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Comparative insecticidal efficacy of five raw African diatomaceous earths against three tropical stored grain Coleopteran pests: *Sitophilus zeamais*, *Tribolium castaneum* and *Rhyzopertha dominica*

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Abstract

Five raw African diatomaceous earth (DE) samples collected from Tanzania, Zimbabwe, Zambia and South Africa were compared in laboratory bioassays at the Natural Resources Institute, UK and the University of Zimbabwe, to determine their potential as locally available grain protectants. A commercially available DE sample, Protect-It®, and an untreated control were included in the study for comparison. In UK, each sample was tested at two concentrations 2,500 ppm and 5,000 ppm, whereas in Zimbabwe an additional concentration of 1,000 ppm was included. These concentrations are higher than those at which commercial DEs are commonly used, but much lower than the concentrations at which farmers in sub-Saharan Africa (SSA) typically admix traditional protectants such as ash and sand with their commodities during storage. The DE samples were admixed with maize or wheat prior to addition of 40-50 14-28 day old insects, and adult mortality assessed after 7, 14 and 28 d of exposure at 27 °C and 55 or 60 % r.h. After the 28-day mortality count, all the adult insects were removed, and the grains retained under the same conditions for F1 progeny emergence assessment 7 weeks from experiments commencement. Mortality data showed highly significant ($p < 0.001$) differences between treatments and between concentrations.

F1 emergence was reduced by at least 72 % in most of the African DE treatments in comparison to the untreated control except when applied at 1,000 ppm. The DE samples were further analysed for crystalline silica content and only the Zimbabwean (Chemutsi) and the Zambian samples had crystalline silica contents of = 3 % compared to Protect-It® or Dryacide® which had = 1 %. These results suggest that if sufficient supplies of the African DEs with the low crystalline content could be easily accessed, then they could offer small-scale farmers in SSA an alternative and possibly cheaper grain protection option to the organo-phosphate-based pesticides many are currently using on their stored grain.

Key words: African diatomaceous earths, grain protection, bioassays, *Sitophilus zeamais*, *Tribolium castaneum*, *Rhyzopertha dominica*, maize, wheat, diatomaceous earths.

Introduction

Following the finding that the imported commercial diatomaceous earths (DEs); Protect-It® and Dryacide®, were effective grain protectants against insect damage for small-scale on-farm storage systems in Zimbabwe (Stathers et al., 2002a; b), further work to evaluate locally-

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occurring insecticidal fossil dusts was initiated. Local deposits of DE exist throughout sub-Saharan Africa (SSA) and may provide a more sustainable and low cost source of DE for grain protection in the region. However, DEs from different geographical locations exhibit differences in efficacy due to differences in physical properties and diatom species forming the DE (Korunic, 1998). Samples of DE from Kagera river, Tanzania; Zambezi valley and Beitbridge, Zimbabwe; South Africa and Zambia were collected and laboratory studies of their efficacy were conducted by Diatom Research & Consulting Inc. in Canada. Using Korunic's rapid assessment method (Korunic, 1997; 1998), none of them were classified as having high potential insecticidal efficacy in comparison to raw DE samples collected from China and other countries (Korunic, 2003a; b). However, given that farmers in sub-Saharan Africa frequently apply traditional protectants such as ash, sand and botanicals at concentrations far higher than the 1,000 ppm used in the rapid assessment study (Golob and Webley, 1980; Golob, 1997), it was decided to study the efficacy of these raw African DEs when applied at higher concentrations of 2,500 ppm and 5,000 ppm.

Materials and methods

The standard protocol for testing diatomaceous earths as described by Fields et al. (2003) was followed with slight modifications. Independent sets of experiments using the same DE samples were conducted, for comparison purposes, at the University of Zimbabwe (UZ) and at the Natural Resources Institute (NRI) in UK.

Test insects and grain

Three insect species, the maize weevil, *Sitophilus zeamais* Motschulsky; the lesser grain borer, *Rhyzopertha dominica* F.; and the rust red flour beetle, *Tribolium castaneum* (Herbst) were used at UZ laboratory while the NRI laboratory used *S. zeamais* only. The insects were reared on

pesticide free grain at 27 ± 2 °C, 60 ± 5 % or 55 ± 5 % r.h.; *S. zeamais* on whole maize, *R. dominica* on whole wheat and *T. castaneum* on 10 % kibbled wheat (by weight); sieved through a 1 mm sieve to remove flour. Uninfested, clean grain of known origin was preconditioned at 27 °C for at least two weeks before starting the experiment. Each replicate had 100 g of grain, with 40 or 50 unsexed 14-28 d old adults held in a 200 ml jar sealed with waxed filter paper. No Fluon (liquid Teflon) was applied to the interior necks of these jars as DEs have been found to stick to it.

Treatments

Five DE samples were tested. These included: a South African sample from Oasis pool products; a Zambian sample from Western Province; a Tanzanian sample from the Nyakanyasi rocks deposit of Kagera river; two Zimbabwean samples: one from Beitbridge deposit, Limpopo Valley and the other from the Chemutsi deposit, Zambezi valley; and a sample of the commercial product Protect-It® (Hedley Technologies Inc., Canada). All samples were coded to enable the tests to be run blind. Each raw DE sample was manually ground in a pestle and mortar, oven dried at 120 °C for two and a half hours, cooled and then sieved to provide particles of less than 100-150 µm.

The DE concentrations tested against *S. zeamais* in UK were 0, 2,500 and 5,000 ppm whereas in Zimbabwe, an additional DE concentration of 1,000 ppm was included against all the test insects. In the *R. dominica* experiment, Actellic Super Dust (ASD) (pirimiphos-methyl 1.6 % + permethrin 0.03 %) was also included as an additional treatment.

In the NRI laboratory, the appropriate weights of DE were added to 300 g of maize grain. The grain and DE were shaken in jars by hand for two minutes. After mixing the grain was divided into three 100 g samples, one for each replicate. In the UZ laboratory, treatment and admixing was per 100 g replicate rather. In all the experiments, untreated grain served as a negative control

(0 ppm) while Protect-It® was a positive control. Each treatment was replicated three times. Following the addition of insects to each jar, the jars were kept at 27 ± 2 °C and at 55 ± 5 % or 60 ± 5 % r.h. for the remainder of the bioassay.

Insect mortality and progeny assessments

After seven and 14 d the contents of each jar were carefully poured onto a tray and the numbers live and dead insects noted and all the contents then returned to each jar. After 28 d, the contents of each jar were carefully poured onto a tray and the number of live and dead adult insects noted and disposed of, and then grain and the DE were carefully returned to the respective jar and further incubated for offspring production. After seven weeks (dated from the beginning of the experiment), the grain in each jar was sieved and the insect progeny assessed.

Analysis for crystalline silica content

Occupational exposure to crystalline silica dust is associated with silicosis, a lung disease (Rosner and Markowitz, 1994; Hughes et al., 1998). Coded samples of all the raw African DE samples were therefore analysed for crystalline silica content using x-ray diffraction (XRD) phase identification analysis by Dr. Rosalind Schwarz of London & Scandinavian Metallurgical Co Limited (LSM) in UK. Total silica content was also determined using X-ray fluorescence analysis in the same laboratory.

Experimental design and data analysis

The main plot was a combination of a 6 (treatment) x 3 (concentration) factorial arranged in a completely randomised design with 3 replications, while the time factor was the subplot. Mortality data were analysed using the GENSTAT statistical package (Genstat Release 6.1, 2002). The data, minus the untreated control or synthetic insecticide (which were treated as dummy variables for the complete set of data), were subjected to split-plot analysis adapted from

the general analysis of variance (ANOVA), after square root transformation to stabilise variance, where necessary.

Results

The raw DEs showed great potential as grain protectants. Generally, efficacy increased with increasing concentration of DE and exposure period (Figures 1-4). Differences between treatments and between concentrations were highly significant ($p < 0.001$). The factors, treatment, concentration, time and their interactions were highly significant ($p < 0.001$) in the split-plot ANOVA except for *S. zeamais*, where the interactions treatment x concentration, and treatment x concentration x time were significant ($p < 0.05$) and non-significant ($p > 0.05$) respectively.

At concentrations of 2,500 ppm and 5,000 ppm and based on adult insect mortality assessments, the following ranking was derived in order of decreasing efficacy: against *S. zeamais* - Protect-It®, Zimbabwean sample from Chemutsi, Zambian sample, Tanzanian sample, Zimbabwean sample from Beitbridge, and South African sample; against *T. castaneum* - Protect-It® = Tanzanian sample > Zimbabwean sample from Chemutsi > Zambian sample > Zimbabwean sample from Beitbridge > South African sample; against *R. dominica* - ASD = Protect-It® > Zambian sample > Tanzanian sample > Zimbabwean sample from Beitbridge > Zimbabwean sample from Chemutsi > South African sample. *S. zeamais* was the most susceptible species followed by *T. castaneum* and lastly *R. dominica*. Of the raw DEs, only the Zambian and Tanzanian samples caused at least 80 % mortality at all concentrations *R. dominica*. All the DEs were highly effective against *S. zeamais* even at the lowest concentration of 1,000 ppm.

Insect progeny emergence was reduced by = 80 % for *S. zeamais*, = 72 % for *T. castaneum* and = 78 % for *R. dominica* after 7 weeks in all the African DE treatments at 2,500 ppm in

comparison to the untreated control (Figures 1-4). The exceptions were the Chemutsi, Zimbabwean sample against *T. castaneum* which had 66 % progeny emergence and South African sample on *R. dominica* which resulted in less than 30 % suppression even at the highest

concentration of 5,000 ppm. Differences were noted between the UK and Zimbabwean laboratory with higher mortalities and lower F1 emergencies being obtained in the latter (Figure 1 and 2).

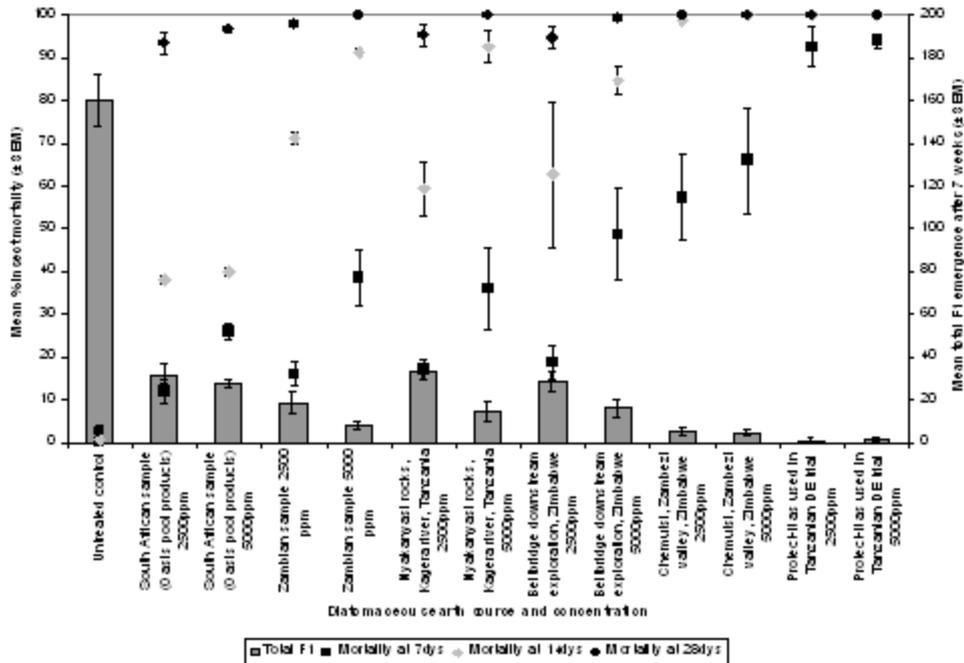


Figure 1. Effect of raw African diatomaceous earths admixed with maize grain on adult mortality and F1 emergence of 50 14-28 day old *Sitophilus zeamais* at 27 °C and 60% r.h, n=3, (NRI, UK).

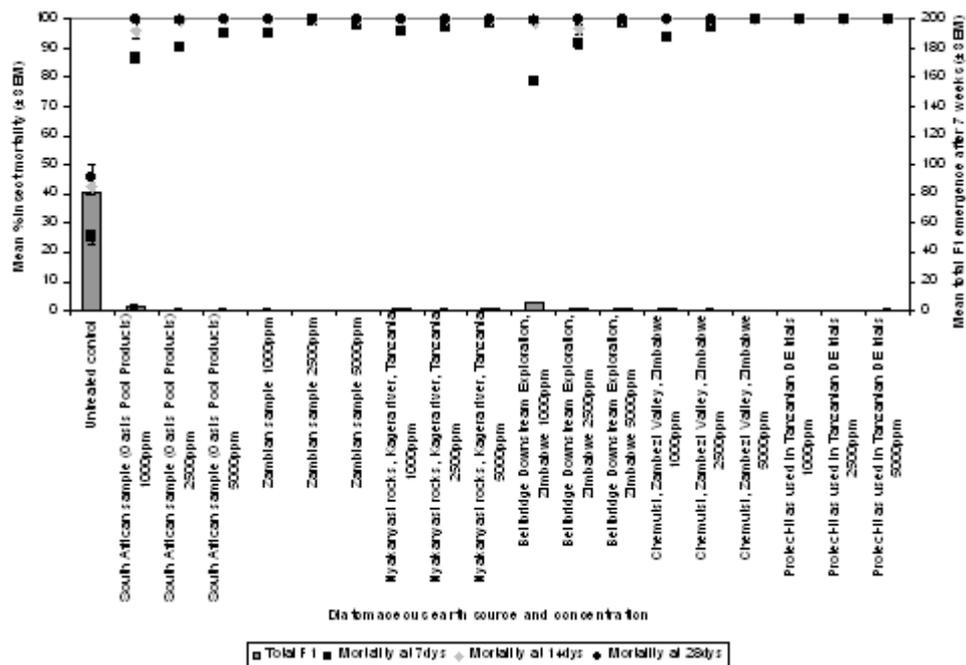


Figure 2. Effect of raw African diatomaceous earths admixed with maize grain on adult mortality and F1 emergence of 40 14-21 day old *Sitophilus zeamais*, at 27 °C and 55 % r.h., n=4 (UZ, Zimbabwe).

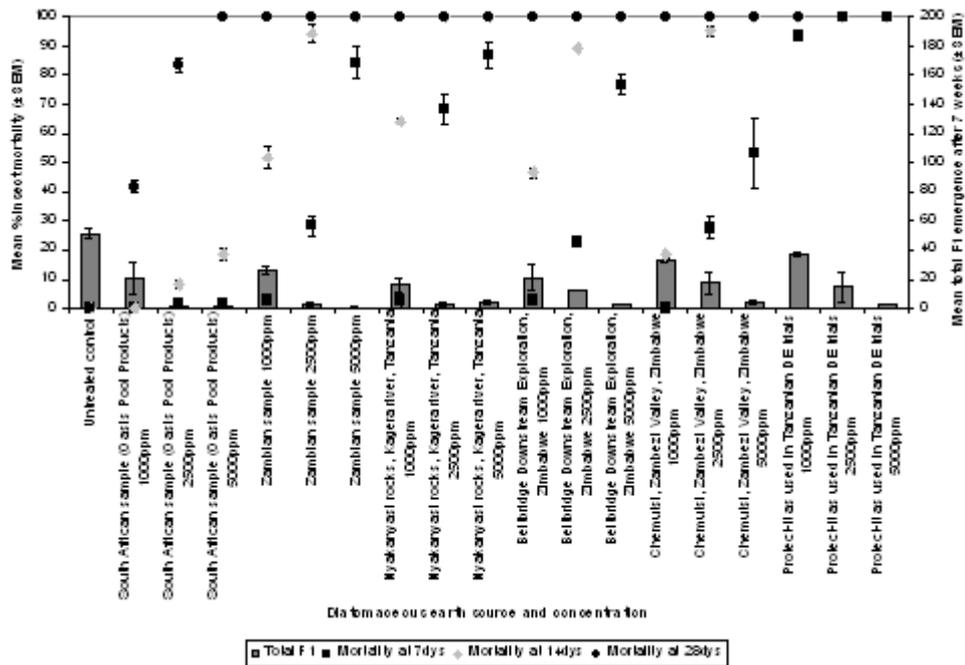


Figure 3. Effect of raw African diatomaceous earths admixed with maize grain on adult mortality and F1 emergence of 40 14-21 day old *Tribolium castaneum*, at 27 °C and 55 % r.h., n=3 (UZ, Zimbabwe).

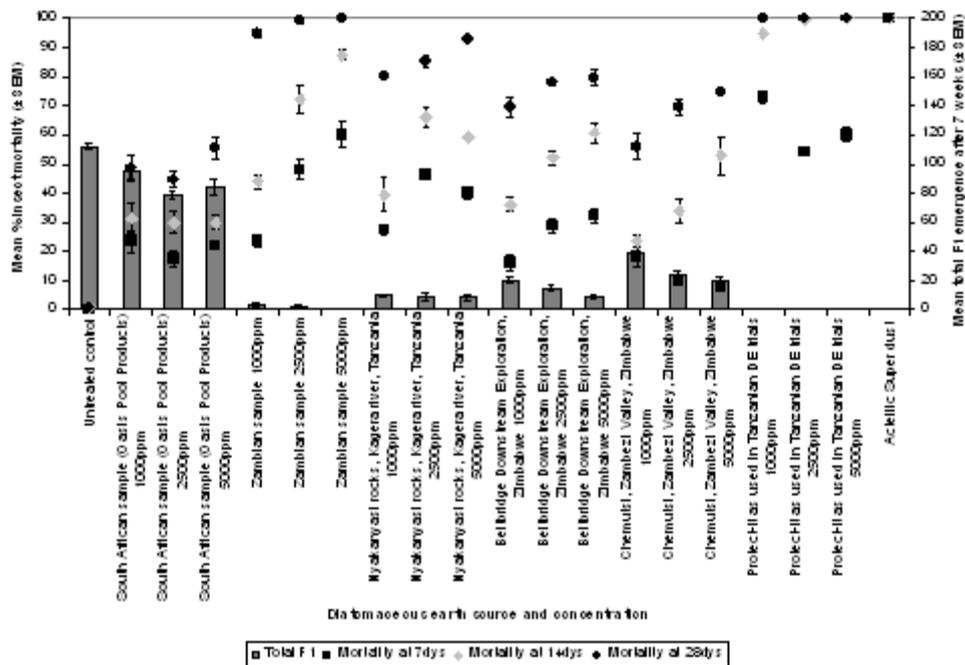


Figure 4. Effect of raw African diatomaceous earths admixed with wheat grain on adult mortality and F1 emergence of 40 14-21 day old *Rhyzopertha dominica*, at 27 °C and 55 % r.h., n=4 (UZ, Zimbabwe).

Comparison of the XRD analysis of the local African diatomaceous earth samples with the samples of commercial DE products, Protect-It® and Dryacide® shows that although the cristabolite, including opal contents of the local African DE samples are < 0.2 % and lower than those of the commercial DEs, the quartz contents of the local African diatomaceous earths were much higher (Table 1). Only the Chemutsi sample from Zimbabwe had a quartz content below < 1 %; all the other samples have quartz contents between 2.6 - 7.6 %, while the commercial DEs have quartz contents of < 0.5 %.

Discussion

The experiments demonstrated that the raw African DEs do have insecticidal potential if used at concentrations as high as 2,500 ppm. The potential can be enhanced through addition of silica aerogel as is the case for many of the registered commercial DEs. Korunic (2003a & b) suggested that all the African DE samples were unsuitable as grain protectants based on both the

rapid assessment method (Korunic, 1997) and actual bioassays at 1,000 ppm. These results were attributed to high levels of contamination found in the samples collected signifying that the samples were not collected from deep enough layers of the DE deposits. The insecticidal value of the DEs could have been higher if samples had been purer. In this experiment, it was not possible for the researchers to control the sampling process.

Differences in DE efficacy were observed between laboratories despite using similar protocols, same sources of DE samples prepared in one laboratory, and on the same commodities. This could be ascribed to differences in insect strains and in maize varieties used. A two-fold difference in susceptibility to DE between *T. castaneum* strains was reported by Rigaux et al. (2001). Grains have different physical and chemical characteristics which affect adherence of DE dust to the kernels and susceptibility to infestation. Grain type and variety (La Hue, 1972; Anthanassiou et al., 2003; Kavallieratos et al., 2005), grain hardness, presence of broken kernels (McGaughey, 1972; Nielsen, 1998) have all been implicated in determining DE efficacy.

Table 1. Silica content of the different samples of African diatomaceous earths.

Sample Code	Sample Identification	Quartz (% w/w)	Cristabolite, including opal (% w/w) ¹	Total crystalline silica (% w/w)	Total silica (% w/w) ²
Tz 03	Dryacide®	0.4	0.3	0.7	76.52
Tz 01 & Zw 02	Protect-It® as used in Tanzanian DE trials (two samples sent)	<0.5	0.4	<1	82.21
Zw 01 & Zw 05	Chemutsi, Zambezi valley, Zimbabwe (two samples sent)	<1	<0.2	<1.2	86.70
Zw 04	Zambian sample	2.6	<0.5	<3.1	85.43
Zw 03	Beitbridge Downstream Exploration, Zimbabwe	7.2	-	7.2	31.98
Tz 02	Nyakanyasi rock, Kagera River, Tanzania	7.6	<0.2	<7.8	76.30

¹ Because of line overlaps, cristabolite could not be distinguished from opal, particularly at low levels

² Includes amorphous, cristabolite, quartz and opal. The % amorphous would be the difference between the total silica and total crystalline silica.

-Because of a line overlap from anorthite, cristabolite could not be determined.

In concurrence with findings by other researchers that bostrichids are tolerant to DEs (Subramanyam et al., 1994; Korunic, 1998; Stathers et al., 2002a), *R. dominica* was the least susceptible insect species in the current study. The Tanzanian and the Zambian sample exhibited good potential against *R. dominica* but only at 5000 ppm and after a prolonged exposure of 28 d.

These results suggest that if these African DEs could be easily accessed in pure form, and the concentration of 2,500 ppm was acceptable to farmers, the Zambian, Tanzania and Zimbabwean (Chemutsi) samples could offer small-scale farmers in SSA a new and possibly cheaper grain protection option. However, only the Zimbabwean sample from Chemutsi had a crystalline silica content of <1.2% which is within the same range as that of the commercial DEs tested (< 1 % w/w) compared to the more than 7 % for the Tanzanian and Zimbabwean sample from Beitbridge. The Zambian sample is in between the two extreme with < 3.1 % (w/w). To the best knowledge of the authors of this paper, there are no formally agreed and internationally accepted safety limits for crystalline silica content of DEs applied as an admix on grain. Currently, many farmers in SSA rely on imported synthetic chemical pesticides for grain protection whose safety and efficacy is now being questioned. For example, in a national post-harvest survey of more than 2,000 farmers, 50 % were found to be using synthetic insecticides incorrectly (Mvumi et al., 1995).

Based on the results of these laboratory experiments and earlier work by Stathers et al. (2004), some of the DE samples were included in field trials conducted in Tanzania (Stathers et al., in prep.) and in Zimbabwe (Mvumi et al., in prep.) during the 2003/2004 and 2004/05 storage seasons. However, the ultimate uptake of the technology based on local DEs will depend on affordability, availability and government policy and regulations.

The impetus for research and development work on local deposits of DEs in Zimbabwe in particular and most likely in the rest of SSA will be driven by the brewing and beverage industries,

plastic and paint manufacturers, as well as the agrochemical industries. In Zimbabwe, DE demand in these industries has to date, been met by imports from Kenya, France and the USA (Tindwa and Sithole, 1988). Thus developing local deposits of DEs could result in considerable savings in scarce foreign currency in terms of imports of both commercial DEs and the active ingredients of synthetic grain protectants.

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