

Evaluation of Beeswax Application for the Control of *Callosobruchus maculatus* in Stored Cowpea

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Abstract - Losses due to *Callosobruchus maculatus* (Coleoptera: Bruchidae) infestation in storage is colossal and sourcing efficient protectants remains unattained. The objectives of this study were to formulate a method that ensures uniform coating of seed testa, determine the impact of bee wax on the mortality and F1 survival of *C. maculatus*, to also determine the impact of the protective treatment on damage, grain loss, grain weight and market value after *C. maculatus* infestation. Bee wax was obtained and processed into a concentrated solution to allow for thorough coating of the seeds. An absolute ethanol was used as the solvent and a positive control and different coating concentrations were used for the experiments. Three different experimental procedures were carried out in accordance with requirement of the objectives. The obtained data were analyzed using ANOVA and probit models with the aid of IBM SPSS software. The findings highlighted a progressive mortality record of *C. maculatus* exposed to different concentrations of bee wax, beewax (WX) at 30% concentration gave the highest value of toxicity (97.5%) compared to the positive and negative control (35.0 and 57.5), the mortality was dose related, and the maximum concentration (30%) of beewax (WX) caused a decline in offspring emergence with the occurrence of fewer eggs on the coated grain as a result leading to very low number of emergence (16%) when compared to positive control (39%) and negative control (26%). Also, Bee wax (WX) at 30% concentration considerably lowered ($p < 0.05$) grain deterioration (6%) and loss in weight (2%) in which the control recorded the highest level of grain deterioration (70%) and weight reduction (43%). The effectiveness of this beehive product displayed a dose-dependent response. The Evaluation of the bioactive components of *Apis mellifera* which include phenol, flavonoid and tannin and also the coating effectiveness of Bee wax due to the presence of oil in its component could be recommended or incorporated into integrated pest management (IPM) practices.

I. INTRODUCTION

Honey bees (*Apis mellifera* L.) are among the most effective and valuable pollinators of flowering crops worldwide (Kevin and Lorna, 2003). Propolis, venom, and bee wax are some useful byproducts of beekeeping. Honeybees gather propolis, a sticky mixture of bee wax and resin, from plant exudates, buds, and leaves (Chittka *et al.*, 1999). The bulk of beeswax consists of fatty acid esters and other long-chain alcohols, which are essential for building honeycomb cell where bees raise their brood and store pollen (Chittka *et al.*, 1999). Numerous studies have reported the therapeutic and economic importance of bee derivatives, including their application as nutraceutical, cosmetic and pharmaceutical materials (Viuda-Martos *et al.*, 2008; Burlando and Cornara, 2013; Cornara *et al.*, 2017).

Bees' wax

Bees' wax is a complex secretion produced in liquid form by specialized glands located in the abdomens of younger worker bees. During the colony expansion phase, particularly in late spring, this secretion is generated in larger quantities to form combs used for hive construction. Pure beeswax is almost white when produced, gradually acquiring a yellowish tint after contact with honey and pollen, over time, and it darkens due to the cocoon residues present in the cells (Hepburn *et al.*, 1991). It resists degradation by acids and digestive fluids in the bees' stomachs and is insoluble in water (Bogdanov, 2004). Since wax formation is strongly influenced by bee lineage and diet, is likely closely linked to bee genetics and diet, the chemical composition of beeswax, contains hydrocarbons such as heptacosane, nonacosane,

hentriacontane, pentacosane, and tricosane), along with free fatty acids and free fatty alcohols, linear wax monoesters, hydroxymonoesters derived from palmitic, 15-hydroxypalmitic, and oleic acids, and complex wax esters containing 15-hydroxypalmitic acid and diols (Münstedt and Bogdanov, 2009). The composition of these constituents may differ across bee species, colonies and geographic regions. According to Diyana *et al.* (2021), beeswax remains one of the most commonly utilized natural waxes in the food and cosmetics industries, largely due to its hydrophobic nature. Additionally, it has been utilized in edible coatings and films composed of gelatin (Zhang *et al.*, 2018), chitosan (Hromiš *et al.*, 2015), starch (Auras *et al.*, 2009; Pérez-Vergara *et al.*, 2020), and gums (Haq *et al.*, 2016; Saurabh *et al.*, 2016). Historically, the use of beeswax dates back to ancient Egypt, as recorded in the Ebers Papyrus (1550 B.C.), where it served pharmaceutical and therapeutic purposes (Rit and Behrer, 1999).

It was a major component in several formulations for producing ointments and lotions, applied to extract plugs, soothe burns and wounds and relieve joint pains. In modern contexts, beeswax coating are recognized for their potential to reduce food spoilage, a significant challenge to sustainability and global food security. These natural coatings help preserve postharvest quality and extend the shelf life of perishable commodities (Formiga *et al.*, 2019, Sousa *et al.*, 2021, Trinh *et al.*, 2022).

Unlike other bee-derived substances, the antimicrobial properties of beeswax have received relatively limited scientific attention until recently. Over the past few years, the bioactive potential of natural bee products has gained more prominence due to their role in controlling microbial growth. Crude beeswax has demonstrated antibacterial activity against several pathogenic bacterial strains and the yeast *Candida albicans* (Ghanem, 2011). Additionally, research and experiments have been done on beeswax's antimicrobial properties in combination with other natural compounds. *Aspergillus niger*, *Salmonella enterica*, *Candida albicans*, and *S. aureus* have all been shown to be inhibited by beeswax crude extracts (Ghanem, 2011). However, no research on its biopesticidal effects has been conducted. Betancur-D'Ambrosio *et al.*, (2024) evaluated the effect of native cassava starch, beeswax and ethanolic propolis extract (EPE) on the mechanical, thermal and inhibitory properties against

the *Aspergillus niger* fungus. Three components were used in an experimental Box-Behnken design: EPE (1–4%v/w), beeswax (0.5–0.9%w/w), and cassava starch content (2–4%w/v). The resulting films had poor mechanical qualities and were opaque. Young's modulus (YM), elongation at break (EB), and tensile strength were shown to be impacted by EPE concentration, while EB and YM were the only parameters impacted by cassava starch content. The cassava starch-beeswax interaction was discovered to have an impact on weight loss in thermal characteristics; the greatest loss happened at high concentrations of these variables in the 200–360 °C temperature range. The films demonstrated a 51% reduction in *Aspergillus niger* growth, with the beeswax-EPE interaction having a notable beneficial impact. The created films' qualities imply that they would be more acceptable as fruit and vegetable coatings.

According to several studies, the cowpea (*Vigna unguiculata* L.), an annual self-pollinating leguminous crop, is the most economically significant native African legume crop (Moussa *et al.*, 2011; Sindhu *et al.*, 2019). Cowpea is widely planted on over 10.6 million hectares in West Africa, with an annual production of 6.1 million metric tonnes, since it can be grown in a region with 300 mm of annual rainfall, unlike other legumes (Boukar *et al.*, 2019). (FAOSTAT, 2018).

Millions of farmers depend on cowpeas for their economic (monetary) and nutritional well-being, making them an important part of their livelihoods (Bolarinwa *et al.*, 2021).

According to Boukar *et al.* (2018), cowpea grains contain 20–32% protein, high levels of essential amino acids (lysine and tryptophan), minerals (zinc, iron, and calcium), vitamins (thiamine, folic acid, and riboflavin), and fiber (6%). Cowpeas are primarily grown for food, fodder, vegetables, green manure, and cover crops. Cowpea grain is a popular weaning food in many African countries due to its excellent nutritional value (Souleymane *et al.*, 2013). It is also a great legume for increasing food security in the nation (Gonçalves *et al.*, 2016).

Despite its importance, cowpea production is continuously plagued with a myriad of biotic stressors, among which pests represent the most economically relevant group (Agunbiade *et al.*, 2013;

Togola *et al.*, 2017; Togola *et al.*, 2020). Since they can cause up to 100% yield losses in cases of severe infestations, especially if no control measures are taken, they pose the most difficult threat to cowpea production and productivity (Souleymane *et al.*, 2013; Mekonnen *et al.*, 2022). Additionally, cowpeas are impacted by numerous insect pests during storage, which can lead to substantial losses (30–90%) after a few months (Gomez, 2004). Damage from their attacks includes mold growth, lower seed germination, and decreased grain weight.

Callosobruchus maculatus. The beetle species Fabricius (Coleoptera: Bruchidae) is also referred to as the cowpea weevil or cowpea seed beetle. They are not actually weevils because they do not have the characteristic weevil snout. Instead, they are members of the bruchid beetle, a subfamily of the Chrysomelidae beetle family. Cowpeas (*Vigna unguiculata* L. Walp) are frequently attacked by insect pests at nearly every stage of their life cycle. This causes significant quantitative and qualitative losses, such as seed perforation and decreases in the weight, market value, and germination ability of seeds, which raises production costs and also results in a lower supply for consumers at higher prices.

Complete losses could result from the attack, which begins prior to harvest and gets worse during storage (Faroni & Sousa, 2006). To preserve the quality of their grains while they are being stored, cowpea producers must employ safe and efficient cowpea preservation techniques (Langyintuo *et al.*, 2003). Nonetheless, the chemical approach has shown to be the most promising, well-liked, and widely accepted of all the existing techniques. Sadly, despite its apparent benefits like speed, effectiveness, and simplicity of use it has a lot of disadvantages. Ekeh *et al.* (2013) state that the most effective way to control *C. maculatus* is to employ chemical pesticides.

However, of all the available methods, chemical approach has been the most promising, acceptable and popular. Unfortunately, it has quite a number of drawbacks despite its seeming advantages such as promptness, efficacy and ease of application. According to Ekeh *et al.* (2013) the use of chemical insecticides gives the best result in controlling *C. maculatus*. According to reports, insecticides were persistent, effective, and efficient methods of control with a rapid knock-action. However, their use by

farmers has been questioned globally (Ahmed *et al.*, 2009). Due to inadequate training, farmers frequently abuse and excessively utilize chemicals used for cowpea preservation (Zhu, 2015). Despite their chemical efficacy, they have certain drawbacks. Since many smallholder farmers in Sub-Saharan Africa cannot afford them, they have significant limitations as a pest management method in the region (Ahissou *et al.*, 2021). Additionally, contaminants in food and fodder from their residues endanger human and animal populations (Jepson *et al.*, 2020).

Furthermore, synthetic pesticides have detrimental effects on non-target creatures like pollinators and natural enemies, as well as the ecosystem services that biodiversity provides in the production of food (Kennedy *et al.*, 2013). They may also cause insect populations to become resistant (Sawadogo *et al.*, 2020). Therefore, it is necessary to look for substitute methods that will be just as effective without any of the disadvantages associated with the use of synthetic chemicals. Particularly when it comes to efficacy, the experiments and trials of biopesticides and inert dusts have not produced the desired outcomes. The current attempt to investigate the possibilities of stable natural products honey bee, such as beeswax, having protective capability against *C. maculatus* infestation in cowpea under storage

II. MATERIALS AND METHODS

Study site

The study was conducted at the Entomology laboratory of Nigerian Stored Products Research Institute, Asa dam, Ilorin, Kwara state, Nigeria. Kwara state lies between the coordinates of Latitude 8° 10' and 19° 50'N and Longitude 3° 10'N and 6° 05'E.

Source of Cowpea.

The experiment utilized clean and meticulously sorted white cowpea grains (*Vigna unguiculata* (L.) Walp) unexposed to chemical obtained from a trusted farmer in Ilorin. Dried and carefully selected grains were put through a sterilization process by freezing at -4°C for 3 days to eliminate all live insects and any existing eggs. This precautionary measure aimed to ensure the absence of pests within the seeds. The pre-treated grains were stored in kilner jars for subsequent use.

Source of bee wax:

500g of *Apis mellifera* wax was collected from a known hives farm in Ilorin. The collected samples were kept in a beaker (wax) until processed.

Insect Culture:

Test insects were cultured following the procedure by Odeyemi *et al.*, (2008) with slight modifications. Adult *C. maculatus* were obtained from pre existing cultures in the insectary of Storage Entomology Laboratory of Nigerian Stored Products Research Institute, Ilorin. And the insects were introduced into kilner jars containing 150 g each of un-infested but susceptible pretreated white cowpea grains. *C. maculatus* was cultured following the procedure by Odeyemi *et al.*, with slight modifications. The culture media was made up of 95% grain sample and 5% *C. maculatus* by weight. The kilner jars containing the culture media were retained and arranged in the insectary at ambient mean temperature and relative humidity of 30.7 °C and 74.5% respectively with the mouth of the jars covered with muslin cloth to allow aeration and prevent insect escape. Adult insects, *C. maculatus* was sieved out after 7 days, the jars were left undisturbed until newly emerged adults showed up (26 days). The newly emerged of mixed sexes of *C. maculatus* were used for the bioassays.

Treatment application technique

A 100g sample of pretreated stocked cowpea was meticulously weighed using a metal weighing balance and placed into 250m³ Kilner jars. Bee wax was prepared at concentrations 8, 16, and 24g per 80ml of ethanol, corresponding to 10%, 20%, and 30% w/v precisely measured were used to coat each of the experimental seeds. This process involved pouring the pretreated seeds for coating into a locked separating funnel, followed by the addition of the desired concentration of bee wax onto the grains and shaking to ensure thorough coating. Subsequently, after 2 minutes, the funnel was drained and the coated grains were recovered and air-dried for 2 hours on a filter paper to ensure the solvent used evaporated.

After the drying period, the coated 100g grains were each transferred from the filter paper into the experimental vials and infested with 20 unsexed 5-day-old cultured *C. maculatus*, adult and thereafter, covered with muslin cloth to allow for aeration. The treatment was replicated four times 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.

Insect Mortality

The 20 adult cowpea weevils introduced to the grains were subsequently assessed for days 3, 5, 7 and 10. Adult beetles were considered dead when no response was observed when poked gently with an entomological pin and they were removed. All living adults after 10days were counted and removed. The data on each assessment day was progressively monitored and summed up. The progressive daily adult mortality was monitored per treatment and their mean values expressed graphically per concentration and treatments. Percentage adult mortality was calculated following the method described by Parugrug and Roxas (2008) and the overall percentage mortality was calculated as

$$\text{Mortality (\%)} = \frac{(\text{Number of dead insects})}{(\text{Total number of insects introduced})} \times 100$$

Insect emergence (F1)

All the experimental vials were subsequently kept after 10 days of exposing to 20 adults and retrieving all insects that were adult until the F1 generation adults started emerging (27days). The timing of emergence initiation was taken note of per rearing tube and emerged adults were counted every 3 days over a period of 10 days, until emergence rate were tending to zero. The monitored emergence data were subsequently documented, plotted against time and totaled per vials and per treatments. These were subsequently compared on the premises of the efficacy of the protect ant in delaying hatching emergence and survival capability of young *C. maculatus* within the grain by comparing directly with the expected eggs laid per vials.

Grain damage assessment.

Grains contained in each vials after F1 emergence assay were assessed for levels of damage in terms of counting the number of perforated seeds and the wholesome ones and recording them. The recorded values were calculated using the following formulae. Means of each of these were calculated per concentration and treatments.

$$\% \text{ perforated seeds} = \frac{\text{Perforated seeds} \times 100}{\text{Total seeds in a jar}}$$

Grain weight loss
 Percentage weight loss was assessed by measuring the initial and final weight of the grain as described

by Ileke and Oni (2011). The powdered sample per vials was carefully collected and weighed per vials i.e. 100g grain in grams.

$$(\%) \text{ loss in Weight} = \frac{(\text{Initial weight of grain} - \text{Final weight of grain}) \times 100}{\text{Initial weight of grain}}$$

3.8.Data Analysis: the data obtained from the laboratory experiments were analyzed using descriptive statistics, such as calculating means and standard deviations. Inferential statistics, ANOVA and was employed to determine significant differences between the various treatments and control groups.

III. RESULTS AND DISCUSSION

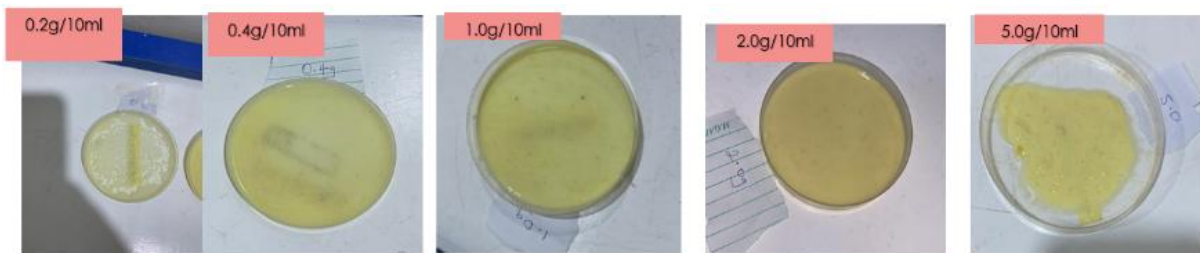


Figure 1: Dissolved dosages of bee wax in ethanol



Figure 2: coated cowpea with the dissolved wax

Table 1: Toxicity rate (%) of *C maculatus* exposed to coating concentration of the bee wax 10 DAT.

Treatment	Toxicity rate					F	p
	Coating concentrations						
WX	10%	20%	30%	Ethanol	Control	f(4, 15) = 66.117	0.00
	86.25±3.75 ^{c3}	92.5±2.5 ^{c3}	97.5±2.5 ^{c3}	57.5±4.78 ⁷²	35.0±2.04 ¹¹		
Ethanol	57.5±4.787 ^b	57.5±4.787 ^b	57.5±4.787 ^b				
Control	35.0±2.041 ^a	35.0±2.041 ^a	35.0±2.041 ^a				
F	f(4, 15) = 35.066	f(4, 15) = 37.820	f(4, 15) = 41.591				
P	0.000	0.000	0.000				

Means with the same alphabet(s) on the same column and under the same heading are not significantly different at ($P > 0.05$) level of probability. WX= wax, Numerical superscript compares concentration along the row. Alphabetic superscript compares product coating along the column.

From the Table above, there are significant difference between the coating concentration and treatments. Bee wax at 30% coating concentration gave the best results followed by 20%, 10%, ethanol (positive control) and lastly negative control.

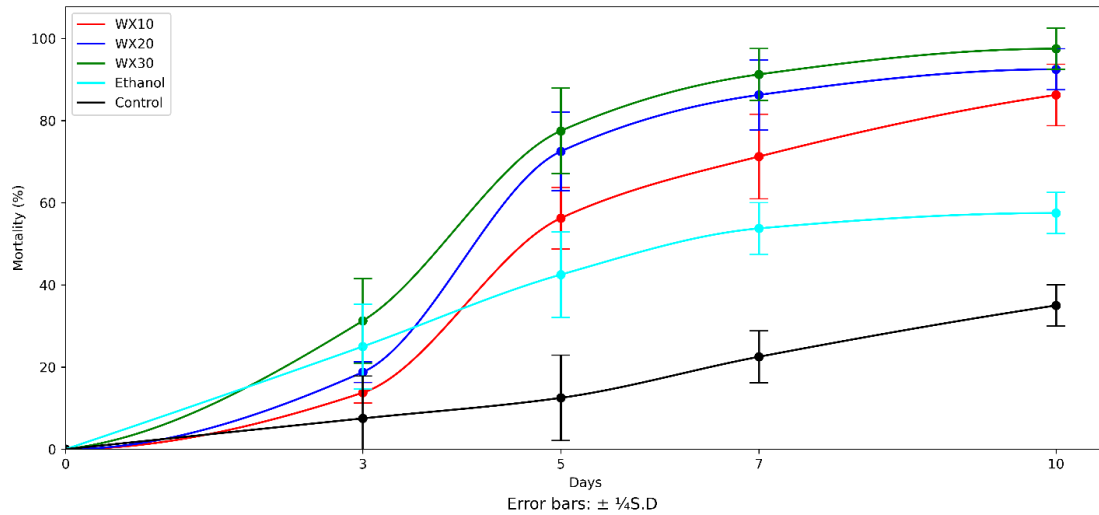


Fig. 2:

Table 2: Effect of beeswax on the emergence of *C. maculatus*

Treatments	Mean Value				
	Coating concentration (%)				
	10%	20%	30%	Control	Ethanol
WX	17±3 ^{a1}	18±3 ^{a1}	16±3 ^{a1}	39±3 ²	26±7 ¹²
Ethanol	26±7 ^{ab}	26±7 ^a	26±7 ^{ab}		
Control	39±3 ^b	39±3 ^a	39±3 ^b		

Means with the same alphabet(s) on the same column and under the same heading are not significantly different at ($P>0.05$) level of probability, WX= wax, Numerical superscript compares concentration along the row. Alphabetic superscript compares product coating along the column.

From the table above, although there is no significant difference between the wax coating concentrations, the one that gave the best result is 30% followed by 10% and lastly 20%. Wax at 10 and 30% were found to be significantly different from negative control respectively.

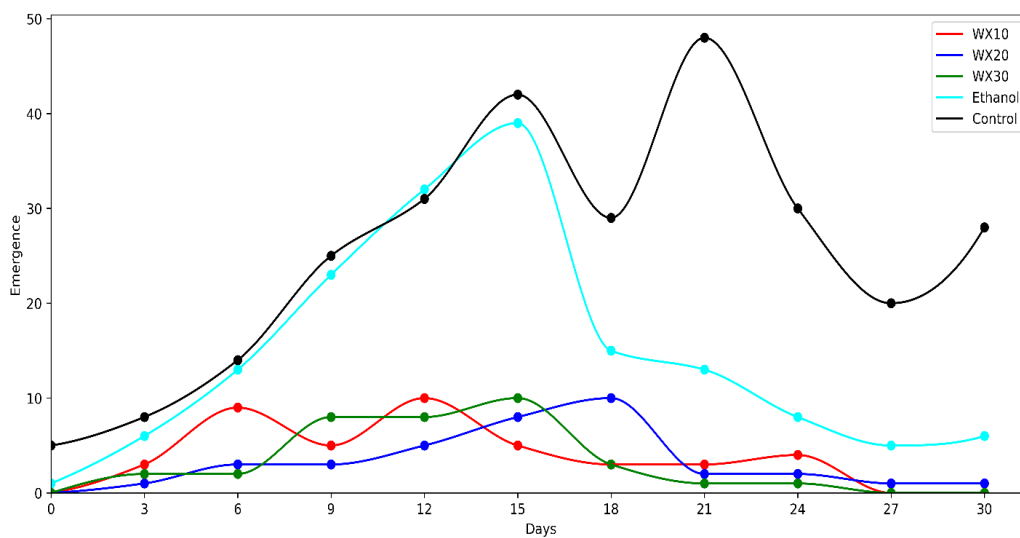


Table 3: Mean percentage of damaged seeds on stored cowpea infested by *C. maculatus*

Treatment	Mean Percentage

WX	Coating concentrations (%).					F	P
	10%	20%	30%	Ethanol	Control		
	8±1 a ¹	7±1 a ¹	6±1 a ¹	50±3 ²	70±1 ³	f(4, 15) = 609.394	0.000
Ethanol	50±3 ^c	50±3 ^c	50±3 ^c				
Control	70±1 ^d	70±1 ^d	70±1 ^d				
F	f(4, 15) = 53.884	f(4, 15) = 68.676	f(4, 15) = 51.756				
P	0.000	0.000	0.000				

Means with the same alphabet(s) on the same column and under the same heading are not significantly different at ($P>0.05$) level of probability, WX= wax, Numerical superscript compares concentration along the row. Alphabetic superscript compares product coating along the column

All coating concentrations proved superior over the treated and untreated controls during the storage period. Negative control had the highest percentage damaged value of (70%) when compared with other treatments. It was also observed that all the

treatments are significantly different ($p>0.05$) across the coating concentration with the inclusive the negative control. Treatment WX with the coating concentration of 30% had the least percentage damaged seeds (6%)

Table 4: Mean percentage weight loss on stored cowpea infested by *C. maculatus*

Treatment	Mean Percentage Coating concentrations						
	10	20	30	Ethanol	Control	F	P
WX	6.66±0.087 ^{a 12}	5.87±0.398 ^{a 12}	2.01±2.348 ^{a 12}	17.64±5.55 ^{3 1}	42.57±6.51 ^{1 2}	f(4, 15) = 9.122	0
Ethanol	17.64±5.553 ^{ab}	17.64±5.553 ^{ab}	17.64±5.553 ^{ab}				
Control	43.35±1.527 ^{c 2}	43.35±1.527 ^{c 2}	43.35±1.527 ^{c 2}				
F	f(4, 15) = 10.361	f(4, 15) = 9.157	f(4, 15) = 8.012				
P	0.000	0.001	0.001				

Means with the same alphabet(s) on the same column and under the same heading are not significantly different at ($P>0.05$) level of probability, WX : bee wax Numerical superscript compares concentration along the row. Alphabetic superscript compares product coating along the column.

There were no significant differences in the percentage weight loss across the coating concentrations but significant differences across the treatments inclusive the negative control. The highest weight loss was recorded in negative control (43%) followed by the positive control (17%). The treatment with the least percentage weight loss (2%) was observed in treatment WX with the coating concentration of 30%.

IV. DISCUSSION

Effects of bee wax on the mortality of *Callosobruchus maculatus* (Coleoptera: Bruchidae) on stored cowpea.

The study's result regarding the impact of Beeswax on the mortality of *Callosobruchus maculatus* in stored cowpea demonstrated a dependency on both concentration and exposure period.

Interestingly, a trend emerged where an increase in concentration corresponded to a higher increase in adult mortality, indicating that higher dosages/concentrations were more effective. All the coating concentrations proved to be more effective

than the two controls (positive and negative) most especially at 30% coating concentration which showed the highest performance, resulting in the highest mortality of the weevil. This is because of the even thicker coating of the dissolved bee wax on the cowpea grains. These findings align with previous reports by Formiga *et al.* (2019), Sousa *et al.* (2021), and Trinh *et al.* (2022), emphasizing the effectiveness of natural beeswax-based coatings in maintaining postharvest quality and extending the storage life of perishable food items. Also, bee wax contains essential oil which was said to be used as pharmaceutical agent for making drugs which dates back to ancient Egypt. It has been reported to serve as the main ingredient in many recipes for the preparation of ointments and creams as reported by Ebers Papyrus (1550 B.C) (Rit and Behrer, 1999). Several studies have found that Essential oils (EOs) are the appropriate substitute for controlling pests in stored grain (Nerio *et al.*, 2009, Vendan *et al.*, 2017). In addition, this finding resonates with the findings of Botushanov *et al.* (2001), who highlighted the efficacy of bee products due to their important chemical components in mitigating various pests. These chemical components are biologically active and possess antioxidant, anti-inflammatory and immunomodulatory attributes (Totan *et al.*, 2001), in which flavonoid, terpenes, phenols, coumarins and vitamins play significant roles (Botushanov *et al.*, 2001), and these constituents are effective in mitigating different types of pests.

Effects of beeswax on adult emergence of *Callosobruchus maculatus* (Coleoptera: Bruchidae). The bee wax significantly reduced adult emergence and progeny development in treated cowpea seeds. The little to zero adult emergence could be due to the insecticidal properties of the beehive product, corroborating the findings of (Bueno-Silva, 2013), who found out that bee hive products contains common phyto-chemicals; 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. The results from the study shows that few eggs were ovipositioned on cowpea seeds treated with Bee wax (WX) but with very few numbers of adult emergences. This could also be as a result of their coating effectiveness, significantly affecting the developmental stages of insects, subsequently influencing emergence. These findings align with previous reports by Formiga *et al.*

(2019), Sousa *et al.* (2021), and Trinh *et al.* (2022), emphasizing the effectiveness of natural beeswax-based coatings in maintaining postharvest quality and extending the storage life of perishable food items.

The effective coating of the grains likely resulted in starvation of adult beetles, potentially leading to reduced egg lying and, consequently, lower hatchability of larvae and final metamorphosis to adults.

Effect of bee wax on grain damaged and weight loss in stored cowpea infested with *C. maculatus*

From tables 3 and 4 above, Bee wax at concentration 30% had the least grain damage and weight loss. Treatment WX₃₀ showed great potential at minimizing the grain damage and weight loss by the cowpea weevil. This could be as a result of the coating effectiveness of bee wax (making the grains inaccessible to the weevils), as seen in the result, the higher the concentration of bee wax the lower the damage and vice versa. Bee wax has other uses such as its effects in inhibiting microbial activities. Betancur-D'Ambrosio *et al.*, (2024), evaluated the effect of native cassava starch, beeswax and ethanolic propolis extract (EPE) on the mechanical, thermal and inhibitory properties against the *Aspergillus niger* fungus. Their finding signifies that bee wax - EPE were able to reduce weight loss and inhibit the properties of *A. niger*. Furthermore, beeswax crude extract has shown inhibitory effects against *S. aureus*, *Salmonella enterica*, *C. albicans* and *Aspergillus niger* (Ghanem, 2011), while effects against pathogenic bacteria and microscopic fungi have been reported for methanol and ethanol extracts (Kacániová *et al.*, 2012). This kind of effects could depend at least in part on beeswax compounds of plant origin (Puleo and Keunen, 1991, Laura *et al.*, 2017). Also, Beeswax is reported to be used as an additive in a variety of industrial products and processes, such as food industry, candles, and cosmetics. In pharmaceutical preparations it plays a role as a thickener, binder, drug carrier and release retardant (Laura *et al.*, 2017).

V. CONCLUSION

The efficacy of bee wax against *Callosobruchus maculatus* presents promising avenues for sustainable pest management in agricultural systems, because it caused 97% mortality, reduced emergence, and lower grain damage and weight loss respectively.

Further research exploring the potency of bee wax against other storage insect pests is recommended.

REFERENCES

- [1] Abd-El Aal, A.M., El-Hadidy, M.R., El-Mashad, N.B. & El-Sebaie., A.H (2007). Antimicrobial effect of bee honey in comparison to antibiotics on organisms isolated from infected burns *Ann Burns Fire Disasters*, 20 (2), pp. 83-88
- [2] Adeyemi, M. A., James, A. O., Julius, A. B. & Yussuf, U. O. (2020). Bee propolis as protectant of stored grains against insect pests' damage in Kwara State, Nigeria. *Technoscience Journal for Community Development in Africa* 1:1, 1 03–108.
- [3] Adeyemi, W. A., & Osipitan, A. A. (2014). Evaluation of the effectiveness of propolis and garlic in the management of maize weevil (*Sitophilus zeamais*) in stored maize (*Zea mays*) grains. *Munis Entomology and Zoology*, 9(1), 117-124.
- [4] Ahmed, A. K., Hoekstra, M. J., Hage, J.J. & Karim. R.B (2003). Honey-medicated dressing: transformation of an ancient remedy into modern therapy. *Ann Plast Surg*, 50 (2) (2003)143-147; discussion 147-148
- [5] Al-Nahari, A.A.M., Almasaudi, S.B., Abd, E.S.M., El-Ghany, Barbour, E., Al Jaouni, S.K. & Harakeh, S. (2015). Antimicrobial activities of Saudi honey against *Pseudomonas aeruginosa*. *Saudi Journal of Biological Sciences*, 22 (5), pp. 521-525.
- [6] Auras R, Arroyo B & Selke S (2009). Production and properties of spin-coated cassava-starch-glycerol-beeswax films. *Starch/staerke* 61(8):463–471. <https://doi.org/10.1002/star.200700701>
- [7] Basualdo, C., Sgroy, V., Finola, M.S & Marioli. J.M (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Vet Microbiol*, 124 (3–4) (2007), pp. 375-381.
- [8] Bergman, A. J. Yanai, J. Weiss, D. Bell, & David, M.P. (1983). Acceleration of wound healing by topical application of honey. *The American Journal of Surgery*, 145 (3), pp. 374-376.
- [9] Bertotto C, Bilck AP, Yamashita F, Anjos O, Bakar Siddique MA, Harrison SM, Brunton NP, & Carpes ST (2022). Development of a biodegradable plastic film extruded with the addition of a Brazilian propolis by-product. *LWT*. <https://doi.org/10.1016/j.lwt.2022.113124>
- [10] Bertotto, C., Bilck, A.P., Yamashita, F., Anjos, O., Bakar. S. M. A., Harrison. S. M., Brunton. N. P. & Carpes, S. T. (2022). Development of a biodegradable plastic film extruded with the addition of a Brazilian propolis by-product. *LWT*. <https://doi.org/10.1016/j.lwt.2022.113124>.
- [11] Betancur-D'Ambrosio, M.C., Pérez-Cervera, C.E. & Barrera-Martinez, C. (2024). Antimicrobial activity, mechanical and thermal properties of cassava starch films incorporated with beeswax and propolis. *J Food Sci Technol* 61, 782–789. <https://doi.org/10.1007/s13197-023-05878-x>
- [12] Bhavin G Visavadia, Jan Honeysett & Martin Danford (2008). Manuka honey dressing: An effective treatment for chronic wound infections. *British Journal of Oral and Maxillofacial Surgery*, 46 (8), pp. 696-697.
- [13] Bodini, R.B, Sobral, P.J.A., Favaro-Trinade, C.S. & Carvalho, R.A. (2013). Properties of gelatin-based films with added ethanol-propolis extract. *Bodini RB, Sobral PJA, Favaro-Trindade CS, Carvalho . LWT* 51(1):104–110. <https://doi.org/10.1016/j.lwt.2012.10.013>.
- [14] Bogdanov S (2004). Quality and standards of pollen and beeswax. *Apiacta* ; 38: 334-341.
- [15] Bogdanov S (2009). Beeswax: production, properties composition and control. *Beeswax book*. Chapter 2. Switzerland: Bee Product Science; quality issues today. *Bee World*; 85: 46-50
- [16] Botushanov, P. I., Grigorov, G. I. & Aleksandrov G. A. (2001). A clinical study of silicate toothpaste with extract from propolis. *Folia Med. (Plovdiv)*, 43(1–2), 28–30.
- [17] Buchwar R, Breed MD, Greenberg AR & Otis G (2006). Interspecific variation in beeswax as a biological construction material. *J Exp Biol* 2006; 20: 3984-3989.
- [18] Bueno-Silva, B., Alencar, S. M., Koo, H., Ikegaki, M., Silva, G. V., Napimoga, M. H. & Rosalen, P. L. (2013). Anti-inflammatory and antimicrobial evaluation of neovestitol and vestitol isolated from Brazilian red propolis. *J. Agric. Food Chemistry*, 61, 4546–4550

- [19] Chauhan, A., Pandey, V., Chacko, K.M., Khandal, R.K. (2010). Antibacterial activity of raw and processed honey. *Electronic Journal of Biology*, 5 (3), pp. 58-66.
- [20] Cokcetin, N.N., Pappalardo, M., Campbell, L.T., Brooks, P., Carter, D.A., Blair, S.E. & Harry, E.J.(2016).The Antibacterial Activity of Australian Leptospermum Honey Correlates with Methylglyoxal Levels *PLoS One*, 11 (12), Article e0167780.
- [21] Coombes, P.E., A.L & J.M. Wilkinson. (2005). Bactericidal Activity of Different Honeys against Pathogenic Bacteria.*Archives of Medical Research*, 36 (5) (2005), pp. 464-467.Henriques et al., 2010
- [22] Diyana ZN, Jumaidin R, Selamat MZ & Suan MSM (2021). Thermoplastic starch/beeswax blend: characterization on thermal mechanical and moisture absorption properties. *Int J Biol Macromol* 190:224-232. <https://doi.org/10.1016/j.ijbiomac.2021.08.201>
- [23] Eddleston, M., Street, J. M., Self, I., Thompson, A., King, T., Williams, N., Naredo, G., Dissanayake, K., Yul, M., Worek, F., John, H., Smith, S., Thiermann, H., Harris, J. B. & Eddie, C. R. (2012). A role for solvents in the toxicity of agricultural organophosphorus pesticides. *Toxicology*. 294, (2-3), 94,.
- [24] Haq M. A, Hasnain A, Jafri F. A, Akbar M. F & Khan A (2016). Characterization of edible gum cordia film: effects of beeswax. *LWT Food Sci Technol* 68:674–680. <https://doi.org/10.1016/j.lwt.2016.01.011>
- [25] Henriques, A.F, Jenkins, R.E, Burton, N.F, & Cooper, R.A. (2010). The intracellular effects of manuka honey on *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis*, 29 (1) (2010), pp. 45-50
- [26] Hepburn, H. R., Bernard, R. T. F., Davidson, B. C., Muller, W. J., Lloyd, P., Kurstjens, S. P (1991). Synthesis and secretion of beeswax in honeybees. *Apidologie* 1991; 22: 21-36
- [27] Horn, L.N. & Shimelis, H. (2020). Production Constraints and Breeding Approaches for Cowpea Improvement for Drought Prone Agro-Ecologies in Sub-Saharan Africa. *Journals of Agricultural Sciences*, 65, 83-91. <https://doi.org/10.1016/j.aos.2020.03.002>.
- [28] Hromiš, N. M., Lazić, V. L., Markov, S. L., Vaštag, Ž .G., Popović, S. Z., Šuput, D. Z., Džinić, N. R., Velićanski, A. S., & Popović, L. M. (2015). Optimization of chitosan biofilm properties by addition of caraway essential oil and beeswax. *J Food Eng.* <https://doi.org/10.1016/j.jfoodeng.2015.01.001>
- [29] Irish, J., Carter, D.A., Shokohi, T & Blair, S.E (2006). Honey has an antifungal effect against *Candida* species *Med Mycol*, 44 (3) (2006), pp. 289-291
- [30] Jaganathan, S. K., Mandal, S.M., Jana, S.K., Das, S. & Mandal, M. (2010). Studies on the phenolic profiling, anti-oxidant and cytotoxic activity of Indian honey: in vitro evaluation. *Natural Product Research*, 24 (14) (2010), pp. 1295-1306
- [31] Jenkins, R., Burton, N., & Cooper, R. (2014). Proteomic and genomic analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) exposed to manuka honey in vitro demonstrated down-regulation of virulence markers. *Journal of Antimicrobial Chemotherapy*, 69 (3) (2014), pp. 603-615.
- [32] Kaluza, B. F., Wallace, H., Heard, T. A., Klein, A. M & Leonhardt, S. D. (2016). Urban gardens promote bee foraging over natural habitats and plantations. *Ecol Evol* 2016; 6: 1304-1316.
- [33] Krell R. (1996). Value-added products from bee keeping. *FAO Agricultural Services Bull. No. 124*. Food and Agriculture Organization of the United Nations, Rome.
- [34] Lu, J., Carter, D.A., Tumbull, L., Rosendale, D., Hedderley, D., Stephens, J., Gannabathula, S., Steinhorn, G., Schlothauer, R.C., Whitchurch, C.B. & Harry, E.J. (2013). The effect of New Zealand kanuka, manuka and clover honeys on bacterial growth dynamics and cellular morphology varies according to the species. *PLoS One*, 8 (2) Article e55898
- [35] Lusby, P.E., Coombes, A.L., & Wilkinson, J.M (2005). Bactericidal Activity of Different Honeys against Pathogenic Bacteria. *Archives of Medical Research*, 36 (5) pp. 464-467.
- [36] Maciejewicz, W. (2001). Isolation of flavonoid aglycones from propolis by a column chromatography method and their identification by GC-MS and TLC methods. *J. Liq. Chromatogr. Relat. Technol.*, 24, 1171–1179.
- [37] Manyi-Loh, C.E., Clarke, A.M., Munzhelele, T., Green, E., Mkwetshana, R.N. & Marcucci, M. C. (1995). Propolis: Chemical composition, biological properties and therapeutic activity. *Apidologie*.26, 83–99.

- [38] Mashhood, A.A., Khan, T.A. & Sami. A.N.(2017). Honey compared with 1% silver sulfadiazine cream in the treatment of superficial and partial thickness burns. *Journal of Pakistan Association of Dermatology*, 16 (1) (2017), pp. 14-19
- [39] Molan, P. & Rhodes. T. (2015). Honey: A Biologic Wound Dressing. *Wounds*, 27 (6) (2015), pp. 141-151
- [40] Nijveldt, R. J., Van Nood, E., Van Hoorn, D. E., Boelens, P. G., Van Norren, K.. & Van Leeuwen, P. A. (2001). Flavonoids: A review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.*, 74, 418–425.
- [41] Ozkara, M., Akyil D. & Konuk M. (2016). Environmental health risk-hazardous factors to living species. *IntechOpen: Pesticides, environmental pollution and health*; p.p. 27.
- [42] Pérez-Vergara, L. D., Cifuentes, M. T., Franco, A. P., Pérez-Cervera, C. E. & Andrade-Pizarro, R. D. (2020) Development and characterization of edible films based on native cassava starch, beeswax, and propolis. *NFS J* 21:39–49. <https://doi.org/10.1016/j.nfs.2020.09.002>
- [43] Saurabh, C. K., Gupta, S., Variyar, P. S. & Sharma, A. (2016). Effect of addition of nanoclay, beeswax, tween-80 and glycerol on physicochemical properties of guar gum films. *Ind Crops Prod* 89:109–118. <https://doi.org/10.1016/j.indcrop.2016.05.003>
- [44] Shears, P. (2000). Antimicrobial Resistance in the Tropics *Trop Doct*, 30 (2) pp. 114-116
- [45] Shin, H.S.U.Z .(2005). Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria. *Food Research international*, 38 (6) (2005), pp. 721-728
- [46] Subrahmanyam, M. (2007). Topical application of honey for burn wound treatment-an overview. *Annals of burns and fire disasters*, 20 (3) p. 137
- [47] Swellamtarek, N.M., Mizuki Onozawa, Kazunori Hattori, Koji Kawai, Toru Shimazui, Hideyuki akaza.(2003). Antineoplastic activity of honey in an experimental bladder cancer implantation model: In vivo and in vitro studies. *Int J Urol*, 10 (4) pp. 213-219
- [48] Tonks A.J, Cooper, R.A., Jones, K.P., Blair, S.J & Parton, A. Tonks (2003). Honey stimulates inflammatory cytokine production from monocytes *Cytokine*, 21 (5) pp. 242-247
- [49] Zhang Y, Simpson, B. K. & Dumont, M. J (2018). Effect of beeswax and carnauba wax addition on properties of gelatin films: a comparative study. *Food Biosci* 26:88–95. <https://doi.org/10.1016/j.fbio.2018.09.011>
- [50] Zhang, C., Huang, S., Wei, W., Ping, S., Shen, X., Li, Y., & Hu, F. (2014). Development of high-performance liquid chromatographic for quality and authenticity control of Chinese propolis. *J. Food Sci.*, 79, C1315–C1322.