

## Insecticidal Activity and Composition of Essential Oil of *Ostericum sieboldii* (Apiaceae) Against *Sitophilus zeamais* and *Tribolium castaneum*

Zhi Long Liu<sup>1\*</sup>, Sha Sha Chu<sup>1</sup>, and Guo Hua Jiang<sup>2</sup>

<sup>1</sup>Department of Entomology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100094, PRChina

<sup>2</sup>Analytic and Testing Center, Beijing Normal University, No. 19, Xijiekouwai Street, Beijing 100875, PRChina

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**Abstract:** In our screening program for new agrochemicals from local wild plants, essential oil of *Ostericum sieboldii* flowering aerial parts was found to possess strong insecticidal activity against the red flour beetle, *Tribolium castaneum* and maize weevil, *Sitophilus zeamais*. The essential oil of *O. sieboldii* was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 42 components of the essential oil were identified. The principal compounds in the essential oil *O. sieboldii* aerial parts were myristicin (30.31%),  $\alpha$ -terpineol (9.92%),  $\alpha$ -cadinol (7.29%) and  $\beta$ -farnesene (6.26%) and linalool (5.94%). The essential oil possessed strong contact toxicity against *S. zeamais* and *T. castaneum* adults with LD<sub>50</sub> values of 13.82  $\mu$ g/adult and 8.47  $\mu$ g/adult, respectively. The essential oil also showed fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC<sub>50</sub> values of 27.39 mg/L air and 20.92 mg/L air, respectively.

**Keywords:** *Ostericum sieboldii*; *Sitophilus zeamais*; *Tribolium castaneum*; essential oil composition; fumigant; contact toxicity.

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### 1. Introduction

The genus *Ostericum* belongs to the family Umbelliferae and was separated from Genus *Angelica* by the presence of high concentrations flavonoids in leaf and mericarp of *Ostericum* [1]. It comprises only about 10 species in the world, of seven are distributed in China [2]. Among them,

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\* Corresponding author: E-Mail: [zhilongliu@cau.edu.cn](mailto:zhilongliu@cau.edu.cn); Phone: +86-10-62732800; Fax: +86-10-62732800.

*Ostericum sieboldii* (Miquel) Nakai is an herbaceous plant distributed mainly in the north of China and in some areas of China (e.g. Hebei, Shannxi, Shandong, Inner Mongolia, Heilongjiang, Liaoning, Jilin province) as well as Japan, Korea, Russia. The young plants are eaten as a spring vegetable, and the roots of *O. sieboldii* have reputed medicinal value as a regional substitute for the traditional Chinese medicine “Radix Angelicae Biseratae” (*Angelica biserrata* or *A. pubescens*) [2, 3]. This medicinal herb was used in traditional Chinese medicine as an analgesic and anti-inflammatory in the treatment of rheumatism and rheumatoid arthritis [4]. During our mass screening program for new agrochemicals from the wild plants, *O. sieboldii* essential oil was found to possess insecticidal activities against the maize weevil (*Sitophilus zeamais* Motsch.) and red flour beetle (*Tribolium castaneum* Herbst). A literature survey has shown that there is no report on the volatile constituents and insecticidal activity of *O. sieboldii*; thus we decided to investigate the chemical constituents and insecticidal activities of the essential oil of *O. sieboldii* against insects for the first time.

*S. zeamais* and *T. castaneum* are the most widespread and destructive primary insect pests of stored cereals [5]. 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 [6]. Fumigation plays 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 because of their easy penetration into the commodity while leaving minimal residues [7]. The currently used fumigants, phosphine and methyl bromide, are still the most effective means for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation. However, repeated use of those fumigants for decades has disrupted biological control by natural enemies and led to resurgence of stored-product insect pests, sometimes resulted in the development of resistance, and had undesirable effects on non-target organisms [7]. Moreover, the use of methyl bromide will be prohibited in the near future because of its ozone depletion potential [8]. These problems have highlighted the need to develop new types of selective insect-control alternatives with fumigant action. Plant essential oils and their components have been shown to possess potential to be developed as new fumigants and they may have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability [9, 10]. They are commonly used as fragrances and flavouring agents for foods and beverages [10, 11]. Lee *et al.* [12] evaluated fumigation toxicity of 20 naturally occurring monoterpenoids against several stored-product pest insects, including rice weevil, *S. oryzae*, *T. castaneum*, sawtoothed grain beetle, *Oryzaephilus surinamensis*, house fly, *Musca domestica*, and German cockroach, *Blattella germanica* and found that ketone compounds were generally more toxic than other monoterpenoids. Recently, 5 monoterpenes (3-carene, 1,8-cineole,  $\beta$ -pinene, terpinene and terpinolene) were evaluated repellent and insecticidal activities against adults of two stored product insects (*T. castaneum* and *S. zeamais*) and  $\beta$ -pinene was the most contact toxic compound and terpinene and terpinolene were consistently the most fumigant toxic compounds [13] while Abdelgaleil *et al.* [14] assessed the contact and fumigant toxicities of 11 monoterpenes against two important stored products insects, *S. oryzae* and *T. castaneum* and found that 1,8-cineole was the most effective monoterpene. Essential oils derived from more than 75 plant species have been evaluated for fumigant toxicity against stored product insects so far [15]. For example, several essential oils from Genus *Artemisia* were found to possess fumigant and contact toxicity against the two grain storage insects [16-18]. Nukenine *et al.* [19] found that *Plectranthus glandulosus* essential oil achieve 100% mortality for the two *S. zeamais* strains within 1 day of exposure at the dosage of 80  $\mu$ L/40 g grain and at the dosage of 20  $\mu$ L/40 g grain, *S. zeamais* F1 progeny emergency was completely inhibited by the oil. Insecticidal formulations based on *Xylopiya aethiopica* essential oil and kaolinite-clay were also developed to control stored product insects [20].

## 2. Materials and Methods

### 2.1. Plant Material

Fresh aerial parts (10 kg of leaves, stems and flowers) of *O. sieboldii* were harvested in August 2009 from Xiaolongmeng National Forest Park (Mentougou District, Beijing 102300). The aerial parts were air-dried for one week and ground to a powder using a grinding mill (Retsch Muhle, Germany). The species was identified by Dr. Liu, Q.R. and the voucher specimen (BNU-zhilongliu-2009-08-29-029) was deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University. The ground powder was subjected to hydrodistillation using a modified Clevenger-type apparatus for 4 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. Essential oil was stored in airtight containers in a refrigerator at 4°C.

### 2.2. Insects

The maize weevils (*S. zeamais*) and the red flour beetle (*T. castaneum*) were obtained from laboratory cultures maintained for the last 15 years in the dark in incubators at 29-30°C and 70-80% relative humidity. The red flour beetles were reared on wheat flour mixed with yeast (10:1, w/w) while maize weevils were reared on whole wheat at 12-13% moisture content in glass jars (diameter 85 mm, height 130 mm) at 29-30°C and 70-80% relative humidity. Unsexed adult weevils/beetles used in all the experiments were about 2 weeks old.

### 2.3. Fumigant Toxicity

A serial dilution of the essential oil (1.31-10.0%, six concentrations) was prepared in *n*-hexane. Whatman filter paper (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). Twenty microliters of an appropriate concentration of the essential oil was added to the filter paper. The solvent was allowed to evaporate for 30 s before the cap was placed tightly on the glass vial (with 10 insects) to form a sealed chamber. *n*-Hexane was used as controls. Six replicates were used in all treatments and controls and they were incubated at 29-30°C and 70-80% relative humidity for 24 h. The mortality was recorded. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC<sub>50</sub> values [21].

### 2.4. Contact Toxicity

A serial dilution (2.6-20%, six concentrations) of the essential oil was prepared in *n*-hexane. Aliquots of 0.5 µL per insect were topically applied dorsally to the thorax of insects, using a Burkard Arnold microapplicator. Controls were determined using 0.5 µL *n*-hexane per insect. Ten insects were used for each concentration and control, and the experiment was replicated six times. Both treated and control insects were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators at 29-30°C and 70-80% relative humidity. Mortality was observed after 24 h. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LD<sub>50</sub> values [21].

### 2.5. Gas Chromatography-Mass Spectrometry

The essential oil of *O. sieboldii* aerial parts was subjected to GC-MS analysis on an Agilent system consisting of a model 6890N gas chromatograph, a model 5973N mass selective detector

(EIMS, electron energy, 70 eV), and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a 5% phenyl-methylpolysiloxane stationary phase, film thickness of 0.25  $\mu\text{m}$ , a length of 30 m, and an internal diameter of 0.25 mm. The GC settings were as follows: the initial oven temperature was held at 60°C for 1 min and ramped at 10°C  $\text{min}^{-1}$  to 180°C held for 1 min, and then ramped at 20°C  $\text{min}^{-1}$  to 280°C and held for 15 min. The injector temperature was maintained at 270°C. The sample (1  $\mu\text{L}$ ) was injected neat, with a split ratio of 1: 10. The carrier gas was helium at flow rate of 1.0 mL  $\text{min}^{-1}$ . Spectra were scanned from 20 to 550  $m/z$  at 2 scans  $\text{s}^{-1}$ . Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature [16-18] or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes ( $\text{C}_8\text{--C}_{24}$ ) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [22]. Component relative percentages were calculated based on normalization method without using correction factors.

### 3. Results and Discussion

The yellow essential oil yield of *O. sieboldii* aerial parts was 0.28% v/w and the density of the concentrated essential oil was determined to be 0.87 g/mL. The chemical compositions of the essential oil were summarized in Table 1. A total of 42 components were identified in the essential oil of *O. sieboldii* aerial parts, accounting for 98.12% of the total oil (Table 1). The main components of the oil are myristicin (30.31%),  $\alpha$ -terpineol (9.92%),  $\alpha$ -cadinol (7.29%),  $\beta$ -farnesene (6.26%) and linalool (5.94%).

The essential oil of *O. sieboldii* flowering aerial parts possessed stronger contact toxicity against *T. castaneum* ( $\text{LD}_{50} = 8.47 \mu\text{g}/\text{adult}$ ) than *S. zeamais* ( $\text{LD}_{50} = 13.82 \mu\text{g}/\text{adult}$ ). Compared with the famous botanical insecticide, pyrethrum extract (25% pyrethrin I and pyrethrin II), the essential oil was 3 times less active against the maize weevils and 23 times less active against the red flour beetle because pyrethrum extract displayed  $\text{LD}_{50}$  value of 4.29  $\mu\text{g}/\text{adult}$  and 0.36  $\mu\text{g}/\text{adult}$ , respectively [17, 23]. The essential oil of *O. sieboldii* also showed strong fumigant activity against *S. zeamais* and *T. castaneum* adults with  $\text{LC}_{50}$  value of 27.39 mg/L air and 20.92 mg/L air (Table 2). The currently used grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* and *T. castaneum* adults with  $\text{LC}_{50}$  values of 0.67 and 1.75 mg/L air, respectively [1]. The essential oil of *O. Sieboldii* was 41 times less toxic to the maize weevil compared with the commercial fumigant MeBr and only 12 times less active against the red flour beetle. However, considering the currently used fumigants are synthetic insecticides, fumigant activity of the essential oil of *O. sieboldii* is quite promising and the essential oil showed potential to be developed as a possible natural fumigant for control of stored product insects. Moreover, for the practical application of the essential oil as novel fumigant/insecticide, further studies on the safety of the essential oil to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost.

**Table 1.** Chemical constituents of essential oil derived from *Ostericum sieboldii*.

RI	Compound	Percent Composition
930	$\alpha$ -Pinene	0.79
952	Camphene	1.64
981	$\beta$ -Pinene	3.22
993	$\beta$ -Myrcene	1.53
1017	$\alpha$ -Terpinene	0.69
1026	$\beta$ -Phellandrene	1.94
1032	1,8-Cineol	0.47
1057	$\gamma$ -Terpinene	0.82
1094	Linalool	5.94
1164	Pinocarveol	0.43
1167	Borneol	3.87
1175	4-Terpineol	0.33
1177	Dihydrocarveol	0.57
1182	$\rho$ -Cymen-8-ol	2.16
1191	$\alpha$ -Terpineol	9.92
1211	Octanol acetate	0.38
1226	<i>cis</i> -Carveol	0.73
1242	Carvone	0.14
1285	Bornyl acetate	0.52
1336	Octyl isobutyrate	0.32
1374	Copaene	0.61
1393	$\beta$ -Elemen	2.57
1403	Methyleugenol	0.62
1420	Caryophyllene	1.42
1432	$\beta$ -Gurjunene	0.33
1438	$\beta$ -Farnesene	6.26
1478	$\gamma$ -Muurolene	0.91
1478	$\alpha$ -Amorphene	0.55
1483	$\beta$ -Selinene	0.87
1492	$\alpha$ -Selinene	0.65
1493	$\beta$ -Ionone	0.38
1498	$\alpha$ -Muurolene	0.63
1504	Cuparene	0.41
1508	$\alpha$ -Farnesene	0.79
1513	Myristicin	30.31
1552	Dihydroactinolide	0.56
1558	Elemicin	1.23
1578	Spatulenol	2.08
1584	Caryophyllene oxide	0.94
1652	$\alpha$ -Cadinol	7.29
1682	Apiol	2.12
2119	Phytol	0.18
	Total identified	98.12

**Table 2.** Toxicity of the essential oil of *O. sieboldii* flowering aerial parts against *Sitophilus zeamais* (SZ) and *Tribolium castaneum* (TC) adults.

Insects	Essential oil	Contact toxicity		Fumigant toxicity	
		LD <sub>50</sub> (µg/adult)	95% fiducial limits	LC <sub>50</sub> (mg/L air)	95% fiducial limits
SZ	<i>O. sieboldii</i>	13.82	12.95-14.82	27.39	25.69-29.48
	Pyrethrum extract	4.29 <sup>a</sup>			
	MeBr	-	-	0.67 <sup>b</sup>	-
TC	<i>O. sieboldii</i>	8.47		20.92	19.17-22.41
	Pyrethrum extract	0.36 <sup>c</sup>	0.32-0.41		
	MeBr			1.75 <sup>b</sup>	

<sup>a</sup> data from Liu *et al.* [17]; <sup>b</sup> data from Liu and Ho [1]; <sup>c</sup> data from Li *et al.* [23]

In the previous studies, the main components of the essential oil were found to possess bioactivities against insects. For example, myristicin was found to possess insecticidal and synergistic activity [24, 25] and  $\alpha$ -terpineol was reported to have insecticidal activity against several insects and mites, e.g. human head louse (*Pediculus humanus capitis*) [26], stored product insects, rice weevil *S. oryzae* adults [27] and cowpea bruchids (*Callosobruchus maculatus*) [28], larvae of the armyworm (*Pseudaletia unipuncta*) and cabbage looper (*Trichoplusia ni*) [29] and two house dust mite (*Dermatophagoides farinae* and *D. pteronyssinus*) [30]. Moreover, (*E*)- $\beta$ -farnesene was demonstrated to be the major component of the alarm pheromone of many aphids [31] and  $\alpha$ -cadinol possessed strong antimite activity against house dust mite (*D. farinae* and *D. pteronyssinus*) [32]. In the previous studies, linalool was shown to have fumigant toxicity against the triatomine bug (*Rhodnius prolixus*) [33] and the house fly with a LC<sub>50</sub> value of 13.6 mg/L air [34]. Moreover, linalool possessed both contact and fumigant toxicity against human head louse [26] and showed a high acaricidal activity by vapour action against mobile stages of *Tyrophagus putrescentiae* [35]. The isolation and identification of the bioactive compounds in the essential oil of *O. sieboldii* are of utmost importance so that their potential application in controlling stored-product pests can be fully exploited.

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