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Full Length Research Paper

Bioactivity of two extracts from *Alpinia officinarum* rhizome against *Tribolium castaneum* (Herbst) adults

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The bioactivities of two solvent plant extracts prepared from *Alpinia officinarum* Hance rhizome, a traditional Chinese herbal plant were investigated against *Tribolium castaneum* (Herbst) adults in the laboratory. The rhizome extracts were prepared by *n*-hexane and methylene chloride extraction. Both extracts had significant repellent activity against *T. castaneum* adults with over 89% at class V at the dosage of 0.10 µl/cm^2 and above after 24 h exposure. The repellent activity significantly increased with increased exposure dosage. Both extracts also had strong fumigant activity against *T. castaneum* adults with over 80.0% mortality at the highest dosage tested of 80 µl/l air after 48 h of exposure. Mortality was dose dependent for both extracts. Regardless of extraction method *A. officinarum* rhizome extracts were demonstrated to have bioactivity against *T. castaneum* adults. These results suggest that the *A. officinarum* rhizome extracts have potential for integrated pest management programs of *T. castaneum* population.

Key words: *Alpinia officinarum* rhizome extract, *Tribolium castaneum* (Herbst), fumigant activity, repellent activity, plant extract.

INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most serious pest insects occurring in stored grains and processed foods worldwide (Lee et al., 2002). Presently, control of *T. castaneum* population is primarily dependent upon repeated use of the fumigant phosphine (White and Leesch, 1995). However, its overuse for decades has led to serious problems, such as insecticide resistance, environmental contamination, rising cost of production, and lethal effects on non-target organisms (Rajendran and Narasimhan, 1994; Jembere et al., 1995; Okonkwo and Okoye, 1996; Yasuhisa, 2007; Jovanović et al., 2007). These increasing concerns over today's synthetic pesticides have led to an intensification of efforts to develop and implement environment-friendly, selective, viable and effective alternatives (Kim et al., 2003; Tapondjoua et al., 2005). Plant-based pesticides have been developed to control stored grain insects since ancient times in China and abroad. However, these methods rapidly declined with the dramatic impact of synthetic pesticides in 1940s and 1950s (Charnley, 1991). Botanical pesticides are natural source of bioactive chemicals with complicated action mechanism, and as a result insects are less likely to develop resistance to these chemicals. Botanical pesticides are also readily biodegradable in the natural environment, and often have less or negligible danger to mammals and the environment if used in suitable amounts compared to synthetic pesticides (Isman, 2006, 2008). Particularly, because of the unacceptable high cost and difficulty of researching and developing new synthetic insecticides, recent research has focused on plant-derived pesticides for insect pest control in developing countries and for organic food production in industrialized countries (Boekea et al., 2004; Isman, 2006, 2008; Liu et al., 2007; Rajendran and Sriranjini, 2008; Paul et al., 2009).

Many Chinese herbal plants have been studied to have strong toxic activity against stored-grain insects (Yang and Tang, 1988; Wang et al., 2006; Liu et al., 2007). *Alpinia officinarum* Hance (Zingiberales: Zingiberaceae)

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rhizome is a traditional Chinese herbal plant, and has been used for many generations by farmers in China to protect stored grains from insect infestation (Lee et al., 2003; Fan et al., 2007). However, bioactivity of the plant extract obtained from *A. officinarum* against *T. castaneum* has not been investigated so far.

In this paper, we evaluated the potential repellent and fumigant activity of plant extract prepared from *A. officinarum* rhizome against *T. castaneum* adults in laboratory.

MATERIALS AND METHODS

Insects

Cultures of *T. castaneum* were maintained in the laboratory without exposure to any insecticide at the Institute of Stored Product Insects of Henan University of Technology. They were reared on wheat flour and rolled oats (6:1, w/w) at 27 ± 2°C, 75 ± 5% r.h., and a 12:12 light:dark photoperiod. Two-week-old adults were randomly chosen for bioassays.

Preparation of the plant extract

The *A. officinarum* rhizome was purchased from a traditional Chinese medicine store. It was identified by the Biology Department of Zhengzhou University. The rhizome was dried at room temperature and then finely ground to powder. Two solvents were selected as extract solvents based on their relative polarity: a non-polar solvent, *n*-hexane, and polar solvent, methylene chloride, which were both double-distilled before use. Fifty grams of the powder was extracted by the Soxhlet method with either 250 ml of *n*-hexane or methylene chloride until the distilled liquid was colorless. The solvent was evaporated under vacuum in a rotary evaporator. The plant extracts were subsequently stored in airtight fuscous glasswares in a refrigerator at 4°C for use in the bioassays.

Repellency bioassay

The repellent effect of the two *A. officinarum* rhizome extracts against *T. castaneum* adults was evaluated using the area preference method. Test areas consisted of Whatman No.1 filter paper cut in half (12.5 cm). An aliquot of either 3.07, 6.14, 12.28 or 24.56 µl of the *A. officinarum* rhizome extract dissolved in 1 ml acetone (analytical purity) was evenly applied on half-filter paper discs using a micropipette corresponding to dosages of 0.05, 0.10, 0.20 and 0.40 µl/cm^2 respectively. The other half of the remaining filter paper was treated with 1 ml acetone alone and used as control. The filter papers were air-dried for about 5 min to evaporate the solvent completely and 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 polytetrafluoro-ethylene (PTFE) on the inside wall to prevent the insects escaping. Thirty unsexed *T. castaneum* adults were then released at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in incubators at 27 ± 2°C, 75 ± 5% r.h., and a 12:12 light:dark photoperiod. Each treatment was replicated five 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, 36, 48 and 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 to 20%), class II (PR = 20.1 to 40%), class III (40.1 to 60%), class IV (60.1 to 80%), class V (80.1 to 100%).

Fumigant activity

Fumigant activity of the plant extracts against *T. castaneum* adults was carried out with 30 unsexed adults exposed in a 250 ml flask tightly sealed with a rubber stopper. The flask contained 10 g wheat at about 13.5% equilibrium moisture content. An aliquot of 0, 2.5, 5, 10 and 20 µl of each plant extract dissolved in 1 ml acetone was evenly applied to a Whatman No.1 filter paper strip (7×9 cm) corresponding to dosages of 0, 10, 20, 40 and 80 µl/l respectively. The filter papers were then dried in air for 10 min prior to being fixed on the rubber stopper by a staple at one end to keep the filter paper suspended in the top of the flask. Care was taken to avoid the filter paper coming into contact with the flask wall. The flasks were placed in the incubators at 27 ± 2°C and 75 ± 5% relative humidity. Five replicates were conducted for each dose. After 48 h exposure, insects were moved into clean vials and mortality determined immediately.

Statistical analysis

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. The LD_{50} value was calculated using probit analysis (Finney, 1971).

RESULTS

Repellent activity

Both the *A. officinarum* rhizome extracts prepared by *n*-hexane and methylene chloride showed potent repellent activity against *T. castaneum* adults after 24 h exposure time, and the repellent activity significantly increased with increased dosage from 0.05 to 0.10 µl/cm^2 but did not increase significantly as dosage was increased further indicating that maximum repellency was reached at 0.10 µl/cm^2. Percentage repellency values were over 90% for the *n*-hexane extract, for the 0.10 µl/cm^2 dosage after 24 h exposure (Table 1). The repellent activity gradually decreased after 24 h for the remainder of the experimental period but even after 72 h the repellency percentage was still above 50% for both extracts at all dosages.

Fumigant activity

Both *A. officinarum* rhizome extracts had strong fumigant activity against *T. castaneum* adults and the fumigant activity progressively increased with increased exposure

Table 1. Repellent activity of the *A. officinarum* rhizome extracts prepared by *n*-hexane and methylene chloride against *T. castaneum* adults.

Plant extract	Dosage (µl/cm^2)	Exposure period (h)				
		12	24	36	48	72
Methylene chloride extract	0.05	52.4 ± 3.8^b	70.6 ± 4.1^b	79.3 ± 5.5^a	81.4 ± 6.2^a	74.9 ± 2.1^a
	0.10	67.7 ±4.4^{ab}	91.4 ± 4.4^a	78.1 ± 5.7^a	72.1 ± 3.5^a	63.5 ± 2.5^a
	0.20	75.8 ± 4.5^{ab}	89.7 ± 2.5^a	79.3 ± 5.5^a	72.1 ± 3.5^a	65.8 ± 3.3^a
	0.40	87.0 ± 3.7^a	95.6 ± 0.9^a	84.3 ± 3.3^a	67.8 ± 3.8^a	66.3 ± 3.8^a
n-hexane extract	0.05	57.7 ± 5.3^b	75.1 ± 4.3^b	81.7 ± 5.2^a	71.1 ± 2.3^a	60.1 ± 4.2^a
	0.10	71.6 ± 5.7^{ab}	91.8 ± 1.8^a	88.2 ± 5.1^a	77.3 ± 3.2^a	74.9 ± 2.1^a
	0.20	82.1 ± 2.9^a	91.6 ± 3.3^a	83.1 ± 5.0^a	68.6 ± 4.6^a	57.3 ± 4.8^a
	0.40	82.1 ± 2.8^a	93.7 ± 2.3^a	75.4 ± 5.6^a	72.5 ± 5.1^a	58.3 ± 4.9^a

Each datum in the table is mean ± SE of five replicates (n=150). The data in a column for each plant extract followed by different letters indicate significant differences tested by Scheffe's test at $P = 0.05$.

Table 2. Fumigant activity of the *A. officinarum* rhizome extract prepared by *n*-hexane and methylene chloride against *T. castaneum* adults after 48 h of exposure at the dosages of 0, 10, 20, 40 and 80 µl/l air based on the flask volume.

Plant extract	Dosage (µl/l)				
	0	10	20	40	80
Methylene chloride extract	1.0±1.0e	24.0±1.6d	45.0±4.4c	59.0±4.4b	80.0±2.8a
n-hexane extract	1.0±1.0e	24.0±1.6d	45.0±2.5c	63.0±3.4b	82.0±5.3a

Each datum in the table is mean ± SE of five replicates (n=150). The data in a row followed by different letters indicate significant differences tested by Scheffe's test at $P = 0.05$.

Table 3. Probit analysis of *T. castaneum* mortality of tested insects after 48 h of exposure to two *A. officinarum* rhizome extracts at dosages of 0, 10, 20, 40 and 80 µl/l.

Plant extract	Regression line equation	LD_{50} (µl/l)	Confidence limit of LD_{50} (µl/l)	Chi-square (χ^2)	Chi-square p value
Methylene chloride extract	Y = 1.67 X + 2.62	26.61	21.96-31.98	0.75	0.69
n-hexane extract	Y = 1.86 X + 2.32	27.20	22.71-32.42	0.24	0.89

dosage ($df = 4$, $P < 0.05$). At a dosage of 80 µl/l air, the two extracts induced over 80.0% mortality of *T. castaneum* adults after 48 h of exposure (Table 2).

From the probit analyses for mortality of *T. castaneum* adults after 48 h exposure to *A. officinarum* rhizome extract prepared by *n*-hexane or methylene chloride, the calculated regression line equations, the LD_{50} values and its confidence limits, the chi-square ($\chi 2$) and its p values are shown in Table 3. LD_{50} values indicate that the *A. officinarum* rhizome extract prepared by *n*-hexane had similar fumigant activity as the *A. officinarum* rhizome extract prepared by methylene chloride.

DISCUSSION

According to our research results, both *A. officinarum* rhizome extracts prepared by *n*-hexane and methylene chloride had strong and similar bioactivity against *T. castaneum* adults. These laboratory experiments suggest that field tests of the *A. officinarum* rhizome extracts may be warranted. Both the non-polar solvent and polar solvents could be used as an extract solvent for preparing the plant extract. In addition, *A. officinarum* is abundant in China, which also makes the plant extract preparation easier and cheaper.

Extracts from other plants have been demonstrated to have bioactivity against *T. castaneum*. The crude seed extracts of *Aphanamixis pofystachya* had strong repellent activity and moderate feeding deterrent activity to *T. castaneum.* The ground leaves, bark and seeds of *A. pofystachya* in a 2.5% mixture reduced F1 progeny of *T. castaneum* in wheat flour (Talukder and Howse, 1995). The essential oil of garlic killed 100% of *T. castaneum*

eggs at 4.4 mg/cm^2 (Ho et al., 1996). *Evodia rutaecarpa* essential oil had strong contact toxicity against *T. castaneum* adults (LD_{50} = 0.118 mg/mg body wt) and larvae (LD_{50} = 0.093 mg/mg body wt), fumigant activity (LC_{50}=11.7 mg/l air), and repellent activity against *T. castaneum* adults (Liu and Ho, 1999). *Elletaria cardamomum* oil significantly reduced the hatching of *T. castaneum* eggs and the subsequent survival rate of the larvae in the concentration range of 1.04 to 2.34 mg cm^{-2} (Huang et al., 2000). Lee et al. (2002) reported that the essential oil from *Rosmarius officinalis* had the most potent fumigant toxicity against *T. castaneum* adults (LD_{50} = 7.8 µl/l air) followed by the oils of *Citrus limonum* (LD_{50} = 16.2µl/l air), *Pimenta racemosa* (LD_{50} = 17.8µl/l air), *Citrus auratifolia* (LD_{50} = 17.9µl/l air), and *Mentha piperata* (LD_{50} = 25.8µl/l air). The essential oil of *Artemisia vulgaris* had a very strong repellent activity at a 0.6 µl/ml (v/v) and high fumigant activity with 100% mortality at 8.0 µl/ml against *T. castaneum* adults (Wang et al., 2006). *Artemisia sieberi* essential oil killed 100% *T. castaneum* adults at the concentration of 37 ml/l and after 24 h exposure (Negahban et al., 2007). The *Alpinia conchigera* essential oils had strong fumigant activity *T. castaneum* adults with LC_{50} values 140, 97 and 73 µl/l air after 12, 24 and 48 h exposure (Suthisut et al., 2011). Moreover, many other essential oils and their constituents also have huge potential as alternatives to currently used synthetic chemical pesticides for the management of *T. castaneum* populations (Shaaya et al., 1991, 1997; Lee et al., 2004; Sahaf et al., 2008; Ogendo, et al., 2008; Nerio et al., 2009).

The observed bioactivity against *T. castaneum* adults demonstrates that *A. officinarum* rhizome extract can be conveniently prepared by non-polar and polar solvents, and may potentially prove to be effective for integrated pest management of *T. castaneum* populations. The activity of *A. officinarum* rhizome extract and its pure constituent level along with structure–activity relationships against different life stages of the *T. castaneum* and other major stored grain insect pests may warrant further investigation. Moreover, provided with a proper formulation and scientific application strategy, *A. officinarum* rhizome extract may be exploited for use to control insect infestation in small-scale farmer's level in developing countries (Isman, 2006, 2008).

Furthermore, as a traditional Chinese medicine, the *A. officinarum* rhizome extract is likely to be safe for human health and ecological system. Therefore, how to appropriately use the *A. officinarum* rhizome extract as a repellent or a fumigant for the integrated pest management of *T. castaneum* in the future should be initiated.

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