Evaluation of inert dusts against phosphine resistant strains of *Cryptolestes ferrugineus*

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Abstract

The relative phosphine resistance of two strains of *Cryptolestes ferrugineus* was measured by the method of knockdown at 2 mg/L of phosphine. The efficacy of five inert dusts (Puliangtai, G-1, G-2, G-3 and G-4) against the adults of two strains (JXCF and YYCF) of *C. ferrugineus* was assessed. The insects were exposed to the five inert dust on filter paper inside petri dishes for 1 day at 30±1°C and 65±1% r.h. Then, the adults were held for 4 days with food at the same conditions without inert dust in surface bioassay. Also, they were placed in 25±1 °C and 65±1% r.h. in grain bioassay. In surface bioassay, the two strains of *C. ferrugineus* were susceptible to the five inert dusts at 0.08 g/m^2 and 0.1 g/m^2. G-3 appeared the most effective against both strains, since its efficacy was 3-6 times higher than the other four dusts tested. YYCF strain was more susceptible than JXCF, with 1-4 times higher mortality level. The five inert dusts were also effective in grain bioassay. This research indicated that inert dusts were effective on phosphine-resistant *C. ferrugineus* populations.

Keywords: Inert dust, *Cryptolestes ferrugineus,* Phosphine resistance

1. Introduction

In the recent years, many relevant reports have been published on phosphine resistance of *Crytolestes. ferrugineus* (Stephens) (Jiang et al., 1995; Liu et al., 2003 ;Lin et al., 2004; Liu, 2004; Wang et al., 2004; Yan. et al., 2004). The efficacy of phosphine is not satisfactory to keep *C. ferrugineus* under control in the high temperature and humidity conditions, where *C. ferrugineus* can survive at phosphine concentrations below 200 mL/m (Lu et al., 2005). Some resistance strains could be killed effectively only when phosphine concentration reaches 550 ppm for 45 days (Pang et al., 2002). Under these circumstances phosphine concentration has to be increased. However, higher concentrations of phosphine causes the insect pests narcosis (Lu et al., 2005); also, the higher concentration of phosphine will increase the risk of phosphine residues in grains. Thus, it is essential to develop alternative methods to phosphine for the control of this pest. This research assessed the efficacy of the inert dust against *C. ferrugineus* as the possible alternative control treatment.

2. Materials and methods

2.1.Insects

Two strains of *C. ferrugineus* collected from Jiangxi Wannianzhongshen State Grain Reserve, JXCF Strain, and from Yiyang Depot, YYCF strain, directly under the Central Grain Reserves.

The insects from the two strains were reared in a mixture of oatmeal, wheat flour and yeast (at ratio of 6:3:1-w/w/w) at 30±1°C and 75±5% r.h. Three days later the adult insects were removed into clean feeding bottle and maintained, and the eggs were maintained in the original bottle until they grew up into adults; then, we chose the adults 2 –weeks after expression as test insects. The strains mentioned above have been maintained for generations in the Stored Product Insect Pests Controlling Laboratory of the Academy of State Administration of Grain.

2.2. Test chemicals

Puliangtai, G-1, G-2, G-3 and G-4 were five inert dusts there were selected by the laboratory mentioned above.

2.3. Test for phosphine resistance

We placed 10 test insects into the fumigated knockdown test bottle of 100-500 mL airtight with a rubber plug. A certain amount of PH_3 gas was discharged into the bottle until the concentration of PH_3 was 2

mg/L. The insects were considered to be in the condition of knockdown when they were in spasms or paralysis. The paralysis duration of each test insects (the value of KT) was recorded. The experiments of each strain were repeated three times (Cao and Zhang, 2000).

2.4. Inert dust surface bioassay

The clean culture dish (the bottom with 70 mm diameter, upper lid of 75mm diameter) was dried and sterilized at 100°C for 2 hours. After cooling, the dish internal bottom was pasted with the round filter paper with the same diameter as the bottom without any gaps. Moreover, the wall of the dish was coated with polytetrafluoroethylene (PTFE) with brush to make the internal wall smooth to prevent the insects from escaping. The dish was dried at 60 °C for 30 minutes before use. The test insecticide was weighed and placed into the dishes. The dish was covered with lid and shaken manually for several times on the test-bed. After the suspending insecticide settling down the lid was removed carefully. The test inert dust was distributed as evenly as possible.

The test insects were taken on the day before the experiment. The insects were placed into the clean petri dish with the filter paper without food in order to remove their foreign substance as their crawling. The insects were placed into two desiccators randomly and exposed in the conditions of 30 ± 1°C and 65 ± 1% r.h. for 12 h. During the test, 20 insects were placed in the prepared inert dust in surface bioassay, and a compared group (three replicates) was put into each desiccators. After treated under the experiment conditional for 1 day, the insects were removed gently to another prepared clean Petri dish without the insecticide with a small brush. With adequate amount of food added, the insects were maintained and observed at the same temperature and humidity for 4 following days. The mortality of the insects was checked and recorded every day.

2.5. The method of inert dust in grain bioassay

The glass can bottle of with 70 mm diameter × 150 mm was dried and sterilized at 100°C for 2 hours, and cooled for use. A circular belt of PTFE of about 2 cm width on the internal wall of the bottle to prevent the insects from climbing up. Then, the bottle was dried and sterilized at 60°C for 30 min and taken out for use. We weighed 100 g wheat (adding 0.5% cracked wheat) and put it into the prepared bottle, then weighed the experimental chemicals by the method of weighing by difference and put it into the bottle with wheat. The bottle was covered with cloth and sealed with rubber band. After this procedure, the bottle was shaken manually for 5 minutes. Each dosage series was repeated three times.

The test insects were taken on the day before the test, and then placed in the clean Petri dish with the filter paper with no food in order to remove the foreign materials as their crawling. The insects were exposed in the environmental conditions of 30 ± 1°C and 65 ± 1% r.h. for 12 hours. During the experiment 20 insects were taken and placed into the bottle with the inert dust in grain, and placed to the desiccators as noted above. Every day the mixed grain was gently poured onto another prepared tray, and the death of the test insects was checked with a brush. The mortality were checked and recorded for the 6 following days.

Toxicity results were analyzed by probit analysis method (Finney, 1971). The calculation of the mean, standard error and multiple comparisons were analyzed by Microsoft ® Excel 2000, DPS data processing software (Tang and Feng, 1996)

3. Results

3.1. Phosphine knockdown test

Table 1 indicated the knockdown duration of 50% insects of each strain of *C. ferrugineus*. KT_{50} was far higher than 30 min. KT_{50} value of the susceptible to phosphine strains was basically correspondingly low by the method recommended by the FAO (1975) while KT_{50} value of phosphine resistance strains was basically large (Wang et al., 2004), which indicated that phosphine resistance of the two strains was very high.

Table 1 Knockdown duration of 50% of two strains of *C. ferrugineus* (KT_{50}).

Insect strains	KT_{50}
YYCF	5 days
JXCF	3 days

3.2. Inert dust surface bioassay

Table 2 indicated that the low concentration of the inert dust was effective against phosphine resistance *C. ferrugineus*. Especially for G-3, its concentration below 0.08 mg/m^2 can completely kill adults of YYCF strain. The effects of inert dusts against pests are better than Puliangtai with the concentration of mg/m^2 except the G-4 against JXCF. Under the same experiment conditions, the mortality was significantly increased, and almost reached 100% when the two strains were treated with dust concentration of 0.1 mg/m^2. *Crytolestes ferrugineus* was very sensitive to the inert dusts, which further proved the results by Liu (2005).

Table 2 Efficacy of inert dusts in surface bioassay against *Cryptolestes ferrugineus.*

Strains	Concentration (mg/m^2)	Inert dust	Mortality rate (%)
#	0.08	Puliangtai	18.0±16.0
		G-1	33.0±18.3
		G-2	31.0±16.8
		G-3	100.0±0.0
		G-4	38.0±27.5
JXCF		Puliangtai	13.0±8.1
		G-1	14.0±9.1
		G-2	29.0±13.1
		G-3	79.0±12.0
		G-4	10.0±6.9
YYCF	0.1	Puliangtai	98.3±1.7
		G-1	96.7±1.7
		G-2	98.3±1.7
		G-3	100.0±0.0
		G-4	96.7±1.7
JXCF		Puliangtai	98.3±1.7
		G-1	100.0±0.0
		G-2	100.0±0.0
		G-3	100.0±0.0
		G-4	100.0±0.0

Compared with result from the same concentration of 0.08 mg/m^2 and the same pesticide, the sensitivity of two strains to the inert dust was different. When the two strains were treated with G-1, G-3 and G-4, the efficacy was significantly different. YYCF strain was more susceptible than JXCF, with 1-4 times higher mortality level

Table 3 The efficacy of inert dusts in 13% m.c. grain at 50 mg/kg, against *Cryptolestes Ferrugineus*, JXCF strain.

Inert dust	Mortality rate of test insects (%)
Puliangtai	40.0±15.3b
G-1	96.7±3.3a
G-2	96.7±3.3a
G-3	100.0±0.0a

3.3. Inert dust in grain bioassay

Table 3 showed the efficacy of inert dust in grain bioassay against *C. ferrugineus*. As can be seen in Table 3, under the condition of 13% moisture, application of inert dusts at 50 mg/kg could control this species, especially G-1, G-2, G-3 and G-4. The efficacy of G-1, G-2, G-3 and G-4 was by far higher than the respective levels reported by Liu (2005). Under the same conditions, G-3 could provide 100 % mortality of *C. ferrugineus* adults.

4. Discussion

Reichmuth (1991) proposed that, when treated with phosphine at 1 mg/L, the insect pests could still act normally, which indicated the insect pests possessed the relative phosphine resistance. When knocked

down within 30 min, the insect pests were determined as the ones susceptible to the phosphine. This essay adopted phosphine of 2 mg / L against YYCF and JXCF, and the KT_{50} values were far higher than 30 minutes proposed by the reference assay, which indicated that the two strains of *C. ferrugineus* have a strong phosphine resistance that could cause fumigation failure.

In the experiment of the inert dust in surface bioassay, under the same conditions, when the concentration was slightly increased from 0.08 mg/m^2 to 0.1 mg/m^2, mortality of *C. ferrugineus* had greatly increased, and reached, in some cases, 100%. Choosing the adequate concentration was very crucial, and inert dust was an effective insecticide as disinfectation line and empty depot disinfectants. In addition, the experiment with the inert dusts in surface bioassay and in grain bioassay indicated that G-3 was the best of the five inert dusts, and should be tested more thoroughly.

Inert dust is effective, broad-spectrum, safe and natural insecticide, that does not leave residues on the final product. Inert dusts can be also used in the case of organic products, and can provide long term protection. The experimental results showed that inert dusts can be used as an alternative to phosphine in a rotation strategy to alleviate the development of the phosphine resistance.

Low concentration and low amount of inert dust could inhibit over 90% of the populations of phosphine-resistant strains of *C. ferrugineus*. The treatment amount for surfaces and grains can be 0.1 mg/m^2 and 50 mg/kg, respectively.

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