

## **Insecticidal efficacy of diatomaceous earth against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on stored maize in Thailand**

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### **Abstract**

The insecticidal and residual effects of diatomaceous earth (DE) against *Sitophilus zeamais* Motschulsky, the maize weevil, on stored maize were evaluated in a laboratory. Adults were exposed on maize treated with DE (CUT 'N DRY™) at five dose rates: 0, 250, 500, 1000 and 1500 ppm and compared to fenitrothion 50% EC at 10 ppm. Exposures were done immediately after treatment and after one, two and three months. The results showed that dose rates and application time significantly affected weevil mortality ( $P < 0.05$ ). All fenitrothion treatments showed 100% mortality of *S. zeamais* adults whereas mortality rates of control treatments (0 ppm) were lower than 2.26%. Mortalities from the same dose rate of DE treatments were constant at longer post-application period intervals. At 250 and 500 ppm of DE mortality was only 3.75-40.25% whereas at 1,000 and 1,500 ppm of DE mortality was 43.25-88.50%. Mortality rates at 250 and 500 ppm of DE immediately, one, two and three months post-application were lower than 40.25% while at 1,000 and 1,500 ppm of DE mortality was 77.00-88.50%, except for 1,000 and 1,500 ppm of DE at one month post-application (43.25-57.00%). No progeny were found in fenitrothion treatments. Progeny ranged from 122-139 at the 250 and 500 ppm DE treatments and 48-116 and 51-123 individuals at the 1,000 and 1,500 ppm, treatments.

Keywords: *Sitophilus zeamais*, diatomaceous earth, maize

### **1. Introduction**

*Sitophilus zeamais* Motschulsky is a serious pest of stored products in Thailand, especially rice and maize because the infestation by this pest results nutrition loss and physical damage physical damage (Rees, 2004; Suthisut et al., 2011). This weevil feeds on all maize such as wheat, rice and other maize products, and it can cause up to 90% damage to maize after 5 months of storage. Residual insecticides or fumigants (methyl bromide, phosphine and sulfur dioxide) have been normally used to control this insect (Subramanyam and Hagstrum, 1995) since fumigants have a broad spectrum of activity and penetrate into the maize kernel (Emekci, 2010). Conversely, fumigation has some drawbacks; such as no long-term protection, safety issues, and development of resistance (Emekci, 2010). Therefore, alternatives to chemical insecticides are needed to control this insect in stored products and to reduce human exposure, development of insecticide resistance and food or environmental contaminations.

Diatomaceous earths (DEs) are fossil skeletons of phytoplankton or diatoms which are found in freshwater or marine sediments (Korunic, 1998). DEs are unicellular algae which were deposited during the Eocene and Miocene periods (Athanassiou et al., 2005; 2011). DEs are a kind of inert dust and they are mainly composed of amorphous hydrated silica and extremely porous (Stathers et al., 2004). They are considered to be alternative natural products for insect management programs in stored maize since they can kill stored-product insects by abrading the wax layer of the insect cuticle, resulting in rapid water loss and desiccation and causing

death within hours or days (Korunic, 1998; Subramanyam and Roesili, 2000). These inert dusts have low toxicity to mammals (oral LD<sub>50</sub> > 5000 mg/kg in rats) (Athanassiou et al., 2011) and they are also used as food additives (FDA, 1995; Arthur, 2000). At present, DEs are already registered for maize protectants in many countries including Australia, Brazil, Canada, Croatia, China, Germany, Indonesia, Japan, Philippines, Saudi Arabia, United Arab Emirates and the USA (Stathers et al., 2004, Vayias and Stephou, 2009). Several DE formulations are commercially available and they have been successfully used as alternative maize protectants to a wide range of stored product insects (Korunic, 1998; Arthur, 2000; Fields and Korunic, 2000; Subramanyam and Roesili, 2000; Athanassiou et al., 2003, 2005, 2006). The objective of this research was to evaluate the efficacy of DE in different dosages applied to control *S. zeamais* in stored maize. This information is important for integrated pest management programs of stored product pests in Thailand.

## 2. Materials and Methods

### 2.1. Insects

*S. zeamais* were obtained from the Postharvest and Processing Research and Development Office, Department of Agriculture (DOA), Ministry of Agriculture and Co-operatives, Thailand and were reared on brown rice (13% moisture content) in cylindrical glass bottles (18 cm high and 8 cm diameter) (Suthisut et al., 2011). Colonies were maintained at room temperature, 30±2°C and 70±10% r.h. in a laboratory at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok campus, Thailand. Adults younger than 14 days old were used for all experiments.

### 2.2. Toxicity test

Four concentrations of CUT 'N DRY™ (Grow Choice Pty. Ltd., Australia) at 250, 500, 1000 and 1,500 ppm were applied as a powder and mixed prepared as follows: 0.25 g/1 kg of maize, 0.5 g/1 kg of maize, 1000 g/1 kg of maize (recommended dose as 1 kg/1 tonne) and 1500 g/1 kg of maize. The control was an untreated lot (0 ppm) and fenitrothion 50% EC at 10 ppm (commercial dose) was applied as wettable powder.

Lots of 4 kg of maize were prepared and thoroughly mixed with the specific dose rate of each insecticide. The treated maize lots were shaken manually for 15 min to achieve equal distribution of the sample, following methods of Subramanyam and Roesli (2000). The mixed lots were divided into 16 bottles, each 250 g of mixed maize was placed into each rearing cylindrical glass bottle (18 cm high and 8 cm diameter) and tightly closed. Then, 100 adults of *S. zeamais* were placed into each bottle at 0, 1, 2 and 3 months after setting up the experiments (4 bottles/treatment) and all samples were kept at room conditions. The same procedure were performed for the post-treatment intervals by preparing new lots. Negative controls (fenitrothion) was done at 10 ppm 10 ppm and positive control lots was sprayed with water only.

### 2.3. Data assessment and data analysis

Adult mortality in all treatments was assessed 14 days of exposure and then all *S. zeamais* adults were removed from the experimental bottles. After the termination of this interval, the rearing bottles remained at the same conditions for an additional period of six weeks. Then, the bottles were opened and the numbers of progeny were recorded. All data were analyzed to compare the number of dead weevils and weevil progeny between each treatment by analysis of variance (one-way ANOVA) with the Tukey's test and the significance was reported when  $P \leq 0.05$  (R version 3.0.1, R Core Development team, 2013).

### 3. Results and Discussion

Adults of *S. zeamais* were exposed on maize treated with CUT 'N DRY™ at four dose rates as 250, 500, 1000 and 1500 ppm compared to fenitrothion 50% EC at 10 ppm and applied for 0, 1, 2 and 3 months before testing. The results showed that dose rates and application periods significantly affected weevil mortalities ( $P < 0.05$ ). All fenitrothion treatments gave 100% mortality of *S. zeamais* adult whereas mortality in control treatments (0 ppm) was less than 2.26%. At 250 and 500 ppm of DE mortality was 3.75-40.25% whereas at 1,000 and 1,500 ppm mortality was 43.25-88.50%. The mortality in 1,000 and 1,500 ppm treatments was significant higher than in other doses ( $P < 0.05$ ), and was 77.00-83.25%. At month post-application, the efficacy of DE in all treatment was lower than mortality immediately after treatment. Especially in the 1,000 and 1,500 ppm treatments where were mortality was 43.25 and 57.00%, respectively. At both two and three months after application, 1,000 and 1,500 ppm of DE treatments had a higher effectiveness ( $P < 0.05$ ) than other concentrations and the mortality rates were 77.00-86.93% (Figure 1).

This study showed that the CUT 'N DRY™ at 1,000 and 1,500 ppm could be successfully used against *S. zeamais* to protect stored maize. Results were similar to Athanassiou et al. (2003, 2005) who found that the DE formulation (SilicoSec®) at dose rates >500 ppm could be used for successful controlling of *Sitophilus* sp., since *S. zeamais* is moderately susceptible to DEs (Stathers et al., 2002). The results also showed that 10 ppm fenitrothion treatments had a higher efficacy than DE for control of *S. zeamais*. Others studies also report efficacy of DE chances of insect reproduction (Fields, 1999; Marsaro Junior et al., 2006; Doumbia et al. 2014). The increase of insect mortality with increasing dosages has been reported as well (Aldryhim, 1990; Arthur, 2002; Marsaro Junior et al., 2006).

As inert materials, DEs have no interaction with the environment and maintain their insecticidal effect for a months, which makes DEs perfect candidates for long-term maize protection (Athanassiou et al., 2005). The application of DEs in certain cases must be more than 1,000 ppm to control certain insect species (Vayias and Athanassiou, 2004; Athanassiou et al., 2005; Vayias et al., 2006). However, the application of DEs at a high dose may cause negative effects on the physical properties of the maize and thus significantly reduce its commercial value (Korunic et al., 1996).

Insect susceptibility to DEs depends on many factors including surface to volume ratios, body setation, cuticle thickness (Bartlett, 1951), life-stage (Subramanyam et al., 1998), insect behavior, application rates (Stathers et al., 2004) and the exposure period (Fields & Korunic, 2000). Moreover, the effectiveness of DEs varies with temperature, r.h. and maize type (Fields and Korunic, 2000; Subramanyam and Roesli, 2000; Vayias and Athanassiou, 2004; Athanassiou et al., 2005, Kavallieratos et al., 2005; Vayias et al., 2006). DE products are very effective at low r.h. and moisture content levels but efficacy decrease significantly as the moisture increases (Arthur, 2000; Fields and Korunic, 2000; Vayias and Athanassiou, 2004). The r.h. level used in the present work (70%) should be considered as relatively high and it is expected to reduce DE efficacy since r.h. affects the rate of water loss by the insect and therefore influences the effectiveness of inert dusts (Aldryhim, 1990; Stathers et al., 2004). High temperatures stimulate movement of insects within the maize mass, allowing greater contact with DE and also increases the respiration rate of insects (Vayias & Stephou, 2009).

The highest progeny production (235-444 individuals) was in control treatments ( $P < 0.05$ ), except at one month post-application. The concentration of CUT 'N DRY™ at 1,000 and 1,500 ppm decreased progeny production compared to controls and the 250 and 500 ppm treatments. The progeny in 1,000 and 1,500 ppm treatments ranged from 48-116 and 51-123 individuals, respectively (Figure 2). Progeny were found in all DE treatments, possibly

because mating and oviposition occurred before the adults died before exposure to the DE (Subramanyam and Roesli, 2000; Arthur and Throne, 2003). However, progeny production increased during storage, as the DE could have absorbed moisture from the atmosphere inside the bottles (Stathers et al., 2004) or absorbed oils from maize seed (Vayias et al., 2006).

The grain type is also a critical factor that affects the insecticidal efficacy of DE products against stored-product pests (Korunic, 1997, 1998; Athanassiou et al., 2003; Vayias and Athanassiou, 2004; Athanassiou and Kavallieratos, 2005; Kavallieratos et al., 2005; Vayias et al., 2006). Efficacy of DE was less on maize than on other grains (Athanassiou et al., 2003; Kavallieratos et al., 2005; Vayias et al., 2006) since the wide spaces between the maize grains allow insects to crawl through and avoid areas where DE concentration is high (Athanassiou et al., 2003; Vayias and Stephou, 2009). The type of DE formulation is also an important factor affecting their insecticidal action (Subramanyam and Roesli, 2000; Kavallieratos et al., 2005; Vayias et al., 2006). Studies have also indicated that DE could be combined with other control methods including entomopathogenic fungi (Michalaki et al., 2007), low doses of insecticides (Athanassiou and Kavallieratos, 2005) or extreme temperatures (Fields and Korunic, 2000) and botanical insecticides (Athanassiou et al., 2006) control stored grain insects.

#### 4. Conclusions

DE is a good alternative for successful control of *S. zeamais* in Thailand but it may not give 100% control since Thailand is located in a humid tropic zone. However, at 1,000 and 1,500 ppm it may be an effective alternative to fenitrothion.

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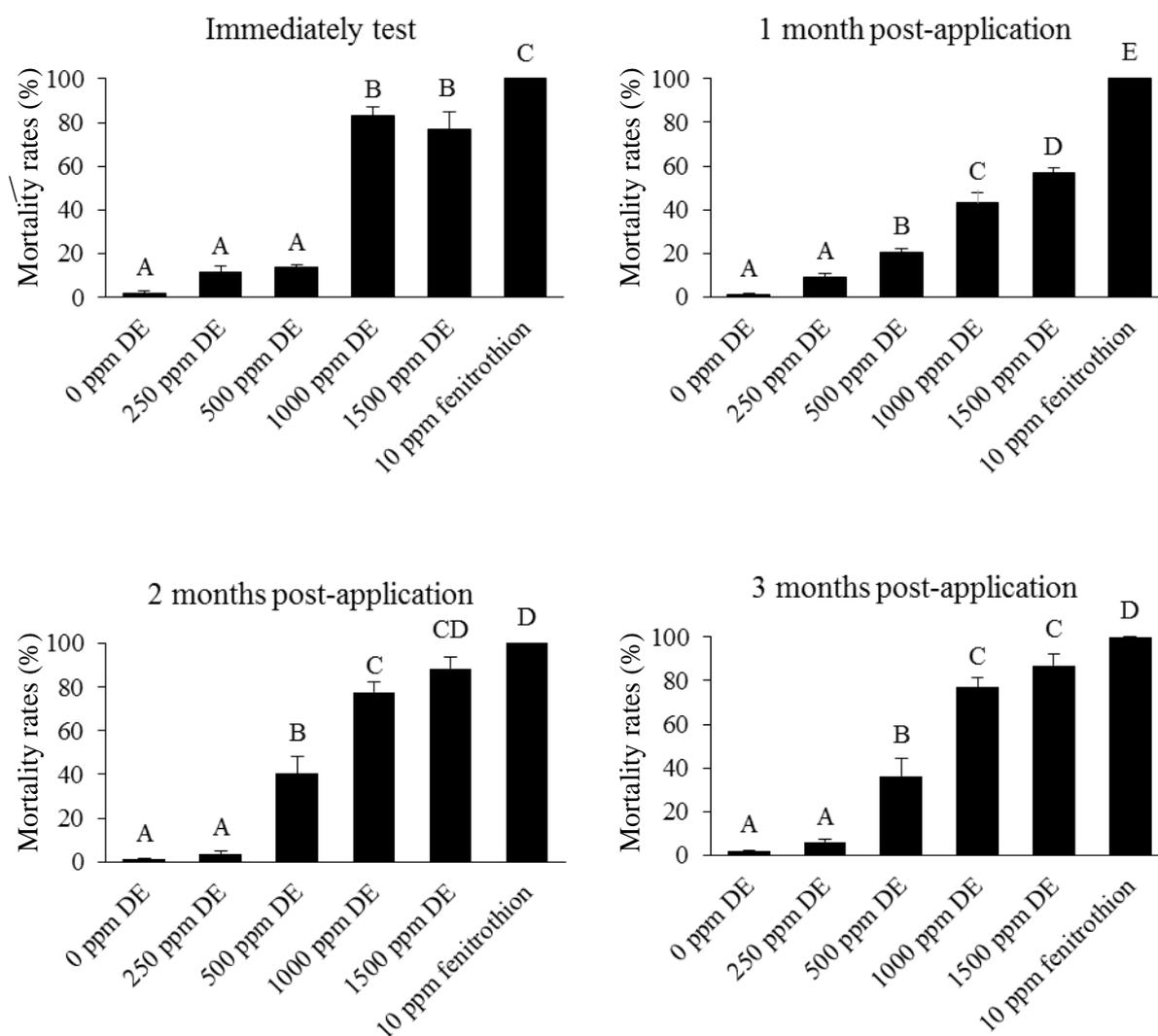
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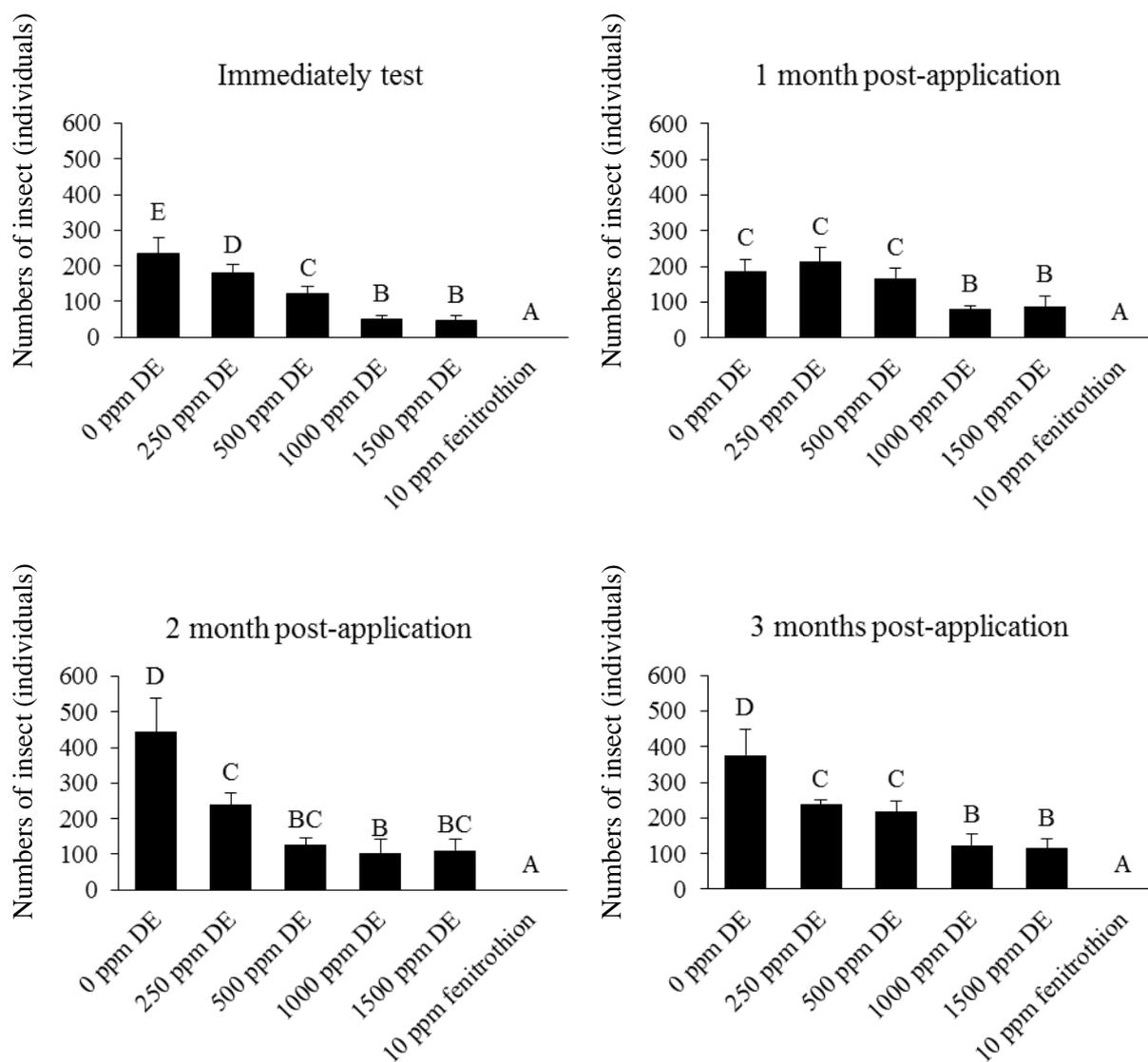
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**Figure 1** Mortality rates of *S. zeamais* on diatomaceous earth (DE) toxicity test at different concentrations (0, 250, 500, 1,000 and 1,500 ppm) comparing to 10 ppm fenitrothion at 14 days after application. The same letters above the bars indicate no significant difference among means at  $P = 0.05$ . Bars show standard error of the means.



**Figure 2** Progeny of *S.zeamais* in diatomaceous earth (DE) treatments at different concentrations (0, 250, 500, 1,000 and 1,500 ppm) comparing to 10 ppm fenitrothion at six weeks after application. The same letters above the bars indicate no significant difference among means at  $P = 0.05$ . Bars show standard error of the means.