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Honeybee propolis protects stored maize grain from damage by maize weevil (*Sitophilus zeamais* Motsch.) and reduces damage by larger grain borer (*Prostephanus truncatus* Horn) in Kenya

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Abstract

The losses caused by larger grain borer (*Prostephanus truncatus* Horn) and maize weevil (*Sitophilus zeamais* Motsch.) on stored maize (*Zea mays* L.) grains range from 9 - 45% and 12 - 20%, respectively, depending on the storage period. These are additional losses to the field losses before harvest, threatening food security status in Kenya. With maize being a staple food consumed daily, using synthetic chemical products to manage these pests has not been easy to implement. Unaffordability of these products also forces resource-poor farmers to sell their produce early at low prices, thus compromising their food security needs. This study examined the effects of honeybee propolis extract in reducing larger grain borer (LGB) and maize weevil (MW) infestation to below economic injury level. The treatments included control, 20% propolis extract, undiluted propolis extract, Actellic super® dust, Kensil F® dust, and 20% propolis extract + Kensil F® dust. Each treatment was applied to 10g of grains, and later, 30 individuals of each insect species were introduced independently. Mortality was measured after 14 days, and all insect individuals were discarded immediately. Progeny emergence was recorded after a further 42 days. Results showed 20% propolis extract caused 48% mortality of LGB but in combination with Kensil F® dust, the extract caused 67% mortality. In contrast, this concentration caused 100% MW mortality. Progeny emergence reduction of 16% - 26% for LGB and 100% for MW was observed in grains treated with propolis extract compared to untreated grains.

Keywords: Propolis ethanolic extract, stored maize, postharvest loss, *Prostephanus truncatus*, *Sitophilus zeamais*

Introduction

In Sub-Saharan Africa, postharvest losses of stored crops are substantial due to infestation by insect pests (Phillips and Throne, 2010; Abebe *et al.*, 2009). The larger grain borer, *Prostephanus truncatus* (Horn) (herein LGB) and maize weevil, *Sitophilus*

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zeamais Motschulsky (herein MW) are among the major pests of stored maize (Asawalam and Emosaire, 2006; Odour *et al*., 2000). The LGB causes losses ranging from 9% to 45% depending on storage period (Mutambuki *et al*., 2011; Gueye *et al*., 2008; Markham *et al.,* 1991), while MW causes grain weight loss of 12% – 20% (Boxall, 2002; Mutiro *et al*., 1992). In areas where smallholder maize production supports the livelihood of majority of the population, such losses threaten food security.

In addition, the optimal time for selling grain depends on the trade-offs between future higher prices offered by the buyers and the higher risk of grain weight loss due to insect damage in storage (Yigesu *et al*., 2010). The risk of failing to prevent grain loss during storage, therefore, forces resource-poor farmers to sell their produce early at low prices.

The commonly used control methods rely mostly on the application of contact synthetic insecticides (Arthur, 1996) and gaseous fumigants such as methyl bromide and phosphine (Taylor, 1989). However, storage insect pests are increasingly becoming resistant to the few existing insecticides (Golob *et al*., 1990; Benhalima *et al*., 2004; Pereira *et al*, 2009). Moreover, fumigants can only be sold and used by pesticide applicators that are certified. The cost of the applicators and sourcing of fumigants is prohibitive to smallholder farmers, who are majority of the producers. The use of many of the chemicals is also under strict regulation (Schoeller *et al*., 1997) due to environmental safety concerns. Alternative control options to protect grain are urgently required. Bee propolis extract is one such option that assures human and environmental safety and reduction in postharvest infestation to acceptable economic level.

Propolis is one of the secondary hive products, the primary product being honey, amassed by bees as an outcome of their foraging and nectar collection processes. Propolis is a complex mixture of various amounts of beeswax and resins gathered by honeybees from plants modified and used by bees as a

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general-purpose sealant, a wind excluder and antibiotic in hives (Markham *et al.*, 1996). It consists of waxes, resins, water, inorganics, phenolics and essential oil (Bonvehí & Coll, 1994; Dobrowolski *et al.*, 1991). The propolis composition is dependent upon the type of plants that the bees forage on (Markham *et al.*, 1996). Among other uses, propolis is utilized in food technology (preservative), medicine (dermatology and dental care), and traditionally as herbal medicine. Extracts of propolis have attracted scientific attention worldwide due to their biological properties and pharmacological activities (Marcucci, 1995) there is great interest in studying its use as a grain protectant.

In this study, the bio-efficacy of propolis extract for the control of LGB and MW in stored maize was evaluated. Adult mortality and reduction of progeny emergence were the parameters used to assess the insecticidal effects of the propolis extract on these pests.

Materials and methods

Sources of propolis, test insects and maize grain

Honeybee propolis was collected from bee-hive boxes belonging to the Project: "Knowledge management of pesticide risk to wild pollinators of high value crops in Brazil and Kenya" at National Agricultural Research Laboratories (NARL), Kabete, 2011-2013. Actellic super® dust was sourced from a local agrochemical store. This product is a mixture of an organophosphate (16% Pirimiphos – methyl), which targets traditional storage insect pests such as MW, and Pyrethroid (0.3% permethrin), which targets exotic pests such as LGB. The DE Kensil F® dust was obtained from the African diatomite Industries (K) Limited at Kariandusi, Gilgil, along the Nairobi - Nakuru Highway. It is a fine creamy white dust composed of silica dioxide (84.0%), aluminium dioxide (4.9%), ferrous dioxide (2.3%), other compounds (8.8%), retained moisture content (12%), and particle size retention of 2.2% when subjected to 106µ (150 mesh) screen analysis. The test insects were obtained from colony

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stocks maintained on susceptible hybrid maize (H513) in a controlled room temperature of 27°C - 30°C and 65% - 70% relative humidity in darkness at the Entomology Section, FCRC Kabete. Adult insects of mixed sexes, 2 – 3 weeks old, were used in the evaluation. Maize grain was obtained from the stock for rearing the insects.

The grains had been disinfested to kill any field infestation by fumigation using phosphine gas for 7 days in an airtight metal drum and aerated for 3 hours before use. The moisture content of the grains was 12.5% (wet weight basis) as determined by forced air oven method at 103±1°C/72 hours (ASAE, 1999).

Preparation of propolis extract

The propolis extract was prepared following a procedure described by Obasa *et al.* (2007) with minor modifications. Briefly, after debris removal, 100g propolis was cut into small pieces (about 5 mm in length) to increase contact surface between propolis and ethanol (solvent) to enhance dissolution. The pieces were put into a 500ml flat-bottomed glass flask, and 200ml of 96% purity ethanol (which was enough to submerge the pieces) was added. Whereas 70% ethanol has been found to extract 50% – 70% propolis constituents, 96% purity of the solvent was chosen because it gives excellent results (Sforcin and Bankova, 2011). The mouth of the flask was then covered with aluminium foil and held securely with rubber bands. The mixture was vigorously hand-shaken for 30 minutes. To allow for thorough extraction of the active ingredients, the mixture was left to stand for 14 days at ambient conditions, with daily vigorous handshaking for five minutes. The resultant extract was filtered through Whatman No. 1 filter paper (Whatman Ltd, Maidstone, England) into a 250ml flat-bottomed glass flask. Ethanol was evaporated from the extract by holding the flask in a hot water bath at 80°C for 10 minutes. The flask was then left to stand overnight at room temperature to aid any residual ethanol to evaporate.

The filtrate (brownish in colour and slightly sticky) was used to prepare

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20% concentration. This was achieved by adding 1ml of undiluted crude extract, with the aid of a plastic 5ml Ebastel® syringe, to 4ml of ethanol in a 10ml glass beaker. The 20% concentration was chosen because it was reportedly effective in controlling *P. truncatus* in the laboratory in Southwestern Nigeria (Adedoyin *et al.*, 2010).

Mortality tests

The experiment consisted of six treatments: T1: control; T2: 20% propolis extract; T3: undiluted propolis extract; T4: Actellic super® dust; T5: Kensil F® dust and T6: 20% propolis extract +Kensil F® dust. The trial was carried out from October to December 2012.

Maize grains (150g) were weighed into each of the six 250ml-capacity glass jars into which the treatments were applied. Untreated grains in the first jar acted as the negative control. The grains in the second and third jars were treated with 20% and undiluted propolis extracts, respectively. Actellic super ® was applied to the grains in the fourth jar at the recommended rate of 50g/90 kg grain was included as positive control while the grain in the fifth jar was admixed with the local DE dust Kensil F® at a dose rate of 0.9% w/w (900 ppm). The dosage of the local DE was chosen because it was reportedly effective against *S. zeamais* (Khakame *et al.*, 2012). The grain in the sixth jar was treated with 20% propolis plus the Kensil F® dust. The mouth of the jars was secured with a plastic lid and the grains were mixed thoroughly by hand for one hour. The lids were removed, and the jars were left at room temperature for two days to allow for evaporation of ethanol used for dilution.

After the two-day allowance for ethanol evaporation, the grains of each treatment jar were divided into eight 10g lots as replicates and put in 2.5cm diameter and 7.5cm height flat-bottomed glass tubes with vented plastic tops. Four 10g lots (replications) of each treatment prepared above were used for *P. truncatus* and the remaining four lots for *S. zeamais*. Thirty adult insects aged 2 - 3 weeks were

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introduced into the treated maize. Following the addition of the insects, the glass tubes were kept undisturbed in a completely randomized design (CRD) on shelves in an incubator (Stuart Scientific, UK) at 27°C and 65% - 70% relative humidity for 14 days when the number of dead insects was recorded. The exposure period was chosen because it was reported to result in satisfactory control of *S. zeamais* (Khakame *et al*., 2012). An insect was considered dead on failure to respond by moving when prodded three times with a small paint brush. After mortality assessment, both dead and live adults were discarded, and the grains were incubated for a further 42 days to assess progeny emergence. Percentage mortality and progeny emergence reduction were calculated as follows:

$$\text{Mortality} = \frac{100 \text{ X No of dead insects}}{\text{Total No. of introduced}}$$

$$\%\ \text{Progeny reduction} = 100 \text{ X } (1 - F_T)/F_C$$

(Arthur and Throne, 2003)

Where F_T and F_C are the mean number of F_1 adults in treated and untreated grains, respectively.

Data analysis

Data on adult mortality and progeny emergence were recorded. The data were subjected to analysis of variance (ANOVA) using general linear model procedure (GenStat software Release 12.1 for windows, 2009), with mortality as the response variable; treatments and insect species as the factors. Significant differences were separated using Fisher's protected least significant difference (LSD) test at p = 0.05% level. Data on mortality was transformed using the formula arcsine √(percentage) while adult progeny emergence was log-transformed to normalize the variation before analysis.

Results

Insect mortality

The percent mortality differed significantly across treatments ($F_{5, 33}$ =406.95, p<0.001) and insect species ($F_{1, 33}$ =763.72, p<0.001). There was a significant interaction ($F_{5, 33}$ =87.13, p<0.001) between treatment and insect species in terms of mortality

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caused by the different factors. The treatments showed varied efficacies against *P. truncatus* (Figure 1). Actellic super® dust was most effective against *P. truncatus* (94.2% mortality) while Kensil F® dust resulted in an unsatisfactory 15.8% mortality of the same pest species. The mean mortality caused by 20% and undiluted propolis extract did not differ significantly (48.3% and 45%, respectively). Application of 20% propolis extract in combination with Kensil F® dust showed an additive (66.7% mortality) but not a synergistic effect. No mortality occurred in the control treatment. Overall, *P. truncatus* was less susceptible to propolis extract compared with *S. zeamais*.

For *S. zeamais,* very good control (> 98% mortality) was achieved on all treated grains (Figure 1) with no significant differences noted between the treatments. No mortality occurred in the negative control treatment. The mean mortality of *S. zeamais* caused by 20% and undiluted propolis extract was the same (100%). The mortality response achieved on grains treated with 20% propolis extract in combination with Kensil F® dust was neither additive nor synergistic. No advantage was thus gained by combination treatment. Overall, *S. zeamais* was more susceptible to propolis extract.

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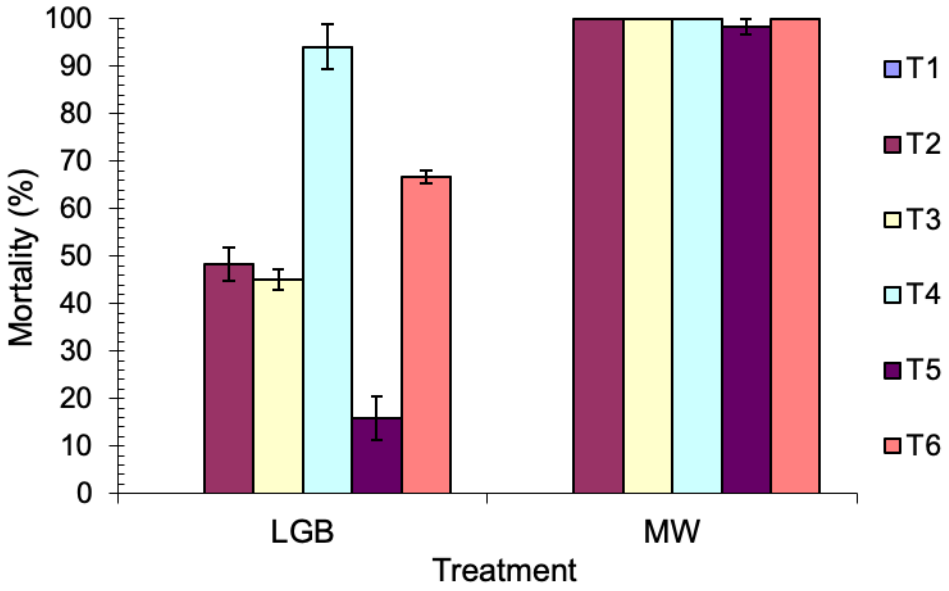

Figure 1: Mortality of *P. truncatus* and *S. zeamais* adults after 14 days of parent exposure to maize grain admixed with the treatments. T1 = control; T2 = 20% propolis extract; T3 = undiluted propolis extract; T4 = Actellic super® dust; T5 = Kensil F® dust; T6 = 20% propolis extract + Kensil F® dust.

NB: no error bars appear where 100% mortality was recorded: Fisher's protected least significant difference test, $p<0.05$.

Progeny emergence

The mean number of progeny production varied significantly with treatment ($F_{5,33}$ =34.11, $p<0.001$) and insect species ($F_{1,33}$ =260.57, $p<0.001$). However, there was significant interaction ($F_{5,33}$ =100.74, $p<0.001$) between treatment and insect species for the effect on progeny emergence. The progeny numbers for *P. truncatus* ranged from 13.3 (Actellic super® dust) to 136.8 (control) and emergence reduction from 0 to 90.3% (Table 1). For *S. zeamais*, no emergence occurred in all treated grains with corresponding percentage reduction of 100% except for the control treatment where 69 adults emerged, representing 0% reduction.

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Table 1: Progeny production of *P. truncatus* and *S. zeamais* after 14 days of parent exposure to maize grain admixed with treatments.

Treatment	Progeny emergence (No.)		Emergence reduction (%)	
	P. truncatus	***S. zeamais***	***P. truncatus***	***S. zeamais***
Control	136.8^{a}	69.0^{de}	0.0^{e}	0.0^{e}
20% propolis extract	100.8^{bc}	0.0^{f}	26.3^{cd}	100.0^{a}
Undiluted propolis extract	114.3^{ab}	0.0^{f}	16.5^{de}	100.0^{a}
Actellic super® dust	13.3^{f}	0.0^{f}	90.3^{a}	100.0^{a}
Kensil F® dust	87.8^{cd}	0.0^{f}	35.8^{c}	100.0^{a}
20% propolis extract + Kensil F® dust	55^{e}	0.0^{f}	59.8^{b}	100.0^{a}

Means within same column followed by same superscript are not significantly different, Fisher protected least significant test, $p<0.05$.

Discussion

This study demonstrated that *P. truncatus* and *S. zeamais* differ in their susceptibility to biological activity of propolis. Although Kensil F® dust was applied at high dosage rate, very low mortality was achieved for *P. truncatus*. The pest's internal feeding behaviour and larval development inside the grain might have resulted in reduced exposure to Kensil F® deposits on the kernel's surface. The combination of the inert dust and 20% propolis ethanolic extract increased the mortality of *P. truncatus* slightly but the effect was additive. The combined application is ergonomic but showed lack of synergistic effect. The mechanism of additive interaction remains unclear. The considerably lower mean mortality obtained in the present study is in discordance with an earlier report (Ositpitan *et al.*, 2010) that showed good control of *P. truncatus* by 20% propolis extract. The difference observed could probably be the consequence of botanical sources and chemical composition in different regions. Bees are known to choose different plants as a source of propolis in a given habitat (Bankova, 2005). Probably, the bees around Nairobi did not find a plant source of promising bioactivity for the control of *P. truncatus*. However, further studies may be done with propolis sourced from other parts of the country to confirm the bioactivity of the propolis

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against this pest. With these findings, it is clear that propolis has potential pest control activity, and this could be explored further to identify methods of packaging the product for easy use by farmers.

All the treatments in the present study achieved satisfactory control of *S. zeamais*. Whereas no significant difference in the control of *S. zeamais* was observed, there were no adult emergences from treated grains. This suggests propolis extract contains compounds that inhibit insect development. No control advantage was gained by a combination of Kensil F® dust and 20% propolis extract.

The high efficacy of propolis extract against *S. zeamais* observed in this study compared to *P. truncatus* remains unclear but could probably be explained by the feeding behaviour of these two insect pests. The *P. truncatus* bores into the grain, generating a lot of flour as it tunnels within the grain. This prevents the pest from direct contact with the propolis extract coated on the outside of the grain (testa). In contrast, maize weevil feeds from the outside, which effectively increases its contact with propolis.

Conclusion

In conclusion, the study has demonstrated that bee propolis extract possesses insecticidal properties useful for the management of storage insect pests and could serve as an innovative alternative to synthetic pesticides.

Recommendations

Based on the findings, it is recommended that propolis from other parts of the country be tested to confirm their efficiency against the two pests. Further, on-farm applications are recommended to ensure the utilization of propolis for the reduction of stored maize pests. A commercial formulation should be sought to ensure propolis is packaged for easier use by farmers.

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