A study of fumigation toxicity of horseradish essential oil against two stored grain insects

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Abstract

Horseradish essential oil is a biological fumigant which was extracted from *Armoracia rusticana* The effects of different concentrations, temperatures and grain types by fumigation using horseradish essential oil were studied in this paper. The oil had significant fumigation efficacy against pests in stored grain under natural conditions. In the absence of grain, a concentration of 2.25 ppm horseradish essential oil could kill *Sitophilus zeamais* and *Rhyzopertha dominica* after 12 h at 25°C . Three ppm of horseradish essential oil could kill all the *S. zeamais* and *R. dominica* on the surface of maize, wheat and paddy during 72 h exposure at 25°C. The mortalities of *S. zeamais* placed under maize, wheat and paddy were 100%, 100% and 98%, respectively, using 24 ppm of horseradish essential oil at exposure for 72 h at 25 °C . The mortalities of *R. dominica* were 100%, 93% and 86%, respectively, under the above conditions. Temperature did not result in significant differences on the fumigation efficacy of horseradish essential oil. Different types of stored grain had a significant influence on the fumigation efficacy of horseradish essential oil. The order of fumigation efficacy were maize, wheat and paddy. We concluded that horseradish oil could be used as a sanitary and environmentally user-friendly bio-fumigant within empty granaries or other storage structures.

Keywords: Horseradish oil, Stored grain pest, Fumigant toxicity, Fumigation

1. Introduction

Almost all countries have been using fumigants to kill pests in stored grain since fumigation is a convenient means to control stored grain pests. Fumigant which can be used in stored grain have special requirements. Therefore only a few fumigants such as methyl bromide and phosphine are being used currently. Due to the involvement of methyl bromide in the depletion of ozone the terms of the Montreal Protocol have classified it as an environmentally controlled substance, and it will be phased-out by 2015 worldwide (MBTOC, 1998). Phosphine is used frequently, but many insects have developed resistance (Benhalima et al., 2004). Finding alternatives to methyl bromide is urgent in the field of stored product (Guo et al., 2004). According to the practices of IPM, an effective fumigation alternative should not only be friendly to the environment and safe to stored grain, but also act effectively against stored-grain pests and have low cost. In recent years, scientists have searched for bio-fumigants. AgraQuest in the United States introduced first fungal fumigant in the world in 2002. This fungal fumigant is a kind of natural volatile substance which is produced by *Mascodor albus* (Wang, 2003). Essential oils extracted from plant can also be used as fumigants in stored grain (Shaaya et al., 1997). Wu et al. (2007) extracted active substances horseradish oil from the horseradish plant and determined the toxicity to stored-grain pests. Horseradish oil was able to kill several species of stored-grain pests. The toxicity of horseradish oil was studied further in this paper, including (1) horseradish oil bioassay in the absence of grain (2) toxicity of horseradish oil to two stored-grain pests in the presence of different grains; (3) toxicity of horseradish oil to two stored-grain pests at different temperatures.

2. Materials and methods

2.1. Insects

All test insects were reared at Wuhan Polytechnic University in electronically controlled incubators 27±1°C and 75±5% r.h. *Sitophilus zeamais* Motschulsky was reared on whole wheat, *Rhyzopertha dominica* (F.) on broken wheat. The wheat to be used was first sterilized at 80°C for 2 h. The moisture content was then adjusted to 13±1%. Adults (7-14 days old) were used in this study.

2.2. Chemicals

Allyl isothiocyanate (85% purity) was provided by Professor Lin Kai-Chun. Acetone was analytical grade (≥97%purity) purchased from Tianjin Basifu chemical Co. Ltd.

2.3. Horseradish oil bioassay in absence of grain

Fumigation in the absence of grain was carried out as described by Xu et al. in 1L airtight jars (Xu et al., 2008). Firstly, 30 test insects were placed in the jar. Secondly, measured quantities of horseradish oil were added to a filter paper (3 ×11cm) which was glued vertically inside the jar. Thirdly, the jar was closed as soon as possible, and the lid was sealed on with parafilm. Finally, the jar was placed in incubators at 25 °C. Each bioassay was carried out with five horseradish oil doses and an undosed control. Each dose or control was repeated three times. Mortality was determined after the treated *S. zeamais* and *R. dominica* were maintained at 25°C for 12 h.

2.4. Horseradish oil bioassay in the presence of grain

Fumigation with grain was carried out as described by He et al. (2008). The adult insects were fumigated in gas-tight 15-L glass desiccators sealed with glass stoppers containing a septum. The 80% of the desiccators' volume were filled by 12% m.c. grain (maize, wheat and paddy respectively). Thirty adult maize weevils and 30 adult lesser grain borers were placed seperately into a ventilated bag. Three replications of each insect were placed under the grain, and three replications were placed on the top of the grain. Horseradish oil was added onto a filter paper in a dish. The container was sealed after the reagent was added. Horseradish oil fumigations were carried at 25±1°C and 75±5% r.h. in electronic controlled incubators with acetone treated as a blank control. The exposure was complete after 72 h, and the desiccators were opened and mortalities were calculated for each replicate.

2.5. Statistical analysis

The mortality results were analyzed statistically using SPSS data processing software (Jia, 2006).

3. Results

3.1. Horseradish oil bioassay in the absence of grain

Horseradish oil was highly toxic to *S. zeamais* and *R. dominica* since low concentration could kill the tested pests rapidly. A concentration of 2.25 ppm horseradish oil killed all *S. zeamais* and *R. dominica* after 12 h exposure at 25°C. The LC_{50} values of horseradish oil to *S. zeamais* and *R. dominica* were 0.64 ppm and 0.69 ppm, respectively (Table 1). The LC_{90} values of horseradish oil to *S. zeamais* and *R. dominica* were 1.60 ppm and 1.71 ppm, respectively (Table 1).

Table 1 Bioassay results of horseradish oil on two test insects after 12 h at 25°C.

Insects	Slope±SE	LC_{50} (95% CI) μg/mL	LC_{90}(95% CI) μg/mL
Sitophilus zeamais	3.16±0.25	0.64 (0.37-0.93)	1.60 (1.11-3.87)
Rhyzopertha dominica	3.27±0.24	0.69 (0.42-0.96)	1.71(1.20-3.61)

3.2. Horseradish oil bioassay in the presence of different grains

Horseradish oil was highly toxic to *S. zeamai*s and *R. dominica* (Tables 2, 3). Three ppm of horseradish essential oil killed all the *S. zeamais* and *R. dominica* on the surface of maize, wheat and paddy for 72 h exposure at 25°C. The mortalities of *S. zeamais* placed under maize, wheat and paddy were 100%, 100% and 98%, respectively, at exposure to 24 ppm of horseradish essential oil for 72 h at 25°C. The mortalities of *R. dominica* were 100%, 93% and 86%, respectively, under the above conditions. The lower mortalities of the pests placed under the grains showed that the presence of grain had a significant influence on fumigation activity. Fumigant efficacy of horseradish oil varied with the type of grain used (Tables 2, 3).

Table 2 Mortalities of adult *Sitophilus zeamais* fumigated with different concentrations of horseradish oil after 72h at 25°C(%), n=3.

		Mortality ± SD (%)				
		Dose (ppm)				
Grain	**Placement in desiccator**	**1.5**	**3**	**6**	**12**	**24**
Maize	top	89.0 ±2.4	100±0	100±0	100±0	100±0
	bottom	75.0±1.9*	88.7±3.5*	100±0	100±0	100±0
Wheat	top	85.0±3.2	100±0	100±0	100±0	100±0
	bottom	68.0±2.7*	82.3±1.5*	98.3±1.5	100±0	100±0
Paddy	top	87.0±4.1	100±0	100±0	100±0	100±0
	bottom	57.0±3.5*	65.3±3.5*	72.3±2.5*	90.0±2.0*	98.2±3.7

*Mortalities of pests is significantly different between top and down of the same grain (P<0.05, Student's t-test).

Table 3 Mortalities adult of *Rhyzopertha dominica* fumigated by different concentrations of horseradish oil after 72h at 25°C(%), n=3.

		Mortality ± SD (%)				
		Dose (ppm)				
Grain	**Placement in desiccator**	**1.5**	**3**	**6**	**12**	**24**
Maize	top	85.0±3.7	100±0	100±0	100±0	100±0
	bottom	70.0±2.5*	56.3±3.5 *	73.7±4.0*	89.0±1.0*	100±0
Wheat	top	86.0±4.1	100±0	100±0	100±0	100±0
	bottom	64.0±2.1*	47.33±3.22*	62.0±3.0*	81.7±1.5*	93.0±1.0
Paddy	top	83.0±5.3a	100±0	100±0	100±0	100±0
	bottom	58.0±2.6*	32.3±2.5*	51.7±3.1*	67.00±1.7*	86.00±1.7*

*Mortalities of pests is significantly different between top and down of the same grain (P<0.05, Student's t test).

3.3. Horseradish oil bioassay different temperatures in the presence of grain

Tables 4 and 5 show the mortalities of *S. zeamais* and *R. dominica* fumigated applying 12 ppm horseradish oil at 16, 24, 32°C. The mortalities of *S. zeamais* and *R. dominica* increased slightly as the temperature increased, but not significantly. This may have been due to the higher concentration of the fumigant used in the test.

Table 4 Mortality adult of *Sitophilus zeamais* fumigated with12 ppm horseradish oil for 72 h at different temperatures. (%)[a]

		Mortality ± SD (%)		
		Temperature (°C)		
Grain	**Placement in desiccator**	**16**	**24**	**32**
Maize	top	100±0a	100±0a	100±0a
	bottom	96.0±3.5a	100±0a	100±0a
Wheat	top	100±0a	100.±0a	100±0a
	bottom	92.3±0.6a*	100±0a	100±0a
Paddy	top	100±0a	100±0a	100±0a
	bottom	86.3±1.5a*	89.0±1.0a*	92.3±2.5a*

[a] Results are the means ± SD (n=3). Means within columns followed by the same letter are not significantly different (P<0.05, LSD Fisher's multiple range test).
* Mortalities of pests is significantly different between top and down of the same grain (P<0.05, Student's t-test).

Table 5 Mortality adult of *Rhyzopertha dominica* fumigated with 12 ppm horseradish oil for 72h at different temperatures (%)[a]

		Mortality ± SD (%) Temperature (°C)		
Grain	**Placement in desiccator**	**16**	**24**	**32**
Maize	top	100±0a	100±0a	100±0a
	bottom	88.7±6.1a*	89.0±6.0a*	90.7±7.5a*
Wheat	top	92.5±10.4a	93.6±8.2a	100±0a
	bottom	78.3±7.5a*	82.3±3.8a*	84.7±5.8a*
Rice	top	98.3±1.9a	99.2±2.9a	100±0a
	bottom	59.7±6.5a*	64.7±3.6a*	66.7±1.5a*

[a] Results are the means ± SD (n=3). Means within columns followed by the same letter are not significantly different (P<0.05, LSD Fisher's multiple range test).
* Mortalities of pests is significantly different between top and down of the same grain (P<0.05, Student's t- test).

4. Discussion

Horseradish, Armoracia rusticana G. Gaertn., B. Mey. & Scherb, is a perennial herbaceous plant in Armoracia genus, which is distributed in eastern Europe, Turkey, Japan, northeast China, north China and other areas with more than 2000 years of cultivation history. Its fleshy root is used for food as a seasoning with special peppery taste welcomed by European, Japanese and Chinese (Delaquis and Mazza, 1995). The isothiocyanates of the horseradish and other cruciferous vegetables vary in their biological activities, such as anti-cancer and tumor, antibiosis and inhibiting platelet aggregation (Zhang et al., 2005). Eighteen essential oils were indentified from 95% of the whole oil in Chinese horseradish.

Of these constituents, allyl isothiocyanate accounted for about 32%, The second constituent was 4 - pentenyl isothiocyanate, which accounted for 26% (Lin et al., 2001). The chemical constitutions of the essential oil from horseradish varied according to the plant distribution area. The essential oil constitutions from the plant in Asia were widely different from that in Europe. But they all possessed allyl isothiocyanate, allyl rhodanate, 4-pentenyl isothiocyanate, butyl isothiocyanate and iso-β-phenyl isothiocyanate (Lin et al., 2001). Wu (2007) determined the toxicity of the pungent essential oils from horseradish to variety of pests, plant pathogens, soil bacteria and nematodes, which indicated that these essential oils could be researched as a potential bio-fumigants. Since a low concentration of 2.25 ppm horseradish oil could completely control *S. zeamais* and *R. dominica* for 12 h exposure at 25°C in the absence of grain, it was suggested that this horseradish oil could be used as a sanitary and environmentally user friendly bio-fumigant within empty granaries or other storage structures.

It had been discussed that grain could absorb and breakdown a fumigant such as ethyl formate (Damcevski and Annis, 1998; Damcevski and Annis, 2006). We also found that the presence of grain decreased the toxicity of horseradish oil to pests. The mortalities of the two pests placed on the top of the grains were 100% after 72 h exposure with the concentration of 3 ppm, but the mortalities of S. zeamais placed under the maize, wheat and paddy were 89, 82 and 65%, respectively, and that of R. dominica were 56, 47 and 32%, respectively, (Table2, 3).

A higher concentration of 24 ppm could kill 100, 100 and 98% of S. zeamais placed under the maize, wheat and paddy, respectively, and that of R. dominica were 100, 93 and 86%, respectively (Tables 2, 3). The higher molecular weight and higher boiling point of the horseradish essential oil might be attributed to the poor penetrability and diffusibility in the grains. Appropriate synergists or gas recirculation equipment could contribute to facilitate diffusion of horseradish oil in the grains.

The type of grain and its amount directly affected the efficacy of horseradish oil. In this paper, horseradish oil gave the best efficacy for maize, good for the wheat and the worst for paddy (Tables 2, 3, 4, 5). This was partly the result of the grain's properties such as size and its surface smoothness. Other factors should also be studied further. We conclude that the horseradish oil could be used with certain grains in small warehouses or the farmers' warehouse under natural fumigation conditions.

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