



## AGE-RELATED RESPONSE OF *TRIBOLIUM CASTANEUM* (HERBST) LARVAE TO DIATOMACEOUS EARTH AT DIFFERENT EXPOSURE PERIODS

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### Abstract

**Context:** Diatomaceous earth (DE) compounds have been recognized as reduced risk and eco-friendly compounds, which can be used in the insect pest management programmes for food storage and processing areas.

**Objective:** To determine the repellent or attractant property of a DE formulation (Mitex) against the larvae of *Tribolium castaneum* (Herbst), a major pest of stored-products.

**Materials and Methods:** The experiment was conducted in choice chambers, which was divided into two equal halves by drawing a line through the midline. One half of the chamber was loaded with untreated flour (control) and the other half was loaded with DE-treated flour. Twenty larvae of a definite age were released in the midline of the two foods, thus providing them an option for choosing either treated or untreated food. The experiment was set separately for different ages of larvae (9-, 12- and 15-day old), doses of DE (2, 4, 8, 16 and 32 mg DE/g of flour) and exposure periods (24-, 48-, 72-, 96- and 120-hours). All the experiments were replicated three times and conducted at  $30 \pm 1^\circ\text{C}$  in an incubator without humidity and light control. Differences of the distribution of the larvae in treated and untreated flour was analyzed by  $\chi^2$  test.

**Results:** The efficacy of a diatomaceous earth (DE) compound (Mitex) as a repellent or an attractant was tested against 9-, 12- and 15-day old larvae of *Tribolium castaneum* (Herbst). Larvae of all ages were significantly ( $p < 0.001$ ) repelled by DE at doses from 8-32 mg/g food at all exposure periods of 24-, 48-, 72- 96 and 120 h. The mature larvae (15-day old) showed tolerance to the low doses like 2 and 4 mg/g food.

**Conclusion:** The present results revealed that DE can be used as a reduced risk repellent compound in the grain and cereal stores, flour mills and grocery shops to manage the larval population of *T. castaneum*.

**Keywords:** Age related response, *T. castaneum*, diatomaceous earth

### Introduction

The management programmes for storage insect pests are in search of reduced risk compounds, which with high insecticidal potentials would be harmless to the environment and human health. In this regard diatomaceous earth (DE) show great potential in the Integrated Pest Management (IPM) programmes in grain and cereal stores (Korunic 1998). The DE compounds were safe for non-target organisms, but very much effective against the insect pests of agriculture and grain stores. The US Environmental Protection Agency allowed DE to be used in the food storage and processing area, and classified them as GRAS (Generally Recognized as Safe) as food additives (Anonymous 1991). DEs are also used in food stores in Australia (Bridgeman 1999).

DEs are soft rock produced from the fossil diatoms, finely ground and are commercialized as insecticides. These are non-toxic to mammals, but cause death to insects mainly due to desiccation and abrasion of the cuticle (Ebeling 1971, Rigaux *et al.* 2001, Arnaud *et al.* 2005). Published reports show that activity of the commercial DE formulations affect growth and development of different species of the stored-product insects, and provide long-term protection to the stored grains (Ulrichs *et al.* 2006). Cook (2003) proposed for using DEs to treat crevices and voids as a part of IPM strategy for flour mills. The present research aims at determining the repellent or attractant property of Mitex (a DE formulation) against the larvae of one of the major stored product pests, *Tribolium castaneum* (Herbst).

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## Materials and Methods

*Test materials:* Larvae of the red flour beetle, *T. castaneum* were chosen for this experiment as a model insect to determine the attractant or repellent property of DE. Adult beetles were collected from the stock culture of reared on standard food (wheat flour : brewer's yeast, 19 : 1, (Park and Frank 1948) in the Entomology Laboratory, Department of Zoology, Rajshahi University. Sub-cultures were established by collecting freshly laid eggs. The sub-cultures were reared on standard food at  $30 \pm 1\text{C}^\circ$  in an incubator without humidity and light control. The eggs hatched 3 - 4 days after laying. The neonates were allowed to feed on the same food and the food was replaced by a fresh one after every three days (Mondal 1984). For this experiment the 9-, 12- and 15 day old larvae were used. Mitex, a commercial product containing DE, marketed by Agril, UK, was used. The experimental doses of Mitex used were 2, 4, 8, 16 and 32 mg<sup>g</sup> of standard food.

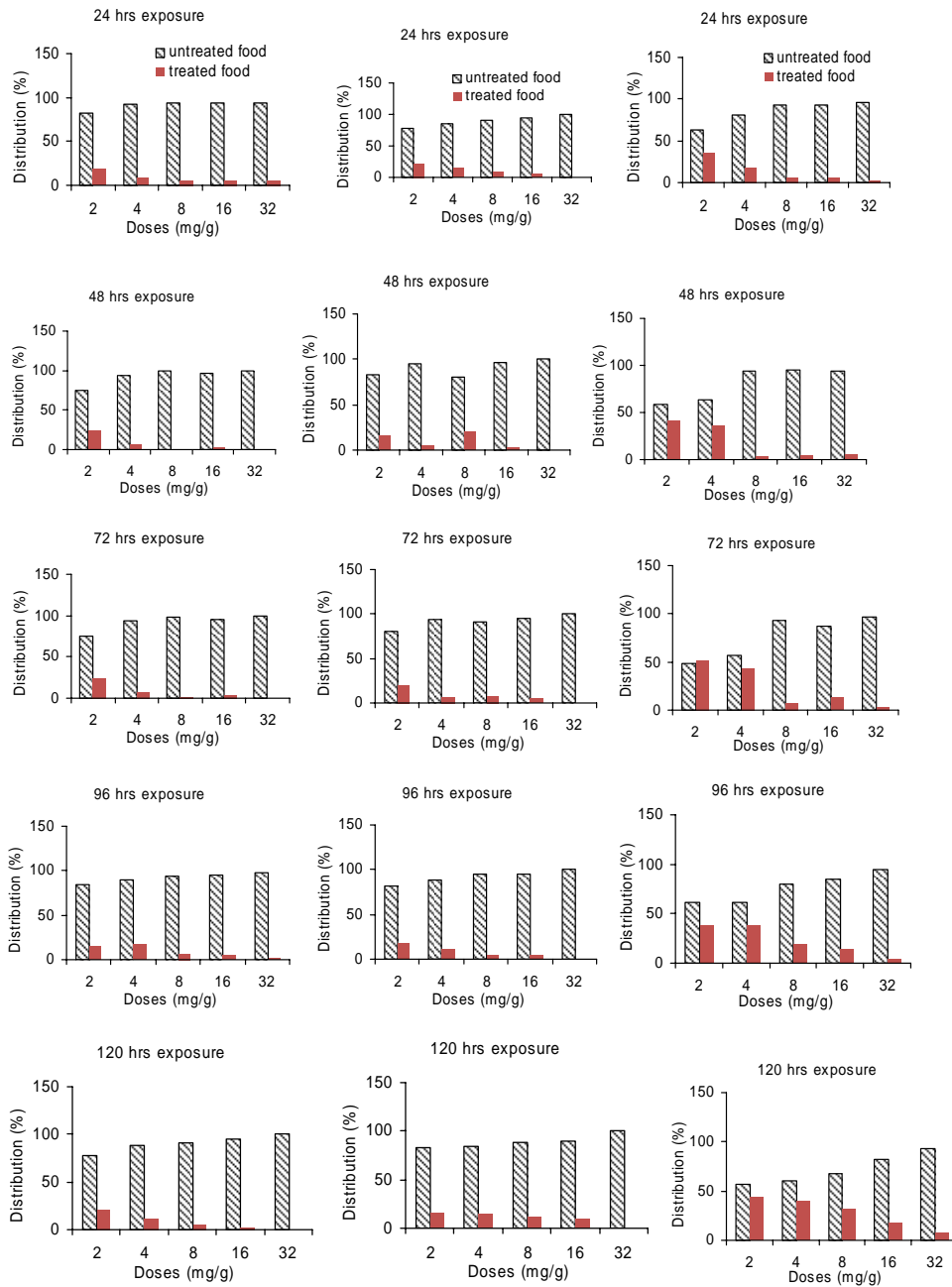
*Experimentation:* The experiment was conducted in choice chamber as described by Mathlein (1967), Mondal (1984) and Parween (1996). The choice chamber was made from a glass petridish (9 cm diameter). The petridish was divided into two equal halves by drawing a line across the middle. By the use of partition one half of the choice chamber was loaded with flour (untreated control) and the other half with treated (DE) flour. Twenty larvae of a definite age were released in the midline of the choice chamber, thus providing an option for the insects to select either treated or control food (Parween 1996). The experiments were set separately for each age of larva, each dose of DE and each exposure period (24, 48, 72, 96 and 120 h). The choice chambers were kept undisturbed at room temperature in the laboratory. After each exposure period, both treated and untreated portions of food were poured separately on separate paper sheets. Then each of the treated and untreated flour was passed through a 500  $\mu\text{m}$  mesh sieve. The number of larvae found on the sieve was counted separately for both treated and untreated foods. The experiment was replicated three times. The number of beetles found in each food section was counted and the percentage was calculated from the average number of three replications. The differences of distribution of beetles on either food section were measured by  $\chi^2$  tests for the level of significance.

## Results

*Response of 9-day old larvae:* The data showed that 9-day old larvae of *T. castaneum* were repelled by DE-treated food at all dose levels at each exposure periods (Fig. 1a). At 24- h exposure, the dose from 8-32mg/g repelled 93.33% larvae ( $\chi^2$  value 37.57,  $p < 0.001$ ). At 48 h exposure, 100% of 9-day old larvae were repelled by the doses of 8 and 32 mg/g (Fig. 1a). Significantly higher percentage of 9-day old larvae showed avoidance to the DE-treated food at doses from 4 - 32 mg/g (Fig. 1a) at 72 h exposure. More than 90% nine day old larvae were repelled by DE at doses over 2 mg/g at 96- and 120 h exposures (Fig.1a).

*Response of 12-day old larvae:* The 12 day old larvae showed similar response to DE treated food like 9 day old larvae at all exposure periods ( $p < 0.001$ ) (Fig. 1b). At 24-h exposure >90% larvae avoided the treated food at doses from 8 - 32 mg/g, whereas the same level of response was found at doses 4 - 32 mg/g at 48 and 72 h exposures. The effect of DE was reduced with the increase of exposure time. More than 90 - 100% larvae were repelled by 8 - 32 mg/g at 96 h exposure, and by 16 and 32 mg/g at 120 day exposures.

*Response of 15-day old larvae:* The larvae of *T. castaneum* ignored 2 and 4 mg/g treated food, or otherwise avoided the treated food at doses from 8 - 32 mg/g (Fig.1c). At 24 h exposure, 15 day old larvae were repelled at all doses of DE except 2 mg/g; but > 90% larvae showed avoidance to 8 - 32 mg/g of treated food. The same aged larvae were equally distributed in untreated and treated foods at 2 and 4 mg/g, at 48 to 120 h exposures. Ignorance to the DE-treated food by the 15 day old larvae was increased with the increase of exposure period but in general, the 15 day old larvae were significantly repelled by DE.



Larval age: 9 days (a)

12 days (b)

15 days (c)

Fig. 1. Distribution of different ages of larvae of *T. castaneum* on DE treated food at different periods.

## Discussion

Results of the experiment revealed that Mitex significantly ( $p < 0.001$ ) repelled the larvae of all ages when exposed for 24 to 120 h by doses of 8, 16 and 32 mg/g. However, the young larvae (9 day old) were highly repelled by DE at doses as low as 2 and 4 mg/g. The older larvae (15 day old) were repelled by the higher doses of DE compared to the younger ones.

Most of the published data on DE efficacy against the stored product insects have been reported on the toxicity of DEs, but not on the repellent or attractant properties of these compounds. According to Carlson and Ball (1962), *Oryzaephilus mercator* is more susceptible to DE than *T. confusum*, because the hairy cuticle of the former species is able to pick up more DE particles than *Tribolium*. Adult *Rhizopertha dominica* and *T. castaneum* have been reported to be tolerant to DE (Subramanyum and Roseli 2000), whereas, starved *Sitophilus* spp. are more susceptible to DE compared to those who were reared on food (Chiu 1939). Potentiality of DEs is related to the storage factors as well as metabolic conditions of the insect pests.

## Conclusion

The present results revealed that DE can be used as a reduced risk repellent compound in the grain and cereal stores, flour mills and grocery shops to manage the larval population of *T. castaneum*.

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