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Chemical composition of an insecticidal extract from *Robinia pseudacacia* L. seeds and it's efficacy against aphids in oilseed rape

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ABSTRACT

Interest in the use of plant extracts to replace chemicals as insecticides is growing. In the present study, the chemical composition and insecticidal activity of *Robinia pseudoacacia* L. seed extract were investigated. The insecticidal extract of *R. pseudoacacia* seeds was analyzed by GC × GC-TOFMS. A total of 51 compounds were identified and the major constituents found in the seed extract were 9,12-octadecadienoic acid (20.9%), 9,12,15-octadecatrienoic acid methyl ester (14.3%) and 9,12-octadecadienoic acid methyl ester (10.9%). The results showed that the petroleum ether fraction of *R. pseudoacacia* seeds exhibited strong insecticidal activity against cotton aphid and cabbage aphid with LD_{50} values of 7.04 ng/insect and 6.87 ng/insect, respectively (at 24 h post-treatment) using a topical application method. The seed extract formulation showed notable efficacy on aphids in a field test, and the mortality was above 95% by 7 days after treatment in the oilseed rape field. The results of a laboratory bioassay and a field experiment have shown that *R. pseudoacacia* seeds could be considered as a potential source for development of a botanical insecticide for controlling aphids.

1. Introduction

Aphids (Hemiptera: Aphididae) are one of the most serious pests that attack a broad range of agricultural crops (De Little et al., 2017). They can cause damage to hundreds of host plants in both the field and under protection (Mardani-Talaee et al., 2016). Aphid control is mainly based on chemical pesticides, including organophosphorus, carbamates, neonicotinoid such as imidacloprid. However, the indiscriminate use of synthetic pesticides may have side effects on both environment quality and human health (Smaili et al., 2014). Furthermore, aphids have developed a high resistance to several pesticides. For example, cotton aphid (*Aphis gossypii* Glover) has developed resistance to organophosphorus, carbamate, and neonicotinoid pesticides (Gore et al., 2013; Herron and Wilson, 2017). Therefore, replacing chemical pesticides with botanical alternatives could be a good choice for overcoming these problems (Dara, 2016; Silva et al., 2016). Until now, several insecticidal plants have been reported, including Asteraceae (García et al., 2007; Tennyson et al., 2015), Meliaceae (Akhtar et al., 2008) and Legumes (Akdeniz and Özmen, 2011) and so on. However, despite current intensive research, suitable commercial botanical insecticides are limited and insufficient (Pavela, 2016). This is due to several reasons. Most research into botanical insecticides lacks chemical characterization, positive controls and field trials (Isman and Grieneisen, 2014). Additionally, the development of suitable formulations for biopesticides need to be given more attention (Pavela and Benelli, 2016).

Black Locust (*Robinia pseudoacacia* L.) is a leguminous deciduous tree, native to North America, and now is cultivated throughout the world (Lee et al., 2004). Several leguminous plants are considered to have insecticidal properties, such as *Sophora alopecuroides* L. (Yang et al., 2014) and *Sophora flavescens* L. (Akdeniz and Özmen, 2011). Phytochemical studies have shown that *R. pseudoacacia* contains a variety of secondary metabolites, such as flavonoids (Veitch et al., 2010), alcohols (Chen and Dai, 2014) and terpenes (Xie et al., 2006), with antioxidant (Katiki et al., 2013) and antifungal activities (Chen and Dai, 2014; Marinas et al., 2014). To the best of our knowledge, there is still no reported laboratory or field experiments on the aphicidal activity of the seed extract from *R. pseudoacacia*.

Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC-TOFMS) is a powerful tool, which has been successfully applied for separation and identification of the chemical

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composition in complex natural samples (Izadmanesh et al., 2017). Compared with the traditional GC and GC-MS methods, GC × GC-TOFMS provides a significant increase in separation and identification (Potgieter et al., 2016). There are also no data on GC × GC-TOFMS analysis of seed extract of *R. pseudoacacia*.

The aim of this research was to determine the chemical composition of the seed extract of *R. pseudoacacia* by GC × GC-TOFMS, and to evaluate the aphicidal activity of the seed extract against aphids in a laboratory bioassay and a field test. This could lead to the identification of a candidate for development of a botanical insecticide for control of aphids.

2. Materials and methods

2.1. Plant material

Seeds of *R. pseudoacacia* were purchased from China National Tree Seed Corporation in August 2013. The plant material was authenticated by Prof. Guijun Zhang from Beijing University of Chinese Medicine. The seeds were dried in the shade and ground into powder and stored at −20 °C.

2.2. Chemicals

HPLC-hexane was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Water was prepared by an ultrapure water system (Purelab Plus, Pall, USA). The standard compound of imidacloprid (purity, 96%) was purchased from National Pesticide Quality Supervision and Inspection Centre (Beijing, China). Alkanes (C_7–C_{30}) were obtained from Supelco (Bellefonte, PA, USA). All other chemicals were purchased from Beijing Chemical Works (Beijing, China). Imidacloprid WP at a concentration of 10% was purchased from Nanjing Red Sun Co., Ltd. (Nanjing, China).

2.3. Insects

Apterous adult aphids were used for insecticidal bioassays. Two species of aphid (*Aphis gossypii* Glover and *Brevicoryne brassicae* L.) were reared on the leaf blades of cotton seedlings and rape seedlings, respectively. The controlled conditions for rearing aphids were: temperature 28 ± 1 °C, relative humidity 70% ± 5% and a long photoperiod of 16 h light.

2.4. Preparation of seed extract of R. pseudoacacia

The dried seed powder (2.5 kg) of *R. pseudoacacia* was extracted with 75% ethanol (25 L × 3) for 6 days at room temperature. The combined extract was concentrated under reduced pressure on a rotary evaporator at 40 °C, and freeze-dried to yield a black-brown powdered extract (525.4 g). This freeze-dried extract (500.0 g) was partitioned successively in hot water (1 L) and extracted with petroleum ether (3 × 0.5 L), ethyl acetate (3 × 0.5 L), and *n*-butanol (3 × 0.5 L). Four fractions were evaporated and freeze-dried to afford a petroleum ether fraction, ethyl acetate fraction, *n*-butanol fraction and water-phase fraction.

2.5. Insecticidal activity in laboratory

The freeze-dried seed crude extract and fractions were dissolved in acetone to a concentration of 500 mg/L. Final concentrations of the seed extract and fractions were set at 31.25, 62.50, 125, 250, and 500 mg/L. Controls were treated with acetone. The leaves of cotton seedlings and rape seedlings were collected, each leaf containing about 50 aphids. Insecticidal activity was evaluated using a topical application method (Jiang et al., 2016; Mohamad et al., 2013; Overgaard et al., 2014; Pavela et al., 2013). Sample solutions were dropped onto the back of each aphid using an auto micro-applicator (900-X, Burkard Manufacturing Co. Ltd., Rickmansworth, UK). After application, these seedlings with treated aphids were placed in covered Petri dishes (9.0 cm i.d.) at controlled temperature (28 ± 1 °C), and incubated at humidity (70% ± 5%) with light: dark (16: 8 h). Imidacloprid was selected as a positive control. Aphid mortality was assessed after 24 h. Three replicates were carried out for each treatment. The corrected mortality rates were measured using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality (\%)} = (M_1 - M_c)/(100 - M_c) \times 100 \quad (1)$$

Where M_1 (%) was the mortality of the treated groups and Mc (%) was the mortality of the control groups.

2.6. GC × GC-TOFMS analysis

The oily matter of petroleum ether fraction (10 μL) was diluted with n-hexane (1 mL). The petroleum ether fraction from *R. pseudoacacia* seeds was analyzed by a GC × GC-TOF/MS (Model Pegasus 4D, LECO Corporation, St. Joseph, MI, USA). The GC × GC conditions were as follows: the first column was a nonpolar Rxi-5 Sil MS 30 m capillary column (0.25 mm i.d. × 0.25 μm film thickness, Restek Corp., Bellefonte, PA, USA) and the second column was a medium polarity Rxi-17 Sil MS 2 m capillary column (0.18 mm i.d. × 0.18 μm film thickness, Restek Corp., Bellefonte, PA, USA). Helium (≥ 99.999%) was used as carrier gas at a flow rate of 1 mL/min. The initial temperature of oven was set at 50 °C for 12 s and up to 280 °C at a ramp rate of 8 °C/min. The secondary column temperature was set at a 5 °C offset above the first column. The injector was set at 240 °C. Sample solution (1 μL) was injected into the GC in split mode, with a split ratio of 50:1.

TOF/MS was operated with an electron energy of 70 eV, using a scan mode in a range of *m/z* 33–550. The detector was set at 1420 V. The ion source temperature and transfer line temperature were set at 250 °C and 280 °C. The modulator temperature offset was set at 15 °C. The acquisition rate was set at 100 spectra/s. The modulation period and hot pulse were set at 4 s and 0.8 s. A NIST library (2011) was selected for identification of compounds.

2.7. Preparation of formulation and its field experiment

The optimum ratios of ethanol extract of *R. pseudoacacia* seeds, emulsifier and antifreeze were screened according to the method as previously described (Roland et al., 2003; Wang et al., 2007; Zhang and Liu, 2011). The optimized seed extract formulation was composed of seed extract (10%, g/v), emulsifier (12%, v/v), antifreeze (3%, g/v), ethanol (12%, v/v), ethyl acetate (6%, v/v), organic silicon (1%, v/v) and water (56%, v/v). The 10% seed extract EW was prepared by using a high-speed shear emulsion equipment (Fluko Hishear FA25, Fluko Equipment Shanghai Co., Ltd., Shanghai, China) to form a homogeneous emulsion liquid. The physical properties of the 10% seed extract EW was evaluated. According to the GB/T1603-2001 and GB/T19136-2003 standards, the dilution stability of the emulsion and thermal storage (54 °C, 12 days) were determined, respectively. According to the GB/T19137-2003 standard, the stabilities at low temperature of the emulsion after cold storage (0 °C, 1 h, 7 days) were tested.

The field experiment of the preparation against aphids was performed in an oilseed rape field (*Brassica napus* L.) in Fanchang County (E 118.14'; N 31.04') of Anhui province. The treated plants were highly infested by the aphids and no chemical pesticides were applied before this research. The cabbage aphid, *B. brassicae* (Hemiptera: Aphididae), is a key pest of oilseed rape (Mirmohammadi et al., 2009). The field experiment was performed according to GB/T17980.79–2004 and NY/T1464.27–2010 of national test guidance in China and the experiment was designed as follows: the prepared seed extract formulation was set at 300 g/ha, 600 g/ha and 900 g/ha (the extract dose), respectively. A total spray volume of 750 L/ha was applied. Water was used as a