K. Aggarwal, and S. Kumar, "Toxicity, feeding deterrence, and effect of activity of 1,8-cineole from *Artemisia annua* on progeny production of *Tribolium castaneum* (Coleoptera: Tenebrionidae)," *Journal of Economic Entomology*, vol. 94, no. 4, pp. 979–983, 2001.
- [34] Z. L. Liu, S. S. Chu, and G. H. Jiang, "Toxicity of *Schizonpeta multifida* essential oil and its constituent compounds towards two grain storage insects," *Journal of the Science of Food and Agriculture*, vol. 91, no. 5, pp. 905–909, 2011.
- [35] W. Q. Li, C. H. Jiang, S. S. Chu, M. X. Zuo, and Z. L. Liu, "Chemical composition and toxicity against *Sitophilus zeamais* and *Tribolium castaneum* of the essential oil of *Murraya exotica* aerial parts," *Molecules*, vol. 15, no. 8, pp. 5831–5839, 2010.
- [36] S. S. Chu, Q. R. Liu, and Z. L. Liu, "Insecticidal activity and chemical composition of the essential oil of *Artemisia vestita* from China against *Sitophilus zeamais*," *Biochemical Systematics and Ecology*, vol. 38, no. 4, pp. 489–492, 2010.
- [37] Z. L. Liu, Q. R. Liu, S. S. Chu, and G. H. Jiang, "Insecticidal activity and chemical composition of the essential oils of *Artemisia lavandulaefolia* and *Artemisia sieversiana* from China," *Chemistry and Biodiversity*, vol. 7, no. 8, pp. 2040–2045, 2010.
- [38] Z. L. Liu, S. S. Chu, and Q. R. Liu, "Chemical composition and insecticidal activity against *Sitophilus zeamais* of the essential oils of *Artemisia capillaries* and *Artemisia mongolica*," *Molecules*, vol. 15, no. 4, pp. 2600–2608, 2010.
- [39] S. Kordali, I. Aslan, O. Çalmaşur, and A. Cakir, "Toxicity of essential oils isolated from three *Artemisia* species and some of their major components to granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)," *Industrial Crops and Products*, vol. 23, no. 2, pp. 162–170, 2006.
- [40] V. Rozman, I. Kalinovic, and Z. Korunic, "Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects," *Journal of Stored Products Research*, vol. 43, no. 4, pp. 349–355, 2007.
- [41] S. A. M. Abdelgaleil, M. I. E. Mohamed, M. E. I. Badawy, and S. A. A. El-Arami, "Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity," *Journal of Chemical Ecology*, vol. 35, no. 5, pp. 518–525, 2009.
- [42] O. Sener, M. Arslan, N. Demirel, and I. Uremis, "Insecticidal effects of some essential oils against the confused flour beetle (*Tribolium confusum* du Val) (col.: Tenebrionoidea) in stored wheat," *Asian Journal of Chemistry*, vol. 21, no. 5, pp. 3995–4000, 2009.
- [43] J. L. Wang, Y. Li, and C. L. Lei, "Evaluation of monoterpenes for the control of *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motschulsky," *Natural Product Research*, vol. 23, no. 12, pp. 1080–1088, 2009.
- [44] C. Regnault-Roger and A. Hamraoui, "Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.)," *Journal of Stored Products Research*, vol. 31, no. 4, pp. 291–299, 1995.
- [45] S. Karaborklu, A. Ayvaz, S. Yilmaz, and M. Akbulut, "Chemical composition and fumigant toxicity of some essential oils against *Ephesia kuehniella*," *Journal of Economic Entomology*, vol. 104, no. 4, pp. 1212–1219, 2011.