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Evaluation of insecticidal efficacy and persistence of Nigerian raw diatomaceous earth against *Callosobruchus maculatus* (F.) on stored cowpea

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DOI 10.5073/jka.2018.463.092

Abstract

The insecticidal efficacy and persistence of Nigerian raw diatomaceous earth (DE) were evaluated in the laboratory on cowpea against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). The raw DE was applied to 1.5 kg lots of cowpea seeds at 0 (untreated control), 250, 500, 750, 1000 and 1500 mg/kg, and a commercial DE formulation (Protect-It®) applied at 1000 mg/kg was included in the test as positive (treated) control. The treated cowpea seeds were kept under ambient laboratory conditions (26 - 34°C and 24 - 93% RH. Bioassays were conducted on samples taken from each treatment at the day of storage and every 30 d for 6 consecutive months. Adult *C. maculatus* were exposed for 3 and 5 d to the samples and adult mortality was assessed over this exposure interval and progeny production and seed damage were assessed after additional 30 d. On freshly treated cowpea, both the raw DE and Protect-It® were highly effective against *C. maculatus* causing 100% adult mortality following 5 d of exposure. In general, the raw DE was less persistent on cowpea providing complete adult mortality only for two months. Protect-It® on the other hand was stable over the 6-month period of storage causing 95.8 to 100% adult mortality. None of the treatments completely inhibited progeny production after 2-3-moths storage period. The results of this study indicated that Protect-It® may provide suitable protection for 6 months against *C. maculatus*, but the raw DE in its present state is not suitable for long-term protection against this insect pest.

Keywords: *Callosobruchus maculatus*, raw diatomaceous earth, cowpea, residual activity

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most economically and nutritionally important indigenous African grain legumes produced throughout the tropical and subtropical areas of the world (Abate et al., 2011). It is a source of relatively low cost, high quality protein, and for many West and Central African farmers a major cash crop (Langyintuo et al., 2003). As production and consumption do not occur simultaneously, producers and traders need efficient storage systems to ensure year round cowpea availability for consumers. Consumers, on the other hand, want to buy cowpeas at the cheapest cost without compromising quality characteristics (Ndong et al., 2012).

Nigeria is the largest producer and consumer of cowpea, accounting for about 45 percent of world's production (Lowenberg-DeBoer and Ibro, 2008) and a per capita consumption of 25 - 30 kg per year (Nurudeen and Rasaki, 2011). The major storage pest of cowpea in Nigeria is *C. maculatus* (Adedire, 2001). As a field to-store pest, the attack, which starts before harvest and intensifies during storage, may cause total losses (Faroni and Sousa, 2006). The damage by *C. maclatus* is caused by oviposition on the surface of grains or pod and subsequent larval penetration into the grains. The attack results in weight loss, nutritional value, reduced level of product hygiene (presence of droppings, eggs, and insects), reduced seed germination resulting in decreased retail value (Almeida et al., 2005). According to Singh et al. (2002), a 5% annual production loss to this bruchid in Nigeria alone would cost about $100 million USD, or a loss of over 40,000 tonnes of cowpea. Fumigants, chiefly phosphine and dichlorvos are the major synthetic insecticides used in controlling *C. maculatus* in Nigeria. The storage conditions available to most farmers enable re-infestation, increasing the frequency of insecticide use. These chemicals may result in deleterios effects ranging from cowpea poisoning, environmental contamination, residues in grain, development of genetic resistance due to improper usage, and hazards to workerIn addition, the high costs of chemicals may also make it difficult for small-scale farmers to access (Lowenberg-DeBoer and Ibro, 2008), accompanied by increased infestation and losses.

The search for alternatives to synthetic insecticides in stored-products for insect pest management has been intensifing. One alternative is the use of diatomaceous earth which has received considerable attention, and are considered among the most promising alternatives to synthetic residual insecticides in stored-grain protection (Athanassiou et al., 2003).. During the last 20 years, DE has been the subject of several review papers with the numerous references cited within each of review. Also DE is now registered as a grain protectant or for structural treatment in several countries (Korunic, 2016). The mode of action of DE is different from the synthetic insecticides. DE absorbs the insect's cuticular waxes, and insects die from desiccation (Korunic, 2013). The advantages of using DE are its low mammalian toxicity, its stability, leaving no toxic residues on grains, control of the synthetic insecticide resistant pests and applied using the same technology for conventional grain protectants (Vayias et al., 2006).

Regional deposits of DE have been shown to be effective against local populations of stored-product insect species. For example varying deposits exist in Croatia (Korunic et al., 2009; Liska et al., 2015), Greece and Romania (Athanassiou et al., 2016) and Iran (Ziaee et al., 2013; 2016). There are also sevaral deposits of DE in Nigeria, however, their insecticidal efficacy has not been widely investigated. Kabir et al. (2011) first reported the insecticidal efficacy of Bularafa DE against *Tribolium castanem* (Herst) then against *Rhyzopertha dominica* (F.) (Kabir et al., 2013). Later, Nwaubani et al. (2014) reported the efficacy of Bularafa and Abakire diatomites agaisnt *R. dominica* and *Sitophilus oryzae* (L.). Information on insecticidal efficacy is important for commercial development of Nigerian DE deposits for use as grain protectant. The objective of this research was to evaluate the insecticidal efficacy and residual activity of Bularafa raw DE to control *C. maculatus* in stored cowpea.

Materials and Methods

Test Insect

Callosobruchus maculatus were obtained from laboratory culture, wich were maintained on cowpea for about a year. Adult insects were used to establish new insect cultures for the experiments. Two (1 litre capacity) glass jars were filled with 400 g of cowpea grains and 100 mixed-sex adults of the test insects were introduced into culture medium to oviposit. Each jar was covered with nylon mesh and secured with rubber bands. Parent insects were removed five days after introduction and the resulting F_1 progeny aged 0-2 days were used for the bioassay. New cultures were set up monthly to ensure availability of adult insects throughout the experiments.

Cowpea seeds

Insecticide free cowpea grains (Var. Borno Brown), were obtained from Borno State Agricultural Development Programme (BOSADP) Maiduguri, Borno State. The grains were cleaned and disinfested according to Kabir (2013), then equilibrated with laboratory condition for 10 days.

Diatomaceous earths

The raw diatomaceous earth (RDE) in the form of soft chalky rock was obtained from mines located 6 km North of Bularaffa village (Latitude: 11^0 8′ 48″ and Longitude: 11^0 49′ 17″ E) in the Gujba Local Government Area of Yobe State, Nigeria. The DE was oven dried, ground and put through a 63 µm sieve. Its pH and tapped density were analyzed in accordance with methods described by Korunic (1997) while its mineral composition was analyzed in the Geology Laboratory, Ahmadu Bello University, Zaria, Nigeria. It has the following properties: tapped density- 312.5 g/L, pH-9.2; mineral composition: SiO_2 - 80.43%, Al_2O_3 -5.02%, CaO – 0.48%, Na_2O – 0.07%, K_2O -0.14%, Fe_2O_3 -0.17%, ZnO - 0.01%, and MnO - 0.01. The commercial formulation of DE (Protect-It®) was obtained from Diatom Research and Consulting Inc., Toronto, Canada. It is an enhanced DE that contains approximately 83.7% amorphous SiO_2, 5.6% Al_2O_3, 2.3% Fe_2O_3, 0.9% CaO, 0.3% MgO and 1.9% other oxides e.g. TiO_2 and P_2O_3), and 3-5% moisture content (m.c.). The median particle size is between 5 and 6 µm with 10% silica aerogel (Athanassiou et al., 2009).

Bioassay Procedure

Adult *C. maculatus* adults were bioassayed at RDE doses of 0 (untreated control), 250, 500, 1,000, 1500 mg/kg RDE and Protect-It at 1000 mg/kg . De's were applied to cowpea grains under ambient conditions (31-34° C and 24 - 30% R.H.). For the acute toxicity test, the appropriate amounts of DE were applied to 50 g of cowpea and placed in 150 ml glass bottles that were tumbled manually for 5 min to achieve an even distribution of the DE on the grains. Then, 30 mixed-sex adult insects were introduced into each bottle, capped with perforated plastic lids and kept on a laboratory shelf. Each treatment combination was replicated four times. Adult mortality was recorded on 3 and 5 d after exposure, while progeny production and grain damage were assessed 40 days after infestation (DAI). The residual toxicity was assessed on 1500 g lots of cowpea grains treated with above mentioned doses and stored in plastic containers for 180 days (from April to October) under laboratory conditions (26-32° C and 33-93% RH). Similar bioassay procedures and observations as described above were conducted at 30 days intervals

Data Analysis

Where necessary, mortality data obtained were first corrected for control using Abbott's (Abbott, 1925) formula and together with data on grain damage were arcsine transformed. Data relating to number of F1 progeny were square root $\sqrt{(x + 1)}$ transformed. All were then subjected to Analysis of Variance (ANOVA Statistix 8.0). Differences between treatment means were separated using Tukey-Kramer Honestly Significant Difference (HSD) test at ($P \leq 0.05$).

Results

There are significant ($P<0.05$) variations in mortality levels of *C. maculatus* adults caused by different doses of RDE, when exposed for 3 days (Fig. 1). Irrespective of storage period, adult mortality increased with increase in raw DE dose. Protect-It was the most effective DE causing 100% adult mortality following 3 days of exposure to freshly treated cowpea grains and on those treated and stored for upto 60 days. With the RDE, similar effects were achieved, however, only on freshly treated seeds. Within each month there were significant declines ($P<0.05$) in mortality levels among raw DE doses.

Adult mortality increased with extended exposure period (Fig. 2). After 5 days of exposure mortality levels recorded for all RDE and Protect-It dosages significantly ($P<0.05$) increased irrespective of

post-treatment storage period. The RDE applied at 1500 mg/kg caused 100% adult mortality only on freshly treated cowpea and after 30 days of storage, whereas with Protect-It caused complete adult mortality for upto 90 post-treatment days of storage. Efficacy of both RDE and Protect-It declined with an increase in post-treatment storage period. In the case of RDE applied at 1500 mg/kg, adult mortality level decreased from 85% after 90 days 58.3% after 180 days post-treatment; and a similar trend was observed for other doses. With Protect-It, however the minimum mortality level caused (92.5% adult mortality) was recorded at 120 days post treatment and did not significantly change thereafter (Fig. 2)

Both DEs had significant impact on progeny production of *C. maculatus*. Effect on progeny production was significantly (P<0.05) influenced by DE dose and storage interval. Throughout the post treatment period, the untreated control supported significantly (P<0.05) higher number of progeny than the treated grains, except on those treated at 250 mg/kg after 60 days post-treatment. Furthermore increase in raw DE dose resulted in increased progeny suppression. Even the highest dose of RDE could not prevent progeny development, although in allcases the number of progeny was less <10. Protect-It was more effective in progeny inhibition inducing complete suppression on grain freshly treated or treated and stored for 30 days. The progeny that emerged thereafter was <3 per bottle (Fig. 3).

Progeny development in all treatments were drastically reduced after 90 days post-treatment. The percent of damaged seeds followed the same trend with number of progeny produced. Significant (P>0.05) differences in grain damage were noted among RDE doses and storage periods (Fig. 4). Higher grain damage was record in the untreated control and grains treated at 250 mg/kg of RDE, where differences were not significant except on freshly treated grains and after 30 post-treatment.

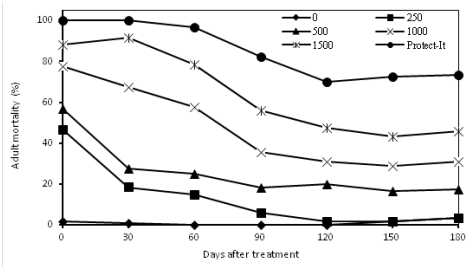


Fig. 1. Mean mortality of *C. maculatus* adults after three days of exposure to cowpea treated with different doses of DE and stored for various periods

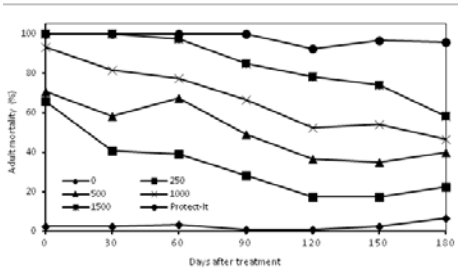


Fig. 2. Mortality of *C. maculatus* adults after five days of exposure to cowpea treated with different doese of DE and stored for various periods

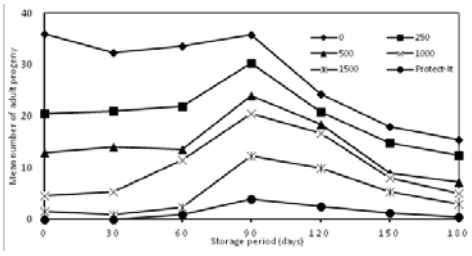


Fig. 3. Mean number of *C. maculatus* F1 progeny after 40 DAI on cowpea treated with different doses of DE and stored for different periods

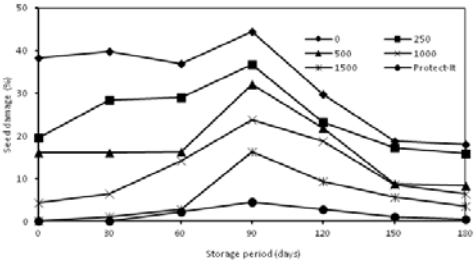


Fig. 4. Seed damage caused by *C. maculatus* 40 DAI on cowpea treated with different doses of DE and stored for different periods

Even on grain treated at 1500 mg/kg grain, damage could not be contained. After about 90 days post treatment, at all DE doses and the untreated control, there was a slight but significant increase in grain damage. Protect–It prevented grain damage on freshly treated seeds and after 30 days post-

treatment; and even where grain damage was recorded they were less than 3% and differences between storage periods were not significant ($P>0.05$).

Discussion

Adult progeny emerged in all DE treatments except in Protect-It treated (freshly treated and 30 days post treatment) grains, possibly because oviposition occurred before the adults died before exposure to the DE (Subramanyam and Roesli, 2000). However, in all treatments and the untreated control, progeny production significantly increased at 90 days post-treatment. This being the period coinciding with, middle of the rainy season in Maiduguri. This period is characterized by lower ambient indoor temperature (26 - 29ºC) and higher relative humidity (>80%) as compared to the first three months of the experimentation (May-July, when the r.h. was bet ween 24 and 58%). DE efficacy is related to relative humidity, temperature and changes in physical proprieties of treated grain (Athanassiou et al., 2005). During this period, it is likely the DE absorbed moisture from the atmosphere (Stathers et al., 2004). Other Studies have also shown that an increase in relative humidity reduces DE efficacy (Fields and Korunic, 2000; Rojht et al., 2010; Beris et al., 2011). Given that DE efficacy is reduced by higher moisture, there are direct consequences of DE effectiveness for grains stored in ventilated structures, especially in humid areas. On the other hand, the relatively higher efficacy of the RDE and Protect-It during the first 60 days of storage (May and June) which coincided with a period of high temperature (32) could be attributed to the fact that at higher temperatures insects are more mobile (Arthur, 2000) increasing contact with the DE particles, thus resulting in greater damage of the insect cuticle and water loss (Athanassiou et al., 2005; Wakil et al., 2010; Athanassiou et al. 2016). These results suggest that raw DE could be more effective in the Sudan and Sahel savannah regions, characterized by long dry season, high temperature and low relative humidity than in the humid areas. Another interesting finding of this study is the general reduction in progeny production including the untreated control after 90 days of storage (Fig. 3). The reason could not be explained. Perhaps cowpea grains became unsupportive of the pest's reproduction. This hypothesis needs to be verified by experiments.

One of the major drawbacks limiting the widespread use of DE is its reduction in efficacy under high moisture storage conditions (Korunic et al., 2016). This limitation could be overcomed in humid areas by thorough grain drying before storage and limiting moistuture equillibration with the sorrounding by using hermetic storage sructures.

In conclusion, this study indicated that the Nigerian RDE may not be suitable for long-term storage of cowpea grains against *C. maculatus* when applied at a dose rates of 1500 mg/kg; perhaps 2000-2500 mg/kg may be effective. Given that DE efficacy decreased during the months with high relative humidity, it is necessary to store DE treated grains in airtight structures or modify storage structures to limit moisture absorption from the surrounding environment in order to increase the benefits of DE treatments. Further studies on different particle sizes, higher dose rates and enhancement of Nigerian RDE are recommended.

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