Repellent and fumigant activity of *Alpinia officinarum* rhizome extract against *Tribolium castaneum* (Herbst)

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The plant extract was prepared by Soxhlet method with anhydrous diethyl ether from *Alpinia officinarum* rhizome, a traditional Chinese herbal plant, and its repellent and fumigant activity was investigated against *Tribolium castaneum* (Herbst) adults. The *A. officinarum* rhizome extract had potent repellent activity against *T. castaneum* adults with over 80% repellency values at the tested concentration (*A. officinarum* extract: acetone = 1:10, v/v) during 48 h of exposure time. *A. officinarum* rhizome extract exhibited strong fumigant activity in a dosage-dependent manner against *T. castaneum* adults with 75% mortality at a dosage of 80 µl/l air after 48 h exposure. These naturally occurring plant extracts could be useful for managing populations of *T. castaneum*.

**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 of stored grains and processed foods throughout the world (Lee et al., 2002a). Currently, control of *T. castaneum* population is primarily dependent upon intensive use of phosphine (White and Leesch, 1995). However, its repeated use for decades has disrupted biological control by natural enemies and led to serious problems including insecticide resistance, environmental and human health concerns, rising cost of production and lethal effects on non-target organisms (Rajendran and Narasimhan, 1994; Jembere et al., 1995; Okonkwo and Okoye, 1996; 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 insecticide-resistant insect pests (Kim et al., 2003; Tapondjoua et al., 2005).

Plant-derived 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 and for organic food production in industrialized countries (Boekea et al., 2004; Isman, 2006, 2008; Liu et al., 2007; Rajendran and Sriranjini, 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 (Lee et al., 2003; Fan et al., 2007), the rhizome of *A. officinarum* Hance (Zingiberales: Zingiberaceae) has also for many generations been used as a traditional method by farmers to protect stored products from insect infestation in China. However, bioactivity of plant extract from *A. officinarum* against *T. castaneum* has not been investigated so far.

Thus, we evaluated the potential repellent and fumigant activity of plant extract obtained from *A. officinarum*.
The repellent effect of the airtight fuscous glassware in a refrigerator at 4° C. vacuum in a rotary evaporator. The plant extract was stored in distilled liquid was colorless. The solvent was evaporated under castaneum equilibrium moisture content. An aliquot of 0, 2.5, 5, 10 and 20 µl of rubber stopper. The flask contained 10 g wheat at about 13.5% unsexed adults exposed in a 250 ml flask tightly sealed with a rubber stopper. The flask was placed in a 12.12 light:dark photoperiod. Healthy, consistent and 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, then dried at room temperature and finely ground to powder. 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 plant extract was stored in airtight fuscous glassware in a refrigerator at 4°C.

Repellency bioassay

The repellent effect of the A. officinarum rhizome extract against T. castaneum adults was evaluated using the area preference method. Test areas consisted of Whatman No.1 filter paper cut in half (Φ12.5 cm). The A. officinarum rhizome extract was dissolved in acetone (1:10, v/v). Then, 1 ml of the solution 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) on the inside wall to avoid the insects escaping. Then 30 unsexed adults of T. castaneum were released at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in incubators at 25 to 29°C and 70 to 80% relative humidity. Each treatment was replicated 4 times and the number of insects present on the control (Nc) and treated (Nt) areas of the discs was recorded after 12, 24, 36, 48 and 72 h, respectively.

Percentage repellency (PR) values were calculated as follows:

\[ PR = \left(\frac{N_c - N_t}{N_c}\right) \times 100\% \]

The mean percentage repellency value was calculated and assigned to repellency classes (Julianna 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%) and class V (80.1 to 100%).

Fumigant activity

A. officinarum rhizome extract had strong fumigant activity in a dosage-dependent manner against T. castaneum adults (df = 4, P < 0.05). At a dosage of 80 µl/l air, the A. officinarum rhizome extract induced 75% mortality of T. castaneum adults after 48 h exposure (Figure 2).

From the probit analyses for mortality of T. castaneum adults after 48 h of exposure to A. officinarum rhizome extract, the calculated regression line equations was \( Y = 2.01X + 1.72 \) (\( \chi^2 = 1.67, p = 0.43 \)) for T. castaneum adults, the LD50 value and its confidence limit were 42.42 µl/l and 35.74-52.67 µl/l, respectively.

DISCUSSION

In our study, A. officinarum rhizome extract showed promise as a repellent and fumigant for the control of T. castaneum adults. Similarly, the crude seed extracts of Aphanamixis polyystachya were strong repellents and moderate feeding deterrents to T. castuneum. The ground leaves, bark and seeds of A. polyystachya in a 2.5% mixture provided some protection for wheat flour by
Figure 1. Repellent activity of the *A. officinarum* rhizome extract against *T. castaneum* adults.

Figure 2. Fumigant activity of the *A. officinarum* rhizome extract against *T. castaneum* adults.

reducing F1 progeny (Talukder and Howse, 1995). Ho et al. (1996) found that the essential oil of garlic killed 100% of eggs at 4.4 mg/cm², using the filter paper impregnation bioassay. *Evodia rutaecarpa* essential oil exhibited strong contact toxicity against *T. castaneum* adults (LD₅₀ = 0.118 mg/mg body wt) and larvae (LD₅₀ = 0.093 mg/mg body wt), fumigant activity (LC₅₀ = 11.7 mg/l air), repellent activity to *T. castaneum* adults (Liu and Ho, 1999). Liu et al. (2007) screened extracts of 40 species of Chinese medicinal herb from 32 different botanical families for bioactivity against *Sitophilus zeamais* and *T. castaneum*. Thirty species of Chinese medicinal herb extracts had insecticidal or feeding-deterrent activities against *S. zeamais* and *T. castaneum*. Specially, extracts of *Artemisia argyi*, *Evodia rutaecarpa*, *Sophora flavescens*, *Litsea cubeba*, *Narcissus tazetta* var. *chinesis*, *Polygonum aviculare*, *Dictamnus dasycarpus*, *Rhododendron molle*, *Stemona sessilifolia*, *Tripterygium wilfordii*, and *Torreya grandis* showed the strongest bioactivity. *Elletaria cardamomum* oil significantly (P < 0.05) reduced the hatching of *T. castaneum* eggs and the subsequent survival rate of the larvae in the concentration range 1.04 to 2.34 mg cm⁻². *E. cardamomum* oil was also drastically reduced of *T. castaneum* adult emergence and totally suppressed its F1 progeny production at a concentration of 5.3 × 10³ ppm (Huang et al., 2000). Lee et al. (2002b) reported that the essential oil from *Rosmarinus officinalis* had the most potent fumigant toxicity against the red flour beetle, *T. castaneum* (Herbst) (LD₅₀ = 7.8 µl/l air) followed by the oils of *Citrus limonum* (LD₅₀ = 16.2µl/l air), *Pimenta racemosa* (LD₅₀ = 17.8µl/l air), *Citrus auratifolia* (LD₅₀ = 17.9µl/l air), and *Mentha piperata* (LD₅₀ = 25.8 µl/l air). The essential oil of mugwort, *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 to *T.*
castaneum adults (Wang et al., 2006). Artemisia sieberi essential oil induced 100% mortality of T. castaneum adults at the concentration of 37 ml/l and an exposure time of 24 h (Negahban et al., 2007). The percentage repellence value for the Ocimum gratissimum oil against T. castaneum after 24 h exposure was 38 to 79%. However, the T. castaneum was more tolerant to the O. gratissimum oil, with only 23% mortality after 168 h treatment with 10 ml/l air (Ogendo et al., 2008). The LC50 with fiducial limits for T. castaneum exposed to Alpinia conchigera essential oils at 12, 24 and 48 h the values were; 140, 105 to 178; 97, 81 to 116 and 73, 64 to 82 µl/l in air (Suthisut et al., 2011). Moreover, many essential oils and their constituents have been studied to possess potential as alternative compounds to currently used insect-control agents for the management of populations of T. castaneum (Shaaya et al., 1991, 1997; Lee et al., 2004; Sahaf et al., 2008; Nerio et al., 2009).

The observed repellent and fumigant activity against T. castaneum adults demonstrates that A. officinarum rhizome extract is a source of biologically active components which may potentially prove to be effective for integrated pest management of stored grain insects. Furthermore, as a traditional pharmaceutical agent, the A. officinarum rhizome extract is also considered to be safe for human being and the environment. Therefore, how to appropriately use the A. officinarum rhizome extract as a control agent for the management of T. castaneum may warrant further investigation.

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