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Preliminary Study of a Method of Stimulating Propolis Collection by Honey Bees (*Apis mellifera*) (Hymenoptera:Apidae) in Bee Hives

[1]A.A. Osipitan, [1]O.Y. Babalola and [2]O.A. Lawal
[1]Department of Crop Protection, University of Agriculture, Abeokuta, Ogun State, Nigeria
[2]Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria

Corresponding Author: A.A. Osipitan, Department of Crop Protection, University of Agriculture, Abeokuta, Ogun State, Nigeria

ABSTRACT

The study evaluated a method termed "top bar spacing" in Kenya top bar hives to stimulate propolis collection by worker bees from tree buds at University of Agriculture, Abeokuta (UNAAB). Ogun State, Nigeria and Olupakun village, Abeokuta, Ogun State, Nigeria. There were four treatments: hives with top bars spaced at 0.2, 0.4, 0.6 and 0.8 cm. Hives with closely-spaced top bars served as control. The studies were conducted between August 2008 and March, 2009 and repeated between August 2009 and March, 2010. The honey yield, propolis yield, weight of dry pressed comb, number of ripe harvested combs and number of unriped combs were significantly ($p<0.05$) higher in year 2010. Likewise, the honey yield, propolis yield, weight of dry pressed comb, number of ripe harvested combs and number of unriped combs were significantly($p<0.05$) higher in Olupakun village. The propolis collected in hives with spaced top bars were significantly ($p<0.05$) higher than propolis in hives with closely-spaced top bars. The honey yield, weight of dried pressed comb and number of ripe harvested combs were significantly ($p<0.05$) lower in hives with differently-spaced top bars. The study shows that a method of "top bar spacing" could stimulate collection of propolis from botanical sources by worker bees. This method could therefore be used by bee keepers with bias for propolis marketing to gather propolis.

Key words: Propolis, worker-bees, bee-hive, bee-colony, honey

INTRODUCTION

Honey bee (*Apis mellifera* L.) (Hymenoptera:Apidae) is a beneficial insect that pollinate crops, produce honey, propolis, beeswax, bee venom and several other bee hive products (Klein *et al.*, 2007; FAOSTAT, 2009). FAOSTAT (2009) reported that the worldwide production of honey is over a million tons, yielding an exchange market worth over US$ one billion. Social bees live in large colony that could consist of over 80,000 colony members depending on the strongness of the colony as dictated by virility of the queen, quality and quantity of nectar and pollens available among other variable factors. The Honey bee (*A. mellifera* L.) is the most widely distributed economic species of bees and accounted for over 90 % of the world honey production and majority of insect's pollination in plants. *A. mellifera* originated from Europe, Asia and Africa and have about 25 races that includes important ones such as European dark bee (*Apis mellifera mellifera*), Asian bee (*Apis cerena*, West African bee (*Apis mellifera adansonii*), China bee (*Apis cerena cerena*), Central and East Africa bee (*Apis mellifera scutellata*), North Africa bee (*Apis mellifera intermissa*), Italian bee (*Apis mellifera ligustica*) (Seegeren *et al.*, 1996). Bees are true social insect that live in colonies and differentiated into three distinct castes namely the queen, the drone and the workers.

The queen bee is the only fertile female in a bee colony and is the mother of all the female worker bees, male drones and virgin queen in the colony. The queen may live up to three years or more and her sole responsibility is to lay eggs; she could daily lay up to 1,500 eggs which could reach peak of 3,000 when the queen is highly prolific. In her life time, the queen could lay up to 500,000 eggs. The eggs laid could be fertile or unfertile. The fertile eggs are laid into small hexagonal cells and eventually hatch into worker bees while the unfertile eggs are laid into the larger hexagonal cells and eventually hatch into drone bees (Seegeren *et al*., 1996).

The worker bees are the second female member of the bee colony; they were produced from fertilized eggs and classified as imperfect or infertile female due to their inability to lay eggs under normal condition. The workers bees have the biggest brain among the bee castes and are responsible for carrying out different activities at different period of their lives as dictated by age when certain glands are active. The worker bees could function as nurse workers that feed the queen and larvae; the house workers that are responsible for indoor duties such as storing of pollens and nectar, wax making and cell building, cleaning and polishing of empty cells in the brood cells for queen to lay eggs, conversion of nectar into honey, clearing of dead colleagues, regulating temperature and humidity in the hive; field workers that function in collection of propolis from tree buds, collection of pollens and nectar, guard the nest from external attack and scout workers that locate appropriate hive or house for bee to live (Farrar, 1967). The worker bees are the most numerous in a bee colony; they could be up to 50,000 in a strong colony. The worker bees are equipped with barbed straight stings that enable them to sting once due to the barbed nature of their sting. The drone bees are twice the size of the worker bee and are the second largest member in the bee colony. The adage “as lazy as a drone” typifies the attitude of the drone in a bee colony. They are few in any bee colony and do not forage for pollens, nectar or propolis. They lack sting apparatus and could therefore not defend themselves or members of the colony. The drones are excited when the virgin queen goes on her nuptial flight; when they perform the singular function of mating the virgin queen to fertilize her in air outside the colony. The drone relies on the workers for their survival as they are fed by them.

Propolis is an important product collected by foraging bees from tree buds. 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 (Arbia and Babbay, 2011). The worker bees that collect propolis in any bee colony are few in number as the assignment demands some expertise. The workers saddled with this responsibility visits tree buds on warm days when the resin is soft and pliable. The resin are collected and missed with wax flakes secreted from special glands on the abdomen of the bee. This mixture is molded into a tiny ball and placed into the pollen baskets on the legs of the bee. The worker never return to the hive with half-filled pollen baskets but gather propolis from other sources until her pollen baskets are full. The house bees usually assist the bees to unload and store the gathered propolis when she returns to the hive.

Propolis 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.*, 2000; Obasa *et al.*, 2007). Propolis have 500 times more flavonoids than the average orange and 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 *et al.*, 2001).

Typical propolis contains approximately 50% resin and vegetable balsams, 30% waxes, 10% essential oils and 5% pollens. 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 and was reported to reduce the chance of cataract in the eyes and 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.*, 1999). 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. Propolis is also used in the attachment of combs to the top and sides of the hive, as well as for filling cracks, reducing the size of the of the hive entrance and embalming intruders (Arbia and Babbay, 2011). Adedoyin *et al.* (2010) reported the potential s of propolis to manage larger grain borer-*Prostephanus truncatus* in stored maize grains. Likewise, its use in canal debridement for endodontic procedure was explored in Brazil (Silva *et al.*, 2004). Propolis is of high demand because of its usefulness as medicine, ingredients in tooth paste, soap, ointment and other industrial usefulness in the making of paints, turpentine or varnishes. This study evaluates a method termed " Top Bars Spacing" to stimulate honey bees to gather propolis.

MATERIALS AND METHODS

Location of study site: The study evaluated "Top Bars Spacing" method to stimulate propolis collection from tree buds by worker bees at two locations in Ogun State between August 2008 and March, 2009 and repeated between August, 2009 and March, 2010. The two locations were University of Agriculture, Abeokuta (UNAAB), Ogun State, Nigeria and Olupakun village, Abeokuta, Ogun State, Nigeria. The two locations were about 50 km distance from each other.

Placement of hives: Thirty Kenya top bar hives were located in each of the locations. The hive is a long trough shaped box with sloping sidewalls covered with 22 top bars of about 28 cm long. It consist of a bottom board, two side walls and a front and a back wall and four slits measuring 1×15 cm in front wall to serve as flight entrance for the bees. The hives were placed on iron stand of 3 m height. Each of the 22 top bars, side walls and the flight entrance were smeared with honey as bait to attract bees to the hives for colonization.

Methodology: Twenty colonised hives were used for the study; the hives were spaced at a distance of 50 m from each other and arranged using Completely Randomized Design (CRD). There were four treatments as follows: hives with top bars spaced at 0.2, 0.4, 0.6 and 0.8 cm. Kenya top bar hives with closely-spaced top bars served as control. The treatments were replicated four times. At six months post-colonisation, the honey combs in the hives was harvested, pressed with a honey comb presser and the following data were taken:

- The weight of honey (kg)
- The weight of propolis (g)

- Dry weight pressed combs (kg)
- Number of harvested ripe combs
- Number of unharvested unripe combs
- Total number of honey combs in hive

Percentage combs harvested was calculated using the formulae:

$$\text{Comb harvested}(\%) = \frac{\text{No. of harvested ripe combs}}{\text{Total No. of combs in hive}}$$

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

RESULTS

Effect of location on mean weight of honey, propolis and pressed comb in differently spaced hives: Table 1 shows the effect of location on the mean weight of honey, propolis, pressed comb and number of harvested and unharvested combs in hives with differently-spaced top bars and hives with closely spaced top bars. In 2009, 2010 and cumulative of the two years, a significantly ($p<0.05$) higher honey yield (27.06, 32.63 and 29.84 kg), respectively was obtained from Olupakun village. The propolis yield was significantly ($p<0.05$) higher at UNAAB in 2009; whereas, in 2010 and cumulatively, the propolis yield were not significantly ($p>0.05$). In 2009, 2010 and cumulative of the two years, a significantly ($p<0.05$) higher pressed combs (10.35, 13.30 and 11.82 kg), respectively was obtained from Olupakun village relative to (7.19, 7.75 and 8.47 kg, respectively obtained from UNAAB.

Effect of location on mean number of harvested and unharvested combs in differently spaced hives: Table 2 shows the effect of location on the mean number of harvested and unharvested combs in hives. In 2009, 2010 and cumulative of the two years, a significantly ($p<0.05$) higher number of harvested combs, combs with brood cells and total combs in hives was obtained from Olupakun village. In 2009, 2010 and cumulatively, the number of harvested combs from Olupakun village was 5.60, 7.80 and 6.70, respectively. Likewise, the number of combs with brood cells in 2009, 2010 and cumulatively (5.25, 7.55 and 6.40, respectively) obtained from

Table 1: Interaction of hives status and year on honey yield, propolis yield and dried weight of pressed combs in hives with differently spaced-top bars

	Honey yield and other yield-related parameters±SE								
	Honey-yield (kg)			Propolis-yield (g)			Dried weight of pressed combs (kg)		
Locations	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
Olupakun	27.06±2.80[a]	32.63±2.52[a]	29.84±2.62[a]	30.90±3.67[a]	37.52±4.18[a]	34.20±3.90[a]	10.35±1.22[a]	13.30±1.44[a]	11.82±1.30[a]
Unaab	21.23±2.36[b]	23.95±2.51[b]	22.58±2.42[b]	34.60±3.02[b]	50.21±14.21[a]	37.40±7.62[a]	7.19±0.83[b]	7.75±1.11[b]	8.47±0.95[b]
LSD values	2.08	1.76	1.47	2.48	28.62	14.55	1.04	1.38	0.90

Significant means were separated using LSD at ($p<0.05$). Mean values with different letter along the column are significantly different from each other at $p<0.05$

Table 2: Interaction of location and year on number of harvested combs, number of combs with broods and total number of combs in hives with differently spaced-top bars

	Honey yield and other yield-related parameters±SE								
	No. of harvested combs			No. of combs with brood cells			Total No. of combs		
Locations	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
Olupakun	5.60±0.70^{a}	7.80±0.66^{a}	6.70±0.67^{a}	5.25±0.56^{a}	7.55±0.47^{a}	6.40±0.49^{a}	10.65±0.46^{a}	15.40±0.53^{a}	13.05±0.46^{a}
Unaab	4.30±0.34^{b}	5.40±0.56^{b}	4.85±0.41^{b}	4.05±0.44^{b}	5.25±0.30^{b}	4.65±0.34^{b}	8.35±0.36^{b}	10.65±0.45^{b}	9.50±0.24^{b}
LSD values	0.64	0.55	0.49	0.41	0.62	0.37	0.90	0.86	0.65

Significant means were separated using LSD at ($p<0.05$). Mean values with different letter along the column are significantly different from each other at $p<0.05$

Table 3: Interaction of hives status and year on honey yield, propolis yield and dried weight of pressed combs in hives with differently spaced-top bars

	Honey yield and other yield-related parameters±SE								
Top bar spacing	Honey-yield (kg)			Propolis-yield (g)			Dried weight of pressed combs (kg)		
(cm)	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
0	40.94±1.67^{a}	44.43±1.60^{a}	42.68±1.51^{a}	3.28±0.14^{a}	3.94±0.20^{a}	3.61±0.15^{a}	16.33±1.50^{a}	20.94±1.42^{a}	18.53±1.25^{a}
0.2	32.73±1.94^{b}	37.16±2.04^{b}	34.94±1.85^{b}	21.46±1.80^{b}	63.63±34.51^{b}	42.54±17.22^{b}	10.86±0.96^{b}	13.28±1.28^{b}	12.08±1.05^{b}
0.4	19.51±1.66^{c}	23.70±2.35^{c}	21.60±1.96^{c}	32.13±1.90^{c}	42.10±1.89^{b}	37.12±1.76^{b}	6.58±0.52^{c}	9.29±0.68^{c}	7.93±0.54^{c}
0.6	14.03±1.18^{d}	18.19±1.56^{d}	16.11±1.16^{d}	40.95±2.21^{d}	55.44±1.66^{b}	48.20±0.99^{b}	5.19±0.51^{c}	6.68±0.62^{d}	5.93±0.51^{d}
0.8	13.53±1.29^{d}	17.95±1.76^{d}	15.74±1.29^{d}	40.91±3.02^{d}	54.21±2.21^{b}	47.57±1.57^{b}	5.09±0.53^{c}	7.44±065^{dc}	6.26±0.57^{d}
LSD values	3.29	2.78	2.32	3.92	45.26	23.01	2.19	1.65	1.43

Significant means were separated using LSD at ($p<0.05$). Mean values with different letter along the column are significantly different from each other at $p<0.05$

Olupakun village was significantly ($p<0.05$) higher than the number of combs with brood cells (4.05, 5.25 and 4.65, respectively) obtained from hives located at UNAAB village. Similarly, the total number of combs at Olupakun village in 2009, 2010 and cumulatively (10.65, 15.40 and 13.05, respectively) was significantly higher than what obtains at UNAAB.

Effect of top bar spacing of hives on mean weight of honey yield, propolis yield and pressed combs in differently-spaced hives: Table 3 shows the effect of hive top bar spacing on weight of honey, propolis and pressed combs. In 2009, 2010 and cumulatively, the honey yield from the hives with no space, 0.2 cm space and 0.4 cm were significantly ($p<0.05$) different from each other. The highest honey yield in 2009, 2010 and cumulatively (40.94, 44.43 and 42.68 kg, respectively) was obtained from hives with tightly-placed top bars (0 cm) and it was significantly ($p<0.05$) higher than honey yield from other hives. The honey yield from hives with top bars spaced at 0.6 and 0.8 cm were not ($p<0.05$) significantly different from each other. In 2009, 2010 and cumulatively, the propolis yield from hives with top bars closely spaced (0 cm) was significantly lower than propolis yield from other hives with top bars spaced differently. Irrespective of year, the propolis yield obtained from hives with top bars spaced at 0.6 and 0.8 cm were not significantly ($p>0.05$) different from each other. In 2009, 2010 and cumulatively, the pressed combs from hives

Table 4: Interaction of hives status and year on number of harvested combs, number of combs with broods and total number of combs in hives with differently spaced-top bars

Top bar spacing (cm)	Honey yield and other yield-related parameters±SE								
	No. of ripe combs			No. of unripe combs			Total No. of combs		
	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
0	8.63±0.89^{a}	11.00±0.71^{a}	9.81±0.78^{a}	1.87±0.23^{d}	4.38±0.38^{c}	3.12±0.28^{d}	10.50±1.07^{a}	15.50±1.04^{a}	13.00±1.03^{a}
0.2	5.13±0.67^{b}	7.75±0.49^{b}	6.44±0.55^{b}	4.13±0.23^{b}	6.75±0.49^{b}	5.43±0.29^{b}	9.25±0.82^{b}	14.50±0.80^{a}	11.87±0.78^{b}
0.4	4.63±0.26^{b}	5.63±0.42^{c}	5.12±0.23^{c}	3.13±0.23^{c}	5.13±0.44^{c}	4.13±0.28^{c}	7.75±0.37^{b}	10.75±0.80^{c}	9.25±0.42^{c}
0.6	3.25±0.25^{c}	4.00±0.57^{d}	3.63±0.32^{d}	7.13±0.40^{a}	7.75±0.70^{a}	7.44±0.51^{a}	10.13±0.44^{a}	11.75±1.22^{bc}	10.93±0.73^{b}
0.8	3.13±0.44^{c}	4.63±0.50^{d}	3.87±0.34^{d}	7.00±0.46^{a}	8.00±0.71^{a}	7.50±0.55^{a}	9.88±0.67^{a}	12.63±1.13^{b}	11.25±0.74^{b}
LSD Values	1.00	0.87	0.78	0.65	0.99	0.59	1.42	1.36	1.03

Significant means were separated using LSD at ($p<0.05$), Mean values with different letter along the column are significantly different from each other at $p<0.05$

with top bars spaced at minimal space of 0.2 cm was significantly ($p<0.05$) higher than pressed combs from hives with top bars at other spaces. Likewise, the pressed combs from hives with closely-spaced top bars (0 cm) was significantly ($p<0.05$) higher than pressed combs from other hives.

Effect of top bar spacing of hives on mean number of harvested and unharvested combs in differently spaced hives: Table 4 shows the effect of hive top bar spacing on the mean number of harvested combs, number of combs with brood cells and total number of combs. In 2009, 2010 and cumulatively, the ripe combs from hives with closely-spaced (0 cm) top bars were significantly ($p<0.05$) higher than the ripe combs from other hives. Conversely, the ripe combs from hives with widely-spaced top bars (0.8 cm) were significantly ($p<0.05$) lower than the ripe combs from other hives, except hives spaced at 0.6 cm.

Conversely, the number of combs with brood cells was significantly lower in hives with closely spaced top bars in 2009, 2010 and cumulatively. It was however, significantly higher in hives with top bars widely spaced (0.8 cm). The number of combs with brood cells in hives with top bars spaced at 0.6 and 0.8 cm were not ($p>0.05$) significantly different from each other. Irrespective of year and cumulatively, a significantly ($p<0.05$) higher total number of combs was obtained from hives with closely spaced top bars. Of the hives with spaced-top bars, the highest total number of combs was obtained from hives with top bars spaced at 0.8 cm.

DISCUSSION

The study indicated the ability of honey bees to gather propolis from tree buds in the forest to seal openings in their hives. Bees gather propolis for three reasons; firstly to keep their hive warmth by sealing cracks and tap holes, secondly to disinfest hive sides, combs and frames and thirdly to cover all the foreign or dead objects in the hive that they cannot remove. Bees use propolis to embalm and prevent decomposition of the objects thereby preventing infection. 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. Similarly, Krell (1996) reported that bees gather propolis to seal open openings in their hives to reinforce the structural stability of their hive, reduce vibration and make the hive more defensible. A significantly higher propolis from hives with the widest top bar spacing suggests a direct relationship between the size of top bar space and propolis gathering activities. However, since bees are likely to suspect exposed hive and swarm, further studies should be conducted to determine the top bars space limit at wish bees would not swarm but gather propolis to seal openings.

Irrespective of hives status, higher honey yield, propolis yield, weight of pressed combs and number of combs were obtained from hives at Olupakun. This may be as a result of the remoteness and virgin nature of the location at Olupakun village, the strongest of the bee colonies in hives, the quality of queens in the hives, abundance of forage at the location and nearness of the hives to forage site. All these factors were not controlled in the study, since the forest was considered as homogenous. The location at Olupakun village has abundant vegetation and trees that provided ready source of pollens, propolis and nectar for bees in hives located in the village. The location at UNAAB is been encroached with developmental activities of the University community that replaces vegetations and trees with buildings; thereby threatening the virginity of the location. Also, the significant higher honey and wax comb yield in 2010 suggests that weather plays a significant role in the activities of bees in the hive and needs to be further investigated.

CONCLUSION

The “top bar spacing” method of stimulating bees to gather propolis could be an additional method to the Loycart method and other methods that uses special frames placed between the supers in hives with moveable frames such as Langstroth. This frame is taken for a foreign object by bees and is covered with propolis. However, most beekeepers in Africa still use Kenya top bar hives and this makes the method of “top bar spacing” relevant. The study also suggests the range of space within which the method of “top bars spacing” could be practiced because when a wide opening exist in the hive, the bees uses wax to seal it rather than propolis. These studies were conducted between August and March. However, quantity and quality of propolis is influenced by the period of the year. Bees normal gather more propolis between April and September and gather propolis with the best quality between June and July. The propolis gather between June and July is of higher quality as all the components such as balsam, resinous compounds, beeswax, ethereal and aromatic oils, pollen, flavonoids, cinnamic acid, cinnamyl alcohol, vanillin, caffeic acid, tetochrysin, isalpinin, pinocembrin, chrysin, galangin and ferulic acid that makes propolis valuable would have been available. Further study should therefore be conducted to determine variation in quality and quantity of propolis within the months of the year.

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