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Full Length Research Paper

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. castaneu* 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*

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rhizome against adults of *T. castaneum* in the laboratory.

MATERIALS AND METHODS

Insects

Cultures of the red flour beetle, *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 25 to 29℃, 70 to 80% relative humidity and 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℃.

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℃ and 70 to 80% relative humidity. Each treatment was replicated 4 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%) and class V (80.1 to 100%).

Fumigant activity

Fumigant activity on *T. castaneum* adults was carried out with 25 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 the 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 air, which was then dried in air for 10 min prior to being fixed on the rubber stopper by a staple at one end. The rubber stopper was tightly stuffed to keep the filter paper suspending in the top of the flask. Care was taken to avoid the filter paper contacting the flask wall. The flask was placed in the incubators at 25 to 29℃ and 70 to 80% relative humidity. Four replicates were conducted. After 48 h exposure, insects were moved into clean vials and mortality determined immediately. Insects showing any movement were considered to be alive.

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

The *A. officinarum* rhizome extract showed potent repellent activity against *T. castaneum* adults during the whole exposure time. Percentage repellency values always kept over 80% at class V at the tested concentration (*A. officinarum* extract: acetone = 1:10, v/v) within 48 h of exposure (Figure 1).

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 LD_{50} 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 pofystachya* were strong repellents and moderate feeding deterrents to *T. custuneum.* The ground leaves, bark and seeds of *A. pofystachya* in a 2.5% mixture provided some protection for wheat flour by

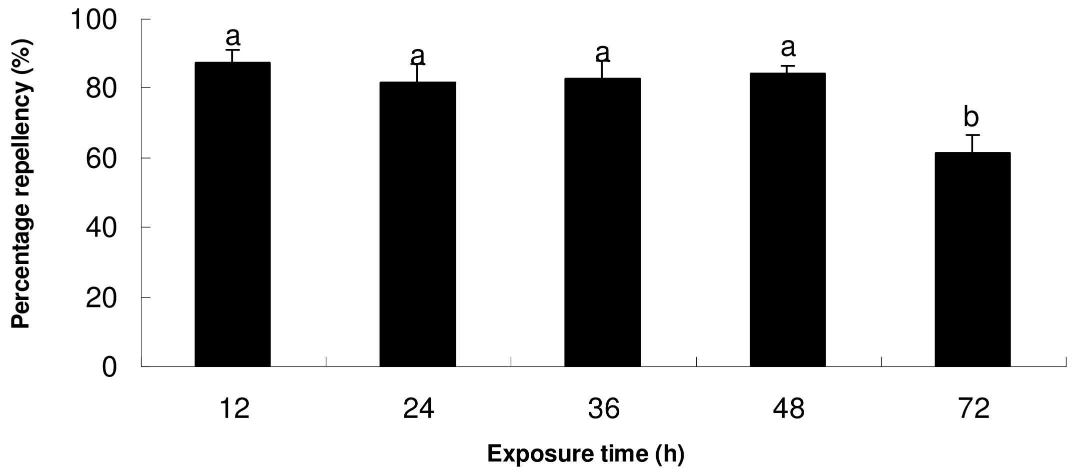

Figure 1. Repellent activity of the *A. officinarum* rhizome extract against *T. castaneum* adults.

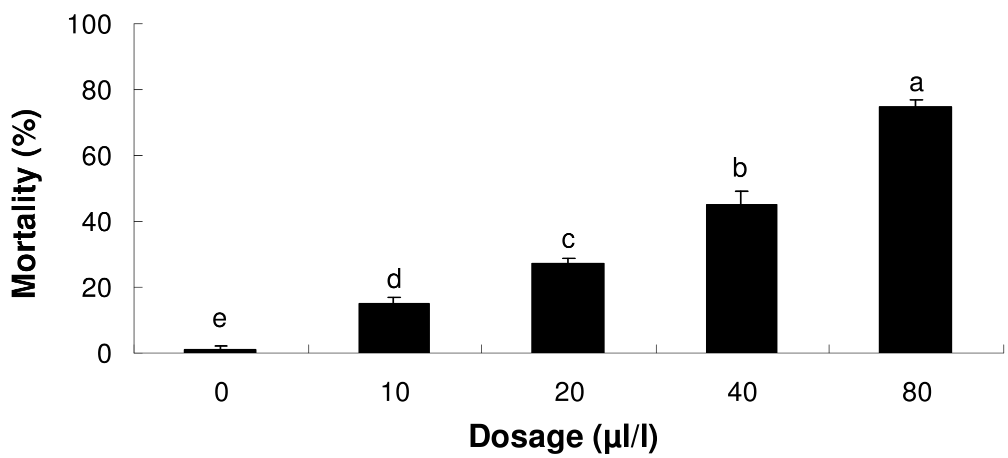

Figure 2. Fumigant activity of the *A. officinarum* rhizome extract against *T. castaneum* adults.

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/cm^2, using the filter paper impregnation bioassay. *Evodia rutaecarpa* essential oil exhibited 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), 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. *chinensis*, *Polygonum aviculare*, *Dictamnus dasycarpus*, *Rhododendron molle*, *Stemona sessilifolia*, *Tripterygium wilfordii*, and *Torreya grandis* showed the strongest bioactivity. *Elletaria cardamomum* oil signifficantly ($P < 0.05$) 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 5.3×10^3 ppm (Huang et al., 2000). Lee et al. (2002b) reported that the essential oil from *Rosmarius officinalis* had the most potent fumigant toxicity against the red flour beetle, *T. castaneum* (Herbst) (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} = 25.8 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 LC_{50} 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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