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Full Length Research Paper

Bioactivity of essential oil from *Ailanthus altissima* bark against 4 major stored-grain insects

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The essential oil was extracted by Soxhlet method with anhydrous diethyl ether from *Ailanthus altissima* Swingle (Sapindales: Simaroubaceae) bark, a traditional Chinese herbal plant and its repellent, contact and fumigant activities were investigated against four major stored grain insects. *A. altissima* bark oil significantly repelled *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae), *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) and *Liposcelis paeta* Pearman (Psocoptera: Liposcelididae) adults, with the repellency value reaching IV grade or stronger during the whole exposure period. *A. altissima* bark oil also possessed strong contact toxicity on *S. oryzae* adults which gradually enhanced with increased exposure time and the corrected percentage mortality reached 76.5% after 72 h treatment. Furthermore, *A. altissima* bark oil had high fumigant activity against *O. surinamensis* and *S. oryzae* adults with the corrected percentage mortality 99.3 and 81.9% within 24 h, respectively.

Key words: *Ailanthus altissima* bark, essential oil, stored-grain insects, bioactivity.

INTRODUCTION

The widespread and intensive use of synthetic insecticides for the control of stored-grain insects has led to serious problems including insecticide resistance, poisoning of workers, rising cost of production and lethal effects on non-target organisms (Jembere et al., 1995; Okonkwo and Okoye, 1996; Liu and Ho, 1999; Jovanovic et al., 2007). Development and implementation of alternative control strategies and integrated pest management systems have recently been considered to be the only solution to combat these increasing insecticide-resistant insect pests (Chen et al., 2002; Kim et al., 2003; Tapondjoua et al., 2005). Plant- based insecticides may provide potential alternatives to currently used insect-control agents because they are natural source of bioactive chemicals with complicated action mechanism, to which the insect pests are difficult to produce resistance, readily biodegradable, often less toxic to mammalian and with less or negligible danger to the environment if used in suitable amounts. Particularly, because of the unacceptable high cost and difficulty of researching and developing new synthetic insecticides, recent research has focused on natural product alternatives for pest control in developing countries (Boekea et al., 2004; Liu et al., 2007; Isman, 2006, 2008; Rajendran and Sriranjini, 2008; Bai, 2008; Nerio et al., 2009; Paul et al., 2009).

Many Chinese herbal plants are potential sources of pesticides and have exhibited potent toxic bioactivity to stored-grain insects (Yang and Tang, 1988; Wang et al., 2006; Liu et al., 2007) . In fact, as a traditional Chinese herbal plant, *Ailanthus altissima* have also for many generations been used as a traditional method by farmers to protect stored products from insect infestation in China (Cheng et al., 2005). However, insecticidal activities of essential oil from A. altissima against stored-grain insects have not been investigated so far.

Thus, the essential oil extracted from *A. altissima* bark was evaluated for its potential repellent, contact and fumigant activity against four major stored grain insects, *Sitophilus oryzae*, *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Liposcelis paeta* adults.

MATERIALS AND METHODS

Insects

The test insects were obtained from laboratory stock cultures maintained in the dark in incubators without exposure to any insecticide at 27 ± 2°C and 75 ± 5% relative humidity at the Institute

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of Stored Product Insects of Henan University of Technology. The food media used were washed, sterilized wheat at about 13.5% equilibrium moisture content for *S. oryzae*, wheat flour, rolled oats and yeast (6:3:1, w/w/w) for *O. surinamensis*, wheat flour and rolled oats (6:1, w/w) for *T. confusum* and wheat flour, degrease milk powder and yeast (1:1:1, w/w/w) for *L. paeta*. Healthy and consistent developed insects were randomly chosen for tests.

Preparation of the essential oil

The *A. altissima* bark was collected in Henan, central China, October 2008, dried at room temperature, ground to powder and silted through 60-eye sieve. Each 50 g of the powder was extracted by Soxhlet method with 250 ml anhydrous diethyl ether until the distilled liquid was colorless. The solvent was evaporated under vacuum in a rotary evaporator. The essential oil was stored in airtight fuscous glassware in a refrigerator at 4°C and evenly diluted with acetone (analytical purity) at the proportion of 1:15 (v/v) (in the remainder of this paper referred to as "the *Ailanthus* oil") for the following bioassays.

Repellency bioassay

The repellent effects of the *Ailanthus* oil against *S. oryzae*, *O. surinamensis* and *T. castaneum* were evaluated using the area preference method. Test areas consisted of Whatman No.1 filter paper cut in half (12.5 cm). One ml of the *Ailanthus* oil was uniformly applied to a half-filter paper disc using a micropipette. The other half of the remaining filter paper was treated with 1 ml acetone alone and used as control. Chemically treated and control half discs were air-dried for about 10 min to evaporate the solvent completely. Full discs were subsequently remade by attaching treated halves to untreated halves with clear adhesive tape. Each remade filter paper disc was tightly fixed on the bottom of a 12.5 cm diameter Petri dish daubed with polytetrafluoroethylene (PTFE) in the inside wall to avoid the insects from escaping. Then 30 unsexed adult insects of each species were released separately at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in incubator at 27 ± 2°C and 75 ± 5% relative humidity. Each treatment was replicated 5 times and the number of insects present on the control (N_c) and treated (N_t) areas of the discs was recorded after 12, 24 and 48 h, respectively.

The repellent effect of the *Ailanthus* oil against *L. paeta* adults was assayed similar to the described earlier method and the only difference was that the diameter of the tested filter paper and Petri dish was 6 cm, the treatment dose was 0.2 ml and 30 adults were put in a Petri dish.

Percentage repellency (PR) values were calculated as follows:

$PR = [(N_c - N_t) / N_c]100\%$

The mean percentage repellency value was calculated and assigned to repellency classes (Juliana and Su, 1983) from 0 to V: class 0 (PR < 0.1%), class I (PR = 0.1 - 20%), class II (PR = 20.1 - 40%), class III (40.1 - 60%), class IV (60.1 - 80%) and class V (80.1 - 100%).

Contact toxicity

Contact toxicity to *S. oryzae*, *O. surinamensis*, *T. castaneum* was assayed by directly applying 0.48 µl of the *Ailanthus* oil using a micropipette on the prothorax backboard of each tested adult insect and the equal amount of acetone was applied alone as control.

Thirty treated unsexed insects were introduced in a clean Petri dish (12.5 cm). The Petri dishes were covered and kept in incubators at 27 ± 2°C and 75 ± 5% r.h. and the number of dead insects was recorded after 24, 48 and 72 h.

Contact toxicity to *L. paeta* was evaluated on filter paper discs by evenly treating a Whatman No.1 filter paper (6 cm) with 0.2 ml diluted *Ailanthus* oil. The filter paper was placed in a clean Petri dish (6 cm) daubed with PTFE on the inside wall to avoid the insects from escaping. Then 30 adults were introduced into a Petri dish which was subsequently covered and kept in incubators at 27 ± 2°C and 75 ± 5% relative humidity. Each treatment was replicated five times and the number of dead insects was recorded after 4 days.

Fumigant activity

Fumigant activity of the *Ailanthus* oil was carried out against *S. oryzae*, *O. surinamensis*, *T. castaneum* and *L. paeta* adults respectively with 30 insects exposed in a 250 ml flask sealed with a rubber stopper. The flask held 10 g wheat. 1 ml the *Ailanthus* oil was evenly applied to a Whatman No.1 filter paper strip (7 × 9 cm), which was dried in air for 10 min and then fixed on the stopper by a staple at one end. The equal amount of acetone was applied alone as control. The stopper was tightly stuffed in the flask to make the filter paper suspend in the top and care was taken to avoid the filter paper from making contact with the walls of the flask. The flask was placed in the incubators at 27 ± 2°C and 75 ± 5% relative humidity. Five replicates were conducted. The number of dead insects was recorded after 24 h.

Statistical analysis

For the above tests, the percentage mortality was corrected by the Abbott (1925) formula. The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at P = 0.05 (SAS Institute, 1994).

RESULTS

Repellency bioassay

The essential oil from *A. altissima* bark exhibited potent repellent activity against *T. castaneum*, *O. surinamensis*, *S. oryzae* adults and *L. paeta* adults (Figure 1) and the repellency value reached IV grade or stronger during the whole exposure period. The *Ailanthus* oil showed the strongest repellent activity against *T. castaneum* adults and the repellency value was always kept at V grade during the observation period, following the *O. surinamensis* adults.

Contact activity

Contact activity of the essential oil from *A. altissima* bark against *S. oryzae*, *O. surinamensis* and *T. castaneum* adults gradually increased with increasing exposure time (df = 2, P < 0.05). Specially, the *Ailanthus* oil showed the stronger contact toxicity against *S. oryzae* adults with the corrected percentage mortality 76.5% after 72 h treat-

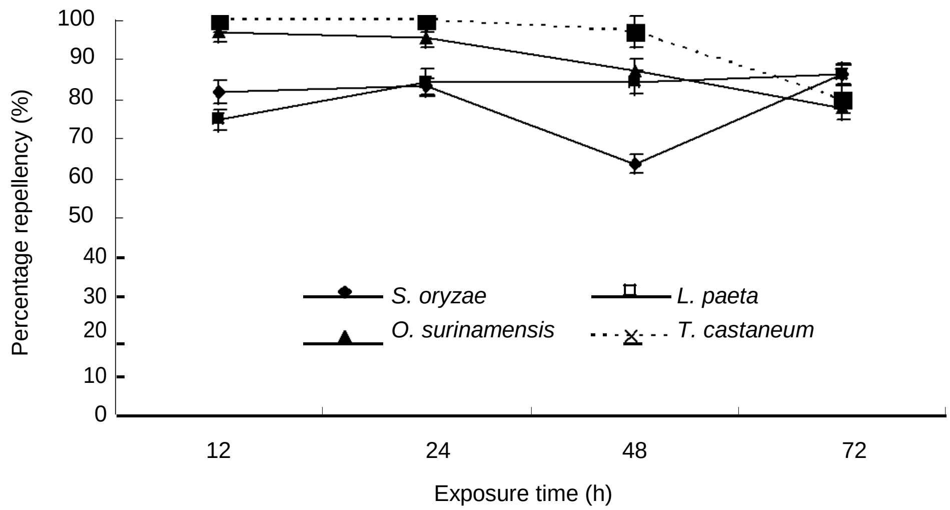

Figure 1. Repellent activity of the essential oil from *A. altissima* bark against 4 major stored-grain insects.

Table 1. The contact and fumigant activity of the *A. altissima* bark oil against 4 major stored-grain insects (the corrected percentage mortality %).

Exposure time	*S. oryzae*	*O. surinamensis*	*T. castaneum*	*L. paeta*	Action mode
24 h	33.7 ± 2.1a	4.6 ± 0.4b	4.6 ± 0.2b	-	Contact activity
48 h	40.8 ± 2.6a	13.6 ± 0.6b	12.2 ± 1.2b	-	
72 h	76.5 ± 1.5a	30.2 ± 2.0b	18.4 ± 0.9c	-	
4 d	-	-	-	3.6	
24 h	81.9 ± 1.1b	99.3 ± 5.6a	5.3 ± 0.3d	29.8 ± 0.5c	Fumigant activity

Each datum represents the mean ± SE of five replicates, each set up with 30 adults (n = 150). Data within a row followed by the same letter are not significantly different at P = 0.05.

ment and weak contact toxicity against *O. surinamensis*, *T. castaneum* adults and *L. paeta* adults (Table 1).

Fumigant activity

The essential oil from *A. altissima* bark showed potent fumigant activity against *O. surinamensis* and *S. oryzae* adults with the corrected percentage mortality reached at 99.3 and 81.9%, respectively and slightly weak fumigant activity against *T. castaneum* adults and *L. paeta* adults (df = 3, $P < 0.05$) (Table 1).

DISCUSSION

Many essential oils and their constituents have been studied to possess potential as alternative compounds to currently used insect-control agents (Shaaya et al., 1991, 1997; Huang et al., 2000; Lee et al., 2001a, b, 2004; Rajendran and Sriranjini, 2008; Batish et al., 2008; Sahaf et al., 2008; Cosimi et al., 2009; Nerio et al., 2009). However, the bioactivities of the *Ailanthus* oil against stored grain insect pests have not been investigated up to now. Previous research testified that *A. altissima* stem bark methanol extract exhibited strong herbicidal effects which contain ailanthone as one of the major herbicidal compounds (Heisey and Heisey, 2003). Our results clearly showed that essential oil from *A. altissima* bark also had strong repellent activity against *T. castaneum*, *O. surinamensis*, *S. oryzae* and *L. paeta* adults and potent fumigant toxicity against *O. surinamensis* and *S. oryzae* adults. Thus, further research on how to use the *Ailanthus* oil as a repellent or fumigant effectively for the control of insects in stored products is necessary and feasible.

In fact, *A. altissima* has been a serious invasive plant in the United States of America which poses a serious threat to biodiversity and economy. Many approaches have been employed to control its spread and prevent from further negative effects by increasing successful collaboration between China and the US (Ding et al., 2006). Positively exploiting the *Ailanthus* oil as a new type of insect-control agent or herbicide will also offer an effecttive method for invasive plants management, because the invasive plants will be a useful resource for researching and developing new types natural pesticides.

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