

Full Length Research Paper

Toxicity of essential oil from *Artemisia argyi* against *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae)

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Accepted 22 August, 2011

The repellent, fumigant effect and contact toxicity of essential oil extracted from *Artemisia argyi* (Asteraceae: Artemisia) plant against *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae) was investigated. The *A. argyi* oil exhibited strong repellent, fumigant effect and contact toxicity against *O. surinamensis* which progressively increased with increased exposure dosage. Repellency percentage of *A. argyi* oil against *O. surinamensis* adults reached IV grade after 48 h exposure period at the dosage of 0.40 $\mu\text{l}/\text{cm}^2$, the corrected percentage mortality for fumigant toxicity reached more than 97% at the dosage of 160 $\mu\text{l}/\text{l}$ air, and the current population inhibition percentage (CPI) and F_1 progeny population inhibition percentage (PPI) reached 77.38 and 96.06% at the dosage of 0.80 $\mu\text{l}/\text{g}$, respectively. The results suggest that the *A. argyi* oil has great potential for effectively controlling *O. surinamensis*.

Key words: Natural product, *Artemisia argyi*, toxicity, *Oryzaephilus surinamensis*, stored grain insect.

INTRODUCTION

The *O. surinamensis* (Linnaeus) (Coleoptera: Silvanidae) is one of the most serious pest insects of stored cereal grains and flour throughout the world. Currently, intensive use of phosphine and other synthetic insecticides for the control of stored products pests has resulted in serious problems including insecticide resistance, environment contamination, unacceptable pesticide residues in food, lethal effects on non-target organisms, and so on (White and Leesch, 1995; Jovanović 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 pesticide-resistant insect pests. Recent research has focused on natural product alternatives for pest control in developing countries and for organic food production in industrialized countries (Isman, 2006, 2008; Liu et al., 2007; Rajendran and Sriranjini, 2008).

Many essential oils and their constituents have been studied to possess potential as alternative compounds to currently used insect-control agents (Shaaya et al., 1997;

Huang et al., 2000; Lee et al., 2004; Boekea et al., 2004; Cosimi et al., 2009; Nerio et al., 2009). In previous studies, *Ailanthus altissima* bark oil (Lü and Wu, 2010), and *Ocimum gratissimum* oil and its constituents (Ogendo et al., 2008) were shown to be repellent and fumigant against *O. surinamensis* population. The LC_{50} value in fumigant toxicity of the essential oil of aerial parts of *Agastache foeniculum* for adults of *O. surinamensis* was 18.781 $\mu\text{l}/\text{l}$ (Ebadollahi et al., 2010). The essential oil of *Thymus vulgaris* gave 100% mortality of *O. surinamensis* at 2,000 and 3,000 ppm (Nesci et al., 2011). The essential oil from *Artemisia scoparia* Waldst et Kit had significant fumigant and repellent activity against three stored product insects, *Callosobruchus maculatus* (Fab.), *Sitophilus oryzae* (L.), and *Tribolium castaneum* (Herbst) (Negahban et al., 2006), and the essential oil from *Artemisia sieberi* Besser also had potent fumigant activity against the above three stored product insects (Negahban et al., 2007). *A. argyi* (Asteraceae: Artemisia) plants are found growing wild and abundantly throughout the temperate zones in China, and its dry plants have ever often been used to control repel mosquitoes and other insects. Here, the paper describes a laboratory study to evaluate the potential

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bioactivity of essential oil from *A. argyi* plants against *O. surinamensis*.

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 of Stored Product Insects of Henan University of Technology. The food media used was wheat flour, rolled oats and yeast (6:3:1, w/w/w). Healthy and consistent developed adult insects (1-2 weeks old) were randomly chosen for tests.

Preparation of the essential oil

The *A. argyi* plant was collected in Henan, central China, June 2010, dried at room temperature, ground to fine powder. Each 50 g of the powder was extracted by Soxhlet method with 250 ml anhydrous diethyl ether at 40°C 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.

Repellency bioassay

The repellent effect of the *A. argyi* oil against *O. surinamensis* adults was evaluated using the area preference method. Test areas consisted of Whatman No.1 filter paper cut in half (Φ12.5 cm). An aliquot of 3.07, 6.14, 12.28 and 24.56 µl of the *A. argyi* oil dissolved in 1 ml acetone (analytical purity) was evenly applied on a half-filter paper disc using a micropipette corresponding to dosages of 0.05, 0.10, 0.20 and 0.40 µl/cm², and the other half of the remaining filter paper was treated with 1 ml acetone alone and used as control. The 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) on the inside wall to avoid the insects 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, 48, 72 h, respectively.

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%), class V (80.1–100%).

Fumigant activity

Fumigation bioassay of the *A. argyi* oil without grain was carried out with 30 unsexed adult insects exposed in a 250 ml glass flask sealed with a rubber stopper. An aliquot of 0, 5, 10, 20 and 40 µl of

the *A. argyi* oil respectively dissolved in 1 ml acetone (analytical purity) was evenly applied on a Whatman No.1 filter paper strip (7×9 cm) corresponding to dosages of 0 (as a control), 20, 40, 80 and 160 µl/l air based on the flask volume, which was dried in air for 10 min and then fixed on the stopper by a staple at one end. The stopper was tightly stuffed in the flask to make the filter paper suspend in the top of the flask, and care was taken to avoid the filter paper contacting the flask inside wall. 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 48 h.

The procedure of fumigation bioassay of the *A. argyi* oil with grain was same as the above except that the flask held 20 g wheat and 2 g rolled oats.

Contact toxicity of *A. argyi* oil, F₁ progeny production in grains

An aliquot of 0, 20, 40 and 80 µl of the *A. argyi* oil dissolved in 1 ml acetone (analytical purity) was evenly mixed with 100 g wheat and 5 g food media in a 350-ml flask corresponding to dosages of about 0 (as a control), 0.20, 0.40 and 0.80 µl/g. Then 30 unsexed adult insects were exposed in the flask tightly sealed with plastic film. The flask was placed in the incubators at 27±2°C and 75±5% relative humidity. The number of living adult insects and the number of F₁ progeny larvae was recorded after 15 d, respectively. Five replicates were conducted.

The current population inhibition percentage (CPI) and F₁ progeny population inhibition percentage (PPI) was calculated using the following formula:

$$CPI = [(C_n - T_n) / C_n] 100$$

$$PPI = [(P_c - P_t) / P_c] 100$$

where C_n is the number of living adults in the control flask, T_n the number of living adults in the treated flask, P_c is the number of F₁ progeny larvae in the control flask, and P_t is the number of F₁ progeny larvae in the treated flask.

Statistical analysis

For the above tests, and 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 *A. argyi* oil had potent repellent activity against *O. surinamensis* adults (Figure 1), and the repellency value significantly increased with increased exposure dosage (df=3, *P*<0.05). The repellency class reached IV grade after 48 h exposure period at the dosage of 0.40 µl/cm².

Fumigant activity

The *A. argyi* oil showed strong fumigant activity against *O. surinamensis* adults under both Flasks with grain and Flasks without grain (Table 1). The fumigant toxicity

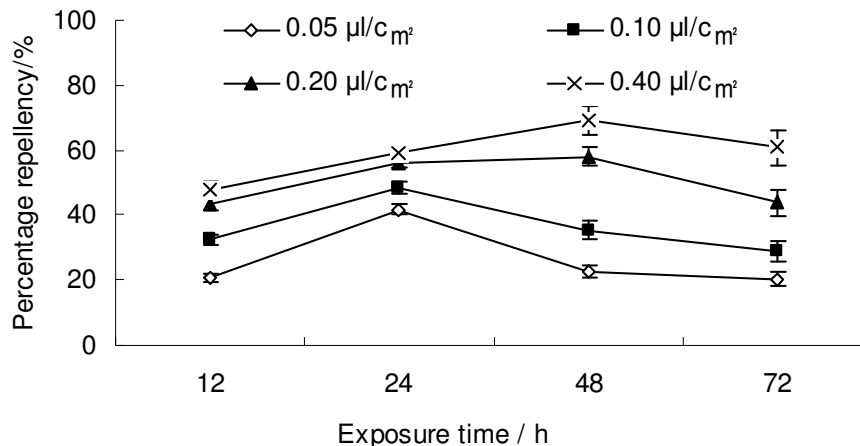


Figure 1. Repellent activity of the *A. argyi* oil against *O. surinamensis* adults.

Table 1. Fumigant activity of *A. argyi* oil against *O. surinamensis* adults.

Dosage (µl/l air)	Corrected mortality (%)	
	Flasks with grain	Flasks without grain
20	43.26±2.36 ^c	48.21±5.33 ^c
40	67.52±5.32 ^b	73.66±3.55 ^b
80	89.63±1.86 ^a	95.38±3.86 ^a
160	97.56±3.47 ^a	100.00±0.00 ^a

Each datum in the table is mean ± SE. The data in a column followed by different letters indicate significant difference tested by Scheffe's test at $P = 0.05$.

significantly increased with increased exposure dosage with the corrected percentage mortality reached 97.56 and 100.00% at the dosage of 160 µl/l air under both Flasks with grain and Flasks without grain, respectively (df=3, $P < 0.05$).

Contact toxicity of *A. argyi* oil, F₁ progeny production in grains

Contact activity of *A. argyi* oil against *O. surinamensis* in grains progressively increased with increasing exposure dosage (df=3, $P < 0.05$). Specially, the CPI and PPI reached 77.38 and 96.06% at the dosage of 0.80 µl/g, respectively (Table 2).

DISCUSSION

Previous research testified that plant-derived essential oils exhibited strong toxic effects on *O. surinamensis* (Tripathi et al., 2000; Negahban et al., 2006, 2007; Ogendo et al., 2008; Ebadollahi et al., 2010; Lü and Wu, 2010). Our results also clearly showed that *A. argyi* oil exhibited strong toxic activity against *O. surinamensis*,

which testified that *Artemisia* essential oil has great potential to control stored product insects (Tripathi et al., 2000; Negahban et al., 2006, 2007; Wang et al., 2006), and were similar to the activity of essential oil from *A. scoparia* (Negahban et al., 2006) and *A. sieberi* (Negahban et al., 2007) against three stored product insects, *C. maculatus* (Fab.), *S. oryzae* (L.), and *T. castaneum* (Herbst). These results suggest that *A. argyi* oil had huge potential as a repellent or a fumigant for the effective control of *O. surinamensis*. Meanwhile, the effect of *A. argyi* oil is safe to consumers because it has been used in many pharmaceutical preparations in traditional Chinese medicine.

Thus, it is necessary to investigate the bioactivity of *A. argyi* oil and their pure constituent level along with structure–activity relationships against different developed stages of the major stored grain insects in the future. In addition, a chlorpyrifosmethyl resistant strain of *O. surinamensis* has been found to have cross-resistance to *Eucalyptus* essential oil and 1,8-cineole fumigant activity (Lee et al., 2000), which has been led to some essential oil-degrading enzymes induced by chlorpyrifosmethyl (Lee, 2002). Therefore, whether the *O. surinamensis* will produce resistance to *A. argyi* oil and their pure constituent should also be further investigated.

Table 2. Contact toxicity of *A. argyi* oil against *O. surinamensis* in grains.

Dosage (µL/g)	The number of living adults	The number of living F ₁ progeny larvae	Current population inhibition rate (%)	F ₁ progeny population inhibition rate (%)
0	28.92±1.83 ^a	25.38±2.45 ^a	/	/
0.20	23.28±1.67 ^b	15.45±1.89 ^{ab}	19.50±4.21 ^b	39.12±3.28 ^b
0.40	13.45±1.15 ^b	7.65±1.21 ^b	53.49±3.16 ^b	69.86±5.42 ^a
0.80	6.56±0.85 ^c	1.00±0.67 ^b	77.38±3.22 ^a	96.06±6.85 ^a

Each datum in the table is mean ± SE. The data in a column followed by different letters indicate significant difference tested by Scheffe's test at $P = 0.05$.

ACKNOWLEDGMENTS

This research was supported by Henan Provincial Key Science and Technology Project (No. 092102110022), and Key Science Foundation of Henan University of Technology (No. 170851).

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