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Composition and Toxicity of Essential Oil of *Illicium simonsii* Maxim (Illiciaceae) Fruit against the Maize Weevils

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Abstract: The essential oil of *Illicium simonsii* fruit was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 40 components of the essential oil were identified. The principal compounds in *I. simonsii* fruit essential oil were β-caryophyllene (10.30%), δ-cadinene (9.52%), methyl eugenol (8.94), β-elemene (5.84%), and α-amorphene (5.20%). The essential oil possessed strong fumigant toxicity against *Sitophilus zeamais* adults with a LC_{50} value of 14.95 mg/L air. The essential oil also showed contact toxicity against *S. zeamais* adults with a LD_{50} value of 112.74 µg/adult.

Keywords: *Illicium simonsii*; *Sitophilus zeamais*; essential oil; fumigant; contact toxicity; caryophyllene

1. Introduction

Illicium simonsii Maxim (Illiciaceae) distributes in the Southwest areas of China. It is one of adulterants of the commonly used non-toxic spice, Chinese star anise, *I. verum* [1]. The fruit of *I.*

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simonsii is also used as a Chinese medicinal herb to relive a cough, eliminate phlegm, expel intestinal parasites, kill insects flea), relive pain and inhibit bactera [1]. For example, ethanol extract of *I. simonsii* fruits possessed strong inhibition of growth of *Salmonella typhi*, *S. paratyphi*, *Staphyloccocus aureus*, and *Vibrio cholera* [1]. Several studies on the chemical constituents of *I. simonsii* have been reported and a number of flavonoids, monoterpenoids, sesquiterpenoids, triphenyl-type neolignans and biphenylneolignans have been isolated from this plant [2-5]. Chemical composition of *I. simonsii* essential oil has also been investigated previously and limonene was the major constituent [6, 7]. The fruits of *I. simonsii* they used in experiments were purchased from Chinese herbs shops and could be stored for some times. Fresh fruits were collected in the present works. However, no reports on toxicity of essential oil of *I. simonsii* fruit against the maize weevils, *Sitophilus zeamais* (Motsch) were available so far. The maize weevil is the most widespread and destructive primary insect pest of stored cereals [8]. Fumigation plays a very important role in insect pest elimination in stored products [9]. 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 [10, 11]. They are commonly used as fragrances and flavouring agents for foods and beverages [11, 12]. Lee et al. [13] evaluated fumigation toxicity of 20 naturally occurring monoterpenoids against several stored-product pest insects, including rice weevil, *Sitophilus oryzae*, red flour beetle, *Tribolium 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, β-pinene, terpinene and terpinolene were evaluated repellent and insecticidal activities against adults of two stored product insects (*T. castaneum* and *S. zeamais*) and β-pinene was the most contact toxic compound and terpinene and terpinolene were consistently the most fumigant toxic compounds [14] while Abdelgaleil et al. [15] 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 [16]. For example, Nukenine et al. [17] 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 μL/40 g grain and at the dosage of 20 μL/40 g grain, *S. zeamais* F1 progeny emergency was completely inhibited by the oil. Insecticidal formulations based on *Xylopia aethiopica* essential oil and kaolinite-clay were also developed to control stored product insects [18]. In this work, we present the chemical composition and toxicity of the essential oil of *I. simonsii* fruit against the maize weevils.

2. Materials and Methods

2.1. Plant Material

The fruit of *I. simonsii* (10 kg) was collected in October 2008 from Yulin City, Guangxi Autonomous Region (Guangxi 537000, China). A voucher specimen (CAU-Zhongyao-Yebajiao-F001) has been deposited in the Department of Entomology, China Agricultural University, Beijing 100094, PRChina. Fruit was air-dried and ground to powder using a grinding mill (Retsch Muhle, Germany) and hydrodistilled for 4 h using a Likens-Nickerson apparatus.

2.2. Insects

A culture of the maize weevil (*S. zeamais*) was maintained for the last 15 years in the dark in incubators without exposure to any insecticide. The 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. The weevils used in the experiments were 2-4 weeks post-eclosion.

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_{50} values [19].

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_{50} values [19].

2.5. Gas Chromatography-Mass Spectrometry

The essential oil of *I. simonsii* fruit 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 µm, 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 min^{-1} to 180°C held for 1 min, and then ramped at 20°C min^{-1} to 280°C and held for 15 min. The injector temperature was maintained at 270°C. The sample (1 µL) was 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 [2, 3] 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 with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [20]. Component relative percentages were calculated based on normalization method without using correction factors.

Table 1. Chemical composition of *I. simonsii* fruit essential oil.

RI	Compound	Percent Composition
931	α-Pinene	0.14
952	Camphene	0.24
977	Sabinene	0.01
981	β-Pinene	0.12
991	β-Myrcene	0.13
1005	α-Phellandrene	0.32
1010	δ-3-Carene	0.12
1029	D-Limonene	2.21
1033	1,8-Cineol	4.18
1057	γ-Terpinene	0.21
1078	Linalool oxide	0.43
1090	δ-Terpinene	0.11
1091	ρ-Cymene	0.12
1094	Linalool	3.96
1109	Fenchol	0.23
1143	*trans*-Verbenol	0.24
1143	Camphor	1.03
1148	Camphene hydrate	0.15
1167	Borneol	1.72
1179	Terpinen-4-ol	3.77
1191	α-Terpineol	4.39
1261	*cis*-Anethol	7.96
1350	α-Cubebene	1.05
1362	3-Allylguaiacol	0.26
1362	Neryl acetate	0.27
1379	Geranyl acetate	1.22
1391	β-Elemene	5.84
1403	Methyl eugenol	8.94
1413	α-Bergamotene	1.38
1420	β-Caryophyllene	10.30
1438	β-Farnesene	4.54
1455	γ-Selinene	3.65
1472	α-Amorphene	5.20
1492	α-Selinene	2.83
1521	δ-Cadinene	9.52
1536	α-Cadinene	2.47
1543	α-Calacorene	1.78
1558	Elemicin	1.80
1577	Spathulenol	3.73
1584	Caryophyllene oxide	3.08
	Total identified	99.65
	Monoterpenoids	31.36
	Sesquiterpenoids	54.47
	Monoterpene oxides	1.92
	Sesquiterpene oxides	3.08

3. Results and Discussion

The yellow essential oil yield of *I. simonsii* fruit was 1.18% v/w and the density of the concentrated essential oil was determined to be 0.93 g/mL. The chemical compositions of the fruit essential oil were summarized in Table 1. A total of 40 components were identified in the essential oil of I. simonsii fruits, accounting for 99.65% of the total oil (Table 1). The main components of the oil are β-caryophyllene (10.30%), δ-cadinene (9.52%), methyl eugenol (8.94), β-elemene (5.84%), and α-amorphene (5.20%). The chemical composition of the essential oil was different from that reported in other studies. For example, the main components of the essential oil of *I. simonsii* fruit collected from Guangxi Autonomous Region, China were limonene (73.42%) and myrcene (9.89%) [6]. However, the principal components of essential oil of *I. simonsii* collected from Sichuan province, China were *trans*-caryophyllene (11.11%), limonene (5.70%) and carvone (4.01%) while the main constituents of essential oil of *I. simonsii* fruit harvested from Guizhou province, China were limonene (39.29%), α-terpeneol (4.47%), and α-pinene (3.27%) [7]. However, those *I. simonsii* fruits they used in the experiments were all purchased from the local Chinese herbs market. The above results suggest that great variations in chemical composition of essential oil of *I. simonsii* fruit maybe due to geographic factors or storage time.

The essential oil of *I. simonsii* fruit possessed contact toxicity against *S. zeamais* adults with a LD_{50} value of 64.54 μg/adult. Compared with the famous botanical insecticide, pyrethrum extract, the essential oil was 15 times less active against the maize weevils because pyrethrum extract displayed a LD_{50} value of 4.29 μg/adult [21]. The fruit essential oil of *I. simonsi* also showed strong fumigant activity against *S. zeamais* adults with a LC_{50} value of 19.42 mg/L air (Table 2). The currently used grain fumigants, methyl bromide (MeBr) and phosphine were reported to have fumigant activity against *S. zeamais* adults with LC_{50} values of 0.67 and 0.006 mg/L air, respectively [1]. The essential oil of *I. simonsii* fruit was 30 times less toxic to the maize weevil compared with the commercial fumigant MeBr. However, considering the currently used fumigants are synthetic insecticides, fumigant activity of the essential oil of *I. simonsii* fruit is quite promising and the essential oil showed potential to be developed as a possible natural fumigant for control of stored product insects. However, for the practical application of the essential oil as novel fumigant, further studies on the safety of the oil to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost.

Table 2. Toxicity of *I. simonsii* fruit essential oil against the maize weevils.

	Contact toxicity		**Fumigant toxicity**	
	LD_{50} (μg/adult)	95% fiducial limits	LC_{50} (mg/L air)	95% fiducial limits
I. simonsii	64.54	61.50-69.33	19.42	17.69-21.18
Pyrethrum extract	4.29 [b]			
MeBr	-	-	0.67 [a]	-
Phosphine	-	-	0.006 [a]	-

[a] data from Liu and Ho [1]; [b] data from Liu et al. [21]

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