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Full Length Research Paper

Fumigant activity of plant essential oil from *Armoracia rusticana* (L.) on *Plodia interpunctella* (Lepidoptera: Pyralidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae)

Haoliang Chen[1], R. O. Akinkurolere[2] and Hongyu Zhang[1]*

[1]State Key Laboratory of Agricultural Microbiology, Institute of Urban and Horticutural Pests, and Hubei Key Laboratory of Insect Resource Application and Sustainable Pest Control, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China.
[2]Laboratory of Food Storage Technology, Biology Department, Federal University of Technology, P. M. B. 704, Akure, Nigeria.

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The fumigant toxicity of essential oil from horseradish plant, *Armoracia rusticana* (L.), was assessed against *Plodia interpunctella* (Hübner) and *Sitophilus zeamais* Motschulsky. *A. rusticana* oil was active against different life stages of *P. interpunctella* and adults of *S. zeamais*. The LC_{50} value for adults was the lowest and that of pupa was the highest. The major compound found by gas chromatography-mass spectrometry was allyl isothiocyanate (97.81%). These results indicate that it may be possible to achieve toxicity levels similar to those of standard chemical fumigants through the applications of essential oils from *A. rusticana*.

Key word: Plant essential oil, *Plodia interpunctella*, *Sitophilus zeamais,* fumigant activity, allyl isothiocyanate.

INTRODUCTION

Insect pests cause a significant nutritional and economic burden to subsistence farmers in developing countries. *Plodia interpunctella* (Hübner) and *Sitophilus zeamais* Motschulsky are two of the major pests of stored products in China and around the world, causing serious losses in stored products (Madrid and Sinha, 1982; Arbogast et al., 2000; Mbata and Osuji, 1983). Fumigation plays a major role in insect pest elimination in stored products. Currently, phosphine and methyl bromide have been the two common fumigants used for stored-product protection. But insect resistance to phosphine is a global issue now, and control failures have been reported in field situations in some countries (Bell, 2000; Chaudhry, 2000; Collins et al., 2005; Pimentel et al., 2007). Methyl bromide use is being phased out because of depletion of the ozone layer and bromine residues in the soil (Ristaino and Thomas, 1998; Kempton and Maw, 1972, 1973).

Recently, other fumigants such as sulphuryl fluoride (Drinkall et al., 1996; Bell and Savvidou, 1999), carbonyl sulphide (Zettler et al., 1997; Ren et al., 2008) and ethyl formate (alone or in mixture with CO_2) (Banks and Hilton, 1996; Haritos et al., 2006) have also been investigated to control insect pests.

However, the use of fumigants is problematic because of their effects on the environment and high costs, which has led to a search for alternative control measures. There is a trend to search for naturally occurring toxicants from plants. Plant essential oils and their components may provide potential alternatives to the currently used stored-product pest control agents because many plants are commonly used as fragrances and flavors in the perfume and food industries (Isman, 2000, 2006). Since the 1980s, there are many reviews that have focused on the fumigant toxicity of essential oils from plants and their components against insect pests of stored products (Isman, 2006; Lale, 1995; Saxena and Koul, 1978; Singh and Upadhyay, 1993; Regnault-Roger, 1997; Shaaya et

*Corresponding author. E-mail: hongyu.zhang@mail.hzau.edu.cn. Tel: 86-27-87281156.

al., 1991,1997), such as the use of *Ocimum gratissimum* L. oil against insect pests in stored food products (Ogendo et al., 2008; Kim et al., 2003), use of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. Stored-product insects vary in their susceptibility to plant compounds (Rajendran and Sriranjini, 2008).

Horseradish, *Armoracia rusticana* (Linn.), is a perennial plant of the Brassicaceae family, which includes mustard and cabbages. The plant is probably native to south-eastern Europe and western Asia, but is popular around the world today. It grows up to 1.5 m (five feet) tall and is mainly cultivated for its large white, tapering root (Nigel, 2004). Park et al., (2006) investigated the fumigant activity of *A. rusticana* essential oil against *Lycoriella ingénue* and found that *A. rusticana* essential oil can evoke 100% mortality at the concentration of 10 µl L^{-1}.

P. interpunctella is a serious and widespread pest of many stored food commodities (Hinton, 1943, Sedlacek et al., 1996). The weevil *S. zeamais* is one of the main pests in stored grains as corn, wheat, rice and sorghum, and it causes large quantity loss in dry weight (Prempeh, 1971, Meagher et al., 1982, Obeng-ofori and Amiteye, 2005). *P. interpunctella* and *S. zeamais* occur in closed areas such as a warehouse. Because of high volatility, plant essential oils would be more effective than non-volatile pesticides to control *P. interpunctella* and *S. zeamais*. In this study, the essential oil from *A. rusticana* (L.) against different stages of *P. interpunctella* and adults of *S. zeamais* by using a fumigation bioassay was assessed.

MATERIALS AND METHODS

Insect culture and source of *A. rusticana* essential oil

P. interpunctella used in this study were obtained from a laboratory colony maintained on artificial diet consisting of cracked wheat (1000 g), wheat shorts (1000 g), wheat germ (100 g), brewer's yeast (80 g), sorbic acid (4 g), methyl-*p*-hydroxybenzoate (4 g), glycerine (240 ml), pure honey (240 ml) and 120 ml of water (McGaughey and Beeman 1988). The insect was reared at 29 ± 1°C, 40 to 60% relative humidity (r.h.), with a 14:10 h light : dark regime. *S. zeamais* were maintained in the laboratory for more than 1 year, and reared on wheat at 30 ± 1°C, 60 to 70% relative humidity (r.h.) and in continuous darkness. 3 weeks old adults were used for the bioassay.

A. rusticana essential oil used in this investigation was obtained during May 2007 from the Wuhan Nature's Favour Bioengineering Co., Ltd. The essential oil was stored in an airtight container at 4°C and dissolved in ethyl acetate to 20% prior to tests.

Fumigation bioassay

Fumigation bioassays method was developed from Yao (2009). It was conducted in a 500 ml glass conical flask (fumigation chamber). An insect cage was placed on the bottom of the conical flask. Essential oil was applied onto a filter paper (1 ×4 cm) and suspended vertically within the chamber by thread. The fumigation chamber was covered with a rubber stopper and sealed with adhesive tape. The test cage for insects used in this experiment was made from wire sieve.

Fumigation of *P. interpunctella* adults

Twenty (20) adults of *P. interpunctella* (0 to 2 days old) were placed in the cylindrical test cage (9 × 3 cm). *A. rusticana* oil was applied at the doses of 0 (control), 2, 4, 8, 16, and 32 µl/l. Percentage insect mortality was recorded after 72 h of exposure to the essential oil gas.

Fumigation of *P. interpunctella* eggs

A black cloth (12 × 12 cm) was placed in a jar with 15 pairs of newly emerged (0 to 24 h old) *P. interpunctella* adults, which were allowed to oviposit on the cloth for 24 h. Afterwards, the cloth bearing the eggs was removed, and the eggs were counted. A black cloth with at least 30 eggs was placed in a 500 ml conical flask. The essential oil was introduced, and, after fumigation, eggs were held at 29 ± 1°C until mortality could be determined by the presence or absence of hatching.

Fumigation of *P. interpunctella larvae*

Newly laid eggs were placed in glass together with small quantity of artificial diet, the bottle was covered with organdy screen and incubated at 29 ± 1°C. The resulting 3rd instar larvae that emerged 2 days after hatching were selected for this experiment. It should be noted that result from a preliminary culture showed that 3rd stadium of *P. interpunctella* were obtained 12 days after hatching. Thirty (30) 3rd instar larvae of *P. interpunctella* were selected from the stock insect culture and placed in the cylindrical test cage (4.5 × 1.5 cm) containing 5 g of artificial diet. The essential oil was introduced, and after fumigation, the gas was released and the larvae were held for 5 days at 29 ± 1°C before the number of dead larvae was counted.

Fumigation of *P. interpunctella pupae*

Thirty (30) 0 to 24 h old pupae of *P. interpunctella* were placed in the test cage (4.5 × 1.5 cm). The cage and the essential oil were introduced into the fumigation chamber as earlier described, but the oil was applied at the doses of 0 (control), 4, 8, 16, 32 and 64 µl/l. After fumigation, the gas was released from the chamber, pupae were held for 7 days at 29 ± 1°C, and then mortality was determined by adult emergence.

Fumigation of *S. zeamais*

Fifty (50) adults of *S. zeamais* (10 to 15-days old) were placed into the cylindrical test cage (4.5 × 1.5 cm). *A. rusticana* oil was applied at the doses of 0 (control), 2, 4, 8, 16 and 32 µl/l. Mortality was recorded after 72 h of exposure to the essential oil gas. Nominal concentrations in the air in the glass conical flask were calculated on the assumption that all of the oil volatilized off the filter paper. 32 µl/l ethyl acetate was also used in bioassays to assess the influence of ethyl acetate on mortality. All treatments were replicated three times.

Gas chromatography-mass spectrometry

The essential oils of horseradish was analyzed on a gas chromatograph (Agilent 6890N)-mass spectrometer (Agilent 5975B)

(GC-MS). The GC column was a 30 m × 250µ m i.d. (0.25 µm film) fused-silica capillary column (J&W Scientific, Folsom, CA). The GC condition was as follows: injector temperature, isothermal at 40°C for 5 min, then programmed to 220°C at 5°C min^{-1} and held at this temperature for 2 min. Helium was used as the carrier gas at a rate of 1.2 ml min^{-1}. Effluent of the GC column was introduced directly into the source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 50 to 550 amu and ion source temperature was 230°C.Compounds were identified by comparison of mass spectra of each peak with those of authentic samples in a mass spectra library (NIST05/WILEY7.0).

Statistical analysis

Data obtained from each dose-response bioassay were subjected to probit analysis (Finney, 1971) in which probit-transformed mortality was regressed against log10-transformed doses; LC50 values, intercepts and slopes were generated (SPSS16.0).

RESULTS

Effect of *A. rusticana* oil on different stages of *P. interpunctella* and adult *S. zeamais*

Figure 1 shows the plot of probit line vs. the observed data. The fumigant activities against different stages of *P. interpunctella* and the adults of *S. zeamais* were dose dependent, with percentage mortality increasing in relation to increase in treatment concentration. Adults of *P. interpunctella* were the most susceptible stage (LC_{50} = 4.543), followed by eggs of *P. interpunctella* (LC_{50} = 9.988), adults of *S. zeamais* (LC_{50} = 14.368), larvae of *P. interpunctella* (LC_{50} = 17.171) and pupae of *P. interpunctella* (LC_{50} = 22.660) (Table 1). The regression line for egg, larvae, pupae and adult of *P. interpunctella* were y = 1.786x - 1.857, y = 2.143x - 2.428, y = 4.000x - 5.400 and y = 2.917x - 2.083, respectively, and for the adult of *S. zeamais,* it was y = 1.333x-2.067. The Chi value for egg, larvae, pupae and adult of *P. interpunctella* were 14.504, 11.185, 21.201and 7.314, respectively, and for the adult of *S. zeamais*, it was 108.400 (Table 1).

Chemical components of *A. rusticana* oil

Figure 2 shows the gas chromatogram of *A. rusticana* essential oil by using a fused-silica capillary column. The main component of *A. rusticana* essential oil was allyl isothiocyanate (97.81%). All the other compounds contained were <1%, so they were not shown here.

DISCUSSION

The essential oil of *A. rusticana* was shown to possess fumigant bioactivity against all stages of *P. interpunctella* and adults of *S. zeamais*. It caused high percentage mortality of all stages of *P. interpunctella* when exposed to 32 µl/l gas vapour of *A. rusticana*. The oil appeared not to have much impact on the adults of *S. zeamais* when the gas vapour was lower than 16 µl/l, however, at 32µl/l, the percentage mortality was 100%. GC-MS showed that the main component of the essential oil from *A. rusticana* was allyl isothiocyanate (97.81%).

The reason for the high fumigant effect of *A. rusticana* oil on *P. interpunctella* and *S. zeamais* could be attributed to its high pungent odour due to the presence of allyl isothiocyanate in the volatile oil. It could be that the volatile oil was able to block the spiracles of the insects by impairing breathing and thereby choking them to death (Park et al., 2006). Its relatively low fumigant effect on pupae might be that the gas vapour could not permeate through the thick wall of the pupal case. Also, the temperature, humidity and the carbon dioxide concentration of the fumigation environment influence the toxic action of fumigants (Bond, 2007).

A number of toxic chemicals produced by plants elicit pungent sensation in mammals (Banvolgyi et al., 2004; Calixto et al., 2005). The efficiency of the WasaOuro® system, an insecticide based on allyl isothiocyanate, the active component responsible for insecticidal action of horseradish and other brassicaceae family, was found to possess fumigant action against *Lasioderma serricorne* and *Tribolium confusum* by disrupting normal reproductive cycles of both insects, resulting in an insect population reduction in grain foods (Worfel et al., 1997). Natural toxins from isothiocyanates, including allyl isothiocyanate, have been shown to have insecticidal activities. Allyl isothiocyanate was reported to increase the production of carbon dioxide in the American cockroach (Tsao et al., 2002). Fumigant activities of horseradish and garlic oils against *Lycoriella ingenua* (Diptera: Sciaridae) have been reported (Park et al., 2006). The result of the GS-MS analysis is in accordance with a previous study by Park et al. (2006).

Increase of problems concerning the use of modem synthetic chemical insecticides, such as persistence of residues, resistance and damage to the environment and human health have generated interest in naturally occurring products. The results presented in this study suggest that *A. rusticana* oil or its major constituents could be efficient fumigants and also could be integrated with other pest management procedures. Further studies are needed to assess the fumigant activity of this essential oil and its constituents to other insects. Also, detailed mechanism of action of allyl isothiocyanate in target pestscould be an interesting area of research.

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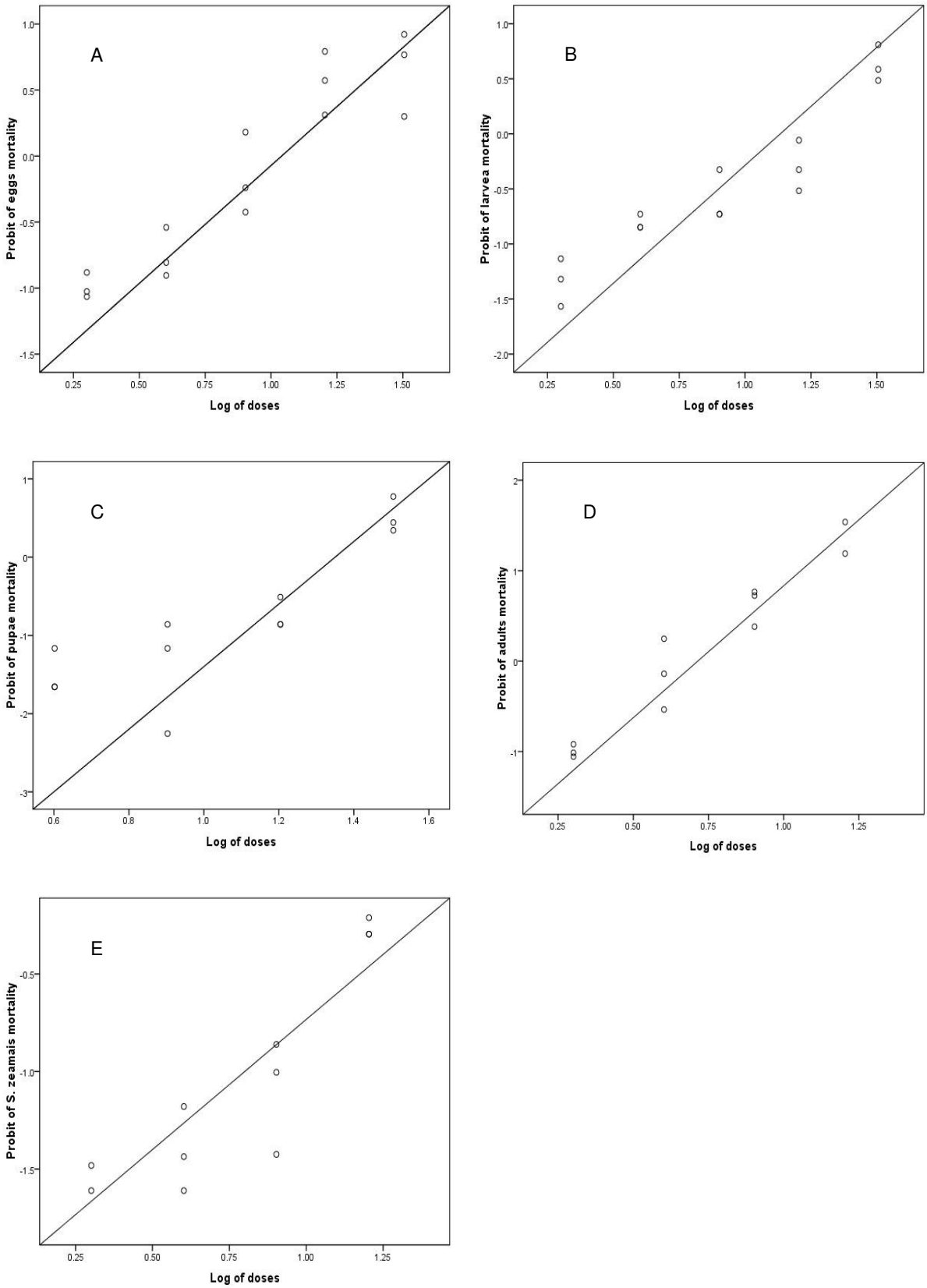

Figure 1. Plot of probit line vs. the observed data. probit line represents relationship between probit of mortality and log of doses. A, B, C, D represent different stages of *P. interpunctella* respectively, E represent the adults of *S. zeamais*.

Table 1. LC_{50} value of *A. rusticana* essential oil against different stages of *P. interpunctella* and the adult of *S. zeamais*.

	Regression line	LC_{50}(µl/l)	95% Confidence Limits		Chi Square
			Lower	Upper	
Eggs of *P. interpunctella*	y=1.786x-1.857	9.988	8.316	12.164	14.504
Larvae of *P. interpunctella*	y=2.143x-2.428	17.171	13.742	22.773	11.185
Pupae of *P. interpunctella*	y=4.000x-5.400	22.660	17.795	26.553	21.201
Adults of *P. interpunctella*	y=2.917x-2.083	4.543	3.789	5.335	7.314
Adults of *S. zeamais*	y=1.333x-2.067	14.368	9.963	20.080	108.400

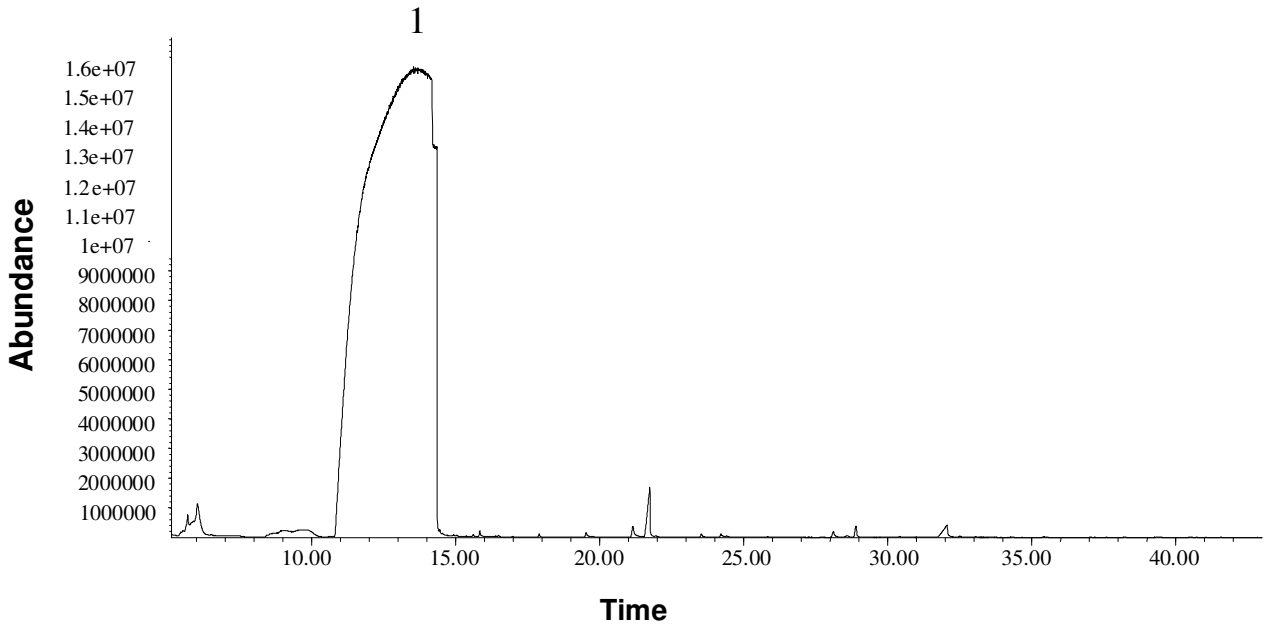

Figure 2. Gas chromatogram of *A. rusticana* essential oil. Peak 1, Allyl isothiocyanate.

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