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Bioefficacy of enhanced diatomaceous earth and botanical powders on the mortality and progeny production of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae), *Sitophilus granarius* (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) in stored grain cereals

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Abstract. Food losses caused by insects during postharvest storage are of paramount economic importance worldwide, especially in Africa. Laboratory bioassays were conducted in stored grains to determine the toxicity of powders of *Eugenia aromatica* and *Moringa oleifera* alone or combined with enhanced diatomaceous earth (Probe-A® DE, 89.0% SiO_2 and 5% silica aerogel) to adult *Sitophilus granarius*, *Tribolium castaneum* and *Acanthoscelides obtectus*. Adult mortality was observed up to 7 days, while progeny production was recorded at 6–10 weeks. LD_{50} and LT_{50} values for adult test insects exposed to plant powders and DE, showed that *A. obtectus* was the most susceptible towards the botanicals (LD_{50} 0.179% and 0.088% wt/wt for *E. aromatica* and *M. oleifera*, respectively), followed by *S. granarius*. *Tribolium castaneum* was most tolerant (LD_{50} 1.42% wt/wt and 1.40% wt/wt for *E. aromatica* and *M. oleifera*, respectively). The combined mixture of plant powders and DE controlled the beetles faster compared to the plant powders alone. LT_{50} ranged from 55.7 h to 62.5 h for *T. castaneum* exposed to 1.0% *M. oleifera* and 1.0% DE, and 0.5% *E. aromatica* and 1.0% DE, respectively. Botanicals caused significant reduction of F_1 adults compared to the control. Combined action of botanical insecticides with DE as a grain protectant in an integrated pest management approach is discussed.

Key words: Toxicity, *Acanthoscelides obtectus*, *Sitophilus granarius*, *Tribolium castaneum*, plant powders, DE

Introduction

To meet the challenge of feeding 9 billion people sustainably by 2050, it is necessary to ensure sufficient, safe and nutritious food for all, while safeguarding natural resources. Globally, insects cause losses of about 5−10% in stored products and as much as 40% in some developing countries (Shaaya *et al.*, 1997; Weaver and Petroff, 2005). Insect damage to grains may result in

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losses in weight, nutrient value, seed viability, and increased susceptibility to contamination by fungi. This deterioration and contamination by insects and insect parts reduces the quality of the grains and lowers their market value. For example, insect infestation of wheat affects the baking quality of the flour in the form of decreased loaf volume, compact and inelastic crumbs, bitter taste and production of off-flavours (Sánchez-Mariñez *et al*., 1997). Insect fragments are also a major concern for the wheat-flour milling industry, because of legal contamination limits (Edwards *et al*., 1991; Perez-Mendoza *et al*., 2003). Postharvest losses constitute a major problem limiting availability of food in most regions of sub-Saharan Africa (Obeng-Ofori 2007; Sugri and Johnson 2009; Adarkwah *et al*., 2010*a*, 2010b; 2011; 2012; 2014). Improving food security through a reduction of postharvest losses is a development imperative for all economies. High postharvest losses reduce real income for all consumers, divert essential income out of farmers' pockets and undermine overall food availability. The poor are particularly affected, as they spend a higher proportion of their income on staple foods. It is, therefore, important that postharvest procedures and measures are given as much attention as production practices. Certainly, the development and implementation of technologies and procedures that can reduce losses at the postharvest stage can play a significant role in increasing global food security (Obeng-Ofori, 2010).

Cereals (such as maize, rice, wheat, sorghum and millet) are important sources of carbohydrates, and grain legumes (such as cowpea and groundnut) are important sources of protein in sub-Saharan Africa (Broughton *et al*., 2003; Adarkwah *et al*., 2014). They are, however, highly susceptible to attack by several species of beetles in the genera *Sitophilus, Tribolium, Acanthoscelides*, and *Callosobruchus* in both field and storage (Lale and Vidal, 2003; Gbaye *et al*., 2011). Dry legume grains are destroyed by *Acanthoscelides obtectus* (Say) and *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) (Gbaye *et al*., 2011). *Sitophilus granarius* (L.) (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) are some of the most destructive pests of durable stored cereal grains worldwide.

Globally, control of these insect pests is primarily dependent upon continued applications of synthetic insecticides, which are still the most effective treatments for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation. Although effective, their indiscriminate use over a long period has disrupted biological control by natural enemies and caused the emergence of new insect species, and has sometimes resulted in the development of resistance (Park *et al*., 2003; Isman, 2006; 2010; Obeng-Ofori, 2010; Adarkwah and Schöller, 2012). There are also serious concerns about environmental degradation and human health. Furthermore, the majority of farmers in Africa are resource poor and have neither the means nor the skills to obtain and handle pesticides appropriately (Obeng-Ofori, 2010).

The body of scientific literature documenting bioactivity of plant derivatives to arthropod pests continues to expand; yet, only a handful of botanical products are used in agriculture in the industrialized world, and there are few prospects for commercial development of new botanical products due to registration requirements (Fields, 2006). However, resource-poor farmers in Asia and Africa have been utilizing plant materials to protect grains against insect infestation for a long time (Hassanali *et al*., 1990; Poswal and Akpa, 1991; Talukder and Howse, 1995; Obeng-Ofori, 2007; Nukenine, 2010). Many rural farmers mix wood ash from various plants with grains as a physical control treatment against insect infestation, while others commonly use smoke from burning plant materials to protect on-farm stored cereal grains against pest infestation. The use of these traditional plant materials has stimulated research to establish the scientific basis for their continued use regarding their efficacy, active constituents and effective application technology (Weaver *et al*., 1991; Schmidt and Streloke, 1994; Bekele *et al*., 1995; Obeng-Ofori *et al*., 1997; Nukenine *et al*., 2010).

Some major obstacles to the use of botanicals in stored product protection are that effective rates of application are much higher than for conventional synthetic pesticides, and users lack experience and appreciation of the efficacy of botanicals for pest control. There are still doubts as to the effectiveness of plant-derived products (both 'home-made' and commercial products), due to their slow action and lack of rapid knockdown effect; difficulty of registration and patenting of natural products and lack of standardization of botanical pesticide products; instability of the active ingredients when exposed to direct sunlight, as well as competition with synthetic pesticides through aggressive advertising by commercial pesticide dealers; and due to the fact that commercially-formulated botanicals are more expensive than synthetic insecticides and are not as widely available. For example, mixing stored cowpea seeds with 1.0 g or more of *Piper guineense* dried fruit or neem kernel powders per 20 g of cowpea seed is recommended for control of *C. maculatus* (Oyeniyi *et al*., 2015). For complete control, large quantities of these insecticidal plant powders are needed, which may not be practicable for large-scale storage.

Clove bud *Eugenia aromatica* species (Myrtaceae) has long been considered to have medicinal properties (such as being a tonic against digestive

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disorders and diarrhoea). The *Eugenia* bud and leaf essential oils contain various compounds, such as acetyleugenol, benzaldehyde, benzyl acetate, benzyl alcohol, α-carophyllene, chavicol, eugenol, R-humulene, m-methoxy benzaldehyde, methyl-n-amyl ketone, methyl salicylate and R-ylangene (Deyama and Horiguchi, 1971). Insecticidal properties and chemical composition of species of *Syngizium aromaticum* (*E. aromatica*) were reported and found to contain eugenol (74.30%), eucalyptol (5.80%), α-cadinol (2.43), β-caryophyllene (18.90%), benzdene-1-ethyl-3-nitro (11.10%) and benzoic acid 3-(1-methylethyl) (8.90%), eugenyl acetate (21.30%), *cis*-ocimene (2.32%), α-thujene (3.36%) and terpinen-4-o-l (0.35%) (Bhuiyan *et al*., 2010). Oboh *et al*. (2015) reported and found chemical compounds of *E. aromatica* to contain α-pinene, β-pinene, neral, geranial, gamma terpinene, *cis*-ocimene, allo ocimene, 1,8-cineole, linalool, borneol, myrcene and pinene-2-ol, α-terpineol, α-copane, *cis*-ocimene, gamma-terpinene, α-thujene, camphene and isoartemisia.

Moringa oleifera Lam. (Moringaceae) also known as 'drumstick tree', 'horseradish' or the 'kelor tree' is widely cultivated and naturalized in tropical Africa, Malaysia, the Philippines, Mexico, the Malabar region and Sri Lanka (Fahey, 2005; Anwar *et al*., 2007; Olufunmilayo *et al*., 2012; Karthika *et al*., 2013). All parts of the *Moringa* tree are edible and have been consumed for many years by humans. Medicinal uses for *M. oleifera* include as an antioxidant (Verma *et al*., 2009), anticarcinogen (Bharali *et al*., 2003), diuretic, anti-inflammatory, antispasmodic, Cáceres *et al*., 1992), and antiulcer, antibacterial and antifungal agent (Cáceres *et al*., 1991). Furthermore, its antinociceptive (Sulaiman *et al*., 2008) properties, as well as its wound healing ability, have been demonstrated (Rathi *et al*., 2006). The main chemical components of leaf extracts of *M. oleifera* showed the presence of phytochemicals, flavonoids, anthraquinones, alkaloids, saponins, steroids, terpenoids, cardiac glycosides, anthocyanin, tannins and carotenoids. Marrufo *et al*. (2013) identified chemical components of *M. oleifera* to be hexacosane, pentacosane, flavonoids, quercetin, luteolin and heptacosane. Quercetin and kampferol are flavonoids, which are compounds of phenolic hydroxyl groups of *M. oleifera* with antioxidant action of potential therapeutic use (Pace-Asciak *et al*., 1995). Other insecticidal compositions from leaves of *M. oleifera* include two nitrile glycosides, niazirin and niazirinin; three mustard oil glycosides, 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]isothiocyanate, niaziminin A, and niazamin B; ethyl 2-hydroxypropanoate (lactate); cyclobuten-3,4-dione; 1-dimethylamino-2-hydroxy-; 2-[(2-acetoxyethyl)-sufinyl]aniline; linalool oxide; 3-buten-2-ol,2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-; 1,2-benzenedicarboxylic acid; bis(2-ethylhexyl) ester; linalool oxide; adenine; and upiol (Karthika *et al*., 2013).

Diatomaceous earth (DE) contains fossilized siliceous remains of diatoms that were deposited during the Cenozoic Era. Diatoms are microscopic unicellular aquatic plants closely related to brown algae that have a fine shell made of silica. DEs offer safer alternatives to synthetic chemicals against storage insect pests (Fields *et al*., 2003; Stathers *et al*., 2004; Athanassiou *et al*., 2005; Demissie *et al*., 2008; Korunic and Rozman, 2010; Adarkwah *et al*., 2012). DE has become one of the most well studied alternatives to synthetic insecticides (Fields and Korunic, 2000; Subramanyam and Roesli, 2000; Mewis and Ulrichs, 2001; Athanassiou *et al*., 2005; Kavallieratos *et al*., 2005; El-Wakeil and Saleh, 2009; Iatrou *et al*., 2010; Athanassiou *et al*., 2011; Kavallieratos *et al*., 2012). However, information on their efficacy in tropical small-scale grain stores is limited (Stathers *et al*. 2002). Some commercially available enhanced DE products include Dryacide®, Protect-It®, Fossil Shield®, Silico-Sec®, Insecto-Sec®, Diatomenerde®, Damol-D1® and Perma-Guard™. These DE products have been found to be effective against various stored product insect pests (Fields *et al*., 2003; Stathers *et al*., 2004; Athanassiou *et al*., 2005; Demissie *et al*., 2008; Korunic and Rozman, 2010; Adarkwah *et al*., 2012; Badii *et al*., 2014).

A major challenge to the practical utilization of botanicals and DE for stored product protection is the large quantity needed for effective control of pests. One approach to address this problem may be to combine insecticidal plant materials that farmers are already familiar with, with DE, but there seems to be limited information in this regard. The objective of this study, therefore, was to assess the toxicity and progeny production of insecticidal dusts containing powders of *E. aromatica* and *M. oleifera* to three major stored product beetles—*S. granarius*, *T. castaneum* and *A. obtectus*. This study also examines if these plant products can be used in combination with DE and what doses would be needed in field evaluation to control these cosmopolitan insects.

Materials and methods

Study site

The study was carried out in the Division Urban Plant Ecophysiology, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Berlin, Germany.

Rearing of insects

Adults of *S. granarius* and *T. castaneum* were cultured in a controlled environment at 25 ± 1

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°C, 65–70% RH and total darkness. They were obtained from laboratory stock culture at the Federal Research Centre for Cultivated Plants (Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany). The strains of insects used are known to have been collected in a grain store in Berlin, Germany in 1968 and have been kept under laboratory conditions since then in the institute. One hundred adults of mixed sex of *S. granarius* or *T. castaneum* were introduced into each glass jar of 500 ml containing 200 g of whole wheat grain, with a moisture content of 14.4%, and germ as feed. The jars were covered with nylon mesh held in place with rubber bands, to provide sufficient aeration and also to prevent escape of the insects. Parent adult beetles were removed after a 14-day oviposition period and insects that emerged were collected and used for the various bioassays. Similarly, *A. obtectus* was maintained on common Bambara groundnut, *Vigna subterranea* (L.) Verdcourt in a climatic chamber under the above same conditions. One hundred and fifty (150) unsexed adults were placed in jars and covered with a nylon net, to prevent the escape of emerging adults. After a 15-day oviposition period, the parental adults were removed and the newly emerged adult bruchids collected (0−3 days) and used for the bioassays.

Collection and preparation of plant materials

The dry fruits of *E. aromatica* were obtained from the Department of Crop, Soil, and Pest Management, Federal University of Technology, Akure, Nigeria. *Eugenia aromatica* was purchased at a local market in Akure, Nigeria in March 2014; therefore, it is unknown exactly when and where it was harvested. The identity was confirmed at the Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The flower buds were oven-dried to a constant weight at a temperature of 400 °F. The fruit was pulverized in the laboratory using the NAKAI NJ-1731 electric blender and further sieved (mesh size 1 mm^2) before being stored in an airtight plastic container. Leaves of *M. oleifera* were collected around Nyankpala during the 2014 harvesting season of April−July in the Tamale Metropolis of the Northern Region of Ghana. The area is located at latitude 9°25′N, longitude 00°58′W and altitude 183 m. The identity of the plant was confirmed at the Department of Agronomy/Botany, Savannah Research Institute of Ghana, where voucher samples were deposited. The leaves of *M. oleifera* were shade dried for 1–2 days and then sent to Germany to be milled into powder using the Retsch MM301 grinding machine obtained from Retsch GmbH, Düsseldorf, Germany. A leaf powder of particle size 150 µm was obtained. Each type of plant powder was immediately put into separate plastic containers with airtight lids and kept under ambient laboratory conditions before using them for the various experiments.

Probe A® diatomaceous earth product

Modified DE Probe-A® is a relatively new DE product that has been tested for its effectiveness against storage beetles in cereal grain admixtures (Mewis and Ulrichs, 2001; Badii *et al.*, 2014). Probe-A® contains 89.0% SiO_2 with mean particle size of 5–7 µm and 5% silica aerogel. The DE Probe-A® product was obtained from Bein GmbH, D-36232 Eiterfeld, Germany.

Toxicity and progeny production of adult beetles in grain

Toxic effects of *M. oleifera* and *E. aromatica* flower bud powders on adult *S. granarius*, *T. castaneum* and *A. obtectus* in stored Bambara groundnut and wheat grains were evaluated in the laboratory.

Ten grammes of disinfested bio wheat (var. Toni) obtained from Erzeugergemeinschaft Biokorntakt GmbH & Co. KG, Berlin, Germany, or Bambara groundnut seeds purchased from Bio-Company in Berlin, Germany were thoroughly mixed with 0.00 (control), 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5% (wt/wt) each of *E. aromatica* and *M. oleifera* and *E. aromatica* flower bud powders in a plastic Petri dish (90 ml). The dosage of each plant powder was measured using the Sartorius sensitive balance obtained from Sartorius AG, Göttingen, Germany. Ten adults each of *S. granarius* (5–10 days), *T. castaneum* and *A. obtectus* (0–3 days) of mixed sex were introduced into each treatment container, which was covered with a perforated mesh lid. The powder and grains were thoroughly mixed for 5 min. The dishes were arranged in a completely randomized design on a laboratory shelf (27 ± 2 °C, 60–80% RH 11:13 light: day) and were not moved throughout the test period to avoid a negative impact on the insects. Each treatment was replicated three times. Dead insects were counted daily for 7 days and then immediately discarded. All insects were confirmed dead when there was no response when their abdomen was gently probed with a camel hairbrush. Eventual morphological changes in adults were observed and recorded. The remaining living adults were removed from the Petri dishes after 7 days. Petri dishes were then kept under the same conditions to monitor the F_1 generation emerging from the seeds. The number of F_1 progeny produced was recorded after 6 weeks for both *S. granarius* and *T. castaneum* and approximately 6 weeks for *A. obtectus.*

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Toxicity of single and combined products to beetles

The effect of *M. oleifera* leaf powder, *E. aromatica* flower bud powder and DE applied singly or as mixtures on the mortality of the three beetle species was evaluated in the laboratory. Twenty grammes of wheat grains or *Vigna subterranea* seeds were treated with 2.0% (wt/wt) of *E. aromatica*, *M. oleifera* powders or DE in glass Petri dishes (9.0 cm diameter). Grains were also treated with a mixture of either *M. oleifera* leaf powder and DE or *E. aromatica* powder and DE at three ratios, namely 1:1 (1.0% wt/wt) plant powder and (1.0% wt/wt) DE, or 2:1 (1.0% wt/wt) plant powder and (0.5% wt/wt DE), or 1:2 (0.5% wt/wt) plant powder and (1.0% wt/wt) DE. There was a control treatment with no insecticidal material. Twenty unsexed adults each of *S. granarius* (5–10 d), *T. castaneum* and *A. obtectus* (0–3 d) were added to the treated and control containers. Adult mortality was observed and recorded daily for up to 5 days posttreatment. Morphological changes in adults were also observed and recorded. The experiment was replicated three times.

Statistical analyses

Statistical analyses were performed using the software package SigmaStat 3.1. Percentage mortality data of *S. granarius, A. obtectus* and *T. castaneum* adults were not corrected for control mortality, because mortality in the treatment was ≤2% (Abbott, 1925). The untreated control population was used as the initial number of offspring. Percentage F_1 progeny production was arcsine (square root (x)-transformed and subjected to two-way analysis of variance (ANOVA) followed by all pairwise multiple comparison procedures (Holm–Sidak method). Probit analysis was applied to estimate the LD_{50} values and their upper and lower confidence interval limits, as well as intercept, by using BioStat Pro version 5.9.8. Lethal exposure times were determined for 50% and 95% mortality (LT_{50} and LT_{95}) graphically using Sigma-Plot version 12.0. Two-way repeated measures ANOVA (two-way RM ANOVA) followed by all pairwise multiple comparison procedures (Holm–Sidak method) were used to compare percentage mortality of beetles among plant powders and DE treatments applied singly or as mixtures.

Results

Toxicity of plant powders to adult beetles

Adult mortality of *S. granarius, T. castaneum* and *A. obtectus* exposed to different dosages of leaf powder of *M. oleifera* and *E. aromatica* flower bud powder is shown in Tables 1–3. In both plant powders, mortality of the three beetle species increased with increasing dosage and exposure period. There was variation in the toxicity of the two plant powders to the three beetle species. Among the three beetle species tested, the plant powders were most toxic to *A. obtectus*. *Tribolium castaneum* was less susceptible to the two plant powders than either *S. granarius* or *A. obtectus*. There was no mortality of *S. granarius* in grains treated with *E. aromatica* flower bud powder after 24 h of exposure, irrespective of dosage applied, but all adult weevils died in grains treated with the highest dosage of 2.5% wt/wt after 7 days (Table 1). However, none of the dosages of both plant powders was able to kill all *T. castaneum* even after 7 days' exposure (Table 2). The highest mortality of *T. castaneum* (86%) was obtained in grains treated with 2.5% wt/wt of *E. aromatica* flower bud powder after 6 days (Table 2). Grains treated with 1.5% wt/wt and above of *E. aromatica* flower bud powder caused 100% mortality of *A. obtectus* after 3 days exposure, but grains treated with 2.0% wt/wt g and above of *M. oleifera* leaf powder had a similar effect after 4 days' exposure (Table 3).

The LD_{50} and LT_{95} for *E. aromatica* flower bud powder and *M. oleifera* leaf powder against all the three beetle species are shown in Tables 4 and 5. Lethal exposure times/doses estimated (LD_{50}, LT_{50}) for the adult test insects to plant powders, showed that *A. obtectus* was most susceptible towards the botanicals (LD_{50} 0.1793% wt/wt and 0.0884% wt/wt for *E. aromatica* and *M. oleifera*, respectively), followed by *S. granarius*. *Tribolium castaneum* was most tolerant (LD_{50} 1.4155% wt/wt and 1.3938% wt/wt for *E. aromatica* and *M. oleifera*, respectively, (Table 4).

Generally, the combined mixture of plant powders and DE controlled the beetles faster than the plant powders alone. LT_{50} ranged from 55.7 h to 62.5 h for *T. castaneum* exposed to 1.0% *M. oleifera* and 1.0% DE, and 0.5% *E. aromatica* and 1.0% DE, respectively (Table 5). For a dose of 2.0% DE alone the LT_{50} for *S. granarius, T. castaneum* and *A. obtectus* was 12.4, 21.4 and 32.5 h, respectively (Table 5). For the combination of different dosages of flower bud powder of *E. aromatica* (*Erfbp*) and DE, i.e. 0.5% wt/wt *Erfbp* +1.0% wt/wt DE, the LT_{95} was found to be 116.6 h for *S. granarius* and 111.4 h for *A. obtectus* (Table 5). For a dose of 1.0% wt/wt *Molp* + 0.5% wt/wt DE, the values of the LT_{95} ranged from 46.5 h for *S. granarius* to 98.3 h for *A. obtectus*. The LT_{95} values of 1.0% wt/wt *Erfbp* + 1.0% wt/wt DE against *S. granarius* and *A. obtectus* were 46.2 and 96.3 h, respectively. At the dosage of 1.0% wt/wt *Molp* + 1.0% wt/wt DE, the LT_{95} was 42.2 h, 56.5 h and 111.4 h for *S. granarius*, *A. obtectus* and *T. castaneum*, respectively

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Table 1. Mortality (%) (± SEM) of adult *Sitophilus granarius* after 1, 2, 3 4, 5, 6 and 7 days exposure to wheat seeds treated with different dosages of *Eugenia aromatica* flower bud powder (*Erfbp*) and *Moringa oleifera* leaf powder (*Molp*) at temperature of 25 ± 2 °C and 60–80% relative humidity

Dosage rate (g/20) g	Mean % mortality (± SEM) in:													
	1 day		2 days		3 days		4 days		5 days		6 days		7 days	
	Erfbp	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.25	0.0 ± 0.0	10.0 ± 5.7	0.0 ± 0.0	13.3 ± 3.3	6.7 ± 6.7	16.7 ± 6.7	10.0 ± 5.8	26.7 ± 3.3	16.7 ± 6.7	30.0 ± 5.8	16.7 ± 6.7	30.0 ± 5.8	43.3 ± 13.3	33.3 ± 3.3
0.5	0.0 ± 0.0	10.0 ± 5.7	0.0 ± 0.0	23.3 ± 8.8	10.0 ± 5.7	40.0 ± 5.8	16.7 ± 6.7	53.3 ± 3.3	40.0 ± 11.5	56.7 ± 3.3	40.0 ± 11.5	60.0 ± 0.0	53.3 ± 3.3	63.3 ± 8.8
1.0	0.0 ± 0.0	13.3 ± 13.3	0.0 ± 0.0	26.7 ± 8.8	20.0 ± 0.0	43.3 ± 3.3	33.3 ± 3.3	53.3 ± 3.3	43.3 ± 6.7	56.7 ± 3.3	43.3 ± 6.7	63.3 ± 8.8	66.7 ± 3.3	66.7 ± 6.7
1.5	0.0 ± 0.0	16.7 ± 8.8	6.7 ± 6.7	30.0 ± 10.0	30.0 ± 15.3	46.7 ± 6.7	50.0 ± 5.8	60.0 ± 0.0	60.0 ± 0.0	63.3 ± 8.8	60.0 ± 0.0	63.3 ± 3.3	73.3 ± 3.3	70.0 ± 5.8
2.0	10.0 ± 10.0	16.7 ± 8.8	16.7 ± 3.3	30.0 ± 10.0	36.7 ± 6.7	46.7 ± 16.7	66.7 ± 8.8	60.3 ± 20.8	66.7 ± 18.6	70.0 ± 17.3	80.0 ± 11.5	80.0 ± 10.0	83.3 ± 3.3	90.0 ± 10.0
2.5	10.0 ± 15.3	23.3 ± 5.8	10.0 ± 15.3	33.3 ± 6.7	43.3 ± 13.3	56.7 ± 12.0	46.7 ± 18.6	70.0 ± 5.8	80.0 ± 11.5	76.7 ± 12.0	66.7 ± 18.6	83.3 ± 16.7	86.7 ± 6.7	90.0 ± 10.0

Note: Data are means ± SE of three replications of 10 insects each.

Table 2. Mortality of adult *Tribolium castaneum* after 1, 2, 3 4, 5, 6 and 7 days exposure to wheat seeds treated with different dosages of *Eugenia aromatica* flower bud powder (*Erfbp*) and *Moringa oleifera* leaf powder (*Molp*) at temperature of 25 ± 2 °C and 60–80% relative humidity

Dosage rate (g/20g)	Mean % mortality (± SEM) in:													
	1 day		2 days		3 days		4 days		5 days		6 days		7 days	
	Erfbp	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.25	0.0 ± 0.0	3.3 ± 3.3	3.3 ± 3.3	6.7 ± 6.7	10.0 ± 5.8	16.7 ± 6.7	3.3 ± 3.3	20.0 ± 5.8	3.3 ± 3.3	23.3 ± 3.3	6.7 ± 6.7	23.3 ± 3.3	13.3 ± 3.3	26.7 ± 3.3
0.5	3.3 ± 3.3	6.7 ± 3.3	6.7 ± 6.7	10.0 ± 5.8	13.3 ± 3.3	23.3 ± 6.7	10.0 ± 5.8	23.3 ± 6.7	16.7 ± 6.7	30.0 ± 10.0	40.0 ± 5.8	30.0 ± 10.0	40.0 ± 5.8	36.7 ± 8.8
1.0	3.3 ± 3.3	10.0 ± 5.8	13.3 ± 3.3	16.7 ± 6.7	16.7 ± 6.7	30.0 ± 10.0	13.3 ± 3.3	30.0 ± 10.0	23.3 ± 3.3	33.3 ± 14.5	40.0 ± 5.8	36.7 ± 14.5	46.7 ± 3.3	46.7 ± 8.8
1.5	6.7 ± 3.3	16.7 ± 3.3	13.3 ± 3.3	30.0 ± 5.8	20.0 ± 0.0	50.0 ± 5.8	20.0 ± 0.0	50.0 ± 5.8	30.0 ± 10.0	53.3 ± 6.7	46.7 ± 12.0	56.7 ± 8.8	53.3 ± 8.8	56.7 ± 8.8
2.0	10.0 ± 10.0	23.3 ± 8.8	16.7 ± 8.8	33.3 ± 14.5	26.7 ± 8.8	50.0 ± 17.3	33.3 ± 12.0	53.3 ± 3.3	33.3 ± 3.3	53.3 ± 3.3	60.0 ± 0.0	56.7 ± 3.3	66.7 ± 3.3	66.7 ± 3.3
2.5	13.3 ± 6.7	23.0 ± 13.3	30.0 ± 10.0	40.0 ± 5.8	43.3 ± 14.5	53.3 ± 3.3	46.7 ± 12.0	63.3 ± 8.8	56.7 ± 14.5	73.3 ± 8.8	86.7 ± 6.7	73.3 ± 8.8	86.7 ± 6.7	76.7 ± 6.7

Note: Data are means ± SE of three replications of 10 insects each.

Table 3. Adult mortality of *Acanthoscelides obtectus* after 1, 2, 3 4, 5, 6 and 7 days exposure to Bambara bean seeds treated with different dosages of *Eugenia aromatica* flower bud powder (*Erfbp*) and *Moringa oleifera* leaf powder (*Molp*) at temperature of 25 ± 2 °C and 60–80% relative humidity

Dosage rate (g/20g)	Mean % mortality (± SEM)													
	1 day		2 days		3 days		4 days		5 days		6 days		7 days	
	Erfbp	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*	*Erfbp*	*Molp*
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.25	73.3 ± 3.3	53.3 ± 12.0	76.7 ± 3.3	53.3 ± 12.0	86.7 ± 3.3	60.0 ± 5.8	86.7 ± 3.3	76.7 ± 8.8	86.7 ± 3.3	76.7 ± 8.8	86.7 ± 3.3	76.7 ± 8.8	86.7 ± 3.3	76.7 ± 8.8
0.5	76.7 ± 3.3	66.7 ± 8.8	80.0 ± 0.0	70.0 ± 11.5	90.0 ± 0.0	80.0 ± 5.8	93.3 ± 3.3	86.7 ± 3.3	93.3 ± 3.3	90.0 ± 0.0	93.3 ± 3.3	96.7 ± 3.3	93.3 ± 3.3	96.7 ± 3.3
1.0	83.3 ± 3.3	70.0 ± 10.0	86.7 ± 3.3	73.3 ± 6.7	90.0 ± 0.0	73.3 ± 6.7	93.3 ± 3.3	90.0 ± 5.8	93.3 ± 3.3	93.3 ± 6.7	93.3 ± 3.3	100 ± 0.0	93.3 ± 3.3	100 ± 0.0
1.5	86.7 ± 3.3	83.3 ± 6.7	93.3 ± 3.3	83.3 ± 6.7	69.7 ± 3.3	83.3 ± 3.3	100 ± 0.0	90.3 ± 5.8	100 ± 0.0	96.7 ± 3.3	100 ± 0.0	100 ± 0.0	100 ± 0.0	100.0 ± 0.0
2.0	90.0 ± 5.8	83.3 ± 3.3	96.7 ± 3.3	83.3 ± 3.3	100 ± 0.0	86.7 ± 8.8	100 ± 0.0	93.3 ± 3.3	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
2.5	96.7 ± 3.3	83.3 ± 3.3	100 ± 0.0	86.7 ± 6.7	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

Note: Data are means ± SE of three replications of 10 insects each.

(Table 5). At the highest dosage 2.0% wt/wt DE alone, the LT_{95} for *S. granarius*, *T. castaneum* and *A. obtectus* was 23.7, 45.8 and 90.3 h, respectively (Table 5).

Effect of combining DE Probe-A® with insecticidal plant powders on insect mortality

The adult beetles irrespective of species suffered significantly higher mortality when exposed to grain treated with mixtures of both leaf powder of *M. oleifera* (*Molp*), *E. aromatica* (*Erfbp*) flower bud powder and DE alone. Mortality was dependent on dosage and duration of exposure (Fig. 1A–F). There was, however, considerable variation in the toxicity of the mixtures of plant powders and DE against the three beetle species. The adults of *A. obtectus* were the most susceptible to the products tested. All the adults of *A. obtectus* died after 4 days' exposure to the different mixtures of plant powders and DE while DE alone caused 100% mortality of the beetles after one day (Fig. 1B). For the adults of *A. obtectus* exposed to *M. oleifera* leaf powder, there was a significant effect of exposure time (DF = 4, *F*-value = 43.797, $P < 0.001$). However, there was statistically no significant interaction between treatment and time (DF = 12, *F*-value = 1.471, $P = 0.203$) (two-way RM ANOVA, DF = 59). All treatments were found effective compared to 0.5% wt/wt *M. oleifera* leaf powder (*Molp*) and 1.0% wt/wt DE (Holm–Sidak method, $P < 0.05$). There was no significant difference in effectiveness between the other treatments (DF = 3, *F*-value = 0.964, $P = 0.468$, Holm–Sidak method, $P < 0.05$). For *S. granarius*, there was both a significant effect of exposure time (DF = 4, *F*-value = 569.061, $P < 0.001$) and treatment ($P < 0.001$). There was also statistically significant interaction between treatment and time (DF = 12, *F*-value = 18.656, $P < 0.001$) (two-way RM ANOVA, total DF = 59). The mixture of *M. oleifera* and DE after 5 days induced the highest mortality of 100% while DE alone killed all the beetles after 4 days (Fig. 1A). There was a significant difference in effectiveness between 1.0% wt/wt *Molp* + 1.0% wt/wt DE and 2.0% wt/wt DE alone (Holm–Sidak method, $P < 0.05$). The highest mixture of 1.0% wt/wt *Molp* + 1.0% wt/wt DE and 2.0% wt/wt DE was both more effective than 0.5% wt/wt *Molp* + 1.0% wt/wt DE and 1.0% wt/wt *Molp* + 0.5% wt/wt DE ($P < 0.001$). A dosage of 1.0% wt/wt *Molp* + 0.5% wt/wt DE was also more effective than 0.5% wt/wt *Molp* + 1.0% wt/wt DE ($P < 0.001$). In the case of *T. castaneum*, grains treated with *M. oleifera* leaf powder and 1.0% wt/wt DE killed all the adults of *T. castaneum* exposed after 4 days while DE alone caused 90% mortality of *T. castaneum* after 5 days (Fig. 1B). There was both a significant effect of exposure time (DF = 4, *F*-value = 37.652, $P < 0.001$) and treatment

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Table 4. The mortality of adults of different stored product insects held on wheat or Bambara groundnut seeds and exposed for 7 days to different dosages of *Eugenia aromatica* flower bud and *Moringa oleifera* leaf powders

Plant product	Insect species	Adult mortality (after 7 days)				
		LD_{50}	LD_{50} SE	LD_{50} LCL	LD_{50} UCL	Intercept
E. aromatica	*Sitophilus granarius*	0.3685	0.2902	–0.6475	1.3844	4.7461
	Tribolium castaneum	1.4155	0.2725	0.4718	2.3593	4.0516
	Acanthoscelides obtectus	0.0884	0.4801	–3.2926	0.221	5.826
M. oleifera	*S. granarius*	0.6863	0.2433	–0.1562	1.5288	4.485
	T. castaneum	1.3938	0.4477	–0.1566	2.9442	4.4316
	A. obtectus	0.1793	0.4543	–2.6705	0.8580	5.6308

Note: The LD_{50} and its standard error was estimated using the probit analysis, lower confidence limits (LCL) and upper confidence limits (UCL) provided from Tables 1–3, analyses based upon 6 concentrations [in % wt/wt] and 10 adults/vial.

Table 5. The survival durations (h) of plant products alone or in combination with diatomaceous earth (% wt/wt)

Eugenia aromatica	*Moringa oleifera*	DE	*Sitophilus granarius*		*Tribolium castaneum*		*Acanthoscelides obtectus*	
			LT_{50}	LT_{95}	LT_{50}	LT_{95}	LT_{50}	LT_{95}
0.5	0	0	162.7	–	–	–	15.5	–
1.0	0	0	131.4	–	–	–	14.6	–
0	0.5	0	91.4	–	–	–	17.2	142.5
0	1.0	0	88.6	–	–	–	17.2	127.4
0.5	0	1.0	42.6	116.6	62.5	–	19.8	111.4
1.0	0	0.5	38.5	90.4	93.3	–	14.7	93.3
1.0	0	1.0	25.1	46.2	63.1	–	14.4	96.3
0	0.5	1.0	42.9	84.7	85.9	–	18.4	112.7
0	1.0	0.5	19.7	46.5	64.6	–	14.7	98.3
0	1.0	1.0	13.4	42.2	55.7	111.4	13.4	56.5
0	0	2.0	21.4	45.8	32.5	90.3	12.4	23.7

Note: The LT_{50} and LT_{95} estimated graphically using SigmaPlot, data from Tables 1–3 after 7 days exposure held on wheat seeds or on Bambara groundnut seeds (for *A. obtectus*) and treated with different dosages of leaf powder of *M. oleifera* (*Molp*) and *E. aromatica* (*Erfbp*) flower bud powder or diatomaceous earth.

(DF = 3, *F*-value = 2.429, $P < 0.001$). There was statistically significant interaction of exposure time and treatment (DF =12, *F*-value = 6.511, $P < 0.001$) (two-way RM ANOVA, total DF = 59). There was no significant difference in effectiveness between 1.0 *Molp* + 1.0 DE and DE alone (Holm–Sidak method, P < 0.05). However, dosage 1.0% wt/wt *Molp* + 1.0% wt/wt DE, and DE alone were both more effective than 0.5% wt/wt *Molp* + 1.0% wt/wt DE, and 1.0% wt/wt *Molp* + 0.5% wt/wt DE ($P < 0.01$). The mixture of 0.5% wt/wt *Molp* + 1.0% wt/wt DE was more effective than 1.0% wt/wt *Molp* + 0.5% wt/wt DE after 5 days posttreatment ($P = 0.163$, Fig. 1C).

For the adults of *A. obtectus* exposed to *E. aromatica* (*Erfbp*) flower bud powder, there was a significant effect of exposure time (DF = 4, *F*-value= 13.863, $P < 0.001$), but not of treatment (DF = 3, *F*-value = 0.157, $P = 0.921$). There was statistically no significant interaction of exposure time and treatment (DF =12, *F*-value = 0.343, $P = 0.971$) (two-way RM ANOVA, total DF = 59). A combination of *E. aromatica* flower bud powder and DE with a dose of 1.0% wt/wt *Erfbp* + 1.0% wt/wt DE was more effective than 0.5% wt/wt *Erfbp* + 1.0% wt/wt DE over time (Holm–Sidak method, $P < 0.05$, Fig. 1E). For the adults of *S. granarius* exposed to *E. aromatica* flower bud powder, there was both a significant effect of exposure time (DF = 4, *F*-value = 55.698, $P < 0.001$) and treatment (DF = 3, *F*-value = 27.672, $P < 0.001$, Fig. 1D). There was also a statistically significant interaction of exposure time and treatment (DF = 12, *F*-value = 6.859, $P < 0.001$) (two-way RM ANOVA, total DF = 59). There was no significant difference in effectiveness between dosages 1.0% wt/wt *Erfbp* + 1.0% wt/wt DE and DE alone, and between 1.0% wt/wt *Erfbp* + 0.5%

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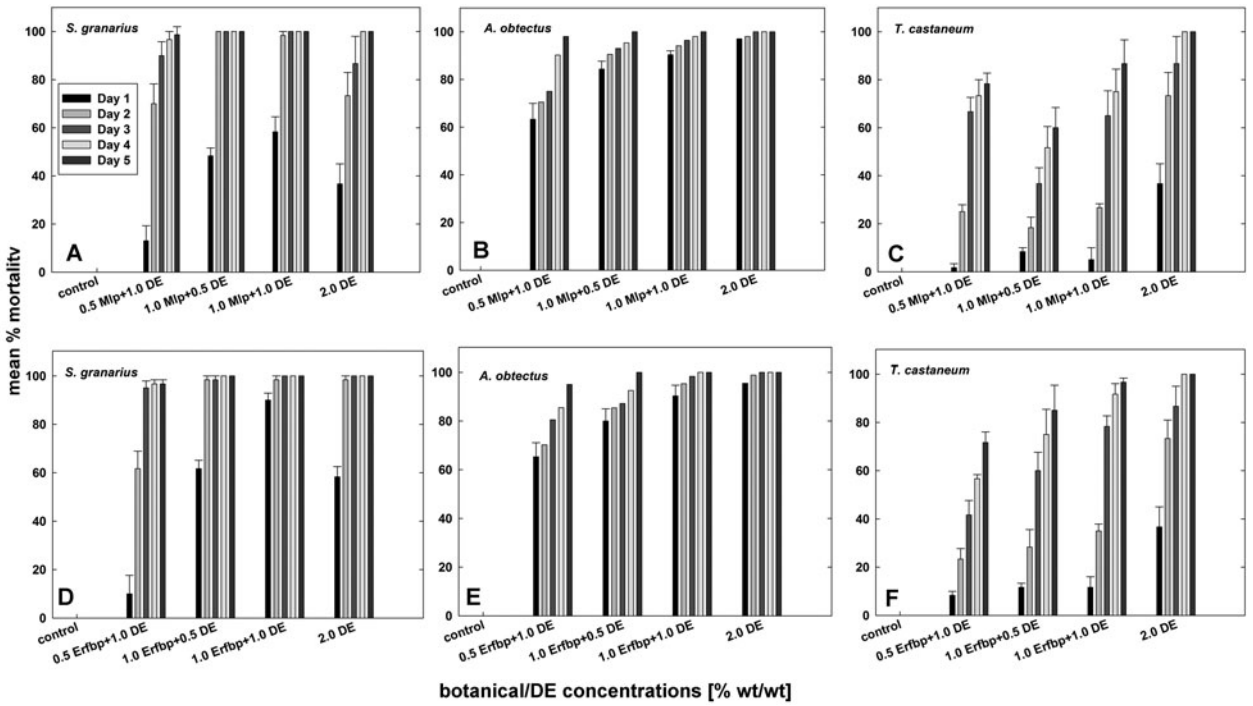

Fig. 1. Cumulative mean percent mortality (± SEM) of adults of *Sitophilus granarius*, *Tribolium castaneum* (on wheat) and *Acanthoscelides obtectus* (on Bambara groundnut seeds) treated with a mixture of different dosages of DE and either of *Moringa oleifera* leaf powder (*Molp*), (A, B, C), or *Eugenia aromatica* flower bud powder (*Erfbp*), (D, E, F), after 5 days of exposure. Data are means of three replications of 10 insects each.

wt/wt DE, and 0.5% wt/wt *Pg* + 1.0% wt/wt DE after 5 days of experimental exposure (Holm–Sidak method, $P > 0.05$). Both 1.0% wt/wt *Erfbp* + 1.0% wt/wt DE, and DE alone were significantly more effective than 1.0% wt/wt *Erfbp* + 0.5% DE, and 0.5% *Pg* + 1.0% wt/wt DE (Holm–Sidak method, $P < 0.05$). The mixture of either *E. aromatica* flower bud powder or DE induced the highest mortality of *S. granarius* of 100% after 5 days (Fig. 1D).

In the case of *T. castaneum*, grains treated with the mixture of 1.0% wt/wt *Erfbp* and 1.0% wt/wt DE caused 97.5% mortality of the beetles after 5 days (Fig. 1F). There was no significant effect of exposure time (DF = 4, *F*-value = 2.538, $P = 0.122$) and treatment (DF = 3, *F*-value = 0.133, $P = 0.880$). There was a statistically significant interaction between exposure time and treatment (DF = 12, *F*-value = 2.288, $P = 0.043$) (two-way RM ANOVA, total DF = 59). On the third day, both 1.0% wt/wt *Erfbp* + 1.0% wt/wt DE caused 78.8% mortality of insects whereas DE alone caused 87.8% mortality. DE alone was significantly more effective than 1.0% wt/wt *Erfbp* + 0.5% wt/wt DE and 0.5% wt/wt *Erfbp* + 1.0% wt/wt DE (Fig. 1F). On the fifth day of experimental exposure, there was no further difference between the treatments 1.0% wt/wt *Erfbp* + 1.0% wt/wt DE and DE alone (Holm–Sidak method, $P < 0.05$).

F_1 progeny production

In all the beetle species tested, fewer adult beetles emerged from grains treated with different dosages of *E. aromatica* flower bud powder and the leaf powder of *M. oleifera*. Results on the first generation (F_1) adults of the three beetle species in grains treated with various dosages of leaf powders of *E. aromatica* or leaf powder of *M. oleifera* (Fig. 2) showed a dose-dependent effect on progeny production (DF = 53, *F*-value = 16.25, $P < 0.001$, two-way ANOVA). Both powders caused a highly significant reduction in the number of F_1 adults produced by all the three tested beetle species compared to the control (*F*-value = 4.53, $P = 0.018$, two-way ANOVA). For the highest dosages of 2.0% wt/wt and dosage 2.5% wt/wt, less than 10% *S. granarius*, *T. castaneum* and *A. obtectus* emerged from the grains treated with *E. aromatica* flower bud powder compared to the untreated control, whereas more than 10% *S. granarius*, *T. castaneum* and *A. obtectus* F_1 adults emerged from the grain treated with different dosages of leaf powder of *M. oleifera.*

Neither powder of *E. aromatica* and *M. oleifera* completely inhibited the progeny production of all the three insects, irrespective of dosage applied. The three beetle species differed significantly in susceptibility towards *E. aromatica* powder. There

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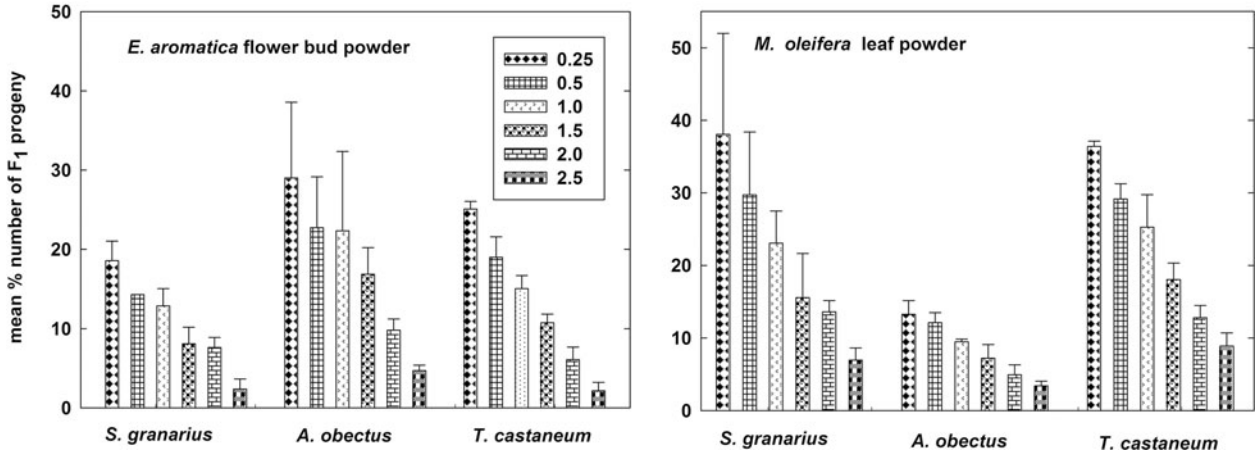

Fig. 2. Mean percent (± SEM) of F_1 progeny of *Sitophilus granarius, Tribolium castaneum* (on wheat) and *Acanthoscelides obtectus* (on Bambara groundnut) expressed as percentage of control, after exposure to *Moringa oleifera* leaf powder and *Eugenia aromatica* flower bud powder at different dosages. Data are means of three replications of 10 insects each.

was no statistically significant interaction between the dosage and beetle species (two-way ANOVA, DF = 10, *F*-value = 0.208, $P = 0.994$). *Acanthoscelides obtectus* was significantly more tolerant in terms of progeny production to *E. aromatica* powder compared to *S. granarius* and *T. castaneum* (Holm–Sidak method, $P < 0.05$) but was more susceptible to *M. oleifera* at a dose of 2.5% wt/wt. *Sitophilus granarius* and *T. castaneum*, however, were significantly more tolerant to *M. oleifera* leaf powder ($P < 0.001$, two-way ANOVA).

After exposure of the three insect species to different dosages of *E. aromatica* flower bud powder, there was a statistically significant difference between the species and dosages (DF = 2, *F*-value = 4.529, $P = 0.018$). However, there was no difference in effectiveness against *A. obtectus* and *T. castaneum* (Holm–Sidak method, $P < 0.05$). The suppression of F_1 production by *E. aromatica* flower bud powder was dosage-dependent, considering all the three beetle species tested (two-way ANOVA, $P < 0.001$). The three beetle species differed significantly in susceptibility to *E. aromatica* flower bud powder (two-way ANOVA, DF = 53, $P < 0.001$). *Acanthoscelides obtectus* and *T. castaneum* significantly differed in F_1 suppression by *E. aromatica* flower bud powder at dosages of 0.5, 1.0 and 1.5% wt/wt. At a dosage of 1.5% wt/wt, all species significantly differed from each other in progeny emergence (Holm–Sidak method, $P < 0.05$).

Discussion

Insects are a problem in stored grain throughout the world, because they reduce the quantity and quality of grain. The majority of farmers in Africa store grains in traditional granaries that are easily attacked by pests, calling for improved, alternative control strategies to ensure food security in Africa. The process must take into consideration the technologies available for the farmers. The farmers will readily accept a concept or technology that builds up or improves upon one that they are used to rather than one that imposes a totally new idea. Synthetic insecticides are commonly used in the management of stored products in Ghana. However, there is increasing concern about the adverse effects this use has on public health and the environment. There are reports on the traditional use of plant powders to control seed beetles attacking stored cowpea seeds (Adarkwah *et al.*, 2017). Plant powder used in the current study caused significant mortality of the adult beetles and the population growth decreased by either hindering oviposition or causing egg toxicity. Plants are rich sources of bioactive compounds that might have a toxic effect on the insect physiological system (Kim *et al.*, 2003; Daoubi *et al.*, 2005). Belonging to various categories (alkaloids, glycosides, tannins, proteic amino acids, steroids, phenols, flavonoids, glucosinolates, quinones, terpenoids, etc.), these bioactive compounds have behavioural and physiological effects on pests that have already been identified (Gonzalo, 2009). Many of these plant compounds have also been recognized to be effective as potential health promoters in humans (Khan *et al.*, 2012).

Eugenia aromatica is lethal to storage bruchids, and so there may be justification for its traditional use by farmers for storing legume seeds (Matsumoto, 1987). The result obtained from this study indicates that *E. aromatica* could be a potential substitute for a synthetic pesticide for the control of *T. castaneum* infestation on wheat. Our results agree with the earlier work of Mahdi and Rahman (2008)

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who reported that black gram seeds treated with *E. aromatica* could suppress F_1 progeny production in *C. maculatus*, which might imply ovicidal action of this plant extract. Adedire and Lajide (1999) also reported that *E. aromatica* powder had significant contact and fumigant action against *C. maculatus*. The mechanism of its action according to the report was by inhibition of oviposition and direct toxicity to adults and eggs (ovicidal). *Eugenia aromatica* has also been reported to retain insecticidal activity for four years after the flower buds were powdered (Ofuya and Dawodu, 2002). The insecticidal activity of the dusts containing powders of *E. aromatica* and *M. oleifera* in different proportions, singly and as mixtures with DE Probe-A® in our study, caused adult mortality and reduction in progeny production of three major stored product beetles. Essential oil constituents isolated from leaf buds of *E. aromatica* (such as eugenol and methyl salicylate) have previously shown significant toxicity potential against *Pediculus capitis* (Phthiraptera: Pediculidae) (Yang *et al*., 2003). Compounds from *E. aromatica* are, therefore, most likely active against a wide variety of insects.

Moringa oleifera powders are thought to have insecticidal effects on the oviposition, eclosion and development of the stored product beetles tested on wheat seed and Bambara groundnut. The present study results indicated that the leaf powder of *M. oleifera* had the highest suppression effect on emerged F_1 beetle adults. *Moringa oleifera* recorded 76% to 100% adult beetle mortality after 7 days of infestation with the highest dosage of 2.5% wt/wt (Tables 1–3). Results also indicated that the powder of *M. oleifera* leaf and *E. aromatica* flower bud showed potential in the control of beetles on wheat seed and Bambara groundnut as bioinsecticides. Generally, the effectiveness of such plant extracts as protectants, is highly dosage-dependent (Upadhyay and Jaiswal, 2007).

Using botanical pesticides, either as crude or formulated extracts, has proved an alternative strategy of insect pest management. *Moringa oleifera* powder failed to cause significant mortality to the adult *T. castaneum* beetles, but discouraged the pest's population growth. The bioactive agent of *M. oleifera* has been shown to be a steroidal glycoside, strophantidin, and the seed powder reduced total microbial and coliform counts by 55% and 65%, respectively (Eilert *et al*., 1981). The 4-(α-L-rhamnosyloxy-benzyl) isothiocyanate was isolated as the active antimicrobial component in *Moringa* seeds (Eilert *et al*., 1981). The chemical constituents of the *M. oleifera* seeds contains 4-(α-L-rhamnosyloxy)-benzyl isothiocyanate, 4-(L-rhamnosyloxy) phenylacetonitrile, 4-hydroxyphenylacetonitrile, 4-hydroxyphenylacetamide, 4-(α-L-rhamnopyranosyloxy)-benzyl glucosinolate, Roridin E, Veridiflorol, 9-octadecenoic acid, *O*-ethyl-4-(α-L-rhamnosyloxy) benzyl carbamate, niazimicin, niazirin, β-sitosterol, glycerol-L-(9-octadecenoate), 3-*O*-(6′-*O*-oleoyl-β-D-glucopyranosyl)-β-sitosterol and β-sitosterol-3-*O*-β-D-glucopyranoside (Fahey, 2005). The insecticidal chemical constituents found in *M. oleifera* leaves and *E. aromatica* flower bud powders might have contributed to the mortality of insects in our study. The assessment of coagulant *M. oleifera* lectin (cMoL) on the flour moth (*Anagasta kuehniella*) suggested that cMoL at 1% w/w could increase the mortality by 27.6%, indicating that the activity of cMoL is a carbohydrate lectin action on the digestive tract (Ramalho de Oliveira *et al.,* 2011). In our study, a dosage of 1% w/w *M. oleifera* leaf powder exposed to *A. obtectus*, *S. granarius* and *T. castaneum* caused 93.3%, 56.7% and 33.3% mortality, respectively after 5 days. The much higher mortality observed in our study compared to that of Ramalho de Oliveira *et al*. (2011) could indicate a more powerful effect on beetles compared to moths.

The *E. aromatica* flower bud powder alone with a dosage of 1.0% wt/wt, therefore, showed longer lasting effects with LT_{50} ranging from 131.4 h for *S. granarius* and 14.6 h for *A. obtectus*, while *T. castaneum* failed to achieve LT_{50}. This result agrees with Ofuya *et al*. (2010) who reported that *E. aromatica* could retain insecticidal activity for as long as four years after the flower buds have been powdered. Generally, the various protectants had low direct toxicity on *T. castaneum*, which agrees with the report of Richards *et al*. (2008) that many species of *Tribolium* at their adult stage are resistant to many insecticides used for their control. The efficacy of these powdered plant extracts was based on high dosages, since lower dosages of *E. aromatica* (0.25% wt/wt, 0.5 % wt/wt) were not as effective compared to 2.5% wt/wt. This corroborates other similar studies (Upadhyay and Jaiswal, 2007; Ojo and Ogunleye, 2013) in which powders of *P. guineense* applied at 5−10.0% w/w caused 100% mortality in *C. maculatus* 4 days posttreatment. This is similar to our findings with *A. obtectus,* in that seeds treated with the lower rates of 0.5−2.5% w/w *E. aromatica* induced 100% mortality after 5 d exposure.

Oyeniyi *et al*. (2015) evaluated the efficacy of *P. guineense* seed powder after 24 h of exposure against *C. maculatus* and recorded LD_{50} values ranging from 0.016 g to 0.679 g, depending on the geographical origin of the seed beetle population. The mortalities obtained for *A. obtectus* in our study were approximately 50% compared to those obtained for *C. maculatus* by Oyeniyi *et al*. (2015), indicating both interspecific and host plant effects may be important for seed beetles. Based on our

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results and those of Oyeniyi *et al*. (2015), we hypothesize that seed beetles are more susceptible than *Tribolium* spp. and *Sitophilus* spp. towards *E. aromatica*. However, some botanical products (essential oils for example) have a broad spectrum of biological toxicity (Bakkali *et al*., 2008), including on mammals. Suthisut *et al*. (2011*a*, 2011b) also found that some natural products were as dangerous to use as synthetic insecticides, because much more of the essential oil is needed to control the insects compared to the synthetic fumigant or contact insecticide. Hence, mixing plant powders with DE to prevent these high dosages was found useful in our studies.

The efficacy of DE against stored product insects is well documented (Subramanyam and Roesli, 2000; Rigaux *et al*., 2001). If less than 1.0% wt/wt DE is applied to bulk grain, one cannot sufficiently control *S. granarius* under field conditions (Schöller and Reichmuth, 2010). Athanassiou *et al*. (2004) used three DE formulations and reported survival of *T. confusum* adults after the application of 1.0% wt/wt DEs. In this study, higher dosages than 1.0% wt/wt DE were applied, to achieve sufficient efficacy within one week. We evaluated the potential synergistic effects of the combination of DE and certain botanical products. Based on the results of the present study, the simultaneous application of both DE and plant powders is feasible, but the results are affected by the target species. Moreover, the insecticidal effect is also affected by the respective dosages of the two components. DEs are slow-acting in comparison with traditional grain protectants (Korunic, 1998). At shorter (3 d) exposures, the speed of kill was generally higher with a mixture of plant powders and DE compared to DE alone. Fast mortality results in prevention of mating and reduction of oviposition; and consequently, in reduced grain damage (Subramanyam and Roesli, 2000; Athanassiou *et al*., 2003). DE mixed with botanical products could be a solution to the effects arising, especially from the slow efficacy of DEs against *Tribolium* species, as *Tribolium* species are classified among the most DE-tolerant stored grain beetles (Arthur, 2000; Vayias and Athanassiou 2004; Adarkwah *et al*., 2012).

The combination of botanical products and DE may be economically attractive, as farmers can plant botanicals to replace the costly DE or synthetic chemicals. On the other hand, the botanical products tested here may not be effective enough if applied alone, and the addition of DE could help to achieve the required effectiveness. In practice, multiple factors may influence the effectiveness of botanical products being used for pest management on stored product beetles, e.g. geographical origin of the beetle strains and cowpea variety, as shown by Oyeniyi *et al*. (2015).

Furthermore, it is unfortunate that in the use of botanical products, issues of exclusivity, licensing and potential profit in the small portion of the agricultural market that postharvest pesticides represent, are serious barriers to further development and bringing these insecticides to the market. There are a number of issues that must to be addressed before botanical products or extracts of plants can be used as grain protectants. These include type of preparation, host commodity, plant species, test insect and how quickly the botanical becomes ineffective under field conditions. A successful grain protectant must have residual activity to protect grain from infestation for several months. High temperature or high grain moisture content increase the degradation of grain protectants; therefore, field trials under different climatic conditions and with different stored products are needed, to verify these laboratory results for recommendation to farmers, for the preservation of wheat seed and Bambara groundnut against infestation.

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