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Evaluation of three enhanced diatomaceous earth formulations for the management of two major storage pests

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Abstract

Major stored product insect pests of maize and rice are controlled by the use of synthetic insecticides which are very expensive for farmers, might affect worker safety if not properly applied due to lack of technical know-how in many developing countries. Moreover, its use might eventually lead to some toxic residues in the end product for human consumption. In an attempt to look for alternatives to synthetic insecticides to control storage pest of cereals, three improved diatomaceous earths (DEs), InsectoSec®, Diatomeenerde Probe-A and Fossil Shield® 90.0 were tested in the laboratory against *Sitophilus zeamais* Motschulsky (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) from Ghana. Application rates on stored maize or rice were 500, 1000, 2000 and 3000 ppm. Adult mortality was recorded after 32 days and, progeny production after 6 weeks. InsectoSec® was most toxic against the adults of *S. zeamais* followed by Fossil Shield and Diatomeenerde Probe-A. For the progeny production InsectoSec® and Diatomeenerde suppressed *S. zeamais* on maize better than Fossil Shield®. In contrast, Fossil Shield® and InsectoSec® were better compared to Diatomeenerde in suppressing progeny production of *T. castaneum*. There was no significant difference in mortality among the highest dosages of the three DE's against *S. zeamais* and *T. castaneum* up to the 14th day, while InsectoSec® and Fossil Shield® were more effective than Diatomeenerde at the lower dosages. Mortality of the adult beetles was dose-dependent. The LT₅₀ ranged from 9 d to 13 d for *T. castaneum* exposed to 1.0% InsectoSec® and 1.0% Fossil Shield®, respectively. LT₅₀ of 5 d, 6 d, and 9 d were recorded for *S. zeamais* exposed to the highest dosage of 3.0% Fossil Shield®, InsectoSec® and Diatomeenerde, respectively. All formulations reduced the progeny

37 emergence compared to the control. The potential of using diatomaceous earth for the bio-
38 rational control of major storage pests in Ghana is discussed.

39 **Key Words:** Diatomaceous earth, InsectoSec[®], Diatomeenerde Probe-A, Fossil Shield[®],
40 mortality, maize, rice

41

42 Introduction

43 Climate change, emerging strains of different species of storage pests, the ever increasing
 44 negative impact of chemical insecticides, its effect on humans, the environment and their
 45 high cost have made the search for alternatives to the use of chemical insecticides imperative
 46 for use in developing countries (Obeng-Ofori, 2010; Kaur and Garg, 2014). The excessive
 47 use of synthetic pesticides to meet ever-increasing needs to protect stored products has
 48 resulted in development of resistance in pest populations (Saglam et al. 2015; Gautam et al.
 49 2016; Cato et al. 2017; Agrafioti et al. 2019; Nayak et al. 2020; Agrafioti and Athanassiou
 50 2018; Sakka and Athanassiou, 2021).

51 Maize and rice are major staple crops in developing countries of Sub-Saharan Africa (SSA)
 52 and Asia which are consumed in many forms including infant foods, snacks and main dishes
 53 (FAO, 2014; Ekpa et al. 2018). These major cereal crops contribute to food security of small-
 54 scale farmers (Leff et al., 2004). The maize weevil *Sitophilus zeamais* Motschulsky
 55 (Coleoptera: Dryophthoridae) and the red flour beetle *Tribolium castaneum* (Herbst)
 56 (Coleoptera: Tenebrionidae) are the most destructive pests of stored maize and rice both in
 57 Ghana and in many countries in SSA (Adarkwah et al., 2010; 2012 a & b). Some other major
 58 storage pests of these crops include *S. oryzae* (Linnaeus, 1763), *Prostephanus truncatus*
 59 (Horn, 1878) (Coleoptera: Bostrichidae) and *Sitotroga cerealella* (Lepidoptera: Gelechiidae)
 60 (Adams and Schulten, 1978; Markham, et al., 1994, Rees, 2004; Kumar and Kalita, 2017).

61 These insects contribute to the contamination of food products with the presence of live
 62 insects, dead insects, insect body fragments and insect products such as chemical excretions
 63 (Phillips and Throne, 2010). Paradoxically, tropical African countries, especially Ghana, face
 64 serious food insecurity challenges (Ngamo and Hance, 2007; Adarkwah et al., 2019).
 65 Postharvest losses continue to undermine the food security and incomes of smallholder
 66 farmers in Sub-Saharan Africa, threatening livelihoods of vulnerable households (FAO, 2017;
 67 Affognon et al., 2015; Abass et al., 2014; Shee et al., 2019; Kumar and Kalita, 2017).
 68 Therefore, the fight against chronic hunger and poverty needs to be intensified if the
 69 Sustainable Development Goals adopted by the United Nations in 2015 are to be realized in
 70 sub-Saharan Africa. Food security could be achieved by increasing agricultural productivity
 71 and reducing pre- and post-harvest crop losses (Oerke, 2006; FAO, 2017; Affognon et al.,
 72 2015; Abass et al., 2014; Shee et al., 2019; Kumar and Kalita, 2017). In SSA where over 307
 73 million people are already affected by severe food insecurity (FAO et al., 2017; Affognon et
 74 al., 2015), the prevalence of undernourishment has recently started increasing again, reaching
 75 22.8% in 2018 (FAO et al., 2019). The region is considered highly vulnerable to the impact
 76 of climate change (Sono et al., 2021) since its population is projected to double to 2.4 billion
 77 people by 2050 (UNDESA, 2017), and is dependent on rain-fed agriculture. The ‘Missing

Food' study estimated that 13.5% of the cereal grain produced across SSA is lost after harvest, equivalent to US\$ 4 billion per year or the annual caloric requirement of 48 million people (World Bank et al., 2011). Post-harvest losses to storage insect pests have been recognized as an increasingly important problem in the tropics (Markham et al., 1994; Abebe et al., 2009). In African countries, these losses have been estimated to range between 20% and 40%, which is highly significant considering the low agricultural productivity in several regions of Africa (Abass et al., 2014).

Sustainable and effective methods for reducing *S. zeamais* and *T. castaneum* damage are therefore urgently needed in developing countries to reduce food insecurity. Currently, the control of these two cosmopolitan insect pests is primarily dependent upon continued application of synthetic contact insecticides and fumigants (Chaubey, 2008), which are often the most effective treatments for the disinfestation of stored food, feedstuffs and other agricultural commodities from insect infestation. Although effective, their repeated use for decades has disrupted biological control by natural enemies and led to outbreaks of other insect species and sometimes resulted in the development of resistance, toxic residues in food with serious health hazards and pollution of the environment (Park et al., 2003; Obeng-Ofori, 2007; Ogendo et al., 2008; Mondal and Khalequzzaman, 2010). These problems have stimulated interest in the development of alternative strategies such as the use of naturally occurring diatomaceous earths that are less harmful to the consumer and the environment (Korunic, 2020). However, relatively few studies are available on the effectiveness against Afrotropical strains of stored product pests and storage environments in SSA (Adarkwah et al., 2017, 2018).

Diatomaceous earth (DE) has been used as an alternative to synthetic chemical insecticides for many years, and as it does not break down rapidly, a single application can be used in an ongoing way to disrupt insect development as long as the commodity remains dry (Korunic and Fields, 2018). The DEs typically comprise approximately 70-90% amorphous silicon dioxide with the balance made up of inorganic oxides and salts (Timlick and Fields, 2010). Diatomaceous earth is fatal to insects because it absorbs their cuticular waxes causing insects to die from desiccation (Nikpay, 2006; Ebeling, 1971; Korunic, 1998). DEs are obtained from geological deposits around the world. They are known to have very low mammalian toxicity. However, DEs cause some unwanted effects on grain related to adversely affect physical and mechanical properties of grain (Korunić, 2020). Therefore, the milling industry is reluctant to accept grain treated with DE because of its abrasive nature and possible damage to milling machinery (Losic and Korunic, 2018). DEs can be applied with approximately the same technology as other powder insecticides (Korunic, 2020; Subramanyam and Roesli, 2000). Several DEs are effective at doses of 500 ppm or higher (Timlick and Field, 2010, Korunic

and Fields, 2018). Apparently, these doses cause unwanted effects on grain surfaces and flowability and their application for direct mixing with grain has limited acceptance by the grain industry (Korunic et al., 1996; Subramanyam and Roesli, 2000).

There is paucity of research on inert dusts in Western Africa, especially diatomaceous earth, compared to the rest of the world. These few studies were carried out during the last decade (Mvumi and Stathers, 2003; Stathers, 2004; Stathers et al. 2002a, b&c; 2003; 2005; Demissie et al., 2008). Therefore, it is essential to develop DE formulations that are effective at lower doses in controlling major strains of storage pests. The aim of our study was to evaluate the insecticidal efficacy of three improved DEs on adult mortality and reproduction in strains of *S. zeamais* and *T. castaneum* from Ghana.

2.0 Materials and methods

All the experiments were carried out at the Alexander von Humboldt Foundation's Bio-control laboratory of the Department of Horticulture and Crop Production, School of Agriculture and Technology, University of Energy and Natural Resources, Dormaa-Ahenkro, Bono Region, Ghana. Temperature and relative humidity conditions for all cultures, experiments and rearing were at 25 ± 5 °C and $70 \pm 5\%$ RH with constant darkness.

2.1 Grain treatment

Maize, *Zea mays* L. and rice, *Oryza sativa* L. grains used in the study were purchased from Dormaa-Ahenkro local market in the Bono region of Ghana. In order to kill any living insects from previous infestations, the grains were kept at -15 °C for 2 weeks. After this period, the grains were kept under experimental room temperature and humidity conditions for 1 week before being used in the experiments. Afterwards, they were sun-dried to 12–13% moisture content (measured by the use of DICKEY-John Mini GMT-plus moisture meter, Gempler's, USA). The grains were conditioned to 25 °C before they were used for the various bioassays.

2.2. Rearing of beetles

Sitophilus zeamais and *T. castaneum* were obtained from Alexander von Humboldt laboratory cultures maintained at Dormaa- Ahenkro. The strains of *S. zeamais* and *T. castaneum* were originally collected from infested stored maize and rice obtained from Dormaa market, and were reared in the laboratory at 25 ± 5 °C and $70 \pm 5\%$ RH. Initially, about 150 pairs of newly emerged (1-24 h old) adults were added to jars containing either maize or rice grains. Pieces of fine nylon mesh fastened with rubber bands covered the jars in order to prevent the escape of the beetles and the contamination of the grains. Mating and oviposition was allowed for a maximum of 7 d. The parental beetles were removed

afterwards, and the grains containing the eggs of each beetle species were transferred to fresh grains. Subsequent progenies produced were used for the bioassays.

2.3 Diatomaceous earth formulations

Three modified DE powders, namely Diatomeenerde Probe-A, Fossil shield® 90.0 S-brown and InsectoSec® were tested. Diatomeenerde Probe-A is a relatively new DE product available commercially, developed by Humboldt University Berlin (Badii et al., 2014). It contains the following chemical properties: 83.7% SiO₂, 5.6 % Al₂O₃, 2.3% Fe₂O₃, 0.9% CaO, 0.3% MgO, other oxides 1.9% and a dry weight of 5%. It has a mean particle size of 10.6 µm and some undefined mineral compounds. Diatomeenerde Probe-A was obtained from Humboldt University Berlin, Germany.

Fossil shield® 90.0 S-brown has a particle size of 9.5 µm and is composed of 87.0% amorphous SiO₂, 3% aerosol and 4.9% Al, 4.7% Fe, 1.3% K, 0.8% Ca, 0.8% Mg, 0.3% Na and has a pH-value of 7.2–8.2. Fossil shield® has previously been tested for its effectiveness against storage beetles in cereal grains (Nielsen, 1998; Mewis and Ulrichs, 2001; Prasantha et al., 2002; Sparagano, 2009). It is distributed by the Fossil Shield Company; Bein GmbH, 36132 Eiterfeld- Germany.

InsectoSec® is manufactured from amorphous diatomaceous earth. It is used as an acaricide mainly to control *Dermanyssus gallinae* (DeGeer) (Mesostigmata: Dermanyssidae) in poultry farms, and under the brand name SilicoSec® also as a plant protection product against stored product insects. InsectoSec® is a marine DE with 10% food-grade bait. It contains 87% (w/w) amorphous SiO₂, with 2-4% moisture content, and approximately 3% Al₂O₃, 1% Fe₂O₃, < 1% CaO, MgO, TiO₃, and P₂O₃, respectively, with a median particle size of approximately 8.2 µm. InsectoSec® diatomaceous earth was obtained from Biofa AG, Münsingen, Germany. Prior to the experiments, all the products were stored in the laboratory for 1 month at ambient conditions.

2.4 Description of bioassay

Effects of different dosages of DE treated maize or rice on adult mortality of *S. zeamais* and *T. castaneum* were assessed in the laboratory. Timlick and Fields (2010) experimental procedure was followed with few modifications. Samples of maize or rice were mixed with different DE products each at 0, 500, 1000, 2000 and 3000 ppm equalling. The dosage of each DE formulation was added to 100 g of the pre-equilibrated maize or rice in a 1-litre glass jar. Each admixture was manually shaken for 2 min for the content of the grains to mix thoroughly with the DE product. After shaking, it was allowed to rest for 30 min for the DE to evenly distribute and properly adhere to the surfaces of the grains. Thirty unsexed adults

each of *S. zeamais* and *T. castaneum* aged 0–3 days were introduced into each jar, both for treatment and untreated control. Each treatment was replicated three times in a completely randomized design placed in a laboratory shelf at 26 ± 5 °C and $70 \pm 5\%$ RH under continued darkness. Dead insects were counted daily for a maximum of 32 d depending on the type of DE, and beetles were immediately discarded. All beetles were confirmed dead when there was no response when their abdomen was gently probed with a camel hair brush. Otherwise, the samples were not touched throughout the test period to avoid a negative impact on the beetles. In order to record the F₁ generation emerging from the maize or rice, the jars were kept under the same conditions except for the thirty parental adults were removed after 7 d. After the incubation period of 7 weeks, the total number of F₁ progeny of *T. castaneum* and *S. zeamais* was recorded.

2.5. Data Analysis

The univariate procedure of SigmaStat was used to control for normality of the data for F₁ progeny emergence and adult mortality before analysis. Percentage mortality data for *S. zeamais* and *T. castaneum* adults were corrected for control mortality, because control mortality was > 2% (Abbott 1925) before it was subjected to a Two-Way Repeated Measures (ANOVA). Mean percentage F₁ progeny of *S. zeamais* (on maize) and *T. castaneum* (on rice) after exposure to different modified diatomaceous earths was expressed as mean percentage F₁ progeny in the untreated. Percentage F₁ progeny production was arcsine (square root (x) - transformed and subjected to a Two-way analysis of variance (ANOVA) followed by all pairwise multiple comparison procedures (Holm–Sidak method).

Lethal exposure times were determined for 50%, 95% and 99% mortality (LT₅₀ and LT₉₅/LT₉₉) graphically using Sigma-Plot version 12.0. Two-Way Repeated Measures ANOVA (Two-Way RM ANOVA) followed by all Pairwise Multiple Comparison Procedures (Holm–Sidak method) was used to compare percentage mortality of beetles among DE treatments.

3. Results

3.1. Efficacy of DE formulations on adult mortality of *S. zeamais*

All the three DE formulations showed insecticidal effectiveness against *S. zeamais* applied at all doses (Table 1). When *S. zeamais* was exposed to stored maize treated with different dosages of the three diatomaceous earths, InsectoSec®, Fossil Shield® 9.0 brown and Diatomeenerde, there were statistically significant differences among the products, and dosages, and their interactions (Two-way RM ANOVA, product df = 2,16, F = 74.99, P < 0.001; dosage df = 4,16, F = 305.90, P < 0.001; product × dosage df = 8,16, F = 8.72, P <

0.001). The effects of duration of exposure (7, 10, 14, 21 or 28 d) and dosage on mortality of *S. zeamais* adults could not be separated, because there was a significant interaction between these two factors (Table 1).

Exposure of *S. zeamais* to InsectoSec® and Fossil Shield® led to complete control after 21 d with the highest dosage of 3000 ppm (Table 1). A maximum mortality of 87% was achieved with Diatomeenerde at this dosage and time. For InsectoSec® and Fossil Shield®, no difference in effectiveness was detected between the highest dosages of 2000 and 3000 ppm after 7, 14 and 21 d, respectively. In the case of Diatomeenerde, 3000 ppm was significantly more effective than 2000 ppm after 7, 14 and 21 d, respectively (Holm-Sidak method, $P < 0.05$) (Table 1). After 21 d and the dosages of 1000 and 2000 ppm, both InsectoSec® and Fossil Shield® were significantly more effective than Diatomeenerde (Holm-Sidak method, $P < 0.05$), but no difference was found between InsectoSec® and Fossil Shield® (Holm-Sidak method, $P > 0.05$). After 21 d, a significant difference between InsectoSec® and Fossil Shield® was only found at the lowest dosage of 500 ppm (Holm-Sidak method, $t = 2.21$, $P < 0.05$). All tested dosages of Diatomeenerde, Fossil Shield® and InsectoSec® recorded 100% mortality of *S. zeamais* at the 32 days of exposure (Table 1).

3.2. Effect of different DEs on adult mortality of *T. castaneum*

The mean percentage mortality of adult *T. castaneum* exposed to different dosages of InsectoSec®, Fossil Shield® 90.0 S-brown or Diatomeenerde Probe-A is shown in Table 2. All the three DE formulations showed insecticidal effectiveness against *T. castaneum* applied at all doses. In all the three DEs tested, mortality of *T. castaneum* increased with exposure time. There was significant interaction between product and dosage, (Two-way RM ANOVA, product df = 2,16, $F = 377.29$; dosage df = 4,16, $F = 548.57$; product \times dosage df = 8,16, $F = 12.34$; $P < 0.001$), therefore dosage and mortality of *T. castaneum* adults could not be separated. After 21 d exposure of *T. castaneum* to InsectoSec® only led to complete control with the highest dosage of 3000 ppm (Table 2). A maximum mortality of 96.67 and 98.89% was achieved with Fossil Shield® and Diatomeenerde, respectively. For InsectoSec® and Diatomeenerde, no significant difference in effectiveness was detected between the highest dosages of 2000 and 3000 ppm after 14 and 21 d, respectively. In the case of Fossil Shield®, 3000 ppm was significantly more effective than 2000 ppm after 7, 14 and 21 d, respectively (Holm-Sidak method, $P < 0.05$) (Table 2). At the highest dosage of 3000 ppm and 21 d of exposure, no difference was found among the three DE-products (Holm-Sidak method, $P > 0.05$). At 2000 ppm, both Diatomeenerde and InsectoSec® were more effective than Fossil Shield® (Holm-Sidak method, $P < 0.05$). At 1000 ppm and 500 ppm, respectively, all the products differed in effectiveness (Holm-Sidak method, $P < 0.05$), with InsectoSec® with the

highest and Fossil Shield® with the lowest effectiveness (Holm-Sidak method, $P < 0.05$). *Tribolium castaneum* exposed to a dosage of 3000 ppm Diatomeenerde resulted in 100% mortality after 32 d (Table 2).

3.3. Effect of different DEs on progeny production of *S. zeamais* in stored maize

In general, InsectoSec® and Diatomeenerde DEs performed better than Fossil Shield® in suppressing the F₁ progenies of *S. zeamais*. Grain treated with lower dosages of 500 ppm and 1000 ppm of Fossil Shield® resulted in 27% and 17% progeny compared to the control, ($df = 3,8$, $F = 20.11$, $P = 0.001$), respectively (Figure 1), whereas Diatomeenerde Probe-A recorded 12% and 8% *S. zeamais* progeny, respectively, compared to the untreated control at these dosages ($df = 3,8$, $F = 99.94$, $P = 0.001$, Figure 1). Treatment with InsectoSec® and Diatomeenerde resulted in at least 92% reduction compared to the untreated control, for InsectoSec® ($df = 3,8$, $F = 1.48$, $P = 0.291$, Figure 1). A significant difference in the number of emerged F₁ insects depending on DE product after 7 weeks of exposure was observed ($df = 2,24$, $F = 8.72$, $P = 0.001$, Two-Way ANOVA, Figure 1). Varying numbers of *S. zeamais* F₁ progeny emerged from the Fossil Shield® diatomaceous earth treated maize. The dosages of 500, 1000 and 2000 ppm had the highest emergence of F₁ progeny of *S. zeamais* ranging from 40%, 33% and 30% of the untreated control, respectively, whereas the highest dosage of 3000 ppm had the least F₁ progeny of 26%. *Sitophilus zeamais* was significantly more tolerant to Fossil Shield® (Holm-Sidak method, $P < 0.05$). There was a significant difference in the effectiveness to suppress progeny production between Fossil Shield® and InsectoSec® (Holm-Sidak method: $t = 4.06$, $P < 0.017$), InsectoSec® performed better than Fossil Shield. However, there was statistically no significant difference in the effectiveness to suppress progeny production between Diatomeenerde Probe A and Fossil Shield® (Holm-Sidak method: $t = 1.20$, $P > 0.05$, Figure 1). The dosages of the three DE formulations ranging from 500 ppm to 1000 ppm had no effect on the number of progeny produced compared to the untreated control (Holm-Sidak method, $P > 0.05$). However, there was a significant difference in efficacy in the dosages of 2000 ppm and 3000 ppm among the DE products. Fossil Shield® and Diatomeenerde (Holm-Sidak method: $t = 2.75$, $P = 0.011$) differed as well as Fossil Shield® and InsectoSec® (Holm-Sidak method: $t = 2.69$, $P = 0.013$), but there was no significant difference at the dosage 2000 ppm between Insectosec® and Diatomeenerde (Holm-Sidak method: $t = 0.06$, $P > 0.05$, Figure 1). For the highest dosage of 3000 ppm there was no difference among InsectoSec® and Diatomeenerde (Holm-Sidak method: $t = 2.25$, $P > 0.05$, Figure 1) and as well among Fossil Shield® and InsectoSec® (Holm-Sidak method: $t = 1.84$, $P > 0.05$, Figure 1). There was a significant interaction between dosages and DE products ($df = 6,24$, $F = 3.61$, $P < 0.011$).

296 3.4. Effect of different DEs on progeny production of *T. castaneum* in stored rice

297 All the DEs recorded less than 10% F₁ *T. castaneum* of the untreated control in all the
 298 dosages tested. The suppression of F₁ production in all the diatomaceous earths treatments
 299 were dosage-dependent. Dosage rates at 3000 ppm generally recorded significantly lower
 300 number of F₁ progeny than those with dosage rates of 500 or 1000 ppm. Fossil Shield® was
 301 the best DE in reducing the adult emergence of *T. castaneum*, (df =3,8, F = 2.53, P = 0.130)
 302 followed by InsectoSec®, (df = 3,8, F = 49.70, P = 0.001), respectively (Figure 2). The lower
 303 dosages of 500 and 1000 ppm Fossil Shield® recorded progeny emergence of 5% and 6%,
 304 respectively, whereas Diatomeenerde recorded 8% and 7% at these dosages, (df = 3,8, F =
 305 2.64, P = 0.121) respectively, (Figure 2). *Tribolium castaneum* progeny was susceptible to all
 306 the DE products especially with higher dosages of 2000 and 3000 ppm. Generally, mean
 307 percentage of F₁ progeny was relatively lower in grains treated with Diatomeenerde Probe-A
 308 with dosages ranging from 1000 to 3000 ppm compared to the lowest dosage of 500 ppm.
 309 There was significant reduction of progeny in all treatments (Two-Way ANOVA, P < 0.001,
 310 df = 2,24, F = 16.19). There was no significant interaction between dosage and DEs (Two-
 311 Way ANOVA, P > 0.177, df = 6,24, F= 1.65). In the effectiveness of different DE products
 312 there was significant difference among Diatomeenerde Probe-A and Fossil Shield® (Holm-
 313 Sidak method: t = 5.54, P < 0.017, Figure 2) and as well among Diatomeenerde Probe-A and
 314 InsectoSec® (Holm-Sidak method: t = 3.90, P < 0.025). There was, however, no significant
 315 difference in the effectiveness between Insectosec® and Fossil Shield® (Holm-Sidak method:
 316 t =1.64, P > 0.05, Figure 2).

317 Moreover, progeny emergence was inversely related to dosage rate. Across treatments,
 318 significant differences (Two-Way ANOVA, P < 0.001, df = 3,24, F = 15.93) were detected
 319 among the various dosages of the DEs. There was a significant difference between the dosage
 320 500 ppm of Diatomeenerde and Fossil shield® (Holm-Sidak method: t = 3.27, P < 0.017), and
 321 InsectoSec® and Fossil shield® (Holm-Sidak method: t = 2.88, P < 0.025) whereas
 322 Diatomeenerde and InsectoSec® showed no significant difference at this dosage (Holm-Sidak
 323 method: t = 0.39, P > 0.05). No significant differences were detected for the 1000 ppm
 324 dosage in all the DEs. At the dosage 2000 ppm, Diatomeenerde and Fossil shield® showed
 325 significant difference (Holm-Sidak method: t =3.31, P < 0.017, Figure 2). For 3000 ppm of
 326 the DE formulations only Diatomeenerde and InsectoSec® (Holm-Sidak method: t = 3.63, P <
 327 0.017) and Diatomeenerde and Fossil shield® (Holm-Sidak method: t = 2.53, P < 0.025)
 328 showed significant differences in the mean number of F₁ *T. castaneum* that emerged (Figure
 329 2).

3.5. Determination of lethal time of DEs on adult *S. zeamais* and *T. castaneum* beetles in grain

Lethal exposure times/doses estimated (LT₅₀) for the adult test insects showed that *T. castaneum* on rice was most susceptible to InsectoSec® whereas *S. zeamais* on maize was most tolerant to InsectoSec®. For the dose of 500 ppm of InsectoSec®, the LT₅₀ for *T. castaneum* and *S. zeamais*, was 10 d and 17 d, respectively, whereas in the highest dosage of 3000 ppm the LT₅₀ for *S. zeamais* and *T. castaneum* were 9 and 4 d, respectively (Table 3). Lethal times (LT₅₀/LT₉₅/LT₉₉) estimates of dosages of InsectoSec® of 500 or 3000 ppm were 17 d, 35 d, 36 d and 9 d, 16 d, 18 d, respectively for *S. zeamais* on maize, whereas for *T. castaneum* on rice 10 d, 23 d, 25 d and 4 d, 17 d, 20 d, respectively (Table 3). Similar lethal exposure times (LT₅₀/LT₉₅/LT₉₉) were estimated for adult *S. zeamais* on maize and *T. castaneum* on rice exposed to Fossil Shield® dosages of 500 or 3000 ppm (Table 3). The LT₅₀/LT₉₅ estimates for Diatomeenerde Probe-A dosages of 500 or 3000 ppm were 32 d, 36 d, and 6 d, 31 d, respectively, for *S. zeamais* on maize whereas for *T. castaneum* 11 d, 31 d, and 6 h, 17 d, respectively, were recorded. Irrespective of the DEs, for the dose of 2000 ppm, the LT₅₀ for *S. zeamais* and *T. castaneum* ranged from 6 d to 12 d, and the LT₉₅ for *S. zeamais* and *T. castaneum* were 18 d and 34 d, respectively (Table 3).

4.0. Discussion

Among DE formulations, InsectoSec® was most effective followed by Fossil Shield® and Diatomeenerde concerning the mortality of *S. zeamais* in our study. Differences in effectiveness between DE formulations and between different studies on a specific DE product may be due, for example, to different strains of an insect species, grain moisture, and storage temperature (Gana et al., 2016; Fields and Korunic, 2013; Prasantha et al., 2015; Vayias and Stephou, 2009; Stathers et al., 2004). Another study on the mortality of *S. zeamais* used the DE product Keepdry® at the dosages of 500 and 1000 g/t in dry and clean maize. Mortality of *S. zeamais* in DE treated samples started from day three. After 14 d of exposure mortality increased to 94% and 98% at 500 and 1000 g/t, respectively. In our current study, after 14 d and 1000g/t mortality was 40% to 60% only. The lower mortality in our study might be attributed to several factors including the fluctuations of temperature and relative humidity of 26 °C ± 5 °C and 70 ± 5% in tropical countries which was difficult to maintain in our study during the entire experimental period compared with a constant temperature and relative humidity of 25°C and 65 ± 10% used by Ceruti et al., (2005). Mvumi et al., (2006) tested five raw African diatomaceous earth (DE) collected from Tanzania, Zimbabwe, Zambia, South Africa and UK in the laboratory using two concentrations 2,500 and 5,000 ppm for the UK samples, whereas in Zimbabwe concentration was 1,000 ppm. The

DEs were admixed with maize or wheat to control *S. zeamais*, *R. dominica* and *T. castaneum*. Adult mortality was assessed after 7, 14 and 28 d and F₁ progeny emergence for 7 weeks at 27 °C and 55 or 60 % r.h. In their study they recorded that, all the DEs were highly effective against *S. zeamais* even at their lowest concentration of 1,000 ppm. Similar results were obtained in our study, although in both studies, 28 d mortality count for *T. castaneum* was observed 100% mortality whereas complete effectiveness against *S. zeamais* was observed on day 32.

Rigaux et al., (2001) studied fourteen strains of *T. castaneum* originally from Nigeria, Abidjan (Ivory Coast), Georgia (USA), Kansas (USA), Waseco county, Minnesota (USA), Maff (UK), Japan, Naphin (Philippines), Pakistan, Vancouver, British Columbia (Canada), Landmark, Manitoba (Canada), Argyle, Manitoba (Canada), and Saint John, New Brunswick (Canada). Protect-It[®] diatomaceous earth used in this study contained 10% silica aerogel, 87% amorphous silicon dioxide, 3% Al₂O₃, 1% Fe₂O₃, less than 1% CaO, MgO, TiO₃, P₂O₃ and particle size of 5.4 µm. In their study they used 25 ± 1°C and a relative humidity of 70 ± 5%. When the strains were exposed to Protect-It[®] diatomaceous earth (Hedley Technologies Inc., Canada) at 600 ppm for seven days in 200 g of wheat, mortalities ranged from 5 to 100%. In our study with *T. castaneum* originating from Ghana, we observed 16 to 39% mortality after seven days when exposed to 500 ppm in 100 g of rice grain. The susceptibility of the strain from Ghana can therefore be rated as intermediate compared to the strains studied by Rigaux et al. (2001).

Marsaro et al. (2006) studied the effectiveness of different dosages of diatomaceous earth ranging from 125, 250, 500 and 1,000 g/t to control *T. castaneum* in corn stored in the state of Roraima, Brazil. The beetles used in the study by Marsaro et al. (2006) originated from Brazil. Mortality was observed from 1-28 d of exposure. They found out that mortality of the adults was influenced by the dosages and the exposure time of insects to diatomaceous earth. It was observed that dosages of 125 and 250 g/t recorded a maximum mortality after 28 days of exposure of 30% and 50 %, respectively. As in our study, Marsaro et al. (2006), found at dosages of 500 and 1,000 g/t diatomaceous earth to be highly effective to control *T. castaneum*, i.e. an effectiveness between 80% and 95%. In a study with a strain of *T. castaneum* originating from Germany. Adarkwah et al. (2017) found a LT₅₀ of 20 h after exposure to Diatomeenerde compared to 9 h in the actual study, i.e. the German strain was much more tolerant than the Ghanaian strain. Consequently, susceptible and tolerant strains of *T. castaneum* are known.

Our results clearly indicate increased mortality values with increased exposure time. Fields and Korunic (2000) reported that Protect-It[®] at 300 mg/kg of wheat 11.8% moisture content at 25° C after 5 d of exposure produced 72% mortality of *R. dominica* and mortality was 90%

after 14 d of exposure. Several published reports document increased mortality of stored-product beetles exposed to DE for increasing time intervals, similar to our findings (Arthur, 2000a & b; Subramanyam and Roesli, 2000; Athanassiou et al., 2003, 2005, 2008). In the current study by Mortazavi et al. (2020), mortality values of *S. granarius*, *R. dominica* and *T. confusum* adults increase at each exposure (days) and with the increase in dosage. There are many published studies indicating exposure time dependence of mortality for DE treatments (Fields and Korunic, 2000; Athanassiou et al., 2003; Stathers et al., 2004; Vayias and Athanassiou, 2004; Chelav and Khashaveh, 2014; Ziaee et al., 2018).

The demand for maize in Sub-Saharan Africa will triple by 2050 due to rapid population growth, while challenges from climate change will threaten agricultural productivity. In Ghana the farmers are expected to readily accept a concept or technology that builds up or improves one which they are used to rather than completely new approaches. Therefore, studies were conducted in the laboratory to evaluate the insecticidal efficacy of three diatomaceous earth formations, i.e. powders which are mixed with the grain. Our results indicate that both InsectoSec® and Fossil Shield® are effective, and can be used with success even at dose rates of 500 ppm on maize and rice to control *S. zeamais* and *T. castaneum* strains in Ghana. The three enhanced DEs are candidates for future use as components of an IPM-based control strategy in Ghana. Therefore, if DEs were to be registered and made commercially available, it would help improve integrated pest management strategies in Ghana.

Declaration of conflict of interest

All authors declare that there is no interest to declare. Also, by this declaration all authors confirm that this manuscript has not been published elsewhere and it is not under consideration by another journal. All authors have approved the manuscript and agree with its submission to Journal of Stored Products Research.

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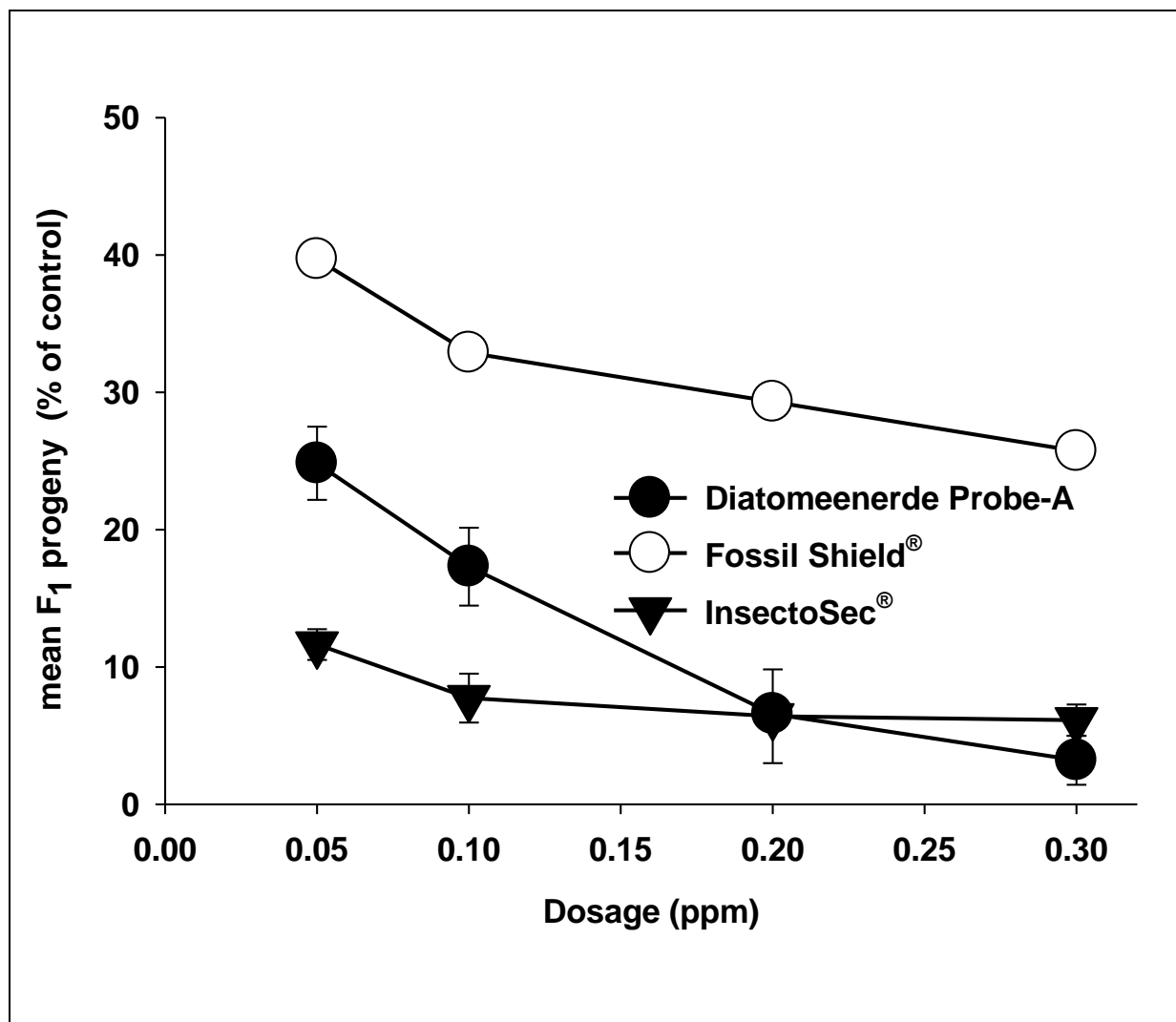
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722 **Figure Caption**

723

724 **Figure 1:** Mean percentage (\pm SEM) of F₁ *S. zeamais* (on maize) expressed as percentage of
 725 control after exposure to modified diatomaceous earths, Diatomeenerde Probe A, Fossil
 726 Shield[®] 9.0 S brown and InsectoSec[®] at different dosages. Data are means of three
 727 replications of 30 parental insects each.

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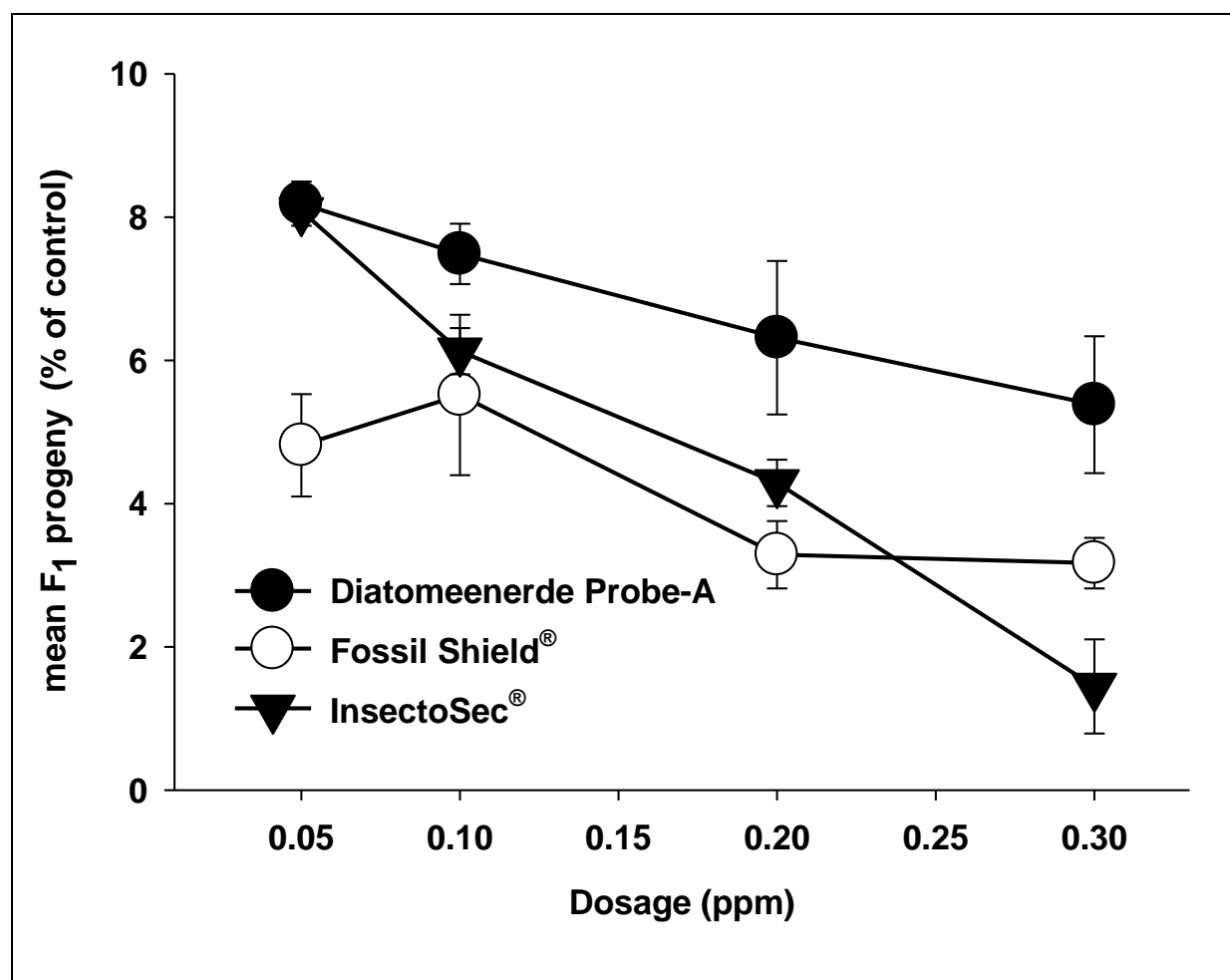
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732 **Figure 2:** Mean percent (\pm SEM) of F₁ progeny *T. castaneum* (on rice) expressed as
 733 percentage of control after exposure to improved diatomaceous earths Diatomeenerde Probe
 734 A, Fossil Shield[®] 9.0 S brown and InsectoSec[®] at different dosages. Data are means of three
 735 replications of 30 parental insects each.

736



737

Table 1: Mean (% \pm SD) mortality of *Sitophilus zeamais* adults after 7, 14, 21, 28 and 32 days of exposure to treated maize grains with different diatomaceous earth products at different dosages, at temperature of $30^{\circ} \pm 2^{\circ} \text{C}$ and 70–80% relative humidity. Data are means of three replications of 30 insects each.

Treatments	Dose (ppm)	Mean (% \pm SD ^a) mortality				
		7 days	14 days	21 days	28 days	32 days
Diatomeenerde	0	0.00 \pm 0.00 d	0.00 \pm 0.00 d	0.00 \pm 0.000 d	2.22 \pm 1.92 d	0.00 \pm 0.00 d
	500	20.00 \pm 3.33 c	23.33 \pm 5.77 c	35.56 \pm 6.94 c	46.67 \pm 6.67 c	100.00 \pm 0.00 a
	1000	22.22 \pm 3.85 c	31.11 \pm 5.09 c	43.33 \pm 12.02 c	48.9 \pm 18.4 c	100.00 \pm 0.00 a
	2000	37.78 \pm 6.94 b	51.11 \pm 3.85 b	68.89 \pm 6.94 b	74.44 \pm 3.85 b	100.00 \pm 0.00 a
	3000	51.11 \pm 13.88 a	68.89 \pm 6.94 a	86.67 \pm 10.00 a	92.22 \pm 8.39 a	100.00 \pm 0.00 a
F		21.03	85.42	48.49	36.94	
P		0.001	0.001	0.001	0.001	
Fossil Shield [®]	0	0.00 \pm 0.00 d	0.00 \pm 0.00 d	0.00 \pm 0.00 d	2.22 \pm 1.92 c	
	500	23.33 \pm 5.77 c	46.67 \pm 5.77 c	71.11 \pm 5.09 c	92.22 \pm 5.09 b	
	1000	47.78 \pm 12.62 b	65.56 \pm 8.39 b	85.56 \pm 3.85 b	100.00 \pm 0.00 a	
	2000	56.67 \pm 3.33 ab	74.44 \pm 5.09 ab	98.89 \pm 1.92 a	100.00 \pm 0.00 a	
	3000	67.78 \pm 1.92 a	82.22 \pm 1.92 a	100.00 \pm 0.00 a	100.00 \pm 0.00 a	
F		53.95	121.43	579.79	935.63	
P		0.001	0.001	0.001	0.001	
InsectoSec [®]	0	0.00 \pm 0.00 d	0.00 \pm 0.00 d	0.00 \pm 0.00 c		
	500	13.33 \pm 0.00 c	34.44 \pm 10.72 c	60.00 \pm 10.00 b		
	1000	22.22 \pm 10.18 bc	58.89 \pm 25.02 b	92.22 \pm 8.34 a		
	2000	28.89 \pm 6.94 ab	80.00 \pm 3.33 ab	97.78 \pm 3.85 a		
	3000	36.67 \pm 8.82 a	83.33 \pm 12.02 a	100.00 \pm 0.00 a		
F		13.6	20.18	145.10		
P		0.001	0.001	0.001		

* Means in the same column within each treatment followed by the same letters are not significantly different (for all the treatments $df = 4$; Holm-Sidak method's, $P > 0.05$).

Table 2: Mean (% \pm SD) mortality of *Tribolium castaneum* adults after 7, 14, 21 and 32 days of exposure to treated rice grains with different diatomaceous earth formulations earth at different dosages, at temperature of 30 ± 2 °C and 70–80% relative humidity. Data are means of three replications of 30 insects each.

Treatments	Dose (ppm)	Mean (% \pm SD)				
		7 days	14 days	21 days	28 days	32 days
Diatomeenerde	0	0.00 \pm 0.00 c	0.00 \pm 0.00 c	0.00 \pm 0.00 d	2.22 \pm 1.93 c	2.22 \pm 1.93 b
	500	38.89 \pm 5.09 b	62.22 \pm 1.92 b	76.67 \pm 5.77 c	87.79 \pm 5.01 b	100.00 \pm 0.00 a
	1000	41.11 \pm 1.92 b	65.56 \pm 6.94 b	83.33 \pm 3.33 b	94.44 \pm 1.93 ab	100.00 \pm 0.00 a
	2000	47.78 \pm 1.92 a	75.56 \pm 1.93 a	93.33 \pm 3.33 a	100.00 \pm 0.00 a	100.00 \pm 0.00 a
	3000	51.11 \pm 5.09 a	81.11 \pm 6.94 a	98.89 \pm 1.92 a	100.00 \pm 0.00 a	100.00 \pm 0.00 a
F		107.41	154.59	411.34	795.39	774.002
P		0.001	0.001	0.001	0.001	0.001
Fossil Shield®	0	0.00 \pm 0.00 e	0.00 \pm 0.00 e	1.11 \pm 1.93 d	1.11 \pm 1.93 c	-
	500	23.33 \pm 3.33 d	34.44 \pm 1.93 d	60.00 \pm 3.33 c	87.78 \pm 1.92 b	-
	1000	33.33 \pm 8.82 c	51.11 \pm 1.92 c	73.33 \pm 6.67 b	94.44 \pm 3.85 a	-
	2000	43.33 \pm 6.67 b	60.00 \pm 6.67 b	81.11 \pm 6.94 b	100.00 \pm 0.00 a	-
	3000	61.67 \pm 2.36 a	75.00 \pm 2.36 a	96.67 \pm 0.00 a	100.00 \pm 0.00 a	-
F		58.61	220.10	188.74	1221.25	
P		0.001	0.001	0.001	0.001	
InsectoSec®	0	0.00 \pm 0.00 d	0.00 \pm 0.00 b	0.00 \pm 0.00 c	2.22 \pm 1.93 b	-
	500	15.56 \pm 5.09 cd	78.89 \pm 6.94 a	91.11 \pm 3.85 b	100.00 \pm 0.00 a	-
	1000	32.22 \pm 5.09 bc	81.11 \pm 3.85 a	91.11 \pm 1.92 b	100.00 \pm 0.00 a	-
	2000	42.22 \pm 8.39 b	77.78 \pm 5.09 a	96.67 \pm 3.33 a	100.00 \pm 0.00 a	-
	3000	75.56 \pm 21.43 a	88.89 \pm 10.18 a	100.00 \pm 0.00 a	100.00 \pm 0.00 a	-
F		21.22	105.36	915.76	774.002	
P		0.001	0.001	0.001	0.001	

* Means in the same column within each treatment followed by the same letters are not significantly different (for all the treatments $df = 4$; Holm-Sidak method's, $P = 0.05$).

748

749 **Table 3:** The survival durations (d) of different beetle species exposed to three different diatomaceous earths (% wt/wt).

DE-type/ Dosage rate (ppm/30g)	LT ₅₀	<i>S. zeamais</i>			<i>T. castaneum</i>	
		LT ₉₅	LT ₉₉	LT ₅₀	LT ₉₅	LT ₉₉
Diatomeenerde-Probe A						
500	32	36	37	11	23	25
1000	29	36	37	9	22	23
2000	12	34	36	8	19	22
3000	6	31	33	6	17	20
Fossil Shield®						
500	14	28	29	17	31	32
1000	8	25	26	13	28	31
2000	6	20	21	9	27	26
3000	5	17	19	5	17	23
InsectoSec®						
500	17	35	36	10	31	33
1000	11	26	34	9	28	30
2000	9	18	26	8	25	27
3000	9	16	18	4	20	21

750

751 *The LT₅₀ LT₉₅ and LT₉₉ estimated graphically using SigmaPlot, data from Figures 1-2 exposure held on maize (for *S. zeamais*) and rice (for *T. castaneum*)

752 seeds treated with different dosages of diatomaceous earths.

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754