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Comparative toxicity of nanostructured alumina and a commercial inert dust for *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) at varying ambient humidity levels

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ABSTRACT

The widespread concern for environmental and human health has raised the need for new reduced-risk control strategies and the search for new chemical classes of pesticides. Recently, a novel type of particulate material, nanostructured alumina (NSA) has been found to induce mortality in insects exposed to wheat treated with NSA dust. Preliminary studies have shown insecticidal activity of NSA particles on two insect species, *Sitophilus oryzae* (L.), and *Rhyzopertha dominica*, (F.), major pests of stored grain. We investigated the toxicity of NSA and Protect-It® diatomaceous earth (DE) using dry dust applications at three different relative ambient humidity levels. Results showed that NSA was more effective in killing *S. oryzae* than Protect-It® and was equally toxic to *R. dominica*. Treatment with both products also reduced progeny production. In addition, *R. dominica* was less susceptible to inert dusts than *S. oryzae*. Our results suggest that NSA might prove a good alternative or complement to DE based products, and encourage further testing with other insect pests and systems, plus experiments on delivery options to further enhance NSA products.

1. Introduction

Synthetic insecticides are applied worldwide for crop protection, to control human and animal diseases and their use has increased steadily over the last 50 years. However, the widespread concern for environmental and human health has increased the need for new reduced-risk control strategies and new chemical classes of pesticides. For example, inorganic insecticidal dusts (inert dusts) are environmentally friendly alternatives for insect pests of field crops, stored products, and animals (Phillips and Throne, 2010). These inorganic dusts are chemically stable, highly persistent and have low mammalian toxicity (Glenn et al., 1999; Subramanyam and Roesli, 2000; Cook et al., 2008). Information on inert dust use for insect and mite control in stored products has been the subject of many reviews and research papers (Ebeling, 1971; Golob, 1997; Cook and Armitage, 1999; and citations within each), leading to new commercial products and uses. Inert dusts contain mainly synthetic silica (silicon dioxide) or natural silica such as diatomaceous earths (Subramanyam and Roesli, 2000) and include kaolinite, silica gel and diatomaceous earth. Diatomaceous earths (DE), which are the fossilized skeletons of diatoms, are predominantly comprised of amorphous or shapeless silica (silicon dioxide) and small amounts of other minerals/elements. The most widely accepted explanation for the mode of action of inert dusts is that they kill arthropods by removing or adsorbing the epicuticular lipid layers, causing excessive water loss through the cuticle (Chiu, 1939a, b; Wigglesworth, 1944; Ebeling and Wagner, 1961). The effectiveness of these insecticides often depends on their capacity to adsorb the oily or waxy outer cuticle layer by direct contact. Their efficacy is related to their specific surface (square meters per gram), and decreases as humidity increases and temperature decreases (Ebeling, 1961; Fields and Korunic, 2000).

Examples of synthetic silica include silica gels, which have a greater oil absorption capacity and thus are more effective insecticides than diatomaceous earth. Another type of silica based material, Kaolinite, ($Al_4SiO_{10}[OH]_8$), controls pests and diseases of agricultural crops by acting as repellent and/or physical barrier (Glenn et al., 1999). Kaolinite inert particle films create a barrier to

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infestation by making the host unrecognizable in the visual or tactile sense, altering behavior, increasing mortality and reducing reproduction (Puterka et al., 2000; Cottrell et al., 2002; Wyss and Daniel, 2004). Arthur and Puterka (2002) observed that kaolinite particles also plugged spiracles and damaged the cuticle of treated insects.

Recently, a novel type of particulate material, nanostructured alumina (NSA) has been found to induce mortality in insects exposed to wheat treated with NSA dust. Preliminary studies have found insecticidal activity of NSA particles on two insect species, *Sitophilus oryzae* (L.), and *Rhyzopertha dominica,* (F.), major pests of stored grain in milling and processing facilities, food warehouses, and foodstuffs (Stadler et al., 2010). Mortality due to NSA was observed at rates comparable to those recommended for commercially available insecticidal dusts. Since then, silica nanoparticles have also been tested against *S. oryzae* (Debnath et al., 2001) indicating the current interest on nanomaterial-based technology for pest management.

The action of inert dusts varies greatly depending on the mineral composition of the dust, type of formulation, insect species, and environmental conditions (Subramanyam and Roesli, 2000; Vardeman et al., 2007). The mode of action of NSA has not yet been elucidated, and detailed toxicity studies are needed to understand how it works and also to determine whether it constitutes a good alternative for insect pest control. Therefore the present study was conducted to compare the toxicity of NSA to that of a commercial enhanced DE, simultaneously and under the same experimental conditions. We chose Protect-It®, given that it is one of the most effective DE based products available on the commercial market (Subramanyam and Roesli, 2000). We investigated the toxicity of these two products at three different relative ambient humidity levels, using two insect species that are pests of stored grain, *S. oryzae*, and *R. dominica.*

2. Materials and methods

2.1. Insecticides

Nanostructured alumina (Al_2O_3) was obtained by glycine–nitrate combustion synthesis technique using a redox mixture, with glycine as fuel and aluminum nitrate as oxidizer (Toniolo et al., 2005). The bulk density of the powder was measured at 0.108 g/cc. Protect-It®, a formulation containing DE and silica gel, was obtained from Hedley Technologies Ltd. (Grand Junction CO, USA). It is a mixture of fresh water DE with 10% silica aerogel (Korunic and Fields, 1998), which contains over 87% amorphous silicon dioxide, 3% Al_2O_3, 1%Fe_2O_3, less than 1% CaO, MgO, TiO_2, P_2O_3, and 3–6% water. The median particle size is reported as 5.4 μm, the bulk density is 0.20 g/cc, and the pH is between 5.5 and 5.7 in a 10% slurry (Korunic et al., 1996). Scanning electron microscopy (Zeiss Supra FE-SEM, Peabody, MA, USA) was performed at Montana State University (Bozeman, MT, USA) to evaluate the particle size and morphology of the two different powders. The particle size distribution of each insecticidal dust was analyzed with a dynamic light scattering system (Malvern Instrument Zetasizer Nano ZS, Malvern, Worcestershire, UK). To avoid particle size reduction associated with ball milling and to more accurately report the effective particle size distribution in the insecticide tests, samples were ultrasonically dispersed for 5 min in water at 20% power (Branson 20 kHz 200 W Sonifier, Danbury, CT, USA) utilizing an ammonium polymethacrylate dispersant (Darvan C-N, RT Vanderbilt Company Inc., Norwalk, CT, USA). Slightly turbid solutions at approximately 1 wt% solids were characterized in disposable polystyrene cuvettes to eliminate cross contamination between samples.

2.2. Test insects

The species tested were adults of grain pests, the rice weevil *S. oryzae* (L.), and the lesser grain borer, *Ryzopertha dominica* (F). Insects were obtained from colonies with no history of exposure to insecticides, reared at Montana State University. Insects were reared at 28° ± 1 °C and 70% ± 5% RH in dark. The desired RH was maintained by using a saturated salt solution of sodium chloride (Winston and Bates, 1960). Adults used in all experiments were 0–6 weeks old, of unknown sex and mating status. Wheat used for the experiments was hard white spring wheat (Clearine cv.). The moisture content of the wheat was determined by a Foss Infratec 1241 Grain Analyzer (Foss North America, Silver Spring, MD, USA).

2.3. Toxicity assessment - dry dust application

Laboratory bioassays were conducted to test the toxicity of NSA using dry dust applications and evaluated at four different concentrations of the product: 1000 ppm, 500 ppm, 250 ppm, 125 ppm, and 62.5 and three different ambient humidity levels. The different levels of ambient humidity were 43, 57, and 75% r.h, and were achieved with chambers containing saturated solutions of potassium carbonate, sodium bromide, sodium chloride, and water, respectively (Winston and Bates, 1960). The humidity chambers were created in 20 cm × 30 cm × 11 cm plastic boxes, with plastic waffle-type grids at the bottom, containing the salt solution below the grid. Temperature and humidity inside the chambers were monitored with HOBO data recorders (Onset Computer, Bourne, MA, USA). The experiments were conducted at 25 ± 1 °C and constant photoperiod of 12:12 (L: D).

For each ambient humidity treatment, the wheat was acclimatized in the corresponding chamber at 25 ± 1 °C for several weeks until it achieved constant grain moisture content. The target grain moisture levels for the lowest, medium and high humidity treatment were 10.7, 12.6, and 14.7 respectively (Wicklow et al., 1998). The bioassay methodology was the same as that reported in Stadler et al. (2010). Briefly, once the grain achieved the target moisture content, it was mixed with nanostructured alumina or Protect-It® and mixed thoroughly to allow an even distribution of the powder through the entire grain mass. Then, dilutions were conducted to achieve the desired concentrations in 30 g of wheat, and placed in Petri dishes. A Petri dish containing 30 g of untreated wheat was used as control. Ten insects were placed in each Petri dish. Each experiment consisted of 9 humidity chambers (three chambers for each humidity level), each one containing 12 Petri dishes (two insecticides with six concentrations per insecticide), and the experiments were replicated twice with each insect species. Adult mortality was assessed 3, 6, 9, 12, and 15 days after continuous exposure to the treated wheat in the corresponding humidity chamber. In each bioassay, after the last (15-d) mortality count and all the remaining live insects were removed, the wheat was returned to the Petri dishes to be incubated for an additional 45 days at 27 °C and 75% r.h.. After this time, the adult F1 progeny were then removed from the wheat by sifting through a #8 (2.36 mm) sieve (Seedburo Equipment Company, Chicago IL, USA), and numbers of adults were recorded.

2.4. Data analysis

The data were analyzed using the Mixed Procedure (PROC MIXED) of the Statistical Analysis System (SAS Institute Inc., 2008) with mortality as the response variable and concentration, product, date of observation and their interaction as main effects. Date of observation was the repeated measure. A separate analysis of the mortality at the end of the bioassay was conducted with PROC

MIXED, where mortality was the response variable and product, concentration and their interaction were the main effects. Progeny was analyzed with PROC MIXED, where number of emerged adults was the response variable and product, concentration and their interaction were the main effects. In all analyses, Petri dish replicates were included as a random factor and each humidity level was analyzed separately. The variance-covariance structure was modeled as compound symmetry. Control mortality was corrected using Abbott's (1925) formula. LSMEANS comparisons were conducted with the Tukey option in SAS.

Standard probit analysis cannot be used on serial time-mortality data, given that observations made on the same group of organisms at different times are correlated (Robertson and Preisler, 1992). Analysis of correlated data differs from typical probit analysis because in addition to their variances, the covariances of the probits also must be estimated to account for correlation among observations (Throne et al., 1995a,b). Therefore, lethal time values (the time required to kill a given proportion of the insects) for a given concentration, were calculated using a probit analysis developed by Throne et al. (1995a, b). They developed a method and computer program for analyzing correlated serial time-mortality data using the complementary log–log, logit, or probit transformation of proportion insects killed. Slopes and intercepts of the regression curves for each humidity level of both products were also compared with a computer program developed by the authors. The log-probit model was chosen as it was the one that fit the data best based on goodness of fit tests.

Regression curves were fitted to the progeny production data using TableCurve 2D software (SPSS, Chicago, IL, USA). This approach provides a means of accurately fitting linear and nonlinear curves to biological data, and has been used in previous publications concerning the response of insect species to ordered concentrations of insecticides (Arthur, 2000, 2001; Chanbang et al., 2007). The software provides the R^2 of the selected model and determines maximum R^2 of any model which could be fitted to the data set.

3. Results

3.1. Particle size & morphology

The average particle diameter of Protect-It® powders as reported by the manufacturer is 5.4 μm. As indicated in Fig. 1 the Protect-It® powders show a majority of large particles approximately 3–6 μm in diameter. The Protect-It® powder further indicates a bi-modal distribution of porosity in the larger particles, ~1.1 μm and 200 nm, which serves to increase the overall surface area of the powder. Particle size analysis in Fig. 3 also indicates the highest volumetric loading of particles close to 5 μm with a distribution of smaller particles peaking at 700 nm.

The nanostructured alumina showed a substantially different morphology than the Protect-It® powder. While the particles appeared far denser, the particle size was substantially smaller as indicated in Fig. 2 and the platelet morphology is readily evident where the thickness of the platelets is observed at 45 nm. Particle size analysis in Fig. 3 also indicates a bi-modal size distribution that was expected given the platelet morphology and stagnant flow conditions used in the analysis for which no alignment is readily induced. The highest loading of large particles is indicated at 1.5 um with a nanosized distribution of smaller particle diameters at 350 nm.

3.2. Overall adult mortality across time and at the end of the bioassay

3.2.1. Adult mortality of Sitophilus oryzae

Adult mortality increased with time (high humidity (HH): $F = 53.47$, d. f. = 4, 44, $P < 0.0001$; medium humidity (MH): $F = 52.48$, d. f. = 4, 44, $P < 0.0001$; low humidity (LH): $F = 48.62$, d. f. = 4, 44, $P = 0.0015$), and concentration (HH: $F = 134.48$, d. f. = 5, 50, $P < 0.0001$; MH: $F = 217.68$, d. f. = 5, 50, $P < 0.0001$; LH: $F = 205$, d. f. = 5, 50, $P = 0.0015$) (Fig. 4). However, there was also a significant interaction between product and concentration on the effect on mortality (HH: $F = 11.98$, d. f. = 5, 50, $P < 0.0001$; MH: $F = 16.36$, d. f. = 5, 50, $P < 0.0001$; LH: $F = 4.83$, d. f. = 5, 50, $P = 0.0011$) for all humidity levels. At the high humidity, NSA was more effective than Protect-It® at the concentrations of 500, 250, and 125 ppm ($P < 0.05$). At the medium humidity NSA was more effective than Protect-It® at the concentrations of 250, 125, and 62.5 ppm ($P < 0.05$). At the low humidity NSA was more effective than Protect-It® at the concentrations of 125 and 62.5 ppm ($P < 0.05$). At the end of the bioassay, and for all the humidity levels tested, there was a significant interaction effect between product and concentration (LH: $F = 4.58$, d. f. = 5, 55, $P = 0.0015$; HH: $F = 18.51$, d. f. = 5, 55, $P < 0.0001$; MH: $F = 15.82$, d. f. = 5, 55, $P < 0.0001$). Tukey comparisons revealed that mortality was very high for all concentrations tested and similar for both products, except for at the lowest concentration, where NSA was more effective than Protect-It® ($P < 0.05$; LH). At the high humidity level, Tukey mean comparisons revealed that NSA was more effective than Protect-It® at the concentrations of 250, 125, and 62.5 ppm ($P < 0.05$). At the medium humidity level, NSA was more effective than Protect-It® at the concentrations of 125 and 62.5 ppm ($P < 0.05$) (Table 1).

3.2.2. Adult mortality of Rhyzopertha dominica

Adult mortality increased with time (HH: $F = 8.99$, d. f. = 4, 42, $P < 0.0001$; MH: $F = 38.94$, d. f. = 4, 44, $P < 0.0001$; LH: $F = 36.88$, d.

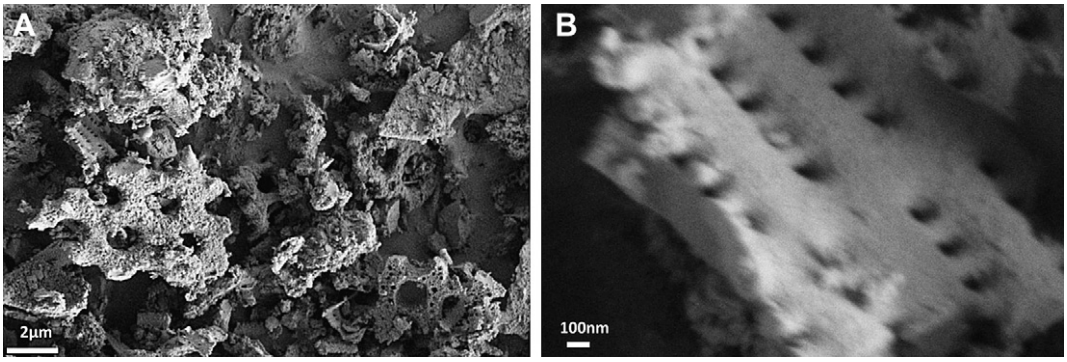

Fig. 1. Protect-It® Scanning Electron Microscope Picture. Extra High Tension (EHT): 1 kV, Working Distance (WD): 4 mm. A) Magnification: 5.50KX; B) Magnification 51.78 KX.

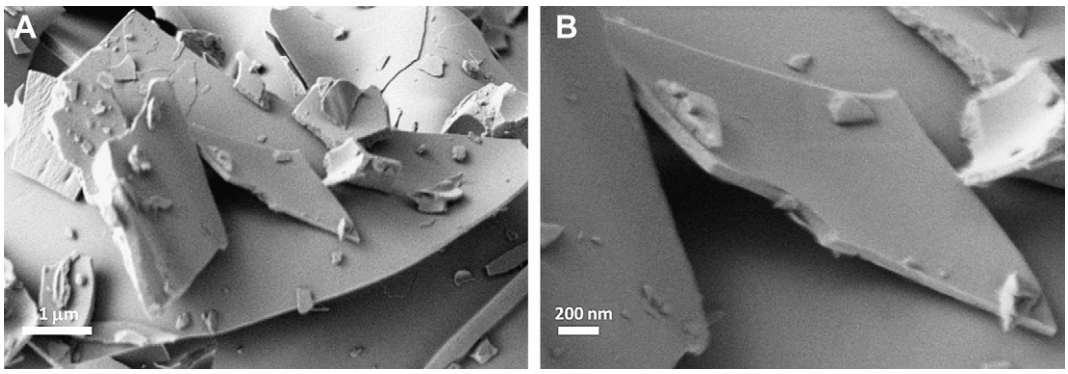

Fig. 2. NSA Scanning Electron Microscope Picture. Extra High Tension (EHT): 1 kV; Working Distance (WD): 3 mm. A) Magnification: 45KX; B) Magnification 120 KX.

f. = 4, 44, $P = 0.0015$), and concentration (HH: $F = 126.99$, d. f. = 5, 45, $P < 0.0001$; MH: $F = 230.23$, d. f. = 5, 50, $P < 0.0001$; LH: $F = 114.75$, d. f. = 5, 50, $P < 0.0001$) (Fig. 5). However, there was also a significant interaction between product and concentration on the effect on mortality (HH: $F = 8.22$, d. f. = 5, 45, $P < 0.0001$; MH: $F = 9.77$, d. f. = 5, 50, $P < 0.0001$; LH: $F = 4.78$, d. f. = 5, 50, $P = 0.0012$) for all humidity levels. Tukey comparisons revealed that both insecticides were very similar in their effectiveness against *R. dominica*. At the end of the bioassay, there was a significant effect of insecticide on adult mortality for the low ($F = 4.57$, d. f. = 1, 55, $P = 0.04$) and medium humidity ($F = 5.32$, d. f. = 1, 55, $P = 0.03$ levels), and no significant interaction between product and concentration (LH: $F = 1.43$, d. f. = 5, 55, $P = 0.23$; MH: $F = 1.66$, d. f. = 5, 55, $P = 0.16$). Tukey comparisons revealed that NSA was more effective than Protect-It® ($P < 0.05$) at the low and medium humidity levels. Also, at the end of the bioassay mortality was greater than the control for all the concentrations tested except for the lowest Protect-It® concentration tested ($P < 0.05$). No significant differences between the two products were observed at the high humidity level ($F = 0.09$, d. f. = 5, 50, $P = 0.77$) and only the 500 and 1000 ppm concentrations caused significant mortality when compared to the control ($P < 0.05$) (Table 2).

3.3. Median lethal times

Lethal time regression slopes were calculated for the concentrations at which probit analysis fit the data, 125 or 250 ppm, depending on the humidity level and species. For *R. dominica*, only 4 sampling times were considered in the analysis: 3, 6, 12, and 15 days. The probit models were not appropriate for other concentrations because mortality was consistently high or low throughout the study.

3.3.1. Lethal time values for Sitophilus oryzae

The lethal time regression slopes were significantly different between the two products (Table 3). At a concentration of 250 ppm and high ambient humidity, NSA killed insects significantly faster than Protect-It®. The LT_{95} for NSA was 10.9 days, while it was 185.8 days for Protect-It®. At a concentration of 125 ppm and medium or low humidity, NSA was also faster than Protect-It® in killing 95% of the test population. LT_{95} for NSA at medium and low ambient humidity were 11.66 days and 10.6 days respectively. LT_{95} for Protect-It® at medium and low humidity were 79.9 days and 19.4 days respectively.

3.3.2. Lethal time values for Rhyzopertha dominica

The lethal time regression slopes were significantly different between the two products when insects were exposed at low or medium ambient humidity (Table 4). However, at a concentration of 250 ppm and high ambient humidity, the LT_{95} for both products overlapped. For NSA the LC_{95} was 25.0 days, and for Protect-It® it was 84. 6 days. LT_{95} for NSA at medium and low ambient humidity were 7.26 days and 2.6 days respectively. LT_{95} for Protect-It® at medium and low humidity were 11.6 days and 251.0 days respectively.

3.4. Progeny

Data for number of F_1 adults emerged with increasing concentration for each product were described by linear and non-linear

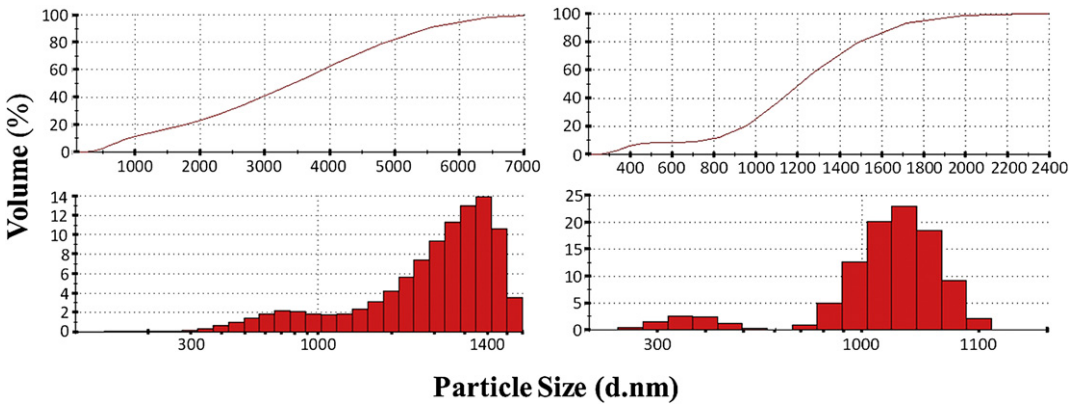

Fig. 3. Laser scattering particle size distributions, as plotted by Zetasizer Nano ZS, in water with ammonium polymethacrylate dispersant (left) Protect-It®, (right) NSA. Top graphs in a linear scale and bottom histograms in logarithmic scale.

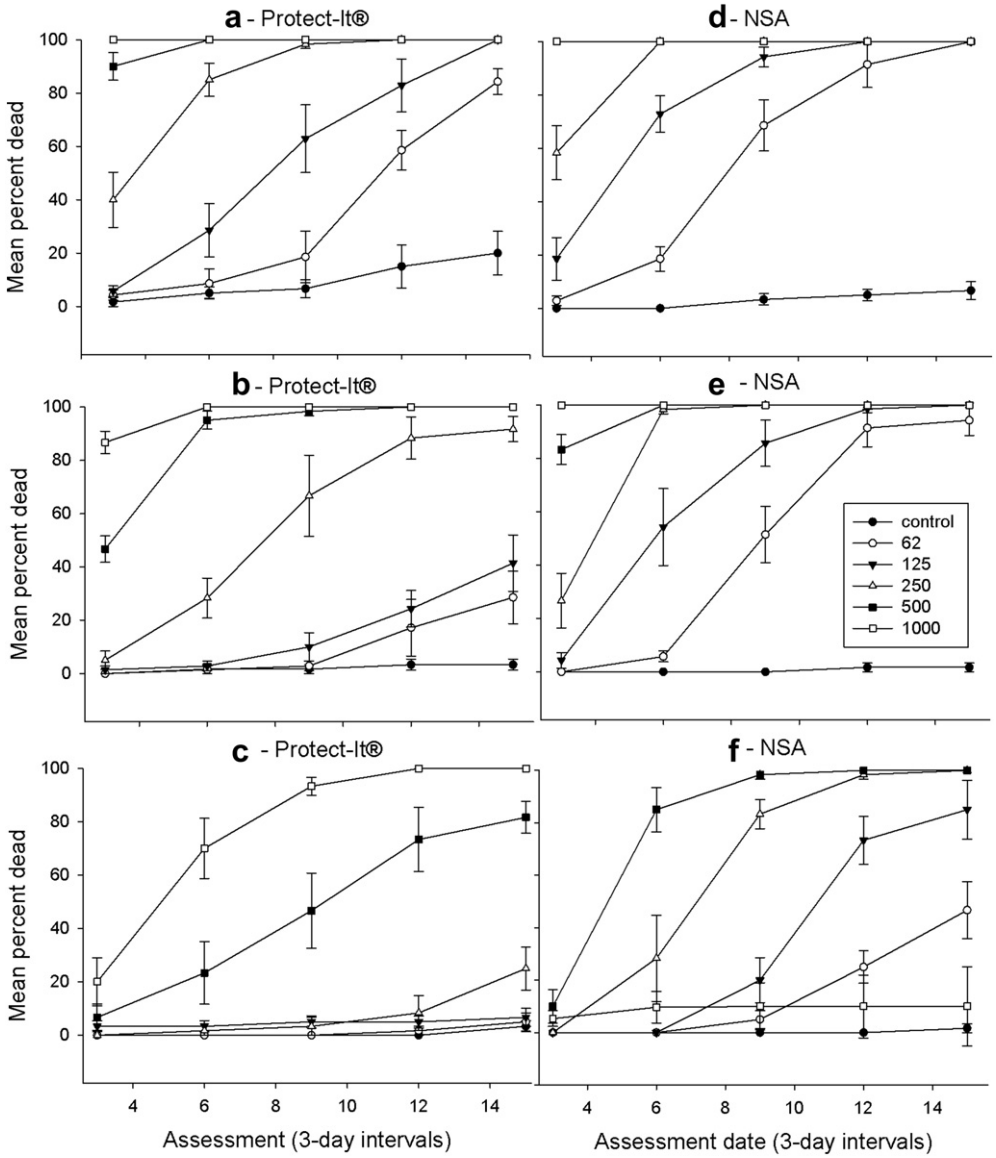

Fig. 4. (a, f). Mortality of *Sitophilus oryzae* over time when treated with 0, 62.5, 125, 250, 500, 1000 ppm of Protect-It® (a, b, c) or NSA (d, e, f). Wheat was held at 27 °C and 43% (a, d), 57% (b, e) or 75% r.h. (c, f).

regression. The simple models were ranked based on the F statistic, and those that explained the data best were chosen (Table 5, Figs. 6 and 7).

3.4.1. *Sitophilus oryzae*

The regression slopes describing the relationship between treatment concentration and number of F_1 adults emerged were steeper for NSA at the high and medium humidity levels, and very similar at the low humidity treatment (Table 5; Fig. 6). At the highest humidity tested, there was a significant interaction between product and concentration in the number of F_1 adults ($F = 6.69$, d. f. $= 5, 55$, $P < 0.0001$). NSA was more effective than Protect-It® in reducing the number of F_1 adults at the two lowest concentrations tested ($P < 0.05$) but there were no differences

Table 1
Adult mortality of *S. oryzae*, after 15 days of exposure to wheat treated with NSA and Protect-It®.

Species	Concentration (ppm)	Percentage of dead insects after 15 days of treatment with NSA			Percentage of dead insects after 15 days of treatment with Protect-It®		
		High	Medium	Low	High	Medium	Low
Sitophilus oryzae	0	1.7 ± 1.7	1.7 ± 1.7	6.7 ± 3.3	3.3 ± 2.1	3.3 ± 2.1	20 ± 8.2
	62.5	46.7 ± 10.8	94.3 ± 0.57	100 ± 0	5.0 ± 3.5	28.6 ± 9.9	84.3 ± 4.8
	125	85 ± 11.2	100 ± 0	100 ± 0	6.7 ± 3.0	41.4 ± 10.6	100 ± 0
	250	100 ± 0	100 ± 0	100 ± 0	25 ± 8.1	91.7 ± 4.8	100 ± 0
	500	100 ± 0	100 ± 0	100 ± 0	81.7 ± 6.1	100 ± 0	100 ± 0
	1000	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0

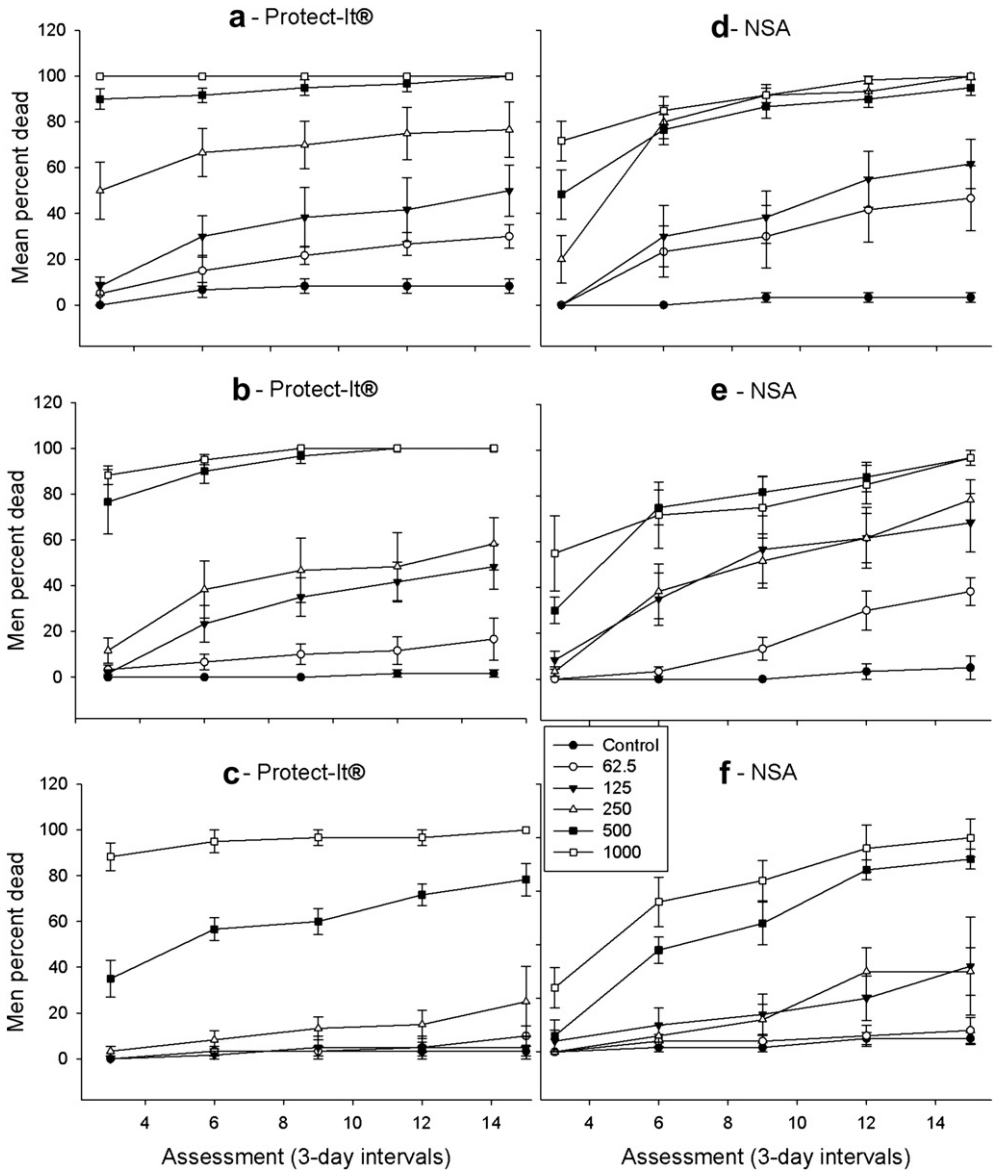

Fig. 5. (a, f). Mortality of *Rhyzopertha dominica* over time when treated with 0, 62.5, 125, 250, 500, 1000 ppm Protect-It® (a, b, c) or NSA (d, e, f). Wheat was held at 27 °C and 43% (a, d), 57% (b, e) or 75% r.h. (c, f).

between the two products at the higher concentrations ($P < 0.05$). At the medium humidity level, there was a significant product effect (product - $F = 6.65$, d. f. = 1, 55, $P = 0.01$), where NSA was more effective than Protect-It®. There was also a significant concentration effect (concentration - $F = 22.78$, d. f. = 5, 55, $P < 0.0001$). There was no significant interaction between insecticide and concentration ($F = 0.93$, d. f. = 5, 55, $P = 0.47$) at the medium humidity level. At the low humidity treatment, there was a significant effect of the insecticide concentration on the number of F_1 adults (concentration - $F = 13.67$, d. f. = 5, 55, $P < 0.0001$), although there were no significant differences between NSA and Protect-It® in the effect (product - $F = 2.51$, d. f. = 1, 55, $P = 0.12$).

Table 2
Adult mortality of *R. dominica*, after 15 days of exposure to wheat treated with NSA and Protect-It®.

Species	Concentration (ppm)	Percentage of dead insects after 15 days of treatment with NSA			Percentage of dead insects after 15 days of treatment with Protect-It®		
		High	Medium	Low	High	Medium	Low
Rhyzopertha dominica	0	5 ± 2.2	5 ± 5	3.3 ± 2.1	3.3 ± 2.1	1.7 ± 1.7	8.3 ± 3.1
	62.5	8 ± 4.9	38.3 ± 6.0	41.7 ± 14.2	10 ± 4.5	16.7 ± 9.2	26.7 ± 4.9
	125	32 ± 18.2	68.3 ± 12.7	55 ± 12.3	5 ± 5	48.3 ± 9.8	41.7 ± 14.0
	250	30 ± 8.9	78.3 ± 8.7	93.3 ± 3.3	25 ± 15.4	58.3 ± 11.4	75 ± 11.5
	500	72 ± 3.7	96.7 ± 3.3	90 ± 3.6	78.3 ± 7.0	100 ± 0	96.7 ± 3.3
	1000	80 ± 7.1	96.7 ± 3.3	98.3 ± 1.7	100 ± 0	100 ± 0	100 ± 0

Table 3
Median lethal time values and regression curve parameters for NSA and Protect-It® at different ambient humidity levels, tested on *Sitophilus oryzae*.

Humidity	Product	Concentration (ppm)	LT50 (CI)	LT95 (CI)	Slope (SE)	Intercept (SE)	Goodness of fit Chi square/*P* value	Slope comparison	Intercept comparison
High	NSA	250	6.84 (6.28, 7.41)	10.90 (9.78, 12.77)	8.11 (0.98)	−6.77 (0.85)	2.32/0.31	**	**
High	Protect-It®	250	36.85 (22.85, 141.82)	185.80 (69.49, 4394.20)	2.34 (0.64)	−3.67 (0.73)	6.37/0.08		
Medium	NSA	125	6.05 (5.39, 6.76)	11.66 (10.06, 14.33)	5.77 (0.63)	−4.51 (0.53)	1.90/0.39	**	N.S.
Medium	Protect-It®	125	19.85 (15.08, 30.87)	79.93 (45.57, 251.26)	2.72 (0.50)	−3.53 (0.55)	6.44/0.08		
Low	NSA	125	4.60 (3.97, 5.30)	10.60 (8.69, 14.19)	4.53 (0.55)	−3.01 (0.40)	0.00004/126.39	N.S.	**
Low	Protect-It®	125	8.11 (7.02, 9.42)	19.35 (15.53, 26.95)	4.35 (0.53)	−3.96 (0.49)	0.60/0.74		

** indicates values within a humidity level and concentration that are statistically different at the 0.01 level.
N.S. indicates values within a humidity level and concentration that are not significantly different at the 0.05 level.

There was no significant interaction between insecticide and concentration ($F = 1.2$, d. f. = 5, 57, $P = 0.32$). As the concentration of the treatments increased the number of F_1 adults decreased.

3.4.2. *Rhyzopertha dominica*

The regression slopes describing the relationship between number of F_1 adults emerged and treatment concentration for both products were very similar (Table 5; Fig. 7). Yet, analysis-of-variance revealed that there was a significant effect of concentration in F_1 emergence (HH: $F = 4.42$, d. f. = 5, 42, $P = 0.0034$; MH: $F = 17.57$, d. f. = 5, 54, $P < 0.0001$; LH: $F = 14.98$, d. f. = 5, 55, $P = 0.25$), but there were no significant differences between the two insecticides on the number of F_1 adults emerged at any of the humidity levels tested (HH: $F = 0.42$, d. f. = 1, 42, $P = 0.52$; MH: $F = 0.27$, d. f. = 1, 54, $P = 0.61$; LH: $F = 1.36$, d. f. = 1, 55, $P = 0.25$).

4. Discussion

We observed a significant delayed mortality for both products at all concentrations tested, similar to results of other studies with insecticidal inert dusts (Subramanyam et al., 1998; Subramanyam and Roesli, 2000; Arthur, 2000, 2002; Athanassiou et al., 2003, 2004; Vayias and Athanassiou, 2004). The concentrations that induced significant mortality to *S. oryzae* and *R. dominica* in our study were comparable to those reported for other commercially available silica based inert dusts, such as silica gel and dust formulations including DE plus silica gel, and was similar to Protect-It®. The recommended rates for DE range from 60 to 5000 ppm (Subramanyam and Roesli, 2000) depending on the source of DE, type of formulation, and environmental conditions. The recommended rate for silica gel in wheat is 250 ppm (Cotton and Frankenfeld, 1949). The recommended dose for Protect-It® is 150 ppm for *S. oryzae*, 200 ppm for *R. dominica*, and 300 ppm for *T. castaneum*. The results from this study agree with these recommendations in the case of low ambient humidity. We found that NSA was as toxic as Protect-It® for *R. dominica*, and slightly more effective at the lowest concentrations, for *S. oryzae*. With regards to progeny, the NSA dust was more effective in eliminating F_1 adults than Protect-It®, for both species of insects tested. NSA reduced F_1 progeny drastically at concentrations as low as 62.5 ppm for *S. oryzae* for all humidity levels, and ranging from 250 ppm to 500 ppm for *R. dominica* depending on the humidity level. These results obtained with NSA are encouraging given that Protect-It® is one of the most effective DE based products in the market (Korunic and Fields, 1998; Subramanyam and Roesli, 2000).

The efficacy of inert dusts decreases as humidity increases and temperature decreases (le Patourel, 1986; Fields and Korunic, 2000). This is probably related to the fact that adsorption of cuticular lipids is slower and less effective under humid conditions (Ebeling, 1961). We also observed reduction in toxicity of NSA as ambient humidity increased. For example, concentrations as high as 500 ppm of NSA were needed to achieve over 50% mortality of *R. dominica* at high ambient humidity while over 80% mortality was achieved with 250 ppm at low ambient humidity. These results suggest that the mode of action of NSA may be very similar to that of other insecticidal dusts based on DE and silica, given the similarity of efficacy between Protect-It® and NSA. If NSA kills insects by adsorbing cuticular lipids, its greater efficacy may be related in part to its smaller particle size and greater surface to volume ratio, which may increase the area of contact between the particle and the insect (Chiu, 1939a, b).

Our results agree with literature data, showing that *R. dominica* is less susceptible to inert dusts than *S. oryzae* (Subramanyam and Roesli, 2000 and references therein). It has been reported that the difference in susceptibility may be due to the fact that *R. dominica* adults are slow moving, are larger than some of the common external feeders in stored grains, and are not as pubescent as the more susceptible species (Fields and Korunic, 2000; Subramanyam and Roesli, 2000). Interestingly, NSA was more effective in killing *S. oryzae* adults than Protect-It® but not adults of *R. dominica*.

Further studies are encouraged to determine whether the stoichiometry in the synthesis of NSA, the particle size, or the agglomerate structure are responsible for an increased efficacy of NSA in *S. oryzae*, and to shed light as to why *R. dominica* is affected similarly by both products. Moreover, NSA is a novel product, and additional efforts can be made on its synthesis and development to enhance it. NSA is based on a naturally occurring compound (Al); is not reactive or expensive and has reduced probabilities of

Table 4
Median lethal time values and regression curve parameters for NSA and Protect-It® at different ambient humidity levels, tested on *Rhyzopertha dominica*.

Humidity	Product	Concentration (ppm)	LT50 (CI)	LT95 (CI)	Slope (SE)	Intercept (SE)	Goodness of fit Chi square/*P* value	Slope comparison	Intercept comparison
High	NSA	250	6.54 (4.68, 13.75)	25.04 (12.45, 167.39)	2.82 (0.69)	−2.30 (0.4)	5.55/0.06	N.S.	N.S.
High	Protect-It®	250	10.24 (5.94, 40.31)	84.58 (6.33, 2678.23)	1.79 (0.46)	−1.82 (0.28)	3.50/0.17		
Medium	NSA	250	2.43 (1.0, 8.30)	7.26 (3.57, 2642.20)	3.46 (0.43)	−1.33 (0.21)	8.38/0.03	**	N.S.
Medium	Protect-It®	250	3.26 (2.36, 4.85)	11.60 (21.46, 68.18)	2.01 (0.34)	−1.03 (0.19)	3.28/0.15		
Low	NSA	250	1.44 (1.25, 1.65)	2.64 (3.18, 4.19)	4.76 (0.58)	−0.75 (0.18)	3.81/0.06	**	**
Low	Protect-It®	250	1 (1, 2.62)	251.02 (33.46, 451000)	0.67 (0.21)	0.02 (0.16)	1.69/0.43		

**indicates values within a humidity level and concentration that are statistically different at the 0.01 level.
N.S. indicates values within a humidity level and concentration that are not significantly different at the 0.05 level.

Table 5
Equation parameters (mean and SE) for equations where y = number of F_1 adult *S. oryzae* or *R. dominica* emerged after exposure of parents for 2 weeks to concentrations of 0, 62.5, 125, 250, 500 or 1000 ppm of Protect-It® or NSA (equations plotted in Figs. 5 and 6).

Species	Humidity	Product	*a*	*b*	R^2	Max attainable R^2
S. oryzae	Low[a]	NSA	20.70 (3.85)	62.63 (27.06)	0.376	0.379
		Protect-It®	36.86 (4.01)	62.41 (14.98)	0.638	0.638
	Medium[a]	NSA	81.34 (6.19)	49.55 (9.54)	0.789	0.792
		Protect-It®	87.82 (10.44)	139.11 (37.38)	0.577	0.582
	High[a]	NSA	212.88 (19.82)	51.44 (11.91)	0.692	0.717
		Protect-It®	227.19 (20.47)	370.78 (90.08)	0.596	0.675
R. dominica	Low[c]	NSA	6.36 (0.91)	−0.15 (0.09)	0.239	0.465
		Protect-It®	8.13 (1.19)	−0.25 (0.16)	0.295	0.518
	Medium[a]	NSA	61.66 (9.90)	290.50 (119.73)	0.2850	0.3295
		Protect-It®	99.58 (11.99)	143.99 (37.44)	0.590	0.591
	High[b]	NSA	63.38 (10.37)	−1.59 (0.57)	0.22	0.282
		Protect-It®	90.79 (15.91)	−2.79 (0.85)	0.257	0.343

Also shown are the possible maximum R^2 (Max R^2) for any equation fitted to the data, and R^2 values of the equations.

[a] Equation $y = a^{\left(\frac{-x}{b}\right)}$.
[b] Equation $y = a + bx^{0.5}$.
[c] Equation $y^{0.5} = a + bx^{0.5}$.

generating resistance in insects. However, nanostructured materials have raised toxicological concerns due to novel properties arising from their size (Borm et al., 2006; Scheufele et al., 2007). Therefore, further studies on the human health effects of NSA are needed before it can be considered for commercialization.

There are several drawbacks to the use of inert dusts in the grain and milling industry. DE dusts have been reported to affect the physical properties of the grain mass (reduce grain test weight and grain flowability) and to damage machinery due to its abrasive properties (Subramanyam and Roesli, 2000; Phillips and Throne, 2010). Although it is likely that these effects may also be observed with NSA, research is needed to test this. One way to minimize the adverse effects of inert dusts is to use a minimum amount that is still effective on insects. Thus, choosing a product

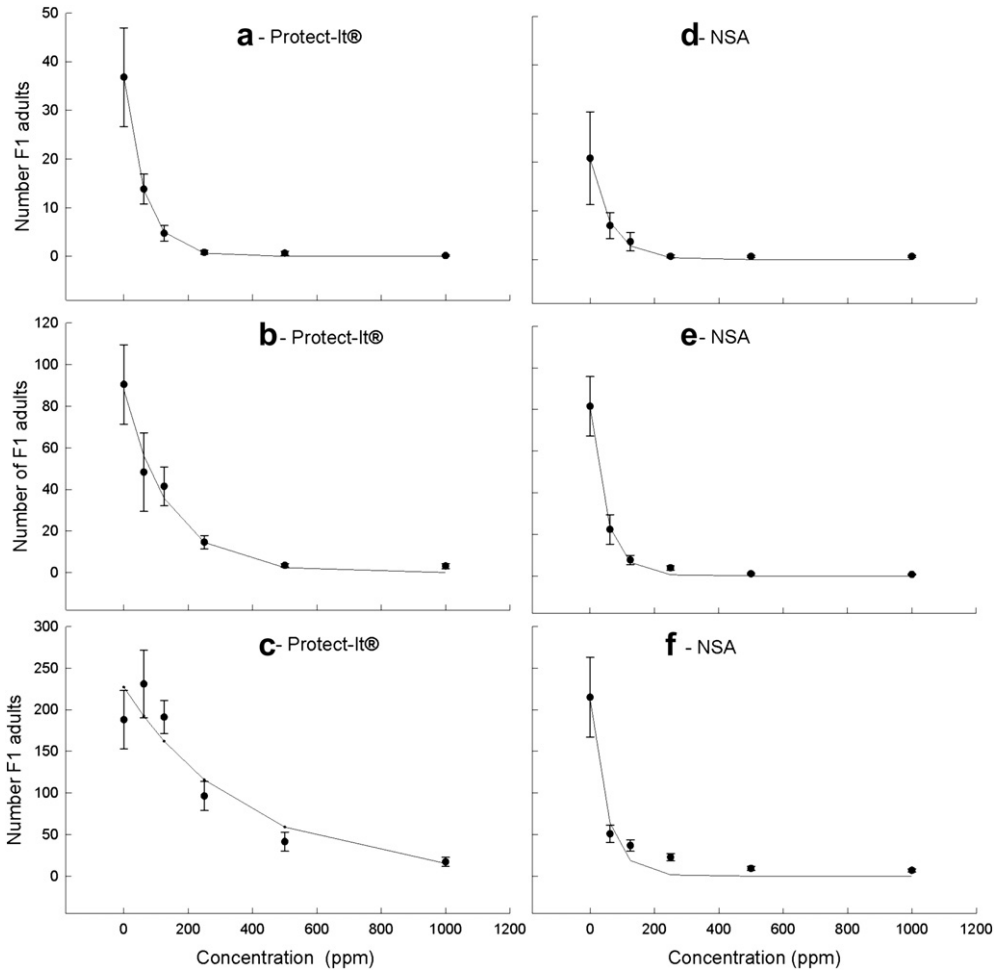

Fig. 6. (a–f) Number of *S. oryzae* F1 adults emerged after 60 days of treatment with 0, 62.5, 125, 250, 500, 1000 ppm of Protect-It® (a, b, c) or NSA (d, e, f). Wheat was held at 27 °C and 43% (a, d), 57% (b, e) or 75% r.h. (c, f).

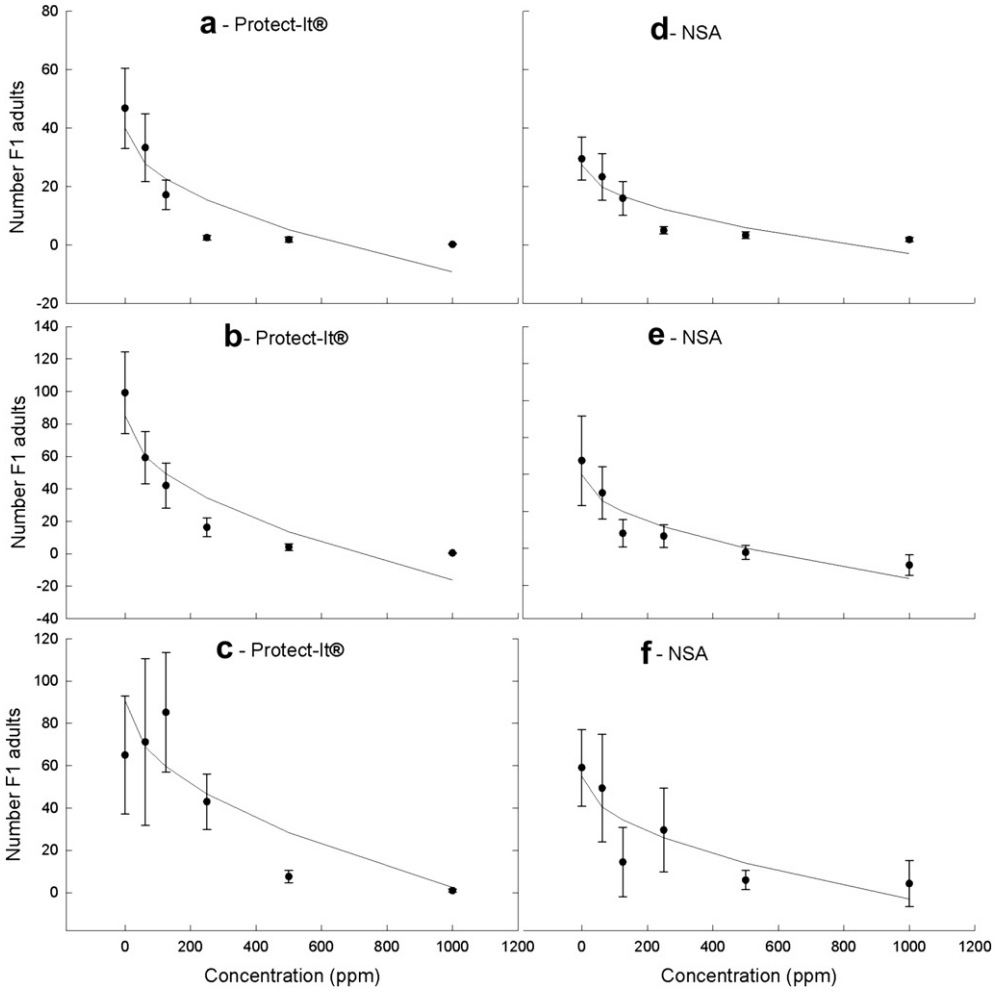

Fig. 7. (a–f) Number of *R. dominica* F1 adults emerged after 60 days of treatment with 0, 62.5, 125, 250, 500, 1000 ppm of Protect-It® (a, b, c) or NSA (d, e, f). Wheat was held at 27 °C and 43% (a, e), 57% (b, e) or 75% r.h. (c, f).

that is effective at lower rates is desirable. Furthermore, lower rates of these dusts could be achieved by using them as surface treatments and in conjunction with other control tactics (Vardeman et al., 2007).

Our results suggest that NSA might prove a good alternative to or as a combination with DE based products, and encourage further testing with other insect pests and food systems. Further studies addressing toxicity and mode of action of this product are encouraged to determine whether particle size, mineral composition, or structure are responsible for the increased efficacy of NSA and why *R. dominica* is affected more similarly by both products.

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