CONTROL OF *TRIBOLIUM CONFUSUM* J. DU VAL BY DIATOMACEOUS EARTH (PROTECT-IT™) ON STORED GROUNDNUT (*ARACHIS HYPOGAEA*) AND *ASPERGILLUS FLAVUS* LINK SPORE DISPERSAL

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ABSTRACT

Environmental and human health problems associated with the use of synthetic pesticides have prompted the demand for non-polluting, biologically specific insecticides. The current study assessed the losses caused by *Tribolium confusum* and its control by diatomaceous earth and the effect on *Aspergillus flavus* spore dispersal during storage of groundnuts. When losses due to *Tribolium confusum* were assessed over a period of 60 days, it was found that an increase in insect population in stored groundnuts resulted in increased weight loss of stored groundnuts. The true weight loss due to insect feeding was 0.60 g per 400 g of stored groundnuts. When diatomaceous earth (DE) was applied to groundnuts followed by the introduction of insects in a compartment (A), increased mortality of insects with increased diatomaceous earth concentration was observed. For a concentration range of 0-2.5 g DE/kg groundnut, 2.5 g/kg treatment was the most effective (only 5 surviving *T. confusum* adults out of 50 were recovered in samples treated with 2.5 g/kg compared to 38 adults in the control samples). Larval emergence from groundnuts treated with DE also declined with increased diatomaceous earth concentration. When groundnuts were inoculated with *A. flavus* spores, followed by DE application and *T. confusum* introduction into compartment A, the transfer of spores between inoculated groundnut samples in compartment A and uninoculated samples in compartment B was reduced. The mean *A. flavus* spore concentration recovered from initially sterile compartment B was 1.08×10^{3}; while it was 45 in the control and in samples treated with 2.5 g/kg dosage respectively. There was a significant difference in the mean numbers of spores recovered from groundnuts in different compartments (A, B) (H = 13.99, df = 4 and P = 0.007). Thus, from this study, losses due to *T. confusum* on groundnuts and fungal spore transfer in storage by this insect can be minimized by the application of diatomaceous earth (Protect-It™) to stored groundnuts.

Key words: Groundnut, *Tribolium confusum*, Diatomaceous earth

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INTRODUCTION

Storage of agricultural produce is part of the post-harvest system through which food material passes on its way from field to consumer. It is generally accepted that 5-15% of the total weight of all cereals, oilseeds, and pulses is lost after harvest [1]. There is a continuous need to protect the stored products against deterioration, especially loss of quality and weight during storage. Quantitative and qualitative losses of stored grains may result from the feeding and waste production by insects, mites, rodents and birds or from the growth of microorganisms all of which are influenced by environmental conditions. Insects, mites and fungi may cause hydrolysis and oxidation and decrease the level of certain nutrients in stored products or even form toxic substances such as mycotoxins. Mould contamination in stored groundnuts has been found to be closely associated with insect infestations [2, 3, 4, 5]. Controlling insects during storage may also contribute towards reducing spread of fungal spores within the storage system. The mean % incidence of *Aspergillus flavus* in insect damaged samples of wheat was much higher (87%) than in insect-free samples (25%) [6]. In damaged wheat kernels, *A. flavus* growth was observed inside the holes caused by the insects [6]. This report is in agreement with the findings on the migration of the mite *Tyrophagus putrescentiae* Schrank and its ability to disperse the toxigenic fungus *A. flavus* from artificially contaminated lots of maize grains [5]. Fungal growth was more marked in compartments containing grains inoculated with both mites and fungi than in compartments containing grains inoculated with fungi only [5]. This is indicative of the fact that mites constantly move within the storage site, and hence improving the dispersal of fungi and other microorganisms that are carried around on their body surfaces or delivered on their faeces.

When investigating the impact of grain pests on seed deterioration and aflatoxins production, fungal growth was observed on legs, antennae, at the joints and even over the whole body surface of insects [4]. In the same study, a decrease in insects, *Tribolium castaneum* Herbst and *Sitophilus oryzae* L., in *A. flavus* infected maize was reported [4]. The decline was attributed to the ability of *A. flavus* to modify a favourable insect growth environment into an unfavourable one. Various storage fungi, including *Aspergilli*, have been found to be valuable dietary supplements to insects found in storage [7, 8]. The insect mortalities may be due to aflatoxins in the contaminated grains [4].

On the contrary insects on mouldy grain may be able to metabolize toxic fungal metabolites [9]. For example, the fungus feeding *Carpohilus hemipterus* L. is unaffected by 25 ppm of aflatoxin B1 in the diet and is ten times more efficient in metabolizing the *Fusarium*-produced 4-monoacetxyscirpenol than non-fungus-feeding caterpillars. Insect damaged seeds tend to have higher levels of aflatoxins than undamaged seeds. The level of aflatoxin B1 on insect-damaged wheat samples varied from 113 to 1248 μg/kg while the two insect-free samples contained 85 and 113 μg/kg [6].

The use of pesticides is one of the ways of controlling storage insect pests. However, the choice of pesticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food. The continuous use of chemical pesticides for control of stored-grain pests has resulted in serious problems such as insecticide resistance [10]. Chemicals used for the control of stored product pests, or as protectants, need also to be compared with the suitability and effectiveness of alternative methods of control. Non-chemical methods are attractive since they neither leave chemical residues in the commodity nor do they cause resistance in insects. In recent years, awareness of the consequences of environmental pollution, the increasing cost of storage insecticides and the growing problem of insect resistance, has led pest management specialists to reappraise inert dusts. Inert dusts include silica aerogels, kaolin, sand and diatomaceous earths (diatomite).

Diatomaceous earths (DE) are the fossilized remains of diatoms, composed mainly of amorphous hydrated silica, but also other minerals including aluminium, iron oxide, magnesium, sodium and lime. Source of these dusts are either of marine or fresh-water origin; the former are said to be more effective [11, 12, 13]. Diatomaceous earths of marine origin are effective against storage insects at about 0.1% (w/w), but commercially available products are often enhanced by other compounds, e.g. ammonium fluorosilicate. Products of fresh-water origin require at least twice the dosage to produce similar levels of insect control [11].

Unlike conventional contact insecticides, DEs like other inert dusts function through their physical properties and are, therefore, generally slower acting [11]. Insect mortality is induced primarily as a result of desiccation. Water loss is a consequence of the destruction of the wax cuticle [12, 13]. Although DEs, having small dense particles of silicon dioxide, were said to abrade the cuticle, they also function by adsorbing the wax [14]. The action of DEs is not dependent on metabolic pathways and therefore, it has been postulated that insects will not be selected genetically by the action of these dusts, leading to physiological resistance. Nevertheless, it may be possible for insects to develop a behavioural response to the dust and avoid contact [11].

Another advantage of DEs over conventional insecticides is their low mammalian toxicity. In the USA, diatomaceous earths are 'Generally Recognised as Safe' by the US Food and Drug Administration and are registered for use as food additives [14].

Studies on the effectiveness of diatomaceous earths have been reported. Insecto, a diatomaceous earth formulation containing 86.7% armophous silica (silicon dioxide), 3% crystalline silica; and 10% food grade additives was shown to effect total mortality of 1st instars of *T. castaneum* when applied to maize, *Zea may*s L. at 0.5 and 1 g/kg rates. At the same rates of Insecto, mortality of *Oryzaephilus surinamensis* L. and *Plodia interpunctella* Hübner 1st instars was 96-97 and 86-97% respectively [15]. Complete suppression in emergence of *T. castaneum* and *P. interpunctella* adults at 1 g/kg Insecto application rate and 98% emergence of *O. surinamensis* have been

reported [15]. The efficacy of Protect-It (Mississauga, Ontario, Canada) formulation of diatomaceous earth (DE) to control internal infestations of rice weevil and maize weevil has been reported [13]. The mortality of *S. oryzae* and *Sitophilus zeamais* Motschulsky emerging from kernels in wheat treated with DE was always greater than controls, and ranged from 56 to 90% at 22^0C and was > 90% at 27 and 32^0C [13]. Diatomaceous earth product Fossil Shield® was shown to be lethal to adult *Tenebrio molito*r L. and *Tribolium confusum* J. du Val [16]. Fossil Shield® was lethal to 1st instar larvae of *P. interpunctella*, but not lethal to older larval stages, while two-week old larvae of *T. confusum* were more sensitive to diatomaceous earth than *P. interpunctella* at the same age [13]. Contact with diatomaceous earth caused adult *Sitophilus granarius* L. to lose weight and reduced their water content, suggesting disruption of 'the water barrier' [16]. Increased mortality of *T. castaneum* and *T. confusum* with increased temperature and exposure interval to diatomaceous earth (Protect-It) has been reported [12]. On- farm trials in Buhera and Binga districts in Zimbabwe demonstrated that maize can effectively be protected from insect damage by admixture with either Protect-It or Dryacide at 1 g/kg of grain [17]. The application diatomaceous earth (Protect-It) to commodities already infested with internal feeders, such as *S. oryzae* and *S. zeamais*, could help eliminate or suppress the infestation [13].

Work on the use of diatomaceous earth to control stored product insect pests and hence suppress fungal spore dispersal is non-existent in Botswana. This fact forms the basis of the research reported here.

MATERIALS AND METHODS

Groundnut samples
Fifty kilograms of shelled, sound, and mature kernels of groundnuts, variety Nyanda were purchased from Botswana Agricultural Marketing Board and used in this study.

Rearing of Insects
One hundred (100) of *Tribolium confusum* adult insects selected previously as the most predominant species in groundnuts were introduced into 6 aquarium boxes (30 x 20 x 20 cm) containing 800g of standard medium (maize/sorghum/glycerine, 8:8:1, w/w/w) [18], for oviposition. The rearing boxes were covered with muslin cloth for aeration. The cultures of *T. confusum* boxes were kept at room temperature range 17.5–26.5^{o}C and 45–72% relative humidity. The ambient readings were taken using a thermohygrograph (Isuzu, Seisakusho Co. Ltd, Japan) in the Insectary of the Department Biological Sciences of the University of Botswana. After four weeks, the introduced adults *T. confusum* were removed using an aspirator to allow for the development of only the newly hatched larvae. Newly emerged adults (one month old) were used in subsequent experiments.

Experimental set-up
Plastic lunch boxes (220 x 110 x 65 mm) with two compartments and a single cover for both compartments were used in the study. The covers were perforated using a

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hardened Entopin (38 x 0.55 mm, Newey Goodman, England) to allow for aeration. The lunch boxes, plus the covers, were sterilized using 1% sodium hypochlorite solution and then wiped with paper towel that had been dipped in 70% alcohol. The two compartments of the lunch boxes were labeled as A and B. Experimental insects were introduced initially in compartment A and none in compartment B, to check migration from A to B.

Loss assessment

For the loss assessment studies, two separate lots of groundnuts (400 g each) were placed in each of the two compartments of the lunch boxes (compartment A and B). The lunch boxes were then kept in the Insectary of the Biological Science Department of the University of Botswana at temperature range 25-33°C and 51–72% R.H. for 2 weeks to allow for moisture equilibration. To determine the moisture content, the kernels were ground using a mortar and pestle and 2 g of the ground samples were dried at 105^{0}C overnight to a constant weight. The loss in weight was calculated as percent moisture. Water activity was determined using the Novasina Thermoconstanter (LABOTECH, Switzerland). The moisture content after equilibration ranged between 6.5 and 7% while the measured water activity ranged between 0.43 and 0.48. After equilibration, 50 newly emerged adults (one month old) of *T. confusum* were introduced in compartment A, four replications were made. Controls were also set up comprising four lunch boxes with 400 g of groundnuts per compartment and no insects were introduced. The lunch boxes were left undisturbed for two months in the Insectary. After 2 months (60 days), the contents of both compartments were separately sieved. The loss in weight of groundnuts for each compartment was calculated as the difference between the initial and the final weights of the groundnuts (taking into consideration the weight of the insects and the weight of the powdery residue).

Control of insects by diatomaceous earths

In the current study Protect-It™ (Hedley Technologies Inc, USA), and inert dust formulation containing 90% diatomaceous earth (DE) and 10% silica gel was tested for effectiveness to control *T. confusum*. The experimental procedures up to the introduction of *T. confusum* into compartment A were repeated. Sets of four replicates were treated as follows: The groundnuts in compartment A of the lunch box were thoroughly mixed with diatomaceous earth (DE) at the following concentrations: (i) 0.05% (w/w), (ii) 0.1% (w/w), (iii) 0.2% (w/w) and (iv) 0.25% (w/w); which are equivalent to 0.2g/400g, 0.4g/400g, 0.8g/400g and 1.0g/400g of groundnuts. Controls containing no DE were set up. Fifty newly emerged *T. confusum* adults (one month old) were introduced in compartment A of all lunch boxes including the controls and kept for two months in the Insectary. After two months (60 days), the number of live insects, larvae and dead insects were recorded. The amount residue formed due to insect feeding was also recorded.

The data were then subjected to ANOVA and Student-Newman-Keuls (SNK) method for pairwise comparison using Sigma Stat Version 2.03 (SPSS Inc. 1992-1997), with

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the different concentrations of DE as main effects and insect and larvae survival as the response variables.

Aspergillus flavus-Tribolium confusum interaction during storage

Groundnuts were sterilized by moistening with distilled water and autoclaving for 15 minutes at 121°C. This was followed by oven drying for 24 hours to eliminate moisture which affects the effectiveness of diatomaceous earth [11]. The experimental set-up was similar to the one described under control of insects by diatomaceous earths except that in addition to insect introduction and DE application to the compartment A, the groundnuts in compartment A were also inoculated with *A. flavus* spore suspension.

Fungal culture

Toxigenic strain BCA22-53141 of *A. flavus* was isolated from Core collection 522. The fungus was cultured on PDA at 25°C until sporulation occurred. Conidial suspension of about 1.1×10^5 conidia/ml; number of conidia counted using a haemocytometer in 0.01 Tween 80 aqueous solution. Eight milliliter was poured into a flask containing 500 g autoclaved white rice and maintained at 25°C for 15 days to mass produce the conidia. The mass culture on rice was air dried for 7 days and then ground to a powder using a grinder. Twenty-five grams of fungal formulation, milled rice and *A. flavus,* were applied to the groundnuts and shaken to evenly cover the groundnuts kernels. The inoculated groundnut kernels were distributed into compartment A of the lunch boxes and separately mixed with the different dosages of DE as specified earlier. Groundnut kernels in compartment B were not mixed with either DE or fungal formulation. Treatments were replicated four times. The controls comprised 1): groundnuts inoculated with fungal formulation plus diatomaceous earth and 2): groundnuts inoculated with fungal formulation plus insects all in compartment A. No diatomaceous earth, fungal formulation or insects were added to compartment B.

Insect infestation

Tribolium confusum adults (one month old) were sterilized by immersion in 0.6% sodium hypochlorite solution for 3 minutes and rinsed with sterile water three times to remove external fungal contaminants [19]. The insects were placed on filter paper and allowed to dry and only insects that recovered were used in subsequent experiments. Fifty active adults were placed into compartment A of each lunch box. The boxes were left undisturbed for two months (60 days) after which damage caused by *T. confusum* and fungal conidia dispersal were evaluated. At the end of the experiment, groundnut kernels from compartment B of each lunch box were examined for fungal contaminations and insect infestation.

Determination of beetles and fungal levels

At the end of the experiment, the groundnut kernels from the compartments of each lunch box were examined for insect contaminations. The contents of each compartment for all the treatments were sieved and the number of live insects, dead insects and live larvae were recorded. Groundnuts (10g) from each compartment of

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lunch box were ground and diluted in 90 ml of sterile distilled water. Serial dilutions up to 10^{-4} (in duplicates) were made and 1 ml of each dilution then plated on a Petri dish containing *Aspergillus flavus* and *parasiticus* agar (AFPA). The plates were incubated at 25^0C and examined after 4 days and plates that contained 15-30 colonies were counted and the results expressed as CFU per gram of sample [5]. The data were analyzed using the ANOVA procedure of the Sigma Stat version 2.03 (SPSS Inc. 1992-1997) with concentrations of diatomaceous earth as main effects and insect survival and fungal levels in the two compartments as response variables.

RESULTS

Loss assessment and insect distribution

Losses caused by *T. confusum* (adults and larvae) were also assessed. The distribution of the insect in the storage compartments at temperature range of 22-29°C and relative humidity (R.H.) range of 45-65% are given in Table 1. In the treatment where *T. confusum* adults were introduced in compartment A, the mean weight of powdery residue formed in compartment A (0.60±0.12g) was greater than its counterpart B (0.36 ± 0.02g). The mean numbers of *T. confusum* adults and larvae in compartment A were also greater than those recorded for the initially sterile compartment B (Table 1). The observed loss in weight in the control treatment (groundnuts only) was only attributable to loss of moisture by the groundnut kernels.

Control of *T. confusum* by diatomaceous earth (Protect-It™)

Groundnuts were treated with different concentrations of diatomaceous earth (DE) before inoculating them with *T. confusum*. The average number of live *T. confusum* adults surviving decreased with increased concentration of DE (Table 2). For example, in samples treated with 2.5 g/kg DE only 5 surviving insects were recovered from compartment A compared to 38 insects in the control samples. The number of *T. confusum* adults reaching the initially sterile compartment B decreased with increased DE concentrations; from approximately 14 *T. confusum* adults in control samples to approximately 2 *T. confusum* adults at 2.0g/kg DE concentration. No *T. confusum* adults were recorded in compartment B of the 2.5g/kg DE concentration.

The number of dead *T. confusum* adults also increased with an increased DE concentration. No dead *T. confusum* adults were recovered from compartment B of the control samples or compartment B of both 2.0g/kg and 2.5g/kg concentrations of DE. However, the number of dead *T. confusum* adults in compartment A of both DE concentrations 2.0g/kg and 2.5g/kg were approximately 37 and 45, respectively. At 2.5g/kg concentration, no larvae were recovered from both compartment A and B as compared to the compartments A and B of the untreated control, from which approximately 42 and 36 *T. confusum* larvae in compartment A and B, respectively were recovered. In general, the number of larvae declined with increased DE concentration.

Analysis of variance revealed significant difference in the numbers of both live and dead insects due to different application rates of DE ($F = 1.08$, $P < 0.001$ for live insects and $F = 4.48$, $P < 0.001$ for dead insects).

Effect of DE on the dispersal of *A. flavus* spores by *Tribolium confusum*

The efficacy of diatomaceous earth (DE) in controlling *T. confusum* in stored groundnuts and its effect on *Aspergillus flavus* spore dispersal was investigated. In general, groundnut samples from compartment A (initially inoculated with *T. confusum*, *A. flavus* with no DE applied) and compartment B (to which *T. confusum* subsequently dispersed) presented greater mean numbers of live *T. confusum* adults and *A. flavus* spores compared to compartment A and B of samples into which DE was applied. For example, at 0.0g/kg DE, the mean number of *T. confusum* adults recovered from compartment B was approximately 42 compared to approximately 0 number of adults recovered from compartment B of samples containing 2.5g/kg DE concentration and the corresponding mean *A. flavus* spore concentration 1.08×10^3 for 0.0g/kg (control) concentration and 45 for 2.5g/kg DE concentration (Table 4.11). At every DE concentration level, the mean number of *A. flavus* spores in compartment A was significantly greater than of its counterpart B (0.0% (w/w): $t = 11.82$, $df = 6$, $P =< 0.001$; 0.05% (w/w): $t = 10.26$, $df = 6$, $P =< 0.001$; 0.1% (w/w): $t = 27.42$, $df = 6$, $P =< 0.001$; 0.2% (w/w): $t = 12.22$, $df = 6$, $P =< 0.001$; 0.25% (w/w): $t = 5.92$, $df = 6$, $P =< 0.001$). Kruskal-Wallis ANOVA on ranks analysis of mean numbers of *A. flavus* spores in compartment B of the different DE concentrations revealed a significant difference between the treatment means ($H = 13.99$, $df = 4$ and $P = 0.007$). Table 3 shows the mean number of *A. flavus* spores recovered from groundnuts which had been treated with different concentrations of DE.

DISCUSSION

Assessment of losses caused by *T. confusum* on stored groundnuts

In the present study on loss assessment in groundnuts, the number of *T. confusum* adults and larvae increased during the storage period in groundnut samples containing *T. confusum*, illustrating the rapid proliferation potential of this species on groundnuts. No *T. confusum* adults or larvae were detected in the control samples containing groundnuts only, confirming the absence of naturally occurring beetles or eggs of beetles in the groundnut kernels selected for investigation, as well as the absence of accidental contamination with beetles from external sources during the study period.

The numbers of adult *T. confusum* and larvae in the initially sterile compartment B were always lower than those recorded for the compartment where they were introduced (compartment A) (Table 1). Similar observations were made for the mite *Acarus siro* L, which, once settled in an appropriate substrate, start to seek new potentially nutritional sites whenever the original population has attained critical level [5]. This is a slow process [5]. In the present study, the lower population densities of *T. confusum* in compartment B as compared to compartment A show that, the nourishing content of the initial substrate in compartment A had not been fully used

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up for the period of study (2 months). If the experiments had been conducted for longer storage period, the numbers of *T. confusum* in compartment A would probably decrease in comparison with those in B.

Increased weight loss and damage caused to groundnuts were related to the adult and larvae populations in the current study (Table 1). The residue due to insect feeding obtained from the two compartments corresponds to the different insect populations found in the two compartments. A greater insect population in compartment A resulted in an increased loss in weight of groundnuts compared to compartment B where *T. confusum* population was smaller. The observed correlation between insect numbers and increased weight loss in the groundnut samples agrees with other observations [20]. When *P. interpunctella* was reared on groundnuts, the weight loss of infested groundnuts was directly correlated with the total number of emerged adults [21]. Losses caused by insect pests of stored products have been assessed [22, 23].

Control of *T. confusum* by diatomaceous earth (Protect-It™)
In recent years, awareness of the consequences of environmental pollution, the increasing cost of storage insecticides and the growing problem of insect resistance conventional insecticides have lead to pest management specialists reappraising inert dusts. Unlike conventional contact insecticides, inert dusts function through their physical properties and are, therefore, generally slower acting [11]. Insect mortality is induced primarily as a result of desiccation. Water loss is a consequence of adsorption of waxy particles from the cuticle surface. In the current study, Protect-It™ (Hedley Technologies Inc., USA), an inert dust formulation containing 90% diatomaceous earth (DE) and 10% silica gel was tested for effectiveness to control *T. confusum*. The results of the study showed that adult mortality of *T. confusum* increased with increased application rates of DE (Table 2). For example, in samples treated with 2.5 g/kg DE only 5 surviving insects were recovered from compartment A compared to 38 insects in the control samples. The number of *T. confusum* adults reaching the initially sterile compartment B decreased with increased DE concentrations; from 14 *T. confusum* adults in control samples to approximately 2 *T. confusum* adults at 2.0g/kg DE concentration. No *T. confusum* adults were recorded in compartment B at 2.5g/kg DE concentration. Generally, the number of live insects, live larvae and dead insects in compartment B of the lunch boxes containing groundnuts were lower than those recorded for the compartment where DE was applied to groundnuts infested initially with *T. confusum* (compartment A) (Table 2). DEs have low mammalian toxicity and are 'Generally Recognized as Safe' by US Food and Drug Administration and are registered for use as food additives. The current dosage levels utilized are within the safe limits.

Various studies on the efficacy of inert dusts have been reported [12, 13, 14, 15, 17, 24, 25]. Attapulgite-based clay dust was shown to control *Corcyra cephalonica*, *T. castaneum* and *Caryedon serratus* when applied to groundnuts at 0.5% (w/w) [24]. Activated kaolin clays (aluminium silicate) have been shown to be effective against a range of stored product beetle pests [25]. A relatively new diatomaceous earth of marine origin, Insecto, has been extensively assessed in the USA [14]. Dosages up to

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0.15% (w/w) gave complete mortality of *T. castaneum* adults [14]. The effectiveness of Insecto applied to shelled maize against various stored-product insect larvae has been investigated. For example, all 1st instars of *T. castaneum* were killed at 0.5 and 1 g/kg rates of Insecto and at these rates; mortality of *O. surinamensis* and *P. interpunctella* 1st instars was 96-97 and 86-97%, respectively [15]. Diatomaceous earths are extremely effective and persistent grain protectants against the major insect storage pests attacking sorghum, maize and cowpeas [17]. Damage levels remained low in all grains treated with 0.5% (w/w) and 0.2% (w/w) of Protect-It™ in contrast to the untreated controls [17].

When evaluating the effects of temperature and relative humidity on the toxicity of diatomaceous earth (Protect-It™) on *T. castaneum* and *T. confusum*, it was reported that mortality of both *T. confusum* and *T. castaneum* after they were held one week, was greater than initial mortality for nearly all exposure intervals, indicating delayed toxic effects from exposure to diatomaceous earth [12]. It has been shown that adults of *S. oryzae* and *S. zeamais* emerging from infested kernels of wheat are susceptible to Protect-It™ [13].

Effect of DE on the dispersal of *A. flavus* spores by *Tribolium confusum*
Groundnut samples from compartment A (initially inoculated with *T. confusum* and *A. flavus*) and compartment B (initially not inoculated with *T. confusum* and *A. flavus* but to which *T. confusum* subsequently dispersed) contained greater numbers of adult beetles and *A. flavus* spores compared to compartments A and B of samples into which DE was applied. The fact that *A. flavus* spores were recovered from initially sterile compartment B, coupled with isolation of *A. flavus* from *T. confusum* adults found in the same compartment confirms that insect pests carry fungal spores which may be transferred to other kernels [6]. On the contrary, the presence of DE led to insect mortality and hence fewer fungal spore recoveries from initially sterile compartment B of all treatments except control (Table 3). This is indicative of the potential of DE for use as an insecticide [12].

CONCLUSION

The present study has shown that *T. confusum* causes damage and losses to stored groundnuts and also disperses fungal spores. Diatomaceous earth (Protect-It™) was found to be effective against *T. confusum*. Thus applying environmentally benign diatomaceous earth to stored food commodities may help control infesting insects and also suppress fungal spore dispersal by insect pests.

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Table 1: Distribution of *T. confusum* in lunch boxes and weight loss caused

Condition	Control*		Groundnuts + *T. confusum*#	
	Compartment A	Compartment B	Compartment A	Compartment B
Initial wt .(Wi) (g)	400.35±0.12	400.21±0.09	400.22±0.10	400.27±0.07
Final wt. (Wf) (g)	400.17±0.15	400.06±0.12	399.59±0.09	399.91±0.15
Wt. Loss due to moisture loss	0.18±0.04	0.15±0.05	a	a
% Moisture loss	0.04	0.04	a	a
Apparent wt. Loss (insect feeding)	-	-	0.63±0.06	0.37±0.08
True wt. Loss (insect feeding) (Wf- Wi)X¶	-	-	0.60±0.12	0.36±0.02
No. live *T. confusum* (adults)	-	-	38 (25-54)b	14 (0-23)
No. live *T. confusum* (larvae)	-	-	42 (23-72)	36 (0-77)
N = 4				

*No insects (*T. confusum*) in control
#50 *T. confusum* adults initially introduced; N = number of replicates.
[a]Weight loss due to loss of moisture in (groundnut + *T. confusum*) treatment was not determined because the loss in weight was also combined with loss due to insect feeding; a moisture correction factor had to be used to calculate the true weight loss due to insect feeding
¶$_X$ = 0.96 (moisture correction factor)
[b]Numbers in parentheses denote ranges

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Table 2: Mean number *T. confusum* after treatment with DE[#]

Dosage (g/kg)	Mean no. live *T. confusum* adults ± S.E.*		Mean no. dead *T. confusum* adults ± S.E		Mean no. live *T. confusum* larvae ± S.E.	
	A	B	A	B	A	B
0.0	38a (25-54)[¶]	14 (0-23)	1a (0-4)	0	42 (23-72)	36 (0-77)
0.5	27ab (16-32)	6 (2-8)	17b (11-24)	0 (0-2)	5 (2-8)	0 (0-2)
1.0	19bc (13-28)	6 (0-13)	22b (18-27)	0 (0-2)	1 (0-5)	0
2.0	12cd (6-17)	2 (0-7)	37c (28-44)	0	3 (0-6)	0
2.5	5d (1-13)	0.00	45d (37-49)	0	0	0

N = 4

[#]50 *T. confusum* adults were initially introduced into compartment A; N = number of replicates.
[¶]Figures in parentheses indicate ranges
*Means within a column for each variable followed by the same letter are not significantly different (P<0.05) Student-Newman-Keuls method for multiple comparison used
A = Compartment A; B = Compartment B

Table 3: Mean number of *T. confusum*[#] adults and A. flavus spores recovered from compartment A and B

Dosage (g/kg)	Mean no. live *T. confusum* adults ± S.E.			*A. flavus*[¶] (CFU/g) ± S.E.	
	A	B		A	B
0.0	27	42		1.42×10^4 ±1100	1.08×10^3 ±110
0.5	32	6		1.23×10^4 ±1180	1.25×10^2 ±20.00
1.0	35	1		1.33×10^4 ±479	1.25×10^2 ±28.90
2.0	22	1		9.25×10^3 ±750	75.00 ±39.26
2.5	5	0		7.50×10^3 ±1260	45.00 ±25.33

N = 4

[#]50*T. confusum* adults were initially introduced into compartment A; no insects introduced in compartment B

[¶]*Aspergillus flavus* spores initially introduced into compartment A

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