Efficacy of four diatomaceous earth formulations against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on cowpea

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Abstract

Laboratory tests were carried out to evaluate the efficacy of four diatomaceous earth (DE) formulations against the cowpea bruchid, *Callosobruchus maculatus* on cowpea. The tests were performed under uncontrolled laboratory conditions (27 - 32°C and 68 - 76% r.h.), by exposing 1 – 48 h old adults to cowpea seeds treated with Celite 209, DiaFil 610, Protect-It and SilicoSec each at four dose rates (250, 500, 1000 and 1500 mg/kg) and an untreated control. Adult mortality was assessed after 3 and 5 d exposure, while number of F1 progeny and percentage seed damage were assessed after 40 d. Test for germination capacity was conducted after 180 d. With all DE formulations adult mortality was significantly higher on treated cowpea than on untreated control and mortality levels increased with increase in DE dose rate and exposure period. With the exception of Celite 209, all DE formulations used at ≥ 1000 mg/kg caused 100% adult mortality after 5 days exposure. Progeny production and percentage seed damage were significantly reduced with increasing DE dose rates, but the former was not completely prevented in any of the treatments. In both treated and untreated cowpea >86% of the seeds germinated. This study showed that the four DE formulations were effective against *C. maculatus* on cowpea, but their efficacy varied in decreasing order Protect-It>SilicoSec> Celite 209 = DiaFil 610.

Keywords: diatomaceous earth, formulation, *C. maculatus*, cowpea, germination capacity

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an important food crop in tropical and subtropical countries especially in Central and West Africa and East Asia. It is estimated that global annual production is over 4.5 million tons; the bulk of cowpea production (40.5%) comes from the drier regions of northern Nigeria (Singh et al., 2002). The Cowpea grain is nutritious and inexpensive and is a source of cheap protein and minerals for both rural and urban consumers in many countries (Bamaiyi et al., 2006).Both the production and storage of cowpea are highly constrained by insect pests, and infestation and losses caused in particular by the bruchid *Callosobruchus maculatus* (F.) is the most serious problem for cowpea storage (Turaki, 2012). *Callosobruchus maculatus* is a primary grain beetle which is widely distributed throughout the world. In Nigeria alone, the dry weight loss due to *C. maculatus* exceeded 2900 tonnes each year. In some cases damage in terms of holes produced by adult emergence from seed can increase to 99% after 6 months of storage (Singh, 2005).

This situation requires the application of control measures in order to minimize the losses caused by this pest. Most attempts to control this pest relied on synthetic insecticides. The ubiquitous issues of pesticide residue and pest resistance to pesticides are becoming increasingly challenging for society to solve (Mucha-Pelzer et al., 2010). Therefore, there is an increased need for alternative plant protection materials.

Diatomaceous earth (DE) products have proven to be suitable alternatives to chemical insecticide treatments and their insecticidal properties have already been established for stored product insect by numerous authors (Korunic, 1998; Arthur, 2000; Fields and Korunic, 2000; Subramanyam and Roesli, 2000; Athanassiou et al., 2003; 2007). Diatomaceous earth products are composed of microscopic fossils of diatoms. Insecticidal activity depends on the DE capacity to damage insects' cuticle and cause water loss from their bodies, so that they die of desiccation (Korunic, 1988). These products are attractive because they have very low mammalian toxicity, are inert, leave no toxic residues on grains, control the insecticide resistant pests and are long-lasting and are applied using the same technology for conventional grain protectants (Vayias et al., 2006; Athanassiou et al., 2007).

There are several commercially available DE formulations which have been successfully evaluated as grain protectants against a wide range of insect species (Subramanyam and Roesli, 2000; Stathers et al., 2004). The efficacy of DE products depends on several parameters, such as insect morphology, type of grains, DE physical parameters, temperature and relative humidity (Korunic, 1998). During the past two decades abundant literature on the efficacy of DE products to control stored product insects have been published. However, only few studies were devoted to *C. maculatus* despite the importance of cowpea and the destructive nature of *C. maculatus*. The purpose of this study was to evaluate the efficacy of four commercially available DE formulations against *C. maculatus* on cowpea.

2. Materials and Methods

2.1. Test insects

The test insects, *C. maculatus* came from laboratory cultures maintained on cowpea seeds at the Entomology Laboratory of the Department of Crop Protection, University of Maiduguri, Nigeria. For this study, new cultures were set up on the cowpea seeds (cultivar Borno Brown). Adults emerging from the cowpea seeds, aged 1 - 48h old were used in the experiments. During the experiments, temperature fluctuated between 28 and 32°C and r.h. ranged from 68 to 76%.

2.2. DE formulations

The DE formulations used in this study were Celite 209, DiaFil 610 Protect-It and SilicoSec. Celite 209(Celite Corporation, USA) is a marine DE containing 87 % SiO_2, 65.0% of particles <12 µm (Korunicand Fields, 2006; Saez and Funtes Mora, 2007). DiaFil 610 (Celite Corporation, USA) is a white fresh water DE containing 89 % amorphous silicon dioxide, 4.0 % Al_2O_3, 1.7% Fe_2O_3, 1.4% CaO, less than 1% of MgO and K_2O. The median particle size is 10 µm, and crystalline silica is >0.1 %. (Korunic and Fields, 2006). Protect-It (Hedley Technologies Inc., Canada) is an enhanced DE that contains 83.7% SiO_2 with 10% silica aerogel. The particle size distribution is between 5 and 6 µm (Fields and Korunic, 2000; Vayias et al., 2006). SilicoSec® is a formulation of diatomaceous earth including 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O. The average particle size is between 8 to 12 µm (Shayesteh and Ziaee, 2007).These DE formulations were obtained from Diatom Research and Consulting Inc., Canada.

2.3. Cowpea

The seeds of the cowpea cultivar Borno Brown was obtained from Teaching and Research Farm, University of Maiduguri, Nigeria. The seeds were cleaned and disinfested by fumigating with phosphine for 120 h and thereafter ventilated for 15 d in a plastic basin covered with muslin cloth to allow entire volatilization of the phosphine gas from the seeds.

2.4. Experimental procedure

The study was conducted under ambient laboratory conditions stated above. Seventeen lots of 200g cowpea were weighed and placed in 1l capacity bottles. One lot was kept as the untreated control. The other lots were treated separately with each of the DE formulations at dose rates of 250, 500, 1000 and 1500 mg/kg.DE was manually mixed thoroughly for 5min with the cowpea seeds. Each lot was divided into four parts of 50 g. One 50 g sample of each treatment combination and the untreated control was kept aside for germination test. The other three samples were separately placed in 150 ml capacity glass bottles to form three replications for each treatment combination. Into each of these jars 30mixed-sex *C. maculatus* adults, 1 - 48 h old were placed and covered with perforated lids. Adult mortality was assessed after 3 and 5 d. This was done by pouring the content of each bottle onto plastic tray gently avoiding loss or damage to eggs. Live and dead adults were counted and recorded. After the 5 d count, all adults were removed and discarded. The seeds were then returned to their respective jar and kept under the same conditions. After additional 35 d, the jars were opened and the following data were recorded: total number of F_1 adults in each jar. In each jar 50 seed were randomly taken and examined for exit hole. The number of seeds with exit holes were termed damaged seeds were expressed as percentage of seeds in the sample. The germination test was also carried out on seeds from different treatments and the untreated control that were stored for 180 d in the laboratory. One hundred and fifty seeds from each treatment were selected at random, divided into three batches of 50 seeds and placed in Petri dishes containing wet cotton. Thus three replications of each treatment and of untreated seeds (control) were set up. The percentage of germination was calculated after 7 d.

2.5. Statistical analysis

Data on adult mortality were first corrected for mortality in the controls using the Abbott's (Abbott, 1925) formula. The corrected mortality data and data on number of progeny, were arcsine-transformed and square toot $\sqrt{(x + 0.5)}$ transformed, respectively. These were then subjected to analysis of variance (ANOVA) using the GLM Procedure of Statistics 8.0 (Statistix Inc. 2005).Differences between treatment means were compared by Tukey- Kramer HSD test at $P \leq 0.05$.

3. Results

The main effects of DE formulation, dose rate and exposure interval on the mortality of *C. maculatus* adults were all significant (P<0.0001). Similarly, of the associated interactions, all were significant except for dose rate x exposure interval (P=0.150). Mortality of *C. maculatus* adults after 3 d of exposure to DE treated cowpea has been shown to increase with increase in DE dose rate (Fig. 1a). Significant differences were noted among dose rates within each DE formulation. Within this exposure interval, the lowest (15.5±4.0%) and the highest (100%) mortality levels were recorded on DiaFil 610 and Protect-It applied at 250 and 1500 mg/kg, respectively. Adult mortality was increased as exposure interval was increased from 3 to 5 d (Fig. 1b). After the 5 d of exposure to treated cowpea, all adults died on cowpea treated with SilicoSec and Protect-It applied at ≥1000 mg/kg and DiaFil610 at 1500 mg/kg. Even at the lowest dose rate of each of the DE formulations more than half of exposed adults died.

The main effects of both DE formulation and dose rate as well as their interaction were significant ($P \leq 0.0001$) for number of progeny and progeny suppression. The mean number of progeny in the untreated control (268.4±18.5) was significantly higher number than the numbers that developed on treated seeds. Progeny production was reduced by increasing DE dose rate. On treated cowpea seeds the lowest number of progeny and the highest progeny suppression were 3.0 and 98.9%, respectively (Table 1).

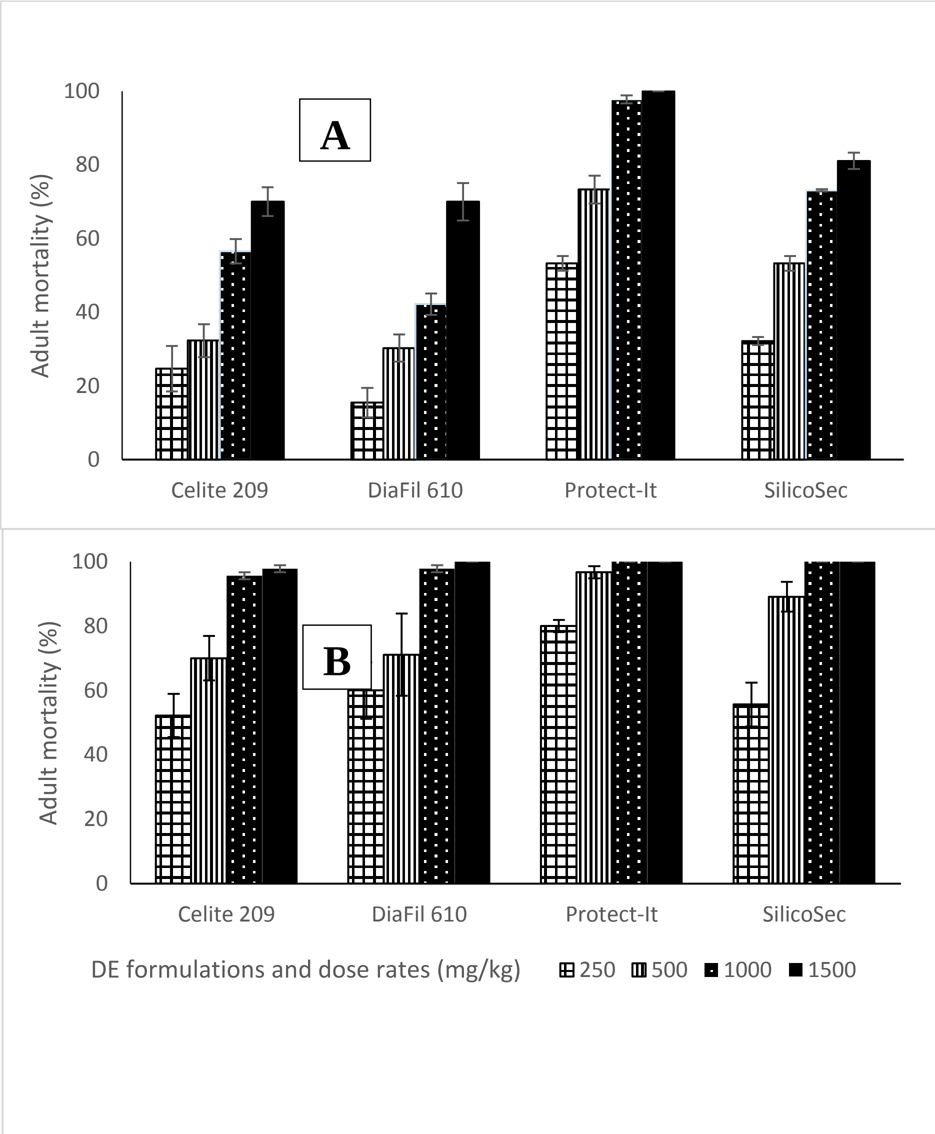

Figure 1 Mortality of adult *Callosobruchus maculatus* after three (A) and five (B) days of exposure to different dose rates of four DE formulations four.

Table 1 Mean (±*SE*) number of *Callosobruchus maculatus* F1 progeny produced and progeny suppression (%±*SE*) after 40 d on cowpea treated with four DE formulations at four dose rates. (Mean number of progeny in the untreated control was 268.4±18.5).

DE dose rate (mg/kg)	DE Formulation				F	*P*
	Celite 209	DiaFil 610	Protect-It	SilicoSec		
	Mean ±*SE* number of F1 progeny					
250	152.7±8.1aA	146.0±13.7aA	70.7±3.0aB	67.3±4.2aB	38.9	<0.0001
500	105.0±10.4bA	101.7±11.7abA	24.6±6.7bB	53.0±5.5aB	21.2	0.0004
1000	66.3±4.1cA	69.0±5.9bA	3.0±1.0cC	20.0±5.0bB	60.6	<0.0001
1500	14.3±1.2dA	12.0±1.7cA	3.7±0.0cB	3.3±1.3cB	16.2	0.0009
F	115	63.8	60.4	48.0	-	-
P	<0.0001	<0.0001	<0.0001	<0.0001	-	-
	Mean ±*SE* progeny suppression (%)					
250	43.1±3.0dB	44.6±6.0cB	73.7±1.1cA	74.9±1.5cA	26.5	0.0002
500	60.9±3.9cB	62.1±4.3bcB	90.8±2.5bA	79.3±2.4cA	19.3	0.0005
1000	75.3±1.5bC	72.3±2.2bC	98.9±0.4aA	92.6±1.9bB	51.1	<0.0001
1500	94.6±0.5aB	95.5±0.7aB	98.6±0.3aA	98.8±0.5aA	14.0	0.0015
F	96.1	43.3	49.1	45.2	-	-
P	<0.0001	<0.0001	<0.0001	<0.0001	-	-

(For number of progeny or progeny suppression, means within a column accompanied by same lower case letter and within row - upper case letter are not significantly different: Tukey Kramer-HSD test; *P* = 0.05)

Percentage of cowpea seeds damaged by *C. maculatus* was significantly affected by DE formulation and dose rate and their interaction (Table 2). The untreated control sustained significantly higher degree of damage (54.0±3.5%). In treated seeds damage decreased with increase in dose rate. Generally, with the exception of DiaFil 610, seed damage was <7% on seeds treated at 1000 or 1500 mg/kg. Protect-It and SilicoSec were more effective completely preventing seed damage when applied at 1500 mg/kg.

Table 2 Percentage seed damage of cowpea treated with four DE formulations at four doses after 40 days. (Mean percentage weight seed damage in the untreated control was 54.0±3.4%)

DE Dose rate (mg/kg)	DE Formulation				F	*p*
	Celite 209	DiaFil 610	Protect-It	SilicoSec		
250	32.3±3.0aA	31.7±2.9aA	15.3±1.5aB	19.7±0.8aB	14.4	0.0014
500	29.3±1.2aA	22.6±1.2aA	5.0±1.2bC	9.6±1.2bb	68.3	<0.0001
1000	6.3±0.9bB	13.7±1.2bA	0.7±0.3cC	3.3=0.6cB	32.0	0.0001
1500	6.3±1.2bA	4.0±1.0cA	0.0±0.0cB	0.0±0.0dB	25.4	0.0002
F	69.8	56.7	56.8	55.3	-	-
P	<0.0001	<0.0001	<0.0001	<0.0001	-	-

Means within a column accompanied by same lower case letters and within a row - upper case letter are not significantly different: Tukey Kramer-HSD test; P = 0.05)

Fig. 2. Illustrates the effect of four DE formulations on the germination capacity of cowpea seeds. Apparently, DE had no effect on the germination capacity of treated cowpea seeds stored for six months. The range of germination capacity was 92.0 to 96.0 % across seeds treated with different doses of the four DE formulations. In the untreated control the mean germination was 93.5±0.6 %. There were no significant difference (*P*>0.05) between the

untreated control and treated seeds or among seeds treated at any dose rates of the four DE formulations.

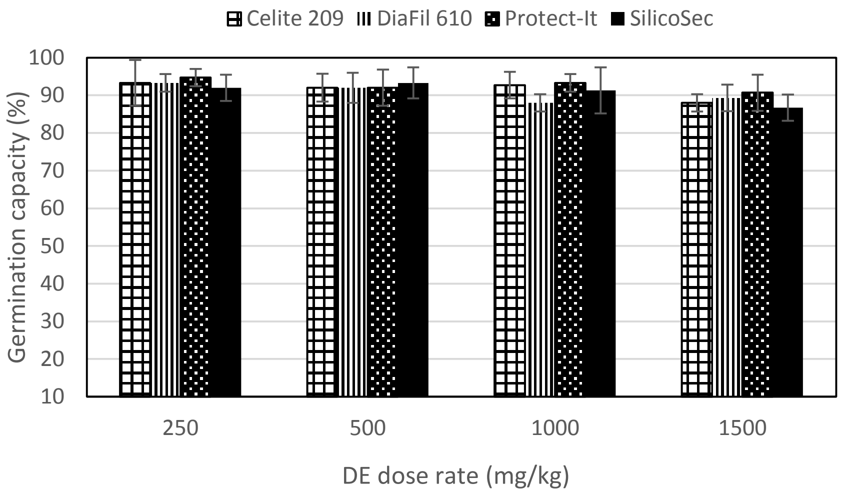

Figure 2 The effect of DE formulations and dose rates on germination capacity of cowpea seeds

4. Discussion

Several studies have documented the efficacy of DEs against several stored product insect species, but there were relatively few published works in respect of *C. maculatus* or efficacy on cowpea. The results presented in this study indicate that DEs are effective in controlling *C. maculatus* on cowpea. Adult mortality increased with increase in dose rate and exposure interval, as reported for other stored product insect species (Vayias and Athanassiou, 2004; Athanassiou et al., 2005; Korunic & Fields, 2006; Shayesteh and Ziaee, 2007; Vayias et al., 2009). In the present study efficacy against *C. maculatus* in terms of all the parameters measured with the exception of germination capacity, varied among DE formulations and were ranked in decreasing order was Protect-It>SilicoSec> Celite 209 = DiaFil.

It is known that DEs differ in species of diatoms (shape), origin (marine or freshwater), particle size distribution, SiO_2 content and so on. Hence, these properties of DEs influence their insecticidal activities (Korunic, 1997, 1998).In another study Kabir and Gaya (2013) found that Protect-It and SilicoSec had same effect on mortality of *C. maculatus* adults and progeny development. And in good agreement with the above results (Kabir, 2013) in a study involving *T. castaneum* on wheat, found that Protect-It was more effective than Celite 209 and DiaFil 610. Higher efficacy of Protect-It may be explained by the fact that it is an enhanced DE containing 10% silica aerogel and has smaller particle size (5 - 6 µ). Ulrichset al. (2006) confirmed that formulations with smaller particle sizes have greater ability to absorb lipids from the insect's epicuticle and thus increase insect mortality. Further, Vayias et al. (2009) reported that the efficacy of DEs is inversely related to their particle size. And the high efficacy of SilicoSec relative to Celite 209 and DiaFil 610 might be attributed to the high (92%) SiO_2 content.

In agreement with observations for adult mortality the number of emerged F1 progeny reduced in proportion with increasing DE dose rate. However, DE treatment could not completely prevent progeny production even where complete parental mortality were observed within five days. Similar observations were made in previous studies involving DE use against *C. maculatus* (Stathers et al., 2004; Kabir and Gaya, 2013). It appears this aspect is common to all internal feeders. For example development of few progenies in trials where complete or substantial parental mortality were observed, have been documented for a number of species: *Sitophilus oryzae* L. (Korunic and Fields, 2006, Nwaubani et al., 2014), *S. zeamais* Mots. (Ceruti et al., 2008), *Rhyzopertha Dominica* F. (Chanbang et al., 2008; Nwaubani et al., 2014), *Prostephanus truncatus* Horn (Stathers et al., 2004; Athanassiou et al., 2007). In the present study, even at dose rate of 1500 mg/kg, the four DE formulations did not prevent progeny emergence.

Our results and those of previous studies suggest that DEs may not have ovicidal effect and even at higher concentrations (1500 mg/kg) cannot prevent oviposition before the onset of death due to desiccation. However, this drawback may be compensated for by reduced fecundity and residual effect on subsequent generations as evidenced by effective progeny suppression (>95%) at the highest dose rate. Also previous studies report that DEs persist in the treated substrate, providing long-term protection against stored-products insect pests that is not possible with the use of residual insecticides (Athanassiou et al., 2005; Vayias et al., 2006). These assumptions, however needs to be verified in experiments involving residual activity over generations to observe whether there will be a downward trend in population. With *C. maculatus* progeny suppression is perhaps more important than adult mortality as the adults are short-lived, do not feed or cause damage but function only to lay eggs. The highest dose rate (1500 mg/kg) used in this study was above the labelled dose rate of 1000 mg/kg for grain commodities. The dose rate was increased in view of the high relative humidity during the wet season when the study was conducted. It has been shown that DE efficacy decreases with increase in relative humidity or grain moisture content (Arthur, 2000; Fields and Korunic, 2000; Stathers et al., 2004).The explanation being that at high relative humidity levels, insects moderate water loss and therefore their survival is increased after exposure in a DE-treated substrate (Fields and Korunic, 2000; Athanassiou et al., 2007). Nevertheless the present results suggest that DE products could be used to control *C. maculatus* under conditions of high relative humidity say 70 -75%, given the levels of adult mortality, progeny suppression and prevention of seed damage achieved. Diatomaceous earth has no adverse effect on the end use quality of treated commodities (Korunic et al., 1996; Shayesteh and Ziaee, 2007). This was confirmed by the absence of any significant effect on germination capacity noted in the present study. Therefore DEs could be ideal candidates for protection of stored cowpea seeds.

5. Conclusions

In conclusion, our result suggest that DEs could be effectively use against *C. maculatus* in stored cowpea. However, efficacy may vary between different formulations at dose rate of 1000 - 1500 mg/kg. This study allowed us to rank the DE formulations in decreasing order of efficacy against *C. maculatus*: Protect-It>SilicoSec> Celite 209 =DiaFil 610. Use of DE even at high dose rate could not completely eliminate F1 progeny production. Therefore additional experiments are required to evaluate residual efficacy on subsequent generations. As DEs do not have adverse effect on germination capacity they could be used with success to protect cowpea seeds.

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References

Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology 18, 265-267.

Arthur, F.H., 2000. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): effects of temperature and relative humidity. Journal of Economic Entomology 93, 526–532.

Athanassiou, C.G., Kavallieratos, N.G., Tsaganou, F.C., Vayias, B.J., Dimizas, C.B., Buchelos C.Th., 2003. Effect of grain type on theinsecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.)(Coleoptera: Curculionidae). Crop Protection 22, 1141–1147.

Athanassiou, C.G., Kavallieratos, N.G., Peteinatos, G.G., Petrou, S.E., Boukouvala, M.C.,Tomanovic,Z., 2007. Influence of temperature and humidity on insecticidal effect of three diatomaceous earth formulations against larger grain borer (Coleoptera: Bostrychidae). Journal of Economic Entomology 100, 599-603.

Athanassiou, C.G., Vayias, B.J., Dimizas, C.B., Kavallieratos, N.G., Papagregoriou, A.S.,Buchelos, C.Th., 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. Journal of Stored Products Research 41, 47-55.

Bamaiyi, L.J., Onu, I., Amatobi, C.I., Dike, M.C., 2006. Effects of *Callosobruchus maculatus* infestation on nutritional loss on stored cowpea grains. Archives of Phytopathology and Plant Protection 39, 119 -127.

Ceruti, F.C., Lazzari, S.M.N., Lazzari, F.A., Pinto-Junior, A.R., 2008. Efficacy of diatomaceous earth and temperature to control the maize weevil on stored maize. Scientia Agraria Curitiba 9, 73-78.

Chanbang, Y., Arthur, F.H., Wilde, G.E., Throne, J.E., 2008. Control of *Rhyzopertha dominica* in stored rough rice through a combination of diatomaceous earth and varietal resistance. Insect Science 15, 455-460.

Fields, P., Korunic, Z., 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. Journal of Stored Products Research 36, 1-13.

Kabir, B.G.J., 2013. Laboratory evaluation of efficacy of three diatomaceous earth formulations against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) in stored wheat. European Scientific Journal 9, 116-124.

Kabir, B.G.J., Gaya, S.S., 2013. Laboratory evaluation of grain protectant efficacy of two diatomaceous earth formulations against cowpea bruchid - *Callosobruchus maculatus* (F.) Nigerian Journal of Experimental and Applied Biology14, 56-61.

Korunic, Z., 1998. Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research 34, 87-97.

Korunic, Z., Fields, P.G., 2006. Susceptibility of three species of *Sitophilus* to diatomaceous earth. In: Lorini, I., Bacaltchuk, B., Beckel, H., Deckers, D., Sundfeld, E., dos Santos, J. P., Biagi, J. D., Celaro, J. C., D'A., Faroni, L. R., Bortolini, L. de O. F., Sartori, M. R., Elias, M. C., Guedes, R. N. C., da Fonseca, R. G. & Scussel, V. M. (Eds) Proceedings of the Ninth International Working Conference on Stored-Product Protection, 15–18 October, 2006, Campinas, Sao Paulo, Brazil. Brazilian Post-harvest Association, Campinas, Brazil.pp. 681-686.

Korunic, Z., Fields, P.G., Kovacs, M.I.P., Noll, J.S., Lukov, O.M., Demianyk, C., Shibley, K.J., 1996. The effect of diatomaceous earth on grain quality. Postharvest Biology and Technology 9, 373–378.

Mucha-Pelzer, T., Bauer, R. Scobel, E., Ulrichs, C., 2010.Insecticidal effects of different application techniques for silica dusts in plant protection on *Phaedon cochleariae* Fab. and *Pieris brassicae* L. Hortscience 45, 1349–1356.

Nwaubani, S.I., Opit, G.P., Otitodun, G.O., Adesida, M.A., 2014. Efficacy of two Nigeria-derived diatomaceous earths against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) on wheat. Journal of Stored Products Research 59, 9-16.

Saez, A., Fuentes Mora, V.H., 2007. Comparison of the desiccation effects of marine and freshwater diatomaceous earths on insects. Journal of Stored Products Research 43, 404-409.

Shayeateh, N., Ziaee, M., 2007. Insecticidal efficacy of diatomaceous earth against *Tribolium castaneum* (Herbst) Caspian. Journal of Environmental Science 5, 119-123.

Singh, B.B., Ehlers, J.D., Sharma B., Freire-Filho, F.R., 2002. Recent progress in cowpea breeding. In: Fatokun, C. A.; Tarawali, S. A.; Singh, B. B.; Kormawa, P. M.; Tamo, M. (Eds). Challengesand opportunities for enhancing sustainable cowpea production. Ibadan: IITA, p. 22-40.

Singh, S.R., Jackai, L.E.N., 1985. Insect pests of cowpea in Africa: their life cycle, economic importance and potential for control. In: Singh, S.R. and Rachie, K.O. (Eds). Cowpea Research, Production and Utilization. John Wiley and Sons, Chichester UK, pp. 217-231.

Stathers, T.E., Dennif, M., Golob, P., 2004. The efficacy and persistence of diatomaceous earths admixed with commodity against four tropical stored product beetle pests. Journal of Stored Products Research 40, 113–123.

Subramanyam, Bh., Roesli, R., 2000. Inert dusts. In: Subramanyam, Bh., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers, Dordrecht, pp.321–380.

Turaki, J.M., 2012. Simulating infestations and losses in storage from egg and adult *Callosobruchus maculatus* (Coleoptera: Bruchidae) sources in cowpea (*Vigna ungiculata* L. Walp.). International Research Journal of Agricultural Science and Soil Science 2**,** 333-340

Ulrichs, C., Entenmann, S., Goswami, A., Mewis, I., 2006. Abrasive and hydrophilic/lipophilic effects of different inert dusts used as insecticide against the stored insect pest *Sitophilus granarius* L. Gesunde Pflanzen 58, 173–181.

Vayias, B.J., Athanassiou, C.G., 2004. Factors affecting efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused beetle *T. confusum* du Val (Coleoptera: Tenebrionidae). Crop Protection 23, 565–573.

Vayias, B.J., Athanassiou, C.G., Kavallieratos, N.G., Tsesmeli, C.D., Buchelos, C.Th., 2006. Persistence and efficacy of two diatomaceous earth formulations and a mixture of diatomaceous earth with natural pyrethrum against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) on wheat and maize. Pest Management. Science 62, 456-464.