

Bee propolis as protectant of stored grains against insect pests' damage in Kwara State, Nigeria

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Abstract: The importance and widespread use of stored grains is potentially negated by insect pests and lack of efficient and environment friendly control methods. In this study we explored the use of propolis extracts as protectant of grains against damage caused by *Sitophilus zeamais* and *Callosobruchus maculatus*. Bee propolis collected from beehives was diluted with ethanol to obtain 0%, 10%, 30%, 50% and 100% concentrations. 5ml each of the concentrations of propolis extract was applied to 100 g each of maize and cowpea grains infested with 10 pairs of tenera maize weevil and cowpea weevil respectively in 250 cm³ kilner jar and left for 35 days. Each treatment was replicated four times and arranged in the laboratory using completely randomised design. Data were collected on percentage grain damage, weight loss and insect mortality. Bee propolis extract significantly reduced ($P < 0.05$) grain weight loss and damage, and enhanced insect mortality. The effectiveness of propolis extract was concentration-dependent. The phytochemical analysis of *Apis mellifera* propolis showed the presence of compound like phenol, flavonoid and tannin. Propolis extract application could be incorporated into integrated pest management (IPM) practices.

Keywords: Stored grains; bee propolis; insect pests; protectant; botanical insecticides

1. Introduction

A grain is a small, hard, dry seed harvested for human or animal consumption. After harvest, dry grains are durable and could be stored for long periods in silos, barns among others. Major global commodity markets exist for maize, rice, cowpeas, soybeans, wheat and other stored grains. Maize is an important staple food crop in parts of the world where it is grown (Nwosu et al., 2015a). Apart from the role maize plays as food, it also serves as cash crop and used as an industrial raw material (Nwosu et al., 2015b). Odeyemi and Daramola (2000) observed decreasing maize production due to some environmental constraints including insect pest invasion. Cowpea is another crop adapted to warm weather and drier regions of the tropics like Nigeria

where other food legumes do not thrive well. It 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). Nigeria is the largest producer and consumer of cowpea, accounting for about 45% of world production while Africa accounts for about 75% (Abebe et al., 2005; Dugje et al., 2009; Brisibe et al., 2011).

Apis mellifera propolis is a resinous substance collected by honeybees from plants particularly from flowers and leaf buds to maintain the hive environment in proper condition (Krell, 1996). Propolis is composed of 30% wax, 50% resin, 10% essential oils, 5% pollen and 5% various organic residues. It is also rich in biochemical constituents including phenolic compounds, vitamins, amino acids, carbohydrate, hydrocarbons and carboxylic

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acids which are mainly responsible for its therapeutic effects (Bankova et al, 1992; Silici, et al., 2007). Bee propolis had been found to contain phytochemicals, such as flavonoids, terpenes, phenolics and their esters, sugars, hydrocarbons and mineral elements. It had been shown to exhibit broad spectrum of biological properties, such as antibacterial, antiviral and anti-inflammatory effects (Nijveldt, 2001; Maciejewicz, 2001; Bueno-Silva, 2013; Zhang et al., 2014). It has also been reported that bee propolis extracts possesses antioxidant activity and displays pesticidal property (Orsi et al., 2005). However, chemical composition of bee propolis can vary greatly depending on the geographical location and botanical origin and bee species (Silici & Kutluca, 2005; Salatino et al., 2011; Toreti et al., 2013).

Sitophilus zeamais Mostchulsky (Coleoptera: Curculionidae) and *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae) are economic pests of stored grains. They cause up to 40% yield loss in stored maize and cowpea grains respectively within six months (Ojo & Omoloye, 2012; Ojo et al., 2016). *Callosobruchus maculatus* alone causes an estimate 12 – 15% grain weight loss in cowpea. Most of the damage is, however, done by the larvae, which develop in the seed causing losses in weight, market value and viability (Taylor, 1977; Ojo et al., 2016). Additionally, they reduce the nutritional composition and alter amino acids content of infested grains, particularly amino acids, lysine, tryptophan (Khan & Mannan, 1991; Odeyemi & Daramola, 2000; Muzemu et al., 2013). In order to manage these pests, the use of synthetic insecticide is commonly employed by farmers due to its effectiveness. Despite the effectiveness of synthetic insecticide, its usage is associated with ecological problems such as toxicity, environmental pollution, pest resistance and resurgence, which are detrimental to human and animal health as well as other components of the ecosystem. Therefore, it is of interest to explore use of natural products as eco-friendly alternative to synthetic insecticides either as a single or as a component of integrated pest management (IPM). Moreover, compared to synthetic pesticides, natural products may have advantages of being low cost, easy to use and non-toxic. This is our motivation for evaluating the insecticidal potency of propolis extracts on stored grain pests, *Sitophilus zeamais* and *Callosobruchus maculatus*.

2. Materials and methods

2.1. Study site

The study was carried out at Kwara State University (KWASU), Zoology Department Laboratory and

KWASU Centre for Beekeeping, Training and Research (CBTR) Malete, Moro Local Government Area, Kwara State, Nigeria. Kwara State lies between latitudes 8° 10' and 19° 50'N and between longitudes 3° 10'N and 6° 05'E. The State falls within the southern savanna zone and partly belong to one of the four major zones into which Keay (1953) divided the savanna regions of Nigeria. The derived savanna zone extends southwards from the southern guinea zone to the forest zone (Adegbola & Onayinka, 1976; Ajao, 2012). The State covers a total land area of about 32,500 square kilometres, a total land size of 3,682,500 hectares and falls within the southern limits of the tropical Savannah zone of Nigeria with mean annual rainfall ranging from 800 mm to 1500 mm, between the months of April and October with two peaks in July and September (Keay, 1953). The mean annual temperature is between 31.5 °C and 35 °C. February to April is the hottest months while June to September has the lowest maximum temperature which coincides with the peak of the dry and wet seasons respectively.

2.2. Source of insect culture and grains

500g each of maize (variety Bende Red) and cowpea (variety IT96 – 610) were procured from Nigeria Stored Product Research Institute (NSPRI), Ilorin, Kwara State, Nigeria. The grains were put in the 4 °C refrigerator for two weeks to kill any incipient eggs and thereafter removed before its use for bioassay. *Sitophilus zeamais* and *Callosobruchus maculatus* adult populations used for the study were obtained from the culture maintained on dried maize and cowpea at NSPRI. Twenty pairs each of adult *S. zeamais* and *C. maculatus* were introduced into 500g maize and cowpea grains with four replicates. These weevils were allowed to feed and oviposit on the grains for fourteen days and thereafter sieved out. Cultures were kept and routinely maintained at ambient mean temperature and relative humidity of 30.7 °C and 74.5% respectively, for 35 days (Nwosu et al., 2015b) prior to check for progeny production. The cluster from these set up served as source of insect used for subsequent bioassay.

2.3. Propolis production and collection

100g of *Apis mellifera* propolis samples were collected from 15 Kenya Top Bar (KTB) and 10 Langstroth (LANG) hives at CBTR apiary by scrapping top bars, internal combs, bottom boards and hive entrances using sharp knife. Collected propolis samples were mixed, pulverised and homogenised using a mortar and pestle. Thereafter, they were weighed and placed in refrigerator until extraction.

2.4. Phytochemical analysis of propolis extract

The phytochemical constituents of the propolis were evaluated following the methods of Harborne, 1973; Shimada et al., 1992; McDonald, 2001 and Chang et al., 2002. Phytochemical analysis of propolis was carried out at NSPRI laboratory, Ilorin, to ascertain its constituents. Antioxidant activity of the extract was measured with the DPPH method (Shimada et al., 1992). Propolis extract was dissolved in methanolic water (9:1) for 72 hours then centrifuged at 30 °C for 10 min at 10000 rpm and different concentration were made by serial dilution method using methanol water as diluents and mixed with 0.16mM methanol DPPH (1:3), incubated at room temperature in the dark for 30 min. Similar protocol was followed for ascorbic acid for comparison and 3:1ml mixture of methanol DPPH and methanol water was employed as negative control. The degree of decolorization of DPPH was determined by measuring the O.D. at 517nm.

Aluminium chloride colorimetric method was used to determine the total flavonoids (Chang et al., 2002). Total flavonoid content was determined using quercetin as standard. The mixture of 10% of 100µl of $AlCl_3$, 100µl $NaNO_3$ (5%), 670µl of 1 millimolar NaOH and 100µl of sample was vortexed and incubated in dark at room temperature for 25 min. The O.D. value was measured using spectrophotometer at 510 nm. The total phenolic content was determined by a Folin-ciocalteu assay using gallic acid (GA) as the standard (McDonald, 2001). The mixture of the propolis extract (1ml), 1ml of Folin-ciocalteu's reagents, 10% Na_2CO_3 (1ml) and distilled water (7ml), was vortexed and incubated for 25 min in dark at room temperature. The quantification of phenolic compounds was performed spectrophotometrically by measuring the absorbance in UV-VIS spectrophotometer at 765 nm against blank which contain a mixture of 1ml methanol water, 1ml of Folin-ciocalteu's reagents and 1ml of 10% Na_2CO_3 . The total phenol content was expressed as Gallic acid equivalents (mg of GAE/g sample) through the calibration curve of Gallic acid. Linearity calibration curve was 10 to 100µg/ml ($r = 0.99$).

2.5. Preparation of propolis extract

Fifty grams raw propolis was cut into small bits and placed in a 250 ml conical flask. 100 ml of ethanol was poured into the conical flask to submerge the propolis. The flask content was vigorously shaken for 30 minutes and left for 7 days to allow for extraction of the active ingredients (Obasa et al. 2007). The extract

was filtered through a Whatman No. 1 filter paper. The ethanol in the light brown filtrate evaporated overnight at room temperature. The light brown, sticky crude extract was thereafter serially diluted with ethanol to prepare 10%, 30%, 50% and 100% (w/vol) ethanolic concentrations of the bee propolis (ECP).

2.6. Testing the insecticidal activity of the propolis extracts on stored grains pests.

50g sample of maize was weighed in 250 m³ kilner jars using Mettler weighing balance. 5 ml of 10% concentration of the propolis extracts was added to the maize grain sample with the aid of a syringe. They were carefully mixed together and left for 1 hour to allow evaporation of the ethanol used for dilution. Thereafter, kilner jar was infested with 10 pairs of 1–5 days old *S. zeamais*. Treatment was replicated four times and arranged in the laboratory using completely randomised design. Then the protocol was repeated for the rest of extract concentrations and control (ethanol). Thereafter the same procedure was repeated for the second grain (cowpea) with *C. maculatus* as pest species. Feeding activities of the maize weevil and cowpea weevil were monitored each for 35 days. Thereafter, insects were removed by sieving, damaged and undamaged grains were sorted and weight loss of the grain as a result of insect feeding activities determined. Data were collected on insect mortality, grain damage and grain weight loss. Inability of the insects to move or respond to three probing using a blunt probe showed that insects had died. The percentage weight loss and percentage grain damage were calculated using the formula (Odeyemi & Daramola, 2000):

$$\% \text{ grain damage} = \frac{\text{Number of damaged grains}}{\text{Total number of grains}} \times 100$$

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2.7. Statistical analysis

Statistical analysis was done using DSAASTAT (Onofri, 2007). Percentage data had zero values, which were non-normal and were arcsine-transformed to homogenise variance before analysis. Data were analysed using descriptive statistics and analysis of variance (ANOVA). Means were separated using Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$.

3. Results

3.1. Effect of propolis extract on *Sitophilus zeamais* and *Callosobruchus maculatus*

Propolis extracts had significant effect ($P < 0.05$) on grain damage and grain weight loss by maize weevil and cowpea weevil (Table 1 and 2) in comparison to the control. There was no significant difference ($P > 0.05$) among the four levels of propolis extract concentrations on the grain weight loss. Propolis treated grain at 100% concentration significantly ($P < 0.05$) prevent grain damage than the control and 10% level of propolis extract concentration. The higher the propolis concentration the lower the grain damage and grain weight loss.

Table 1: Percentage grain damage ($\pm$ SEM) of *Sitophilus zeamais* and *Callosobruchus maculatus* on propolis treated maize and cowpea grains

Treatment	<i>Sitophilus zeamais</i>	<i>Callosobruchus maculatus</i>
0%	46.67 $\pm$ 0.39 ^a	54.29 $\pm$ 0.63 ^a
10%	36.67 $\pm$ 0.45 ^{ab}	49.98 $\pm$ 0.52 ^a
30%	33.33 $\pm$ 0.47 ^{ab}	46.43 $\pm$ 0.40 ^{ab}
50%	30 $\pm$ 0.49 ^{ab}	43.57 $\pm$ 0.54 ^{ab}
100%	26.67 $\pm$ 0.52 ^b	30.71 $\pm$ 0.88 ^b

Means in columns sharing common letters are not significantly different at $P < 0.05$ (Tukey's HSD test)

Table 2: Percentage grain weight loss ($\pm$ SEM) of *Sitophilus zeamais* and *Callosobruchus maculatus* on propolis treated maize and cowpea grains

Treatment	<i>Sitophilus zeamais</i>	<i>Callosobruchus maculatus</i>
0%	28.39 $\pm$ 0.68 ^a	30.4 $\pm$ 0.49 ^a
10%	4.62 $\pm$ 0.96 ^b	7.5 $\pm$ 1.03 ^b
30%	2.36 $\pm$ 0.22 ^b	7.35 $\pm$ 0.41 ^b
50%	2.25 $\pm$ 0.49 ^b	5.85 $\pm$ 0.45 ^b
100%	2.12 $\pm$ 0.75 ^b	3.55 $\pm$ 0.52 ^b

Means in columns sharing common letters are not significantly different at $P < 0.05$ (Tukey's HSD test).

The application of propolis extracts to *S. zeamais*' and *C. maculatus*' mortalities showed a strong concentration response (Table 3). There were significant differences ($P < 0.05$) in percentage mortalities of *S. zeamais* and *C. maculatus* induced by the treatment application of propolis extracts. The mortality induced as a result of propolis extract application on maize and cowpea grains were significantly different ($P < 0.05$) from each other and in comparison to the control. The treatment, 100% concentration exhibited high mortalities of 98.75%

and 97.5%, whereas untreated control had very low mortalities of 2.5% and 12.5% in *S. zeamais* and *C. maculatus* respectively.

Table 3: Mean percentage (%) mortality ($\pm$ SEM) of *Sitophilus zeamais* and *Callosobruchus maculatus* on propolis treated maize and cowpea grains

Treatment	% mortality	
	<i>Sitophilus zeamais</i>	<i>Callosobruchus maculatus</i>
10%	16.25 $\pm$ 1.19 ^a	17.5 $\pm$ 2.71 ^a
30%	36.25 $\pm$ 0.80 ^b	38.5 $\pm$ 1.20 ^b
50%	61.25 $\pm$ 1.09 ^c	57.5 $\pm$ 0.85 ^c
100%	98.75 $\pm$ 0.25 ^d	97.5 $\pm$ 0.29 ^d
Control (0%)	2.5 $\pm$ 1.83 ^a	12.5 $\pm$ 2.71 ^a

Means in columns sharing common letters are not significantly different at $P < 0.05$ (Tukey's HSD test).

3.2. Phytochemical composition of bee propolis

A total of eight (8) phytochemicals were identified in the bee propolis used in this study (Table 4). These phytochemicals include tannin, phenol, flavonoid, steroid, cardiac glycoside, saponin, alkaloid and terpenes. Of all the phytochemical constituents identified, terpenes, alkaloid, saponin and cardiac glycoside respectively had higher concentrations.

Table 4: Phytochemical composition (Mean $\pm$ SEM) of the bee propolis

Component	Values
Tannin ($\mu\text{g/ml}$)	2177.01 $\pm$ 0.14 ^f
Phenol ($\mu\text{g/ml}$)	8165.20 $\pm$ 0.05 ^g
Flavonoid ($\mu\text{g/ml}$)	852.37 $\pm$ 0.15 ^e
Steroid ($\mu\text{g/ml}$)	480.87 $\pm$ 0.06 ^d
Cardiac Glycoside (mg/g)	41.38 $\pm$ 0.06 ^a
Saponin (mg/g)	108.49 $\pm$ 0.04 ^b
Alkaloid (mg/g)	127.16 $\pm$ 0.07 ^b
Terpenes (mg/g)	425.34 $\pm$ 0.03 ^c

Means in column sharing common letters are not different at $P < 0.05$ (Tukey's HSD test)

4. Discussion

The study revealed the efficacy of propolis extracts in mitigating the *S. zeamais* and *C. maculatus*. Extracts from propolis showed great potential at minimising the grain damage and grain weight loss induced by maize weevil and cowpea weevil. These findings related to the

report of Osipitan et al., 2010 who recorded 5.74% grain weight loss and 24.91% grain damage of maize treated with propolis extract at 20% concentration against larger grain borer. Similarly, Adeyemi & Osipitan, 2014 reported 22% weight loss and 0% grain damage of maize treated with propolis extract at 15% concentration against maize weevil. The effectiveness of their propolis extracts were also concentration-dependent, the higher the concentration the lower the grain damage and grain weight loss.

Pest population reduction is another reason why propolis extract is effective as it was toxic to the two pest species. Previous findings on the application of propolis in managing another coleopteran - larger grain borer in maize grain (Osipitan et al., 2010) demonstrated the pesticidal potency of propolis extract for pest control; this also corroborated our findings in this study. The action of propolis extract causing high mortality on grain pests was due to the phytochemicals in the propolis. This action was similarly reported by Marcucci (1995) and Bueno-Silva et al. (2013) that tropical bee propolis is a honeybee product with a broad spectrum of biological properties. These chemical components are biologically active and possess antioxidant, anti-inflammatory and immunomodulatory attributes (Totan et al., 2001), in which flavonoid, terpenes, phenols, cumarins and vitamins play significant roles (Botushanov et al., 2001), and these constituents are effective in mitigating different types of pests. Our findings on chemical constituents of propolis was also corroborated by Nijveldt (2001) and Maciejewicz (2001) who similarly observed that bee propolis contains common phytochemicals - flavonoids, terpenes, phenolics and their esters, sugars, hydrocarbons and mineral elements with a broad spectrum of biological properties such as antioxidant, insecticidal, antibacterial and antiviral effects (Bueno-Silva, 2013). The flavonoids in propolis are capable of scavenging free radicals and thereby protecting the cell membrane against lipid peroxidation (Kolankaya, et al., 2002; Zhang, et al., 2014).

Generally, botanical insecticides are pest-specific, biodegradable and environmentally friendly and non-toxic to non-target organisms. In contrast to the commonly used synthetic insecticides which are highly toxic, harmful to both target and non-target organisms and pest developing resistance against it over time. Incorporating use of propolis extracts into integrated pest management practices should be encouraged. This is as a result of the presence of hundreds of chemical constituents which serve different roles and purposes as a potential biopesticide.

5. Conclusion

In conclusion, infestation of maize by *S. zeamais* and cowpea by *C. maculatus* results in substantial weight losses and grain damage. *Apis mellifera* propolis extract was effective in the control of pests of maize and cowpea. Propolis and other botanicals should be employed singly or integrated with other control management strategy, to overcome challenges associated with the use of synthetic insecticide.

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