

Palynological Analyses of Honeys Produced in Honey-flow Season in Oyo State, Nigeria

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Abstract

This study was conducted to evaluate the pollens source of honey harvested in the honey flow season in Oyo State, Nigeria. A total of 19 samples of honey were collected from 19 bee hives from two different villages in the January, 2013, 2014 and 2015. The experiment was carried out at Tede Atisbo Local Government of Oyo State and Ladoja and Sons Plantation and Apiculture farm located at Saki-West Local Government of Oyo State. All samples of honey collected were analysed palynologically in the Main Laboratory of the College of Health Sciences, Ahmadu Bello University, Zaria, Nigeria. Result shows the presence of fifteen and twelve pollen samples in Atisbo and Saki West Local Government Areas respectively annually. The result revealed that all the honey tested were multifloral. The prominent pollens include: Ipomea batata (Convolvulaceae), Ocimum spp (Lamiaceae), Launea taraxacifolia (Compositae), Anarcadium occidentale (Anarcadiaceae), Gliricidia sepium (Fabaceae), Elaeis guinensis (Palmaceae), Glycine max (Fabaceae), Glycine max (Fabaceae), Helianthus annuus (Compositae), Brassica spp (Brassicaceae), Moringa oleifera (Moringaceae), Sophora japonica (Fabaceae), Cocos nucifera (Palmae), Psidium guajava (Myrtaceae), Gossypim spp (Malvaceae), Mimosa pudica (Fabaceae), Hymenocardia acida (Phyllanthaceae), Piliostigma thoningi (Fabaceae), Eucalyptus spp (Myrtaceae), Psidium Spp (Myrtaceae), Magnifera indica (Anarcadiaceae), Parkia biglobosa (Fabaceae).

Keywords: Palynology, honey, Oyo State, Pollen, Honey-flow season

Introduction

Honeybees and flowering plants are mutually dependent; as honeybees need flowering plants for food in the form of pollen and nectar, whereas plants need bees for pollination. Honey contains pollen grains which are collected by honeybees while foraging the flowers for pollen and nectar. The microscopic analysis of pollen (palynology) is the

standard method and an effective tool to understand the distribution and abundance of floral nectar sources in any given region.

Palynological analysis is used to determine the characteristics, types and quality of honey. Determination of the geographical and floral origins of honeys has been carried out by microscopical analysis of pollen types (Maurizio and Louveaux, 1965; Low *et al.*, 1989; Battesti and Goeury 1992). Low *et al.* (1989) and Lutier and Vaissière (1993) showed the importance of a standardized method that minimizes mistakes in characterization with the pollen analysis of honey obtained from different parts of the world.

Studying the pollen in honey greatly contributes to the understanding of the geographical and botanical origin of honey, as the honeybees are known to visit more than 3 km in the search of forage. Knowledge of botanical source of honey is a prerequisite for beekeepers to undertake migratory beekeeping for increasing honey production and pollination. Honey characterization is essential when its commercial quality must be assessed. The honey production of an area mainly depends on the area's flora and climatology, because the flowering and nectar production season can be different for the same species in different areas (Zamarlicki, 1984). Also palynological analyses have been carried out to characterize mono and multifloral honeys (Battesti and Goeury 1992; Seijo and Jato, 1998; Valencia-Barrera *et al.*, 1994, 2000).

The identification of honey sources in an ecological zone is germane for commercial beekeeping aimed at boosting honey production. Knowledge of honeybee plants and time of pollen and nectar flow greatly influence the brood rearing activity and the functioning of honeybee colonies and production of honey and other hive products (Ostrowsha, 1998). Although studies have been made on honeybee in Oyo State of Nigeria but there is no documentation on the botanical formulation or origin of the honey produced in the State. This study was conducted to analyse the pollen content of honey produced in the honey flow season to ascertain the botanical origin, quality and grade of honey produced in Oyo state, Nigeria.

Materials and Methods

Study Site

In this study 19 samples of honey were collected annually from 19 bee hives from two different villages in January, 2013, 2014 and 2015. The honey samples were collected from Tede Atisbo Local Government of Oyo State and Ladoja and Sons Plantation and Apiculture farm located at Saki-West Local Government of Oyo State. The location climate is notably with dry and wet seasons with relatively high humidity. The dry season lasts from November to March while the wet season starts from April and ends in October. Average daily temperature ranges between 25°C and 35 °C in the years. Thirteen samples were obtained from apiaries in Tede, Atisbo Local Government and 6 from Saki in Saki West Local Government Area of Oyo State annually. All the samples of honey collected were analysed palynologically in the Main Laboratory of the College of Health Sciences, Ahmadu Bello University Zaria, Nigeria.

Method of Qualitative and Quantitative Palynological Analysis

Samples of honey for pollen analysis were prepared following the harmonized methods of qualitative and quantitative analyses. There are two method of sample preparation i.e. the Acetolytic and the one prescribed by the International Commission for Plant-bee Relationship (Louveaux *et al.*, 1978). The frequency of each pollen type in the samples was expressed as percentage of the total pollen.

10 g of honey was weighed into a pointed glass centrifuge tube (capacity 50 ml), 20 ml of distilled water (20- 40 ° C) was used to dissolve the honey and the solution was centrifuged for 10 min. The supernatant liquid was decanted and 20 ml of distilled water was used to completely dissolve the remaining sugar crystals and then centrifuged for 5 min at 1000 g, the supernatant liquid was decanted to the last drop and a texture was placed upside down an angle 45 ° C so as to allow the remaining excess liquid to be taken up on absorbent paper.

A heating plate was heated to 40 ° C and liquefy the glycerin jelly, a water proof marker was used to drawn a square of 22 × 22 mm in the microscope slide and into the heating plate, the sediment was thoroughly mixed by a pasture pipette and then spread evenly with a micro spatula over the marked area of 22 × 22 mm, the slide was left on the heating plate until the sediment get chide, a drop of glycerin jelly was applied into the cover slip so as to form a large cross diagonally, the preparation was left on the heating plate for a period of 5 minutes. The examination was carried out at the magnification of 600x, after a first general check so as to ascertain the main types and density of pollen grain in groups of 100 following 10 parallel equidistance lines uniformly distributed from one edge of the cover slip to the other. With the above procedures both identification of pollen and percentage was able to be traced to the plant used.

Determination of Relative Frequency of Pollen in Honey Samples

The image of each result of the slide was captured by the compound microscope for pollen types (botanical genus and species) that were identified. The cover glass was later sealed with paraffin wax. The pollen types contained in each samples, were identified with the help of standard slides prepared from the local flora by matching each type of pollens assessed with pollen slides of flowering plants within the vicinity of where the hives were sited. This identified pollen was analyzed for their respective counts and percentage frequency categorized in percentage.

Results

Table 1 shows the presence of 15 types of pollen in Saki West Local Government Area. The total pollen grain count of 5577 were established and identified to family, generic and species levels. The palynology result of the 10 honey samples shows that the entire honey samples were multifloral. The pollen types identified were *Parkia biglobosa*, *Psidium guajava* which were present in all samples at the range of (6.2 to 11.5%) and (5.4 to 11.2%) respectively. Others are *Gossypium* spp which appeared in samples A,B,C,D and H (4.5 to 12.0%), *Sophora japonica* was encountered in C, F and H (6.1 to10.0%), *Anarcadium occidentale* occurred in A,B,C,F and G (6.2 to 13.1%),*Ocimum* spp was observed in A,B and G (2.5 to 3.4%), *Gliricidia sepium* appeared in D and J (4.0 to 4.1%), *Launea taraxacifolia* appeared in A,C and E (1.0 to 4.5%), *Ipomea batatas* was encountered in A,B,D and J (4.2 to 6.1%), *Glycine max* appeared in B,D,H,I and J (2.0 to 4.0%), *Piliostigma thoningi* was encountered in A,B,C,G and H (2.9 to 6.2%), *Eucalyptus* spp appeared in D,F and J (1.5 to 3.5%), *Mimosa pudica* was observed in D and H (1.5 to 2.1%), *Juglans regia* appeared in (2.5 to 4.0%), *Moringa oleifera* occurred in (5.5 to 9.2%). The total sum of pollen count ranged between 505 and 608.

Table 1: Pollen content (%) of honey produced in Saki West Local Government Area

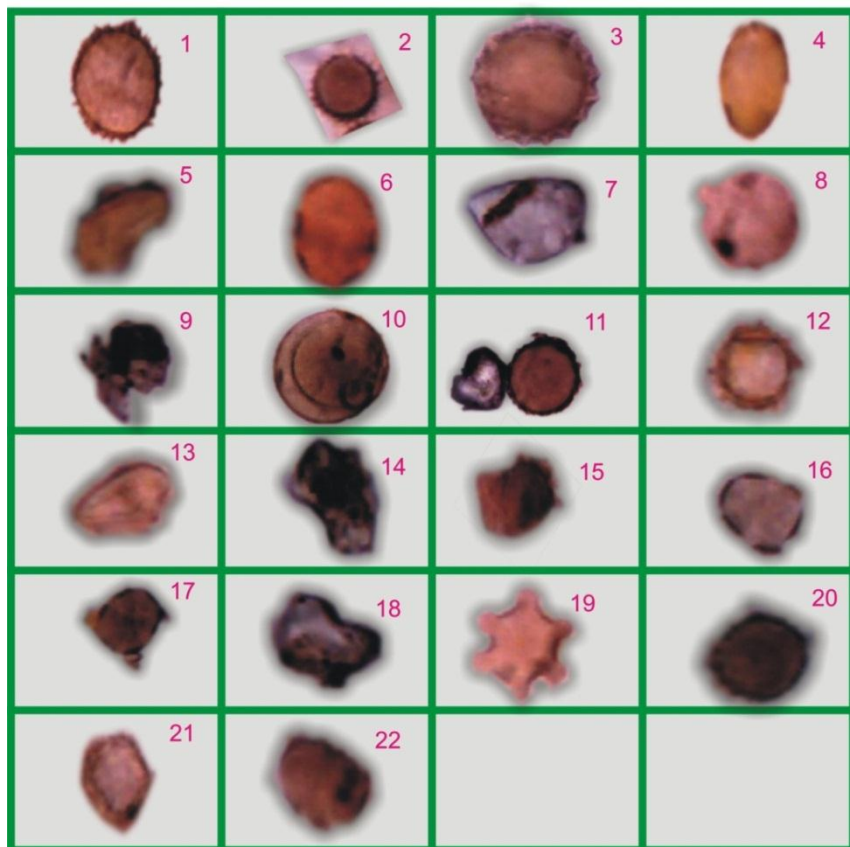
Pollen sample	A%	B%	C%	D%	E%	F%	G%	H%	I%	J%
<i>Parkia biglobosa</i>	10.2	10.4	11.5	8.0	11.5	12.0	9.5	8.0	10.5	6.2

<i>Psidium guajava</i>	8.2	6.5	8.5	6.0	10.1	11.2	8.0	10.5	9.5	5.4
<i>Gossypium spp</i>	12.0	8.0	10.5	7.5	-	-	-	4.5	-	-
<i>Sophora japonica</i>			6.1	-	-	7.2		10.0	-	-
<i>Anarcadium occidentale</i>	9.5	6.5	11.5	-	-	6.2	13.1	-	-	-
<i>Ocimum spp</i>	2.5	3.4	-	-	-	-	3.0	-	-	-
<i>Gliricidia sepium</i>	-	-	-	4.0	-	-	-	-	-	4.1
<i>Launea taraxacifolia</i>	1.0	-	3.2		4.5	-	-	-	-	-
<i>Ipomea batatas</i>	5.0	4.2	-	6.1	-	-	-	-	-	5.0
<i>Glycine max</i>	-	2.0	-	4.0	-	-	-	3.5	2.5	3.0
<i>Piliostigma thoningii</i>	3.4	5.6	2.9	-	-	-	6.2	4.5	-	-
<i>Eucalyptus spp</i>	-	-	-	1.5	-	3.5	-	-	-	2.0
<i>Mimosa pudica</i>	-	-	-	2.1	-	-	-	1.5	-	-
<i>Juglans regia</i>	-	-	-	-	4.0	2.5	-	-	3.0	-
<i>Moringa oleifera</i>	5.5	-	-	7.0	-	-	2.9	-	-	-
Total pollen (P)	568	608	617	505	580	545	596	485	528	545

Table 2 shows the presence of 12 types of pollen in Atisbo Local Government Area. The total pollen grain count was 5314. The palynology result of the 10 honey samples were Multifloral. The pollen types identified were *Anarcadium occidentale*, *Magnifera indica* which were present in all samples at the range of (5.4 to 11.4%) and (4.9 to 13.0%) respectively. Others are *Cocos nucifera* appeared in A,D,G and I (2.0 to 9.5%), *Juglans regia* appeared in C,F and J (1.5 to 4.1%), *Hymenocardia acida* was encountered in C,F and I (3.0 to 3.2%), *Brassica spp* was observed in only A(2.3%), *Piliostigma thoningi* A,D,E,F and I (3.2 to 4.6%), *Capsicum annum* occurred B,C,F and J (3.0 to 4.5%), *Glycine max* appeared in B,C,D and J (2.7 to 4.5%), *Helianthus annus* appeared in G and H (1.8 to 2.6%), *Moringa oleiracea* occurred in A,D and G (5.5 to 9.2%).The total sum of pollen count ranges between 490 to 600. Plate 1 shows the pictures of all the different pollens encountered in the study.

Table 2: Pollen content of honey produced in Atisbo Local Government Area

Pollen sample	A%	B%	C%	D%	E%	F%	G%	H%	I%	J%
<i>Anarcadium occidentale</i>	10.5	5.4	9.5	10.2	11.0	8.5	6.5	10.4	9.5	11.4
<i>Magnifera indica</i>	8.6	4.9	9.6	13.0	7.5	5.2	5.8	6.6	8.9	10.0
<i>Cocos nucifera</i>	2.0	-	-	7.0	-	-	9.5	-	2.5	-
<i>Juglans regia</i>	-	-	1.5	-	-	4.1	-	-	-	3.2
<i>Hymenocardia acida</i>	-	-	3.2	-	-	3.0	-	-	3.0	-
<i>Brasica oleiracea</i>	2.3	-	-	-	-	-	-	-	-	-
<i>Piliostigma thoningi</i>	4.6	-	-	3.2	4.0	3.5	-	-	4.2	-
<i>Capsicum annum</i>	-	3.3	4.5	-	-	3.5	-	-	-	3.0
<i>Glycine max</i>	-	3.7	3.0	2.7	-	-	-	-	-	4.5
<i>Helianthus annus</i>	-	-	-	-	-	-	2.6	1.8	-	-
<i>Moringa oleifera</i>	4.3	-	-	6.1	6.5	-	-	5.0	-	-
<i>Parkia biglobosa</i>	6.5	-	-	-	10.0	7.8	5.0	-	-	6.0
Total pollen sum (P)	506	510	490	575	600	510	518	530	555	520

**Key:**

1. *Ipomea batata* (Convolvulaceae)
2. *Ocimum* spp (Lamiaceae)
3. *Launea taraxacifolia* (Compositae)
4. *Anarcadium occidentale* (Anarcadiaceae)
5. *Gliricidia sepium* (Fabaceae)
6. *Elaeis guinensis* (Palmaceae)
7. *Glycine max* (Fabaceae)
8. *Glycine max* (Fabaceae)
9. *Helianthus annuus* (Compositae)
10. *Brassica* spp (Brassicaceae)
11. *Moringa oleifera* (Moringaceae)
12. *Sophora japonica* (Fabaceae)
13. *Cocos nucifera* (Palmae)
14. *Psidium guajava* (Myrtaceae)
15. *Gossypim* spp (Malvaceae)
16. *Mimosa pudica* (Fabaceae)
17. *Hymenocardia acida* (Phyllanthaceae)
18. *Piliostigma thoningi* (Fabaceae)
19. *Eucalyptus* spp (Myrtaceae)
20. *Psidium* Spp (Myrtaceae)
21. *Magnifera indica* (Anarcadiaceae)
22. *Parkia biglobosa* (Fabaceae)

Plate 1: Photomicrograph of Pollens Found in the Analysed Honey Samples**Discussion**

The results of the palynological analysis of the honey from the two studied areas demonstrated the abundant and diversified pollen composition of the honey samples examined. The excess of honeybees visiting or foraging many plants may be connected to the fact that pollen is the only protein food within the beehive; as a consequence, it plays an important role in feeding the colony. In fact, pollen is used for feeding the larvae and the young bees. It contributes to body growth in general and is a determining factor in the development and the functionality of certain organs such as the adipose body, ovaries and in particular the hypopharyngeal glands; these glands play an important role in royal jelly secretion; royal jelly is used for feeding the larvae for the first three days of their life and provides the queen bee with necessary nourishment.

In line with this, most of the families of plants recorded in this study have been reported to be visited by honeybees (Shubharani *et al.*, 2013). It is shown in this study that bees visit plants regardless of their habits and habitat. The same notion was earlier reported by Adeonipekun (1989). In addition honeybees prefer to visit plants with good nectar and attractive flora (Ige and Apo, 2007); though there are taxa that are non-nectariferous in nature. The investigation of 19 honey samples shows 22 plants belonging to 13 families were useful for honeybees as they provide food in form of pollen and nectar. The result showed that all honey samples were Multifloral, family Fabaceae consist of 7(Spp), Compositae (2Spp) and the remaining families have a specie each which are Moringaceae, Convolvulaceae, Solanaceae,

Lamiaceae, Palmaceae, Brassicaceae, Juglandaceae, Arecaceae, Myrtaceae, Anarcadiaceae, Malvaceae and Phyllanthaceae

Conclusion

The pollen composition of the honey samples in this study has shown that honeybees travel a considerable distance in search of suitable food materials (nectar) for their survival and production of honey. The presence of the pollen grains in the honey samples is a clear indication that the honeys are not adulterated in the state and that they are multifloral.

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