



Application method and effectiveness of Bee-propolis in reducing grain damage and weight loss in cowpea

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Abstract

Cowpea (*Vigna unguiculata* L. Walp) is a crop adapted to Savannah and drier regions of the tropics like Nigeria where other legumes don't thrive well. Cowpea is highly vulnerable to infestation by *Callosobruchus maculatus* across all developmental stages. This pest-induced damage results in substantial quantitative and qualitative losses, characterized by seed perforation, decreased seed weight, reduced germination potential, and diminished market value. This study aimed to evaluate the efficacy of bee propolis in inhibiting cowpea damage and weight loss during storage. Propolis was processed into concentrated solutions for seed coating using ethanol as a solvent. A 100 g sample of cowpea was coated with bee propolis solutions (10%, 20%, 30% w/v) by mixing them in a separating funnel, shaking for thorough coating, and then air-dried for 24 hours. The coated grains were thereafter infested with 20 unsexed adult *C. maculatus*, covered with muslin cloth, and replicated four times. Controls were uncoated grains and ethanol-treated grains. The results show that propolis is effective in reducing grain damage and weight loss of cowpea. The highest concentration of propolis at 30% reduced both grain damage and weight loss compared to both positive and negative controls. This approach offers a solid framework for evaluating the protective efficacy of bee propolis against *Callosobruchus maculatus* on stored cowpea, suggesting its potential integration as a biopesticide.

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a crop adapted to Savannah and drier regions of the tropics like Nigeria where other legumes do not thrive well. It is one of the most economically and nutritionally important indigenous African grain legumes produced throughout the tropical and sub-tropical areas of the world (Adeyemi *et al.*, 2020; 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). Cowpea is highly susceptible to infestation by the insect pest *Callosobruchus maculatus* at all stages of its life cycle. This infestation leads to significant quantitative and qualitative losses, evidenced by seed perforations reduced weight, lower market value, and diminished germination capacity. Consequently, these damages increase food losses and wastage and reduce supply to consumers, often at higher prices.

The attack may result in complete losses since it begins prior to harvest and intensifies during storage (Faroni and Sousa, 2006). Despite the availability of various chemical pesticides, the persistence of *C.*

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maculatus infestation remains a significant challenge in cowpea storage, leading to substantial economic losses for farmers. Furthermore, the indiscriminate use of synthetic pesticides raises concerns regarding environmental contamination and human health risks. Exploring alternative methods could address these challenges by providing effective pest control while minimize adverse environmental and health impacts. Therefore, to preserve the quality of grains while they are being stored, cowpea farmers must employ safe and efficient cowpea preservation methods (Langyintuo *et al.*, 2003).

There is a lack of comprehensive research on the effectiveness and optimal application techniques of honey bee products specifically against *C. maculatus* infestation in stored cowpea grains. Therefore, there is a need to investigate the potential of honey bee products (Propolis) as natural grain protectant.

Apis mellifera propolis is a significant resinous substance collected by honeybees from plants particularly from flowers and leaf buds which has long piqued the interest of researchers (Adeyemi *et al.*, 2020). It is a resinous, wax-like substance collected by bees from tree buds and other botanical sources. They utilize it as cement to fill up gaps or seal cracks in their hives. It contains biological properties, and depending on where it came from, it can be green, brown or red in color. At temperatures over room temperature, it becomes sticky; lower temperatures it becomes rigid and extremely brittle (Orsi *et al.* 2005). Bee propolis is rich in bioactive compounds, including phenolics and flavonoids, and exhibits notable antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Bodini *et al.*, 2013; Bertotto 2022). It has been demonstrated to have a major impact on decreasing grain weight loss, damage and increasing insect mortality against pests like *Sitophilus zeamais* and *Callosobruchus maculatus* (Adeyemi *et al.*, 2020). Adeyemi and Osipitan, 2014 reported 100% mortality of *Sitophilus zeamais* in grains treated with propolis and garlic, with significant reductions in grain damage and loss. This study aimed to evaluate the effectiveness of bee propolis as a natural control agent against grain damage and weight loss caused by *Callosobruchus maculatus* in stored cowpea.

Materials and Methods

Study site and insect culture: The study was carried out at Entomology Laboratory, Nigerian Stored Products Research Institute (NSPRI). 500g of cowpea was procured from known farmer, Ilorin, Kwara State, Nigeria. The grains were put in the refrigerator for 72 hours, to kill any incipient eggs and thereafter removed before its use for bioassay. *Callosobruchus maculatus* adult used for the study were obtained from the culture maintained on dried cowpea seeds in the entomology laboratory.

Twenty unsexed adult *C. maculatus* were introduced into 500g of 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 25 days prior to check for progeny production. The F1 progeny from the culture set up served as source of insect used for subsequent bioassay.

Sample preparation: A 100 g sample of pretreated cowpea (cowpea seeds cold shocked for 72 hours to kill any existing eggs or larva) was meticulously weighed using a metal weighing balance and placed into 250 ml Kilner jars. Bee propolis, solution prepared at concentrations 8g, 16g, and 24g per 80 ml of ethanol, corresponding to 10%, 20%, and 30% w/v precisely measured was used to coat each of the experimental seeds.

Method of application: This process involved pouring the pretreated (cowpea seeds cold shocked for 72 hours to kill any existing eggs or larva seeds for coating into a locked separating funnel, followed by the addition of the desired concentration of propolis onto the grains and shaken to ensure thorough coating. Subsequently, after 2 minutes, the funnel was drained and the coated grains were recovered and air-dried for 24 hours on a filter paper to ensure the ethanol has fully evaporated. After the drying period, the coated 100 g grains were transferred into the experimental vials and infested with 20 unsexed 5-day-old cultured adult *Callosobruchus maculatus*. Thereafter, it was covered with muslin cloth to allow for aeration. The treatment

was replicated four times per concentration of propolis and arranged in the laboratory using a completely randomized design. The protocol was repeated for both the negative and positive controls i.e. the uncoated grains and the grains treated with ethanol respectively. This technique is in accordance with (Adeyemi *et al.*, 2020) with modifications.

Determination of grain damage and weight loss:

Grains were assessed for levels of damage in terms of number of perforated seeds. The grains were examined for insect infestation by sorting out the perforated grains and weighing them using the electronic weighing balance. Also, the wholesome grains were weighed and recorded. The formula below was used to determine the level of damage.

$$\text{Grain damage (\%)} = \frac{\text{weight of perforated seeds}}{\text{total weight of seeds in vial}} \times 100$$

Percentage weight loss was assessed by weighing the grains before the treatment application and recorded, thereafter the final weight of grains was measured and recorded at the end of the experiment. This was done as described by Ileke and Oni (2011).

$$(\%) \text{ loss in Weight} = \frac{\text{initial weight of grains} - \text{final weight}}{\text{initial weight of grains}} \times 100$$

Statistical analyses

Data collected were analyzed using means and standard deviations for descriptive statistics, while analysis of variance (ANOVA) was employed for inferential statistics to determine significant differences between treatment and control (using IBM SPSS Statistics 25).

Results and Discussion

Table 1: shows that statistically, there are no significant differences between the coating concentrations of PP. Propolis (PP) coatings at concentration 30%, 20%, and 10% exhibited significant differences compared to both ethanol and negative control. Furthermore, a significant difference was observed between the ethanol treatment and the negative control.

Table 1: Percentage damage of propolis treated cowpea grains ($\pm$ SEM) by *Callosobruchus maculatus*

Treatments	Mean
PP 10%	27 $\pm$ 6 ^b
PP 20%	24 $\pm$ 5 ^b

PP 30%	23 $\pm$ 4 ^b
Ethanol	50 $\pm$ 3 ^c
Control	70 $\pm$ 1 ^d

PP= Propolis (10%, 20%, and 30% w/v concentrations), Means with the same alphabet(s) on the same column are not significantly different at ($P>0.05$) level of probability.

From the table 2, statistically there are no significant differences between the coating concentrations of PP. Statistical analysis revealed no significant difference between the propolis (PP) and ethanol treatments. However, both PP and ethanol showed significant differences when compared to the negative control.

Table 2: Mean percentage weight loss on propolis treated cowpea grain infested by *Callosobruchus maculatus*

Treatments	Mean
PP 10%	14 $\pm$ 3 ^b
PP 20%	12 $\pm$ 6 ^b
PP 30%	11.3 $\pm$ 0.5 ^b
Ethanol	17.64 $\pm$ 5.553 ^{ab}
Control	43.35 $\pm$ 1.527 ^c

PP= Propolis (10%, 20%, and 30% w/v concentrations), Means with the same alphabet(s) on the same column are not significantly different at ($P>0.05$) level of probability.

From the results above, treatment with Propolis showed great potential at mitigating the grain damage and weight loss caused by the cowpea weevil (*C. maculatus*). These findings relate to Adeyemi *et al.*; (2020) whose result also shows lower grain damage and weight loss with 100% propolis extract treated cowpea. Also, Osipitan *et al.*; (2010) recorded that, when compared to untreated maize grains, propolis at all tested doses decreased the population of larger grain borer, grain weight loss and grain damage of maize treated with propolis extract. Similarly, Adeyemi and Osipitan (2014), reported 22% weight loss and 0% grain damage of maize treated with propolis extract at 15% concentration against maize weevil. Bee propolis is rich in bioactive compounds, such as phenolics and

flavonoids, and they exhibit notable antibacterial, antifungal, anti-inflammatory, and antioxidant properties. The flavonoid compounds that work as insecticides are rotenoids. Rotenoids are toxins that inhibit metabolism and the nervous system which works slowly. It is suspected that insects that die as a result of starvation could be due to paralysis of the mouth apparatus or other organs (Khairunnisa *et al.* 2023).

Conclusion

The efficacy of propolis (at 30% concentration) against *Callosobruchus maculatus* presents promising avenues for sustainable pest management in agricultural system. Furthermore, from the findings of this study, it is clear that the effectiveness of propolis is dependent on the coating concentrations which at 30% was effective in reducing grain damage and weight loss. Thus, increasing the concentration of propolis coating corresponded with a reduction in grain damage and weight loss.

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