

# PROPOLIS PRODUCTION BY HONEY BEE *APIS MELLIFERA* (HYMENOPTERA: APIDAE) AND ITS POTENTIAL FOR THE MANAGEMENT OF THE LARGER GRAIN BORER *PROSTEPHANUS TRUNCATUS* (HORN) (COLEOPTERA: BOSTRICHIDAE) ON MAIZE GRAINS

Osipitan Adebola Adedoyin<sup>1\*</sup>, Ogunbanwo Ibikunle Ayotunde<sup>1</sup>,  
Adeleye Issa Gbotemi<sup>2</sup> Adekanmbi Dende Ibrahim<sup>3</sup>

<sup>1</sup>Department of Crop Protection, College of Plant Science and Crop Production,  
University of Agriculture, P.M.B. 2240, Abeokuta, Ogun State, Nigeria

<sup>2</sup>Department of Silviculture, Ministry of Forestry, P.M.B. 2008, Abeokuta, Ogun State, Nigeria

<sup>3</sup>Department of Regulation and Utilization, Ministry of Forestry, P.M.B. 2008, Abeokuta, Ogun State, Nigeria

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**Abstract:** The aim of the study was to evaluate a possibility of propolis production by honey bee *Apis mellifera* (Horn) (Hymenoptera: Apidae) and its potential for the management of the larger grain borer (LGB) *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) on maize grains. Bee propolis was collected from hives kept on the field for twelve months; they were thereafter diluted with ethanol to obtain 5%, 10%, 15% and 20% concentrations. 5 ml each of the concentrations was applied to 150 g clean maize grains infested with 10 pairs of 1–5 days old LGB in 250 cm<sup>3</sup> Kilner jars. The control jars were treated with ethanol. At 90 days post-infestation, data were collected on percentage of weight loss, percentage of grain damage, weight of frass generated (g), number of pupae, larvae and adult LGB. The results indicated that monthly propolis yield was significantly ( $p < 0.05$ ) lower in the period of honey flow (December–March) relative to other periods. Propolis at all the concentrations tested reduced the population of LGB in propolis-treated maize grains relative to non-propolis treated-ones. The highest effects of propolis was shown at the highest concentration of 20% and it was significantly ( $p < 0.05$ ) different from its effects at other concentrations. Bee propolis may be integrated with other ecological friendly control methods to manage LGB infestation in maize.

**Key words:** *Apis mellifera*, ethanol, propolis, *Prostephanus truncatus*, maize grain

## INTRODUCTION

Honey bees *Apis mellifera* L. (Hymenoptera: Apidae) are the most widely distributed beneficial insects considered as the most economical. Although, beekeeping is renowned for honey yield; other bee hive products such as propolis was reported to possess medicinal or antibiotic values (Sforcin *et al.* 1995; Krell 1996). Propolis is an important product collected by bees gaining a lot of recognition and attracting public interest over the years. It is a wax-like resinous substance collected by bees from tree buds or other botanical sources and used as cement to seal cracks or open spaces in hives. It has biological properties and its colour varies from green to brown and red depending on the origin source. It is sticky at a temperature higher than room temperature. At lower temperatures it becomes hard and very brittle (Orsi *et al.* 2005). Despite the variation in active constituents of propolis from different plant origin, they have the same effect (Markham *et al.* 1996)

In hives, propolis reinforces structural stability, reduces vibration, makes hives more defensible by sealing alternate entrances and prevents them against diseases and parasites (Krell 1996). Propolis was a subject of recent scientific investigation due to its biological properties such as antibiotics, antifungal, anti-inflammation, anesthetic, healing, immunomodulatory, antioxidant and cacinostatic properties (Sforcin *et al.* 1995; Obasa *et al.* 2007). It consist of more than 200 constituents in its waxes and resins that made it a “veritable cascade of aromatic nutrient” remarkable for combating all type of pathogens (bacteria, viruses, parasites and fungi) (Botushanov 2001). Typical propolis contains approximately 50% resin and vegetable balsams, 30% waxes, 10% essential oils and 5% pollens Orsi *et al.* 2005. Orsi *et al.* (2005) analyzed propolis from the province of Henan in China and reported sinapic acid, isoferulic acid and caffeic acid as compounds showing anti-bacterial properties. Propolis is used for several purposes as traditional medicine

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\*Corresponding address:  
osipitan1@yahoo.com

and was reported to reduce the chance of cataract in the eyes and to be effective in relief of many inflammations, healing of superficial bumps or scalds, curing of viral diseases, ulcer, treatment of allergies and sore throat and improvement of heart health (Orhan *et al.* 2005). It was also reported to be effective in the treatment of skin burns (Gregory *et al.* 2002) and canker sore diseases (Samet *et al.* 2007). Park *et al.* (1998) reported that propolis actively protected against caries and other forms of oral diseases due to its antimicrobial properties. Likewise, its use in canal debridement for endodontic procedure was explored in Brazil (da Silva *et al.* 2004).

The larger grain borer (LGB), *Prostephanus truncatus* is an introduced insect pest of maize and dried cassava in Africa (Wright *et al.* 1993). It is a member of the family *Bostrichidae*, the false powder beetles which contains about 500 species spread in tropical areas. The insect was reported as a major pest of stored maize grains in Mexico and Central America for many years (Chittenden 1911) before its accidental introduction to Africa. Already, in 1981, it was causing severe loss in Tanzania (Dunstan and Magazini 1981). It was also recorded in Togo (Harnisch and Krall 1984), Republic of Benin (Anonymous 1986), Nigeria (Pike *et al.* 1992) and other African countries such as Ghana, Guinea, Burkina Faso, Malawi, Zambia, Niger, South Africa, Kenya, Burundi, Rwanda and Uganda (Opolot and Odong 1999). LGB is a highly voracious species and can cause up to 40% yield loss in stored maize grains within six months and 75% in fermented cassava roots within four months (Hodges *et al.* 1985). In addition to causing weight loss, LGB can also reduce the nutritional composition of infested grains, particularly amino acids, lysine, tryptophan and level of grain viability (Torreblanca *et al.* 1983). Adult beetles and their larval stages cause damage to a wide range of commodities including, cereals, pulses, cocoa, coffee, groundnut and wooden structures (Espinal *et al.* 1996).

Grains of maize (*Zea mays* L.), the major host of LGB are produced by one of the oldest and most widely cultivated cereals that provides food for man and feed for livestock. It is a staple food and is sometimes grown on a garden scale where it cannot be grown as a farm crop. It is an important source of carbohydrate and vitamin C (CGIAR 1996). Maize can be consumed fresh by cooking and can also be stored as dry maize for planting or for processing into other products such as pap (ogi), custard and other industrial uses such as popcorn and corn flakes. Maize production is however threatened by several insect pests such as *Busseola fusca* (Fuller), *Eldana saccharina* (Walker), *Sessamia calamistis* (Hampson), armyworm – [*Spodoptera exempta* (Walker)], leaf hopper [*Cicadulina mbila* (Naude)] and ear worm – *Musidae nigrivenella* (Ragonot), *Sitophilus zeamais* (M), the lesser grain borer – *Rhizopertha dominica* (F), rust red flour beetle- *Tribolium castaneum* (H), saw tooted grain beetle – *Oryzaephilus surinamensis* (L). The addition of LGB to the complex of the insects infesting maize has further exacerbated the threat to maize production by insect pest. The voracity and destructive tendencies of LGB; coupled with the drawbacks associated with the use of insecticides such as contamination of grain, persistence, drift, phytotoxicity, high cost and

biomagnifications prompted evaluation of the insecticidal potential of propolis that is of botanical origin with no reported incidence of hazard and human toxicity nor harmful effect on the environment. This study evaluates propolis production by honey bee and its potential for the management of the larger grain borer *P. truncatus* on maize grains.

## MATERIALS AND METHODS

### Evaluation of bee hives for propolis production

Four Kenya top bar hives were located in a farm at University of Agriculture, Abeokuta (UNAAB), south western Nigeria. The hive is a long trough shaped box with sloping sidewalls covered with bars of about 28 cm long. It consists of a bottom board, two side walls and a front and back wall and four slits measuring 1x15 cm in the front wall to serve as a flight entrance for the bees. The hives were placed on an iron stand of 3 m height. The top bars of the hives were not closely spaced, but placed at 1cm from each other to stimulate gathering of propolis by the honey bees. The flight entrance and top bars were baited with honey on weekly basis until colonization. Two of the hives were colonized by the honey bees two weeks after placement and the propolis were collected from the top bars and the flight entrance on weekly basis with the aid of a hive tool for twelve months between January and December 2008. The propolis were weighed and kept in covered jars in a refrigerator.

### Assessment of propolis for management of *Prostephanus truncatus* (Horn)

#### Sources of insect culture, maize and propolis

*P. truncatus* used for the study was obtained from the culture maintained on dried cassava chips in 250 cm<sup>3</sup> jars in the laboratory at temperature and relative humidity of 28±1°C and 79–82% R.H. Several LGB adult of mixed sexes of unknown ages were introduced into the culture media. Frass generated by feeding activities of the insects was sieved out on weekly basis using sieve of mesh size 0.25 mm to prevent excessive grain moisture content and growth of mould. Culture media were rejuvenated monthly to replace depleted ones, and adults were sieved out to set up new culture to guarantee regular source of insect. Grains of maize used for this study, Solo was obtained from Ogun State Agricultural Development Programme (OGADEP), Idi aba, Abeokuta, Ogun State. The kernels were disinfected in the deep freezer at temperature of –20°C or 48 hours to get rid of any insect or pathogen (Osipitan 2005). They were allowed to acclimatize for 48 hours before usage. The bee propolis was obtained from the two hives located in the University of Agriculture, Abeokuta.

#### Preparation of propolis extract

100 g raw propolis was weighed using Mettler weighing balance. The propolis was thereafter cut into small bits of about 5–10 mm and placed in a conical flask. 100 ml of ethanol was poured into 250 conical flask to submerge the propolis. The outlet of the flask was covered with a foil

paper and held tightly with rubber bands; the mixture was vigorously shaken for 30 minutes and left for 7 days to allow for extraction of the active ingredient in the mixture (Obasa *et al.* 2007). The resultant extract was filtered through a Whatman No. 1 filter paper into a 250 conical flask. 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 5%, 10%, 15% and 20% ethanolic extracts of propolis (EEP) respectively.

### Experimental procedure

The sample of 150 g of clean and disinfested maize grains were weighed in 250 cm<sup>3</sup> kilner jars using Mettler weighing balance (Mettler Toledo). 5 ml of the various concentration of the propolis extracts i.e. (5, 10, 15 and 20%) and ethanol (control) were applied to the grains with the aid of a syringe, mixed thoroughly and left for 1 hour to allow evaporation of the ethanol used for dilution. Each of the jars was then infested with 10 pairs of 1–5 days old *P. truncatus*. Each treatment was replicated three times and arranged on work table in the laboratory using complete randomized design. The control glass

jars contained 150 g maize grains and were infested with 10 pairs of 1–5 days old LGB and treated with ethanol. 150 g clean and disinfested grains were weighed into the jars to monitor change in weight of grains as a result of moisture loss or gain (Hurlock 1967). Insects were sexed using the method of Shires and McCarthy (1976). At 90 days after infestation of the jars, the insects and dust they generated by their feeding activities were sieved out of the grains and the grains were separated into damaged and undamaged and the following data were taken:

- I. Number of adult *P. truncatus*
- II. Weight of dust (g)
- III. Number of damaged and undamaged grains
- IV. Final weight of grain
- V. Number of pupae
- VI. Number of larvae

Insect that did not move or respond to three probings with a blunt probe were considered dead (Obeng-Ofori *et al.* 1997). The percentage weight loss and the percentage damage respectively were calculated using the formula, according to Baba-Tierto (1994).

$$\% \text{ grain weight loss} = \frac{\text{Initial weight of grain} - \text{final weight of grain} \times 100}{\text{Initial weight of grain}}$$

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

### Statistical analysis

Statistical analysis of data was based on SAS's general linear models procedure (SAS institute 1998). Analysis of variance (ANOVA) was generated for all variables, and significant means were separated using Duncan Multiple Range Test (DMRT) and Least Significant Difference (LSD) at  $p = 0.05$ .

## RESULTS

### Yield of propolis from hives

Table 1 shows the monthly propolis harvest from the hives for four months. A total of 456 g of propolis was harvested from two hives in twelve months. In hive 1, the propolis yield in the honey flow period (December–March) was 5 g, 3 g, 5 g and 10 g respectively. They were however not significantly ( $p > 0.05$ ) different from each other, but were significantly ( $p < 0.05$ ) lower than yield in other periods, except the yield in March that was not statistically ( $p > 0.05$ ) different from propolis yields in April, May and November. The highest propolis harvest (36 g) took place in August and it was significantly ( $p < 0.05$ ) higher than harvest in the other months except in June and July. In hive 2, the lowest propolis harvest of 4 g was in December and January and it was not significantly ( $p > 0.05$ ) different from propolis harvest in February. The propolis yield during these months (December–February) was however significantly ( $p < 0.05$ ) lower than yield in other periods. The highest mean propolis harvest from two hives was in August and it was significantly ( $p < 0.05$ )

higher than the harvest in other months except in June and July.

### Mean number of LGB in propolis-treated grains infested with LGB

Table 2 shows the mean number of LGB in propolis-treated grains infested with LGB. The mean number of larvae, pupae, and adults from propolis-treated maize grains at all concentration used except 5% for the number of pupae was significantly ( $p < 0.05$ ) lower than mean numbers from the control. The lowest mean number of larvae (20.00) was from grains treated with propolis at 20% concentration and it was significantly ( $p < 0.05$ ) lower than mean numbers from the other propolis-treated grain and the control. Also, the lowest mean number of pupae (12.33) was from grains treated with 20% concentration propolis and it significantly ( $p < 0.05$ ) differed from 20.33, 23.66 and 33.33 pupae from grains treated with 15%, 10% and 5% concentration of propolis respectively. A significantly ( $p < 0.05$ ) lower mean number of adult LGB (44.33) was from grains treated with 20% concentration of propolis which differed significantly ( $p < 0.05$ ) from the adult population on the other propolis-treated maize grains and the control.

### Mean % grain damage, % weight loss, and weight of dust in propolis-treated grains infested with LGB

The mean % grain damage, % weight loss and mean weight of dust at all the concentrations of propolis were significantly ( $p < 0.05$ ) lower than the means of the control

Table 1. Yield of bee-propolis from hives located in University of Agriculture, Abeokuta

Propolis yield [g] Months/Honey production period															
	honey flow period				dearth period			build-up period		nectar flow period					
Hives	Dec	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	total	LSD	value
1	5	3	4	10	15	19	30	35	36	25	20	15	218	109	9.54
2	4	4	5	15	20	25	35	38	39	22	18	13	238	119	8.95
Mean	4.5	3.5	5	12.5	17.5	17	32.5	36.5	37.5	23.5	19	14	456	228	8.50

Significant means were separated using the Least Significant Difference (LSD) at  $p = 0.05$

Table 2. Mean number of LGB on maize grains treated with various doses of propolis

Mean number of LGB±SE			
treatments	larvae	pupae	adult
20%	20.00±0.58 c	12.33±0.88 c	44.33±3.7 e
15%	23.33±1.86 c	20.33±0.67 b	100.67±1.45 d
10%	76.66±4.48 b	23.66±0.88 b	118.33±1.76 c
5%	82.00±2.3 b	33.33±2.40 a	191.66±3.18 b
0% (Control)	89.66±3.18 a	36.33±4.10 a	348.33±4.81 a

Means followed by different letter within a vertical column are significantly different by Duncan Multiple Range Test (DMRT) at  $p < 0.05$   
LGB – Large grain borer

Table 3. Mean % grain damage, % weight loss, and weight of dust in maize grains treated with propolis

Damage Indices±SE			
treatments	weight of dust [g]	% grain damage	% weight loss
20%	7.73±3.71 d	24.91±1.24 e	5.74±1.11 e
15%	23.93±1.31 c	30.86±0.51 d	17.71±0.83 d
10%	31.55±0.72 b	46.94±0.98 c	24.03±1.31 c
5%	34.92±3.18 b	61.90±0.27 b	40.68±0.59 b
0% (Control)	53.22±2.60 a	70.47±1.08 a	70.47±1.08 a

Means followed by different letter within a vertical column are significantly different by Duncan Multiple Range Test (DMRT) at  $p < 0.05$

(Table 3). The lowest mean weight of dust (7.73 g) was from grains treated with propolis at 20% concentration and it was significantly ( $p < 0.05$ ) lower than mean weight of dust from 15% (23.93 g), 10% (31.55 g), 5% (34.92 g) propolis concentration and control (53.22 g). However, the weight of dust at 10% and 5% concentrations were not significantly ( $p > 0.05$ ) different from each other. Also, the lowest mean % grain damage (24.91) was from grains treated with 20% propolis concentration, and it was significantly ( $p < 0.05$ ) lower than mean % grain damage of maize grains treated with propolis at the rest concentrations and control. Likewise, a significantly ( $p < 0.05$ ) lower mean % weight loss (5.74) was from grains treated with 20% concentration propolis which differed significantly ( $p < 0.05$ ) from % weight loss on the other propolis-treated maize grains and the control.

## DISCUSSION

The study indicated the ability of honey bees to gather propolis from trees in the forest of Abeokuta in south

western Nigeria. Banskota *et al.* (2001) reported propolis as a wax-like resinous substance collected by honey bees from tree buds or other botanical sources and used as cement to seal cracks or open spaces in the hive. Krell (1996) reported that propolis also reinforce the structural stability of hive, reduce vibration and make a hive more defensible. A significantly high propolis (74 g) was gathered by the bees from botanical sources during the build up period. This may likely be as a result of towered activities of gathering propolis by worker bees to seal openings and repair hives during this period. Seegeren *et al.* (1996) reported that the worker bees are responsible for collection of propolis, guarding the flight entrance of the colony, maintaining the temperature of the brood combs at 35°C, collection of nectar among other functions. A significantly lower propolis gathered by bees during the honey flow period (December–March) may likely be the result of increased nectar gathering for honey production during this period.

The large number of chemical components in propolis may justify its many biological activities. In this study,

the biological activities of propolis reduced all stages and adult LGB in propolis-treated maize grains. Propolis at 20% concentration reduced larvae and pupae build-up in treated maize grains by over 300% and adult build-up in the control was about 8-fold greater. This result related to the findings of Sforcin *et al.* (2002) which reported the biological activities of propolis that favoured it to be used as antiviral, antibacterial, antibiotic, anti-inflammatory, anesthetic, healing, immunomodulatory, antioxidant and carcinostatic. Likewise, Banskota *et al.* (2001) reported propolis as a folk medicine possessing a broad spectrum of biological activities. Also, Totan *et al.* (2001) reported that caffeic acid phenethyl ester (CAPE), a biologically active component of propolis from honey bee hives has potent antimutagenic, anticarcinogenic, immunomodulatory, anti-inflammatory and antioxidant properties. Botushanov *et al.* (2001) reported that propolis has over 200 identified constituents in its waxes and resins that made it veritable cascade of aromatic nutrients. The many active principles present give it remarkable properties in combating all types of pathogens (bacteria, viruses, parasites, fungi). Its flavonoids, organic, phenolic and aromatic acids and cumarins in the presence of numerous mineral elements and vitamins have strong antioxidant, anti-inflammatory, antiseptic and painkilling effects.

Since propolis is a natural product with no reported detrimental effect on the soil, air, and water environment. It could therefore be explored singly or integrated with other control management to manage the population of LGB in maize. This will go a long way in overcoming drawbacks such as pest resistance, resurgence, toxicity, bio-magnification, high cost and development of new breed of pest associated with the use of synthetic insecticide for management of LGB.

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## POLISH SUMMARY

### PROPOLIS – PRODUKT WYTWARZANY PRZEZ PSZCZOŁĘ MIODNĄ *APIS MELLIFERA* (HYMENOPTERA: APIDAE) I JEGO PRZYDATNOŚĆ DO ZWALCZANIA KAPTURNIKA OLBRZYMKĄ *PROSTEPHANUS TRUNCATUS* (HORN) (COLEOPTERA: BOSTRICHIDAE) NA ZIARNIE KUKURYDZY

Celem prowadzonych badań była ocena wytwarzania propolisu przez pszczołę miodną *Apis mellifera* oraz możliwości jego wykorzystania do zwalczania kapturnika olbrzymka *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) na ziarnach kukurydzy. W ciągu 12 miesięcy propolis pobierano z uli pszczelich umiejscowionych na polu. Zgromadzony materiał rozcieńczano etanolem w celu uzyskania koncentracji: 5, 10, 15 i 20%. 150 g zdezynfekowanych ziarniaków kukurydzy umieszczano w słoikach Kilnera i traktowano 5 ml rozcieńzonego propolisu stosując każdą z testowanych koncentracji, a w kombinacji kontrolnej zastosowano sam etanol. Potraktowane ziarna kukurydzy zasiedlono 10 parami dorosłych owadów (1–5 dniowych). Po 90 dniach żerowania kapturnika olbrzymka określano: procentowy ubytek wagi ziarna, procent uszkodzenia ziarna, wagę resztek po żerowaniu (g) oraz liczbę poczwerek, larw i owadów dorosłych. Stwierdzono, że miesięczne wielkości wytwarzanego propolisu w okresie od grudnia do marca były istotnie niższe ( $p < 0.05$ ) w porównaniu do pozostałych okresów w ciągu całego roku. Propolis zastosowany we wszystkich koncentracjach ograniczał populację kapturnika olbrzymka na ziarniakach kukurydzy w porównaniu z kombinacją kontrolną. Najlepszy efekt uzyskano przy zastosowaniu 20% koncentracji propolisu, istotnie wyższy w porównaniu do pozostałych trzech badanych koncentracji. Stosowanie propolisu może być zalecane wraz z innymi przyjaznymi środowisku metodami zwalczania kapturnika olbrzymka na ziarnie kukurydzy.