JSR Publications

Available Online

J. Sci. Res. **4** (3), 783-788 (2012)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Short Communication

Repellent Action of Diatomaceous Earth against the Adult Red Flour Beetle *Tribolium castaneum* (Herbst)

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Received 3 February 2012, accepted in revised form 26 August 2012

Abstract

Tribolium castaneum (Herbst) adults were released to choice chambers containing untreated flour in one half and diatomaceous earth (DE)-treated flour in the other half. The doses of DE used were 2-32 mg/g. Females, males and unsexed adults were exposed separately to each dose for 24-, 48-, 72-, 96- and 120-h. DE at 32 mg/g strongly repelled ($P<0.001$) both sexes and unsexed beetles at all exposure periods. The males showed comparatively more avoidance to DE-treated flour at longer exposures.

Keywords: Repellent; Diatomaceous earth; *Tribolium castaneum.*

doi: http://dx.doi.org/10.3329/jsr.v4i3.9637 J. Sci. Res. **4** (3), 783-788 (2012)

1. Introduction

The present day insect pest management measures for the stored grain and cereal commodities are relied on the use of techniques which will prohibit infestation by the insects rather than to kill them within the commodities. The consumers avoid those commodities which contain any kind of infestation, either by the presence of dead or live insects or their body parts. the hazards of chemical residues in food and development of resistance by a wide range of insect species to different groups of insecticides have generated a sustained research to find out non-toxic, environmentally friendly, reduced-risk products for the aforementioned purpose.

The diatomaceous earth (DE) produced from the fossil diatoms are known as potential insecticides against a wide range of insect species [1], and are used for the protection of stored grain from an ancient time by the Chinese and the Red Indians. DEs of various physical formulations added with or without pesticides are used in Integrated Pest Management (IPM) programmes [2], because they are harmless to mammals [3]. DEs cause mortal effect in the treated insects by desiccating both cuticle and the digestive tract

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[4,3]. The US Environmental Protection Agency (EPA) allowed the use of DEs in the food storage and food processing areas [5] and classified them as GRAS (Generally Recognized as Safe) as food additives [5]. DEs are also used in food stores in Australia [6].

The Red flour beetle, *Tribolium castaneum* (Herbst) is a major pest of a wide variety of stored foods and became resistant against almost of all groups of insecticides. *T. castaneum* have been reported to be less susceptible to DE [7]. However, the storage conditions are important factors which play role in DE-toxicity against the insect pests. Potentiality of DEs as attractant or repellent against the storage insect pests is scarcely reported in the literature. It is reported that DE product (Mitex) significantly repelled the larvae of *T. castaneum* when exposed for different periods [8]. Mitex was also found to control the population buildup in both *T. castaneum* and *Sitophilus oryzae* [9].

The present research is aimed to observe the activity of Mitex (a DE formulation) as either attractant or repellent, against the sexed and unsexed adult *T. castaneum*.

2. Materials and method

2.1. *Collection of T. castaneum*

Large number of adult *T. castaneum* was collected from the stock culture of the beetles, reared in the Entomology Laboratory, Department of Zoology, Rajshahi University. The adults were placed on standard food (1:9 whole wheat flour: brewer's yeast) [10] in a 500ml beaker, and was covered with fine meshed cloth to avoid escape of the beetles. After 24 hours the food was passed through a 250µm mesh sieve to collect the eggs. Hundred of the eggs were then placed on standard food in glass Petri dishes and reared until the pupae were formed. After every three days the food was replaced by a fresh one to avoid conditioning by the beetles [11].

The 2-day old pupae were sexed by examining the exogenital process of the females under microscope [12]. The male and female pupae were kept separately. While a number of pupae were left unsexed. The pupae were allowed to emerge as adults.

2.2. *Test material and doses used*

Mitex, a commercial product containing diatomaceous earth (DE), marketed by Agril, UK was used in this experiment. The experimental doses of Mitex used were 2, 4, 8, 16 and 32 mg/g of standard food.

2.3. *Experimentation*

The experiment was conducted in a choice chamber as described by Mathlein [13]. The choice chamber was made of a plastic Petri dish (9 cm diameter), which was divided into two equal halves by drawing a line across the middle.

By the use of a partition one half of the choice chamber was loaded with untreated wheat flour and the other half with DE-treated flour. Twenty 7-day old adults were released in the middle line of the choice chamber, thus providing the beetles an option for choosing either treated or untreated food. The experiment was set separately for male, female and unsexed adults of *T. castaneum.*

The experiment was set separately for male, female and unsexed beetles, for each dose of DE, and each exposure period of 24-, 48-, 72-, 96- and 120 hr. After each exposure period, both treated and untreated flour was carefully poured into separate Petri dishes and then passed through a 500 µm sieve to separate beetles from the flour. Number of the adults found on each half of the choice chamber was counted and recorded separately. The experiment was conducted at room temperature with three replications for each set of experiment.

2.5. *Statistical analysis*

The percentage of differences of the beetles in the treated and untreated food was measured by chi-square test to determine the level of significance.

3. Results and Discussion

At 24 and 48-h exposures the female *T. castaneum* was significantly repelled by the dose of 32mg/g of DE only (χ^2 = 14.2 and 16.06 respectively, P<0.001). The males were distributed equally in untreated and treated flour at all the doses except the lowest (2 mg/g, χ^2 = 10.89, P<0.001) and the highest (32 mg/g, χ^2 = 8.00, P<0.05) ones at 24-h exposure (Table 1). But at 48-h exposure the males were found to be susceptible to the doses of 16 and 32mg/g of DE When unsexed *T. castaneum* adults were exposed for 24-h, they were significantly repelled by the dose of 32mg/g (χ^2 = 9.39, P<0.05) only, but at 48-h exposure the beetles avoided the treated flour at doses of 16 and 32mg/g (χ^2= 8 and 9.39 respectively, P<0.05) (Table 1).

When the females and unsexed beetles were exposed to the treated flour for 72-h they showed avoidance to 16 and 32mg/g DE treated flour (χ^2 = 10.89 and 20.05 respectively, P<0.00, females; χ^2 = 6.72 and 9.39, P<0.05, unsexed beetles), but they were equally distributed in untreated and treated flour at all other lower doses. The males were repelled only by 32mg/g DE treated flour (χ^2 = 14.22, P<0.001) when they were exposed for the same period (Table 1).

All the doses except 32mg/g DE, did not change the normal distribution of female (χ^2 = 12.50, P<0.001) and unsexed (χ^2 = 8.00, P < 0.05) and *T. castaneum* in untreated and treated flour when they were exposed to the treated flour for 96-h. However, the males significantly avoided the treated flour at all dose levels except the 4 mg/g, where the males were equally distributed at two halves of the choice chamber (Table 1).

The females were repelled significantly by the doses 16 and 32mg/g DE, when they were continuously exposed for 120-h (χ^2 = 9.89, P<0.05, and 14.22, P<0.001

respectively). The males and were found to be susceptible to doses 8-32mg/g, and 75% of them were repelled by the 32 mg/g of DE-treated flour. The unsexed beetles were repelled only by the 32 mg/g dose (χ^2 = 9.39, *P*<0.05), but attracted by 2 mg/g of DE (χ^2 = 9.38, *P*<0.05) (see Table 1).

Table 1. Percentage distribution of adult *T. castaneum* in DE-treated flour

Exposure period (h)	Dose (mgDE/gF)	Female		Male		Unsexed	
		% in treated flour	λ^2-value (significance level)	% in treated flour	λ^2-value (significance level	% in treated flour	λ^2-value (significance level
24	2	38.33	2.72 (NS)	26.66	10.89***	55.00	0.50
	4	38.33	2.72 (NS)	46.66	0.22	53.33	0.22
	8	35.00	4.50 (NS)	43.33	0.89	46.66	0.22
	16	46.33	0.89	40.00	2.00 (NS)	53.33	0.22
	32	23.33	14.22***	30.00	8.00*	28.33	9.39*
48	2	45.00	0.50	43.33	0.89	50.00	0.00
	4	46.66	0.22	46.66	0.22	40.00	2.00 (NS)
	8	45.00	0.50	41.66	1.39 (NS)	45.00	0.50
	16	41.66	1.39 (NS)	23.33	14.22***	30.00	8.00*
	32	21.66	16.06***	23.33	14.22***	28.33	9.39*
72	2	41.66	1.39 (NS)	46.66	0.22	68.33	6.72*
	4	35.00	4.50 (NS)	35.00	4.50 (NS)	51.66	0.05
	8	35.00	4.50 (NS)	36.66	3.56 (NS)	36.66	3.56 (NS)
	16	26.66	10.89***	36.66	3.56 (NS)	28.33	9.39*
	32	18.33	20.05***	23.33	14.22***	28.33	9.39*
96	2	41.66	1.39 (NS)	28.33	9.39*	60.00	2.00 (NS)
	4	51.66	0.05	45.00	0.50	46.66	0.22
	8	35.00	4.50 (NS)	30.00	8.00*	41.66	1.39 (NS)
	16	40.00	2.00 (NS)	30.00	8.00*	45.00	0.50
	32	25.00	12.50***	21.66	16.06***	30.00	8.00*
120	2	45.00	0.50 (NS)	40.00	2.00 (NS)	71.66	9.38*
	4	43.33	0.89 (NS)	56.66	0.89 (NS)	50.00	0.00
	8	45.00	0.50 (NS)	31.66	6.73 *	51.66	0.05
	16	28.33	9.39 *	30.00	8.00*	40.00	2.00 (NS)
	32	23.33	14.22***	25.00	12.50***	28.33	9.39*

Note: NS = not significant, * = *P*<0.05, *** = *P*<0.001.

The results of the present experiment showed that DE mixed food would repel *T. castaneum* adults of both sexes at dose levels of ≥ 32mg/g at exposure from 24-120 h.

Distribution patterns of the adults in treated flour at doses from 2-16mg/g normally showed no effect on the food choice of the beetles, but the males were found to be a little bit reactive to doses like 8 and 16mg/g DE at longer exposures (<24-h). However, the unsexed beetles were attracted to the dose 2mg/g DE at longer exposures (72-120h).

DEs have proved as effective grain protectants [4], and also as structural treatments to storage facilities [6,14]. The toxic effects of DE are regulated by temperature and humidity [15], the substrate [16] and depend on insect species, strains and life stages [15,17,18]. These results can be explained as DE doses <32mg/g did not affect either the cuticle or the gut lining of the adults at short exposure, but at longer exposure it might cause abrasion to cuticle and desiccate the beetle even at 8mg/g dose level. The exposure time and sex had no effect on the distribution patterns of the beetle at low doses of DE. A higher percentage of distribution of unsexed beetles in DE at 2 mg/g dose at longer exposures as observed, might be due to their sudden presence in the treated food while wondering around, when they were sieved. The report of Chiu [19] pointed out that when DE is mixed with food, the desiccation action of the material can be replenished by ingesting and metabolism of food by the insects.

4. Conclusion

The present results revealed that DE (Mitex) can be used as a reduced risk repellent compound in the grain and cereal stores, flour mills and grocery shops to resist infestation by *T. castaneum.*

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