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Evaluation of insecticidal activity of the essential oil of *Eucalyptus robusta* Smith leaves and its constituent compound against overwintering *Cacopsylla chinensis* (Yang et Li) (Hemiptera: Psyllidae)

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ABSTRACT
Water-distilled essential oil from *Eucalyptus robusta* Smith leaves was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Thirty-six compounds, accounting for 96.21 % of the total oil, were identified and the main components of the essential oil of *E. robusta* leaves were α-pinene (30.18%), 1,8-cineole (26.08%), and spathulenol (5.31%), followed by globulol (4.44%), and viridiflorol (3.97%). The essential oil of *E. robusta* leaves possessed contact toxicity against the overwintering adults of Chinese pear psylla (*Cacopsylla chinensis*) with a LD_{50} value of 10.61 μg/adult. The two main constituent compounds, α-pinene and 1,8-cineole exhibited strong acute toxicity against the overwintering Chinese pear psylla with LD_{50} values of 1.34 μg/adult and 11.76 μg/adult, respectively.

Keywords: *Eucalyptus robusta*; *Cacopsylla chinensis*; essential oil; contact toxicity; α-pinene; 1, 8-cineole.

1. Introduction

Chinese pear psylla, *Cacopsylla chinensis* (Yang et Li) (Hemiptera: Psyllidae) is one of the main pests of pear trees in China. The most notable characteristics of the adults of *C. chinensis* are seasonal morphological dimorphism, that is, during the growing season they are small, light-colored (summer form), then become large and dark (winter form) in later autumn and winter [1]. It overwintered mainly by the adults in the north of China. Control of the overwintering adults was the most important strategy because nymph of this species excretes a large amount of honeydew and it is very hard to control during this period [2]. At present, the control of *C. chinensis* is dependent on the use of synthetic insecticides such as imidacloprid, thiamethoxam, fenvalerate, acetamiprid, amitraz and avermectins, especially in growing season. However, there were many drawbacks caused by heavy application of synthetic insecticides, such as toxic residues in fruits; disruption of the natural biological control systems and sometimes resulted in the widespread development of resistance as well as undesirable effects on non-target organisms [3-5]. Thus, there is an urgent need to develop safer, environmentally friendly and efficient alternative that have potential to replace synthetic pesticides and are convenient to use. Plant essential oils and their components have been shown to have potential for development as new insecticides and may have advantages over conventional insecticides in terms of low mammalian toxicity, rapid degradation and local availability [4, 5]. Many essential oils derived from Chinese medicinal herbs have been evaluated for insecticidal toxicities and repellency against insects [6-15]. During our screening program for new agrochemicals from Chinese medicinal herbs, *Eucalyptus robusta* Smith (Family: Myrtaceae) was found to possess strong insecticidal activity against Chinese pear psylla.

E. robusta is native to Eastern Australia and now widely cultivated in China and has been used as a traditional Chinese medicinal herb [16]. The known chemical constituents of this species include monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, phloroglucinol derivatives, flavonoids, and tannins [17-25]. Chemical composition of the essential oil of *E. robusta* leaves has also been widely determined [26-31]. *E. robusta* essential oil possessed strong repellency to *Blattella germanica* [32] and exhibited strong fumigant and larvicidal activity against *Aedes aegypti* [33].

The essential oil derived from *E. robusta* was demonstrated to exhibit strong antimicrobial, antifungal, and antimalarial activities [28-30, 35]. However, literature survey has shown that there is no reports on insecticidal activity of the essential oil of *E. robusta* leaves against overwintering *C. chinensis*, thus we decided to investigate chemical composition of *E. robusta* essential oil and to evaluate acute toxicity of the essential oil and its two main constituents against the adults of overwintering Chinese pear psylla.

2. Materials and Methods

2.1 Insects

Chinese pear psylla (*C. chinensis*) overwintering adults were trapped from Weishanzhuang pear orchard (39.43° N latitude and 116.02° E longitude, Daxing District, Beijing 100026) by using corrugated paper trappers (Shaanxi Jinggong Technology, Co., Ltd. http://www.jgkj.com.cn). The corrugated trappers were surrounded near the pear trees about 100 cm above the ground to attract overwintering pear psylla in November 20, 2011 and collected in February 20, 2012 [2].

2.2 Chinese medicinal herb and essential oil extraction

Dried leaves of *E. robusta* (5 kg) were purchased from Anguo Herb Market (Anguo, Hebei Province, China). The sample was identified and a voucher specimen (CMH-DaYeAn-Guang Xi-2011-09) was deposited in the Department of Entomology, China Agricultural University. The herb was firstly grinded to powdered form using a grinding mill (Retsch Muhle, Germany) and was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in airtight containers in refrigerator at 4 °C for subsequent experiments. (+)-α-Pinene and 1, 8-cineole (99%) were purchased from Sigma-Aldrich Chemical Co. (P.O. Box 14460, St. Louis, MO 63178, USA). Avermectins was purchased from Aladdin-reagent Company (Shanghai). Pyrethrum extract (25% pyrethrin I and pyrethrin II) was purchased from Fluka Chemie.

2.3 Contact toxicity using topical application

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil and its constituent compounds. Five concentrations (1,300-10,000 ppm, in acetone) and five replicates of each concentration were used to determine LC_{50} values. The overwintering pear psylla were removed from the paper trappers to glass vials (80 mm in diameter, 130 mm in height, about 60 adults per vials) and the glass vials were maintained in incubators at 28-30 °C and 70-80% relative humidity for 24 hr. Then the pear psylla were firstly anesthetized by using ether and transferred to Petri dish which was placed on the ice. Aliquots of 0.5 μl of the dilutions were applied topically to the dorsal thorax of the insects. Six replicates were used in all treatments and controls. Both treated and control insects were then transferred to glass vials (10 insects/vial) and kept in incubators at 28-30 °C and 70-80% relative humidity. All the treatments were replicated three times. Acetone was used as a negative control and pyrethrum extract and avermectins were used as positive controls. Mortality of insects was observed after 24 hr.

2.4 GC-MS analysis

Analyses of volatile constituents were determined using an Agilent 5973 GC-MS system operating in the EI mode at 70 eV [equipped with a 30m HP-5MS column (0.25 mm ×30 m × 0.25 μm) and coated with 5% phenyl-methylpolysiloxane using a HP-5MS (df = 0.25 μm) (Agilent J&W Scientific, USA)]. The temperature program used for the analysis was as follows: initial temperature at 60 °C, held for 1 min, ramped at 4 °C /min to 290 °C and held for 0.5 min. Helium was the carrier gas at 1.0 ml/min; the sample (1 μl diluted to 1/100, v/v, in hexane) was injected in the split mode (1:5). The injector and detector temperatures were preformed at 230 °C and 300 °C, respectively. The Kovats retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C_8-C_{24}. Quantification was performed using percentage peak area calculations and the identification of individual compartments was done using the Wiley/ NBS Registry of Mass Spectral Database and NIST MS Search, literature [36] and several authentic compounds. The relative concentration of each compound in essential oil was quantified based on the peak area integrated by the analysis program.

2.5 Statistical analysis

The observed mortality data were corrected for control mortality using Abbott's formula. The results from all replicates were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LC_{50} values with their fiducial limits [37]. Samples for which the 95% fiducial limits did not overlap were considered to be significantly different.

3. Results and Discussion

3.1 Chemical composition of the essential oil

The water distillation for 3 h of *E. robusta* leaves afforded essential oil (yellow) with a yield of 0.17% (v/w) and the density of the concentrated oil was 0.84 g/ml. Thirty-six compounds were identified and the main components of the essential oil of *E. robusta* leaves were α-pinene (30.2%), 1,8-cineole (26.1%), and spathulenol (5.3%), followed by globulol (4.4%), and viridiflorol (4.0%) (Table 1). The results were quite different from the previous reports [22-28]. For example, the essential oil of *E. robusta* leaves collected from Brazil mainly contained α-pinene (73.0%) followed by limonene (8.3%) and β-pinene (6.8%) [30] while 1,8-cineole (50.0%), α-pinene (22.2%), *trans*-pinocarveol (13.0%), globulol (5.7%), and pinocarvone (5.4%) were the major constituents in the essential oil of *E. robusta* leaves grown in Algeria [27]. However, *trans*-pinocarveol (26.6%), α-pinene (13.0%), pinocarveol (6.4%) were the major constituents in the essential oil of *E. robusta* leaves harvested from Australia [35] and the major compounds identified in the essential oil of *E. robusta* leaves collected from the Democratic Republic of Congo were ρ-cymene (27.3%), myrtenal (12.8%), β-pinene (6.3%) and α-terpineol (6.3%) [29, 38]. Moreover, the essential oil of *E. robusta* leaves collected from Mali mainly contained α-pinene (23.9%), ρ-cymene (23.2%), 1,8-cineole (14.5%), α-phellandrene (12.0%) and β-pinene (8.6%) [31]. The above findings suggested there were great geographic variations in chemical composition of the essential oil of *E. robusta* leaves or the great variation maybe due to different varieties or populations of *E. robusta*. For practical use, it is necessary to standardize the essential oil of *E. robusta* leaves.

3.2 Contact toxicity of the essential oil and its constituents

α-Pinene possessed stronger contact toxicity (LD_{50} = 1.34 μg/adult) than 1,8-cineole (LD_{50} = 11.76 μg/adult) against overwintering *C. chinensis* adults (no overlaps in 95% fiducial limit, Table 2) while the crude essential oil had an LD_{50} value of 10.61 μg/adult. Compared with the positive control, pyrethrum extract (LD_{50} = 1.47 μg/adult), the essential oil exhibited 7 times less activity against *C. chinensis* and α-pinene showed the same level of acute toxicity as the positive control while 1,8-cineole exhibited only 7 times less activity against *C. chinensis*. The essential oil also exhibited less toxicity against *C. chinensis* adults than garlic essential oil (LD_{50} = 1.42 μg/adult) [2]. However, compared with avermectins (commercial insecticide, $LD_{50} = 2.16 \times 10^{-3}$ μg/adult), α-pinene exhibited only six hundred times less toxicity against *C. chinensis*. In the previous reports, α-pinene has been known to show contact and fumigant toxicity as well as repellency against several species of insects and mites [39-47], e.g. grain storage insects, *Sitophilus zeamais*, *S. oryzae* and *Tribolium castaneum* [43, 44, 47], German cockroaches, *Blattella germanica* [32, 40, 42, 46], mosquitoes, *Culex pipiens molestus* [39], and *Pediculus humanus capitis* [41].

α-Pinene exhibited acetylcholine esterase (AChE) inhibition activity against female German cockroaches with an IC_{50} value of 0.28 mg/mL [46]. However, it also attracted several scolytids and associated beetles [48, 49]. The above findings suggest that acute toxicity of *E. robusta* essential oil and its constituent compounds especially α-pinene are quite promising. They show potential to be developed as possible natural insecticides for control of Chinese pear psylla by considering currently used synthetic insecticides because of their high toxicity to humans and other non-target organisms. However, for the practical application of the essential oils and their constituent compounds as novel insecticides, further studies on the safety of the essential oils/compounds to humans and plants are needed. A further study is also necessary to determine the toxicity of these essential oils and their constituent compounds on other economically important pests and their natural enemies in greenhouse conditions where pest management depends on chemical applications. Owing to their volatility, *E. robusta* essential oil and its active components have limited persistence under field conditions, therefore, although natural enemies are susceptible via direct contact, predators and parasitoids reinvading a treated crop one or more days after treatment are unlikely to be poisoned by residue contact as often occurs with conventional insecticides [4, 5]. Moreover, when *E. robusta* essential oil-based products are sprayed to control overwintering adults of Chinese pear psylla in early spring, those natural enemies and pollinators are still in the overwintering state and rarely contact the *E. robusta* essential oil-based products. Further studies on the development of formulations are also necessary to improve the efficacy and stability and to reduce cost.

Table 1: GC-MS analysis of the essential oil derived from *Eucalyptus robusta* leaves

Compounds	RI	Composition (%)
α-Pinene*	931	30.18
β-Pinene*	974	0.56
β-Myrcene*	991	0.77
δ-3-Carene	1008	0.32
Limonene*	1029	1.23
1,8-Cineole*	1033	26.08
γ-Terpinene	1059	0.45
Linalool oxide	1078	0.40
Fenchone	1088	1.01
Linalool*	1094	1.54
Octen-1-ol acetate	1104	0.34
trans-Pinocarveol	1138	3.01
(+)-Camphor	1147	0.56
Borneol*	1167	2.32
4-Terpineol*	1179	1.18
α-Terpineol*	1188	0.65
Estragole*	1195	0.32
ρ-Menth-1-en-8-ol	1208	3.12
cis-Carveol	1226	0.23
Bornyl acetate*	1287	0.12
Eugenol*	1356	0.53
Methyleugenol*	1403	0.21
β-Caryophyllene*	1426	1.76
Aromadendrene	1437	2.16
allo-Aromadendrene	1458	0.81
Germacrene D	1485	0.54
δ-Selinene	1492	0.89
trans-Nerolidol*	1567	0.76
Spathulenol	1575	5.31
Globulol	1581	4.44
Viridiflorol	1592	3.97
α-Cadinol	1640	0.44
Total identified		96.21

RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons; *Identification by co-injection of authentic compounds.

Table 2: Insecticidal activity of the essential oil of *Eucalyptus robusta* leaves and its two main constituents against *Cacopsylla chinensis* overwintering adults

Treatment	LD_{50} (μg/adult)	95% fiducial limits	Slope ± SD	χ^2
Eucalyptus robusta	10.61	(9.52-11.74)	4.25 ± 0.43	12.88
α-Pinene	1.34	(1.20-1.49)	3.67 ± 0.36	9.84
1,8-Cineole	11.76	(10.38-13.15)	3.74 ± 0.39	11.04
Avermectins	2.16×10^{-3}	$(1.65\text{-}2.62)\times10^{-3}$	3.52 ± 0.32	15.64
Pyrethrum extract	1.47	(1.16-1.71)	4.18 ± 0.67	12.48

LD_{50}, median lethal dosage; SD, standard deviation.

4. Conclusions
The essential oil of *E. robusta* leaves and its two constituent compounds demonstrated some acute toxicity against the overwintering *C. chinensis* adults. They showed potential to be developed as a possible natural insecticide for control of Chinese pear psylla but needs to be further evaluated for safety in humans and to enhance its activity.

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