



Melissopalynological Characterization of Honey Samples from Southeastern, Nigeria

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ABSTRACT

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The identification of plants in which the bees forage is key in establishing bee farms and increasing honey production. In this study pollen analysis of honey samples from the southeastern part of Nigeria was carried out to ascertain their floral sources and ecological origin. The honey samples were acetolyzed and microscopically studied to determine the pollen types. A total of seventy-one pollen types belonging to forty-one families of plants were identified. The honey samples were dominated by pollen grains from the families of Arecaceae, Euphorbiaceae, Myrtaceae, Irvigiaceae, Fabaceae, Combretaceae/Melastomataceae, and phyllanthaceae. Some of the dominant pollen grain identified include *Elaeis guineensis*, *Alchornea cordifolia*, *Hymenocardia acida*, *Ocimum gratissimum*, *Syzygium guineense*, *Nauclea latifolia* and *Azelia africana*. Out of the six samples studied Njikoka sample was monofloral having *Mimosa pigra* as predominant pollen while Ayamelum, Ekwusigo, Nsukka, Ezeagu, and Udeno samples are multifloral containing *Elaeis guineensis*, *Phyllanthus* sp., *Piliostigma reticulatum*, *Irvingia* sp., *Alchornea cordifolia*, and *Lansea* sp. as major secondary pollen. All the samples analyzed have *Elaeis guineensis* in common except Ezeagu, indicating that these plants are present in all five locations. These results can also be used as a tool in the geographical identification of Southeastern Nigeria honey from other geopolitical zones.

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Introduction

The practice of beekeeping in the production of honey is an old agricultural system in southeastern Nigeria, it has helped in improving the standard of living of people both in rural and urban areas. Although, pests and diseases have been reported to cause about a 15% decline in honey bee colony establishment, as well as the regular absconding and aggressiveness of the honeybees (Adekanmbi et al., 2019). Honey's medicinal, therapeutics, and nutritional properties have made it a sought-after commodity both in domestic and international markets, by providing employment and room for the adulteration of honey through the demand and supply gap. There is a need for honey products to be subjected to these parameters; floral type, precise place of origin, and quality to check their authenticity in Nigeria. Most markets sell adulterated honey Made of brown sugar, which could affect our health and increase the sugar level in humans.

The composition of honey varies according to the source of flowers used by bees, the harvest period, and the

geo-climatic conditions of the regions concerned (Yédomonhan, 2009). Studies of pollen analysis will assist in bee management and in the development of beekeeping. It provides reliable information on floral and geographical sources of honey along with the relative preference of the bees among the diverse assemblage of plant species flowering at the same period. The wind and insect-pollinated taxa found in a honey sample will often produce a pollen spectrum that is unique for the specific geographical region where it was produced. Pollen analysis of honey has important commercial value because honey made from some plants commands a premium price.

According to Ige and Obasanmi, (2014), analysis of pollen in honey dates as far back as the nineteenth century, starting with the pioneering work of Pfister (1895). Since this period, a lot of studies (Agwu and Njokuocha, 2004; Atanassova et al., 2004; Fagundez and Caccavari, 2006; Sadia et al., 2008; Adekanmbi and Ogundipe, 2009; Forcone, 2014; Moar; 2014; Aino, 2016; Njokuocha et al.,

2019) have been carried out by several researchers around the world to examine the pollen contents of honey from various countries. In Nigeria, available literature on the pollen contents of honey from various parts of Nigeria has all revealed the floral sources utilized by bees in honey production. Fifty-six (56) honey plants which were characteristic flora of tropical rainforest and mosaic of Low land rainforest taxa were recorded by Nnamani and Uguru (2013) from the study of honey samples collected from Southern Nigeria. Emuobosa (2017) recorded various honey plants from the comparative study of the pollen content of honey collected from the apiary and open markets in Nigeria and the Bénin republic. Similarly, Njokuocha et al. (2019) determined the pollen spectrum of *Apis mellifera* honey from different locations in Nigeria.

However, in Enugu and Anambra state where honey production is a popular business in many communities, apart from the works of (Agwu et al., 1989; Agwu and Abaeze, 1991; Njokuocha and Nnamani, 2009; Njokuocha and Ekweozor, 2007; Njokuocha and Osayi, 2015; Njokuocha et al., 2019) on the pollen content of Nsukka honey, reliable information on floral sources of honey produced in this area until now are limited. These studies help to differentiate monofloral honey from multiflora and specific types of honey which are of high commercial value. The aim of this research, therefore, was to examine the pollen grains contained in honey from these States to provide more information on the botanical and geographical origins of the honey.

Materials and Methods

Honey Sample Collection

The honey samples used were collected from the beekeepers in the Ayamelum, Ekwusigo, Ezeagu, Njikoka, Nsukka, and Udeni in Anambra and Enugu States, South-eastern, Nigeria (Figure 1).

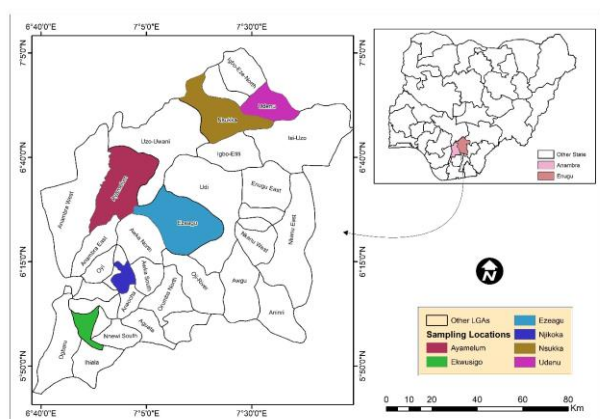


Figure 1. Showing the sampling locations of the honey samples

Pollen Analysis

Pollen analysis was done according to the guidelines given by the International Commission of Bee Botany (Louveaux et al., 1978). Honey samples were diluted with 35 ml of warm acidified water and centrifuged at 5000 rpm for 10 minutes to dissolve the colloidal matters and sugars. The supernatant was carefully decanted and 10 ml glacial

acetic acid was to remove the water before acetolysis. Honey samples were acetolyzed following the procedure of Erdtman (1969). Polliniferous residue was mounted on glycerine jelly and observed under a compound microscope with 400X magnification. The pollen grains were identified with the help of descriptions and photomicrographs in books and Journals (Y'bert, 1979; Bonnefille and Rioulet, 1980; Agwu and Akanbi, 1985; Gosling et al., 2013). They were also compared with reference slide collections in the Palynology Laboratory, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Pollen Count

The characterization of pollen was based on percentages of each pollen type: the pollen grains were placed into one of the following pollen frequency classes: Predominant (> 45% of the total pollen grains counted); Secondary (16% - 45%); Important Minor (3% - 15%) and Minor pollen types (<3%) (Jones and Bryant, 2004).

Results and Discussion

The detailed pollen record of each of the samples is presented in (Table 1a,b). Microscopic examination of honey samples from Anambra and Enugu revealed a total of twenty-seven thousand four hundred and seventy pollen grains (27,470). A total of 71 pollen types belonging to 41 families of plants were recorded in the honey samples. The identified species belong to varying genera of herbs, shrubs, grass, and trees. The colours of the samples after dilution were amber, golden yellow, and yellowish-brown for the honey samples collected from Ayamelum, Ekwusigo, and Njikoka Local Government Areas in Anambra State as well as dark amber, light brown, and amber for the samples from Ezeagu, Nsukka, and Udeni Local Government Areas in Enugu State (Table 2). Amongst all the samples analyzed, one was found to contain predominant pollen type (> 45%) in occurrence (Table 3a,b). In Anambra State, the honey sample collected from Njikoka Local Government Area was dominated by pollen of the *Mimosa pigra* (Table 4). All the honey samples from Enugu State were multi-floral honey (Table 4). Generally, *Elaeis guineensis* was present in all the honey samples. Also, present in the samples as secondary pollen types (16 - 45%) were *Lannea* sp., *Alchornea cordifolia*, *Phyllanthus* sp., *Piliostigma reticulatum*, *Irvingia* sp., *Syzygium guineense*, and Combretaceae/Melastomataceae. The pollen types were classified as predominant (>45%), secondary pollen (16-45%), important minor (3-15%), and minor (<3%) (Tables 3).

The presence of an array of predominant and important pollen types in the honey samples confirmed that they were of botanical origins and a true indication of their geographical origin. The honey samples collected from Anambra and Enugu State were high in pollen diversity and were found to originate from two main sources: wild plants and cultivated crops. The dominant pollen identified from the honey samples includes *Elaeis guineensis*, *Nauclea latifolia*, *Alchornea cordifolia*, *Syzygium guineense*, *Irvingia* sp., *Piliostigma reticulatum*, *Bridelia ferruginea*, *Mimosa pigra*, and *Ocimum gratissimum* which are made up of both wild and cultivated crops (Table 4).

Table 1a. Percentage composition of honey samples collected from Anambra and Enugu States

Sn	Pollen types	Anambra State			Enugu State		
		Ayamelum (%)	Njikoka (%)	Ekwusigo (%)	Nsukka (%)	Eziagu (%)	Udenu (%)
1	Amaranthaceae	0.2	-	0.4	0.4	1.2	-
2	Ampelidaceae	-	-	-	-	-	-
	<i>Cissus doeringii</i> Gilg. and Brandt.	-	-	0.2	-	0.3	-
3	Anacardiaceae	-	-	-	1.9	0.8	-
	<i>Anacardium occidentale</i> Linn.	0.2	-	-	-	-	-
	<i>Mangifera indica</i> Linn.	-	-	3.7	-	-	-
	<i>Lannea</i> sp.	-	1.3	-	0.9	-	26.2
	<i>Spondias mombin</i> Linn.	1.9	-	-	-	-	-
4	Annonaceae	-	-	-	-	-	-
	<i>Monodora</i> sp.	-	-	-	-	0.2	-
5	Apiaceae	-	-	0.6	0.4	-	-
6	Apocynaceae	-	-	-	-	0.2	-
7	Arecaceae	-	-	-	-	-	-
	<i>Elaeis guineensis</i> Jacq.	23.3	35.1	26.3	41.0	-	27.8
8	Asteraceae	1.7	-	-	4.6	0.5	-
9	Bombacaceae	-	-	-	-	-	-
	<i>Bombax buonopozense</i> P. Beauv.	0.2	-	-	-	-	-
10	Boraginaceae	-	-	-	-	-	-
	<i>Cordia</i> sp.	-	-	0.2	-	-	-
	<i>Heliotropium indicum</i> L.	0.4	-	-	-	-	-
11	Burseraceae	-	-	-	-	-	-
	<i>Canarium schweinfurthii</i> Engl.	-	-	1.6	-	-	-
	<i>Commiphora</i> sp.	9.0	-	-	-	-	-
12	Capparidaceae	0.3	-	-	-	-	-
	<i>Cadaba</i> sp.	-	-	-	-	0.6	-
13	Celastraceae	-	-	-	-	-	-
	<i>Hippocratea africana</i> (Wild.) Loes	0.7	-	-	-	-	-
14	Combretaceae/Melastomataceae	-	0.5	-	1.5	19.0	-
	<i>Combretum</i> sp.	-	-	-	-	0.4	-
15	Cyperaceae	-	0.2	-	0.9	0.3	-
16	Ebenaceae	-	-	-	-	-	-
	<i>Diospyros</i> sp.	0.2	-	-	-	-	-
17	Ephorbiaceae	2.7	-	-	-	-	-
	<i>Acalypha</i> sp.	-	-	-	1.4	-	-
	<i>Alchornea cordifolia</i> (Shum. & Thonn) Mull. Arg.	-	4.8	25.3	1.8	9.0	23.6
	<i>Securinea virosa</i> (Rosb. Ex Wild.) Baill.	3.8	-	-	-	-	-
	<i>Antidesma</i> sp.	-	-	-	-	0.3	-
18	Fabaceae	-	-	-	-	-	-
	Caesalpinioideae	-	-	-	-	-	-
	<i>Afzelia africana</i> Sm.	0.2	-	-	-	-	-
	<i>Albizia</i> sp.	-	-	-	-	0.1	-
	<i>Cassia</i> sp.	0.5	-	-	0.3	-	-
	<i>Delonix regia</i> (Boj. Ex Hook.) Raf.	-	-	-	-	0.4	4.2
	<i>Dialium guineense</i> Wild.	-	-	-	0.3	-	-
	<i>Piliostigma reticulatum</i> (Dc.) Hochst	21.7	-	-	-	-	-
	Mimosoideae	-	-	-	-	-	-
	<i>Cleome</i> sp.	-	-	-	-	0.2	-
	<i>Mimosa Pigra</i> Linn.	-	53.5	-	-	-	-
	Faboideae	-	-	-	-	-	-
	<i>Crotalaria pycnostachya</i> Benth.	-	-	0.6	-	-	-
	<i>Pterocarpus</i> sp.	-	-	-	0.4	0.4	-
19	Hymenocardiaceae	-	-	-	-	-	-
	<i>Hymenocardia acida</i> Tul.	-	0.3	0.2	7.9	13.0	-
20	Irvingiaceae	-	-	-	-	-	-
	<i>Irvingia</i> sp.	1.8	0.4	27.5	-	-	-
21	Lamiaceae	-	-	-	-	-	-
	<i>Ocimum gratissimum</i> L	6.3	-	-	-	-	-
22	Liliaceae	4.5	1.0	-	0.3	0.3	-
23	Loganiaceae	-	-	-	-	-	-
	<i>Anthocleista vogelii</i> Planch.	-	-	-	-	0.3	-
24	Loranthaceae	0.2	-	-	-	-	-
25	Meliaceae	-	-	-	-	-	-
	<i>Trichilia</i> sp.	0.2	-	-	0.4	-	-
	<i>Khaya senegalensis</i> (Desr.) A. Juss	-	-	-	-	1.3	-
26	Moraceae	0.4	0.1	0.2	0.4	-	-

Table 1b. Percentage composition of honey samples collected from Anambra and Enugu States

Sn	Pollen types	Anambra State			Enugu State		
		Ayamelum (%)	Njikoka (%)	Ekwusigo (%)	Nsukka (%)	Eziagu (%)	Udenu (%)
27	Myrtaceae						
	<i>Syzygium guineense</i> Engl.	-	0.6	6.9	18.0	1.7	8.9
	<i>Psidium guajava</i> L.	-	-	-	2.5	-	-
28	Ochnaceae	-	-	-	-	-	-
	<i>Lophira lanceolata</i> Van Tiegh. Ex Keay	-	-	-	0.9	-	-
29	Passifloraceae	1.2	-	-	-	-	-
30	Phyllanthaceae	-	-	-	-	-	-
	<i>Phyllanthus</i> sp.	0.2	-	1.2	-	24.4	-
	<i>Bridelia ferruginea</i> Benth.	10.3	-	-	0.4	0.4	-
31	Poaceae	0.2	0.1	0.6	-	4.2	-
32	Proteaceae	-	-	1.4	-	-	-
	<i>Protea angolensis</i> Welw.	-	-	-	-	0.3	-
33	Rhamnaceae	-	-	-	-	-	-
	<i>Ziziphus</i> sp.	-	-	-	1.0	-	4.7
34	Rubiaceae	-	-	-	-	-	-
	<i>Nauclea latifolia</i> Sm.	7.3	-	0.6	-	0.1	-
	<i>Crossopteryx febrifuga</i> (Afzel. Ex G. Don) Benth.	-	-	-	-	16.7	-
	<i>Mussaenda erythrophylla</i> Schum & Thonn.	-	-	-	1.4	2.2	-
35	Rutaceae	-	-	-	-	-	-
	<i>Fagara xanthoxyloides</i> (Lam.)	-	-	0.4	-	-	-
36	Sapindaceae	-	-	-	4.2	-	-
	<i>Blighia sapinda</i> Konig	0.3	-	-	-	-	-
	<i>Paullinia pinnata</i> Linn.	0.1	-	-	-	-	-
37	Sapotaceae	-	-	-	-	-	-
	<i>Mimosop andogensis</i> Hiern.	-	-	-	-	0.2	-
38	Scrophulariaceae	-	-	-	-	-	-
	<i>Striga</i> sp.	-	-	-	-	0.2	-
39	Sterculiaceae	-	-	-	-	-	-
	<i>Sterculia tragacantha</i> Lindl.	-	-	-	-	0.3	-
40	Solanaceae	-	-	-	-	-	-
	<i>Solanum</i> sp.	-	-	-	-	0.3	-
41	Ulmaceae	-	-	-	2.3	-	-
	<i>Celtis</i> sp.	-	-	-	-	0.6	-
	Indeterminate/unidentified	0.11	0.1	0.1	0.2	0.1	0.3
	Total	4428	6295	5104	3939	6940	764

These are plants that are either cultivated or conserved specially for their economic benefits as commercial or subsistence crops in the areas where the honey was produced. This result agrees with the findings of Njokuocha et al. (2019).

The fact that the samples were collected from the wild gives the honey bee access to many plant species which may have contributed to the high pollen diversity. Ige and Apo (2007), are of the view that the more the source of nectar/pollen available to the bees for collection, the more pollen type and this automatically means the more the richness of the honey. The age of the bees is also an important factor when it comes to pollen diversity and abundance. Adeonipekun (2012), observed that an old and defensive colony of bees recorded a higher abundance of pollen grains, while a young colony recorded lesser pollen grains but had higher diversity a reflection of the difference in their experience and nature.

These honey samples generally rich in pollen displayed a vivid landscape of the bee foraged plants growing in the area where the honey samples were collected. Even though bees are species-specific, they still collect pollen from readily available flowers. The pollen types came from nectariferous and non-nectariferous plants.

The dominance of *Elaeis guineensis* pollen in Ayamelum, Njikoka, Ekwusigo, Nsukka, and Udenu honey samples means that the oil palm is readily available and these could be attributed to the fact that the palm is used in commercial agriculture in the production of palm oil. It is a non-nectariferous plant, but the honey bees' feeds on the juice of their fruits in the absence of nectar may be due to their high-calorie level. It also serves as a haven for the bees, since they are usually found in the riverine forest or in freshwater swamps which are usually quiet and free from regular human encroachment. These indicators make *Elaeis guineensis* their preferred choice in an area where the plants are abundant (Table 4). According to Okereke et al. (2016), the predominant vegetation in Anambra State is a mosaic of forest savannah vegetation or secondary re-growth where the oil palm is continuously present, together with some selectively preserved economic trees.

In Ayamelum honey samples *Piliostigma reticulatum* was also found to be dominant, but they were absent in Njikoka, Ekwusigo, Nsukka, Ezeagu, and Udenu samples (Figure 2), there is an indication of its shelterbelt in homesteads when in full foliage, which serves as a haven for bees.

Table 2. The weight of pollen sediment and colour of the honey samples after dilution

S.n.	Sample	Colour	Weight
1	Ayamelum	Amber	2.56
2	Ekwusigo	Golden yellow	1.88
3	Njikoka	Yellowish brown	3.45
4	Ezeagu	Dark amber	4.23
5	Nsukka	Light brown	4.44
6	Udenu	Amber	1.91

Table 3a. Frequency class of pollen types in the honey samples

Sn	Pollen types	Anambra State			Enugu State		
		Ayamelum (%)	Njikoka (%)	Ekwusigo (%)	Nsukka (%)	Eziagu (%)	Udenu (%)
1	Amaranthaceae	M	-	M	M	M	-
2	Ampelidaceae	-	-	-	-	-	-
3	<i>Cissus doeringii</i> Gilg. and Brandt.	-	-	M	-	M	-
	Anacardiaceae	-	-	-	M	M	-
	<i>Anacardium occidentale</i> Linn.	M	-	-	-	-	-
	<i>Mangifera indica</i> Linn.	-	-	IM	-	-	-
	<i>Lannea</i> sp.	-	M	-	M	-	S
	<i>Spondias mombin</i> Linn.	M	-	-	-	-	-
4	Annonaceae	-	-	-	-	-	-
	<i>Monodora</i> sp.	-	-	-	-	M	-
5	Apiaceae	-	-	M	M	-	-
6	Apocynaceae	-	-	-	-	M	-
7	Arecaceae	-	-	-	-	-	-
	<i>Elaeis guineensis</i> Jacq.	S	S	S	S	-	IM
8	Asteraceae	M	-	-	IM	M	-
9	Bombacaceae	-	-	-	-	-	-
	<i>Bombax buonopozense</i> P. Beauv.	M	-	-	-	-	-
10	Boraginaceae	-	-	-	-	-	-
	<i>Cordia</i> sp.	-	-	M	-	-	-
	<i>Heliotropium indicum</i> L.	M	-	-	-	-	-
11	Burseraceae	-	-	-	-	-	-
	<i>Canarium schweinfurthii</i> Engl.	-	-	M	-	-	-
	<i>Commiphora</i> sp.	IM	-	-	-	-	-
12	Capparidaceae	M	-	-	-	-	-
	<i>Cadaba</i> sp.	-	-	-	-	M	-
13	Celastraceae	-	-	-	-	-	-
	<i>Hippocratea africana</i> (Wild.) Loes	M	-	-	-	-	-
14	Combretaceae/Melastomataceae	-	M	-	M	S	-
	<i>Combretum</i> sp.	-	-	-	-	M	-
15	Cyperaceae	-	M	-	M	M	-
16	Ebenaceae	-	-	-	-	-	-
	<i>Diospyros</i> sp.	M	-	-	-	-	-
17	Ephorbiaceae	M	-	-	-	-	-
	<i>Acalypha</i> sp.	-	-	-	M	-	-
	<i>Alchornea cordifolia</i> (Shum. & Thonn) Mull. Arg.	-	IM	S	M	IM	S
	<i>Securinega virosa</i> (Rosb. Ex Wild.) Baill.	IM	-	-	-	-	-
	<i>Antidesma</i> sp.	-	-	-	-	M	-
18	Fabaceae	-	-	-	-	-	-
	Caesalpinioideae	-	-	-	-	-	-
	<i>Afzelia africana</i> Sm.	M	-	-	-	-	-
	<i>Albizia</i> sp.	-	-	-	-	M	-
	<i>Cassia</i> sp.	M	-	-	M	-	-
	<i>Delonix regia</i> (Boj. Ex Hook.) Raf.	-	-	-	-	M	IM
	<i>Dialium guineense</i> Wild.	-	-	-	M	-	-
	<i>Piliostigma reticulatum</i> (Dc.) Hochst	S	-	-	-	-	-
	Mimosoideae	-	-	-	-	-	-
	<i>Cleome</i> sp.	-	-	-	-	M	-
	<i>Mimosa Pigra</i> Linn.	-	P	-	-	-	-
	Faboideae	-	-	-	-	-	-
	<i>Crotalaria pycnostachya</i> Benth.	-	-	M	-	-	-
	<i>Pterocarpus</i> sp.	-	-	-	M	M	-
19	Hymenocardiaceae	-	-	-	-	-	-
	<i>Hymenocardia acida</i> Tul.	-	M	M	IM	IM	-
20	Irvingiaceae	-	-	-	-	-	-
	<i>Irvingia</i> sp.	M	M	S	-	-	-

Table 3b. Frequency class of pollen types in the honey samples

Sn	Pollen types	Anambra State			Enugu State		
		Ayamelum (%)	Njikoka (%)	Ekwusigo (%)	Nsukka (%)	Eziagu (%)	Udenu (%)
21	Lamiaceae	-	-	-	-	-	-
	<i>Ocimum gratissimum</i> L	IM	-	-	-	-	-
22	Liliaceae	IM	M	-	M	M	-
23	Loganiaceae	-	-	-	-	-	-
	<i>Anthocleista vogelii</i> Planch.	-	-	-	-	M	-
24	Loranthaceae	M	-	-	-	-	-
25	Meliaceae	-	-	-	-	-	-
	<i>Trichilia</i> sp.	M	-	-	M	-	-
	<i>Khaya senegalensis</i> (Desr.) A. Juss	-	-	-	-	M	-
26	Moraceae	M	M	M	M	-	-
27	Myrtaceae	-	-	-	-	-	-
	<i>Syzygium guineense</i> Engl.	-	M	IM	S	M	IM
	<i>Psidium guajava</i> L.	-	-	-	M	-	-
28	Ochnaceae	-	-	-	-	-	-
	<i>Lophira lanceolata</i> Van Tiegh. Ex Keay	-	-	-	M	-	-
29	Passifloraceae	M	-	-	-	-	-
30	Phyllanthaceae	-	-	-	-	-	-
	<i>Phyllanthus</i> sp.	M	-	M	-	S	-
	<i>Bridelia ferruginea</i> Benth.	IM	-	-	M	M	-
31	Poaceae	M	M	M	-	IM	-
32	Proteaceae	-	-	M	-	-	-
	<i>Protea angolensis</i> Welw.	-	-	-	-	M	-
33	Rhamnaceae	-	-	-	-	-	-
	<i>Ziziphus</i> sp.	-	-	-	M	-	IM
34	Rubiaceae	-	-	-	-	-	-
	<i>Nauclea latifolia</i> Sm.	IM	-	M	-	M	-
	<i>Crossopteryx febrifuga</i> (Afzel. Ex G. Don) Benth.	-	-	-	-	S	-
	<i>Mussaenda erythrophylla</i> Schum & Thonn.	-	-	-	M	M	-
35	Rutaceae	-	-	-	-	-	-
	<i>Fagara xanthoxyloides</i> (Lam.)	-	-	M	-	-	-
36	Sapindaceae	-	-	-	IM	-	-
	<i>Blighia sapinda</i> Konig	M	-	-	-	-	-
	<i>Paullinia pinnata</i> Linn.	M	-	-	-	-	-
37	Sapotaceae	-	-	-	-	-	-
	<i>Mimosop andogensis</i> Hiern.	-	-	-	-	M	-
38	Scrophulariaceae	-	-	-	-	-	-
	<i>Striga</i> sp.	-	-	-	-	M	-
39	Sterculiaceae	-	-	-	-	-	-
	<i>Sterculia tragacantha</i> Lindl.	-	-	-	-	M	-
40	Solanaceae	-	-	-	-	-	-
	<i>Solanum</i> sp.	-	-	-	-	M	-
41	Ulmaceae	-	-	-	M	-	-
	<i>Celtis</i> sp.	-	-	-	-	M	-

M: Minor, S: Secondary, IM: Important minor, P: Predominant

Apart from their medicinal properties, they are also prolific producers of pollen grains which makes the bees seek after them. The extant plants of *Mimosa pigra* were found to be dominant in the Njikoka community due favourably weather conditions, they are an important pollen source for Apis foragers since they are prolific producers of pollen grains (Figure 2). Their pollen concentration in the honey sample was very high due to the proximity between the beehives and where the plant was growing.

Pollen of *Irvingia* sp and *Ocimum gratissimum* was dominant in the honey sample from Ekwusigo and Ayamelum, these an indication that the bees foraged cultivated areas within the forest zone. These plants could only be found in farmland or conserved area. *Alchornea cordifolia* was also found to be dominant in Ekwusigo, Ezeagu, Njikoka, and Udenu Honey samples which is indicative of forest regrowth.

Syzygium guineense pollen was slightly dominant in Ekwusigo, Nsukka, and Udenu Honey samples. While Asteraceae and Poaceae were dominant in honey samples from Ezeagu and Nsukka. This may be an indication of an increase in deforestation and expansion of agricultural landscapes which promoted increase and extension in agricultural weeds. Pollen of *Phyllanthus* sp., Combretaceae/Melastomataceae, *Lannea* sp. and *Crossopteryx febrifuga* were found to be common in Ezeagu and Udenu honey samples. Since these plants are prolific producers of pollen and would reduce competition during the foraging period among bees when its flowering makes them a preferred plant for foraging.

These pollen types are comparable to the ones identified in the present study. The pollen from both wind and insect-pollinated taxa present in a honey sample will often create a pollen spectrum that is unique for the specific geographical region or micro-vegetation area where it was produced (Ige and Obasanmi, 2014). Honey sample from Ayamelum,

Ekwusigo, Nsukka, Ezeagu, Udenu were classified as multiflora as dominant pollen types were within (6.3-23.3%), (6.9-27.5%), (4.2-41.0%), (9-24.4%), (4.7-27.8%) respectively. While, pollen types from Njikoka honey sample were found within (4.8-53.5%), which was classified as monofloral with *Mimosa pigra* dominating the sample with a record high of 53.5% (Table 4). The pollen spectrum revealed the common plant species foraged by the honey bees in this zone for pollen and nectar. Generally, the honey samples were dominated by plant species that reflect the forest savannah mosaic of vegetation.

Conclusion

The pollen spectra of the honey samples revealed the plants utilized by the bees for honey production, which indicates that the honeys were pure and not adulterated and provides possibility of utilizing this rich bee flora of the region for the development of apiculture and increased honey production of Nigeria. The pollen contents of studied honeys revealed the characteristic floristic composition of the ecological regions of the source areas.

Table 4. Predominant pollen types, percentage occurrence and classification of honey samples collected from Anambra and Enugu States

Location	Class of honey	Pollen types	Percentage occurrence (%)
Ayamelum	Multi floral	<i>Elaeis guineensis</i> Jacq.	23.3
		<i>Ptilostigma reticulatum</i> (Dc.) Hochst	21.7
		<i>Bridelia ferruginea</i> Benth.	10.3
		<i>Commiphora</i> sp.	9.0
		<i>Nauclea latifolia</i> Sm.	7.3
		<i>Ocimum gratissimum</i> L	6.3
Njikoka	Monofloral	<i>Elaeis guineensis</i> Jacq.	35.1
		<i>Mimosa pigra</i>	53.5
		<i>Alchornea cordifolia</i> (Shum. & Thonn) Mull. Arg.	4.8
Ekwusigo	Multi floral	<i>Elaeis guineensis</i> Jacq.	26.3
		<i>Irvingia</i> sp.	27.5
		<i>Alchornea cordifolia</i> (Shum. & Thonn) Mull. Arg.	25.3
		<i>Syzygium guineense</i> Engl.	6.9
Nsukka	Multi floral	<i>Elaeis guineensis</i> Jacq.	41.0
		<i>Syzygium guineense</i> Engl.	18.0
		<i>Hymenocardia acida</i> Tul.	7.9
		Asteraceae	4.6
		Sapindaceae	4.2
Ezeagu	Multi floral	<i>Phyllanthus</i> sp.	24.4
		Combretaceae/Melastomataceae	19
		<i>Crossopteryx febrifuga</i> (Afzel. Ex G. Don) Benth.	16.7
		<i>Hymenocardia acida</i> Tul.	13
		<i>Alchornea cordifolia</i> (Shum. & Thonn.) Mull. Arg.	9
		Poaceae	4.2
Udenu	Multi floral	<i>Elaeis guineensis</i> Jacq.	27.8
		<i>Lannea</i> sp.	26.2
		<i>Alchornea cordifolia</i> (Shum. & Thonn.) Mull. Arg.	23.6
		<i>Syzygium guineense</i> Engl.	8.9
		<i>Ziziphonphus</i> sp.	4.7

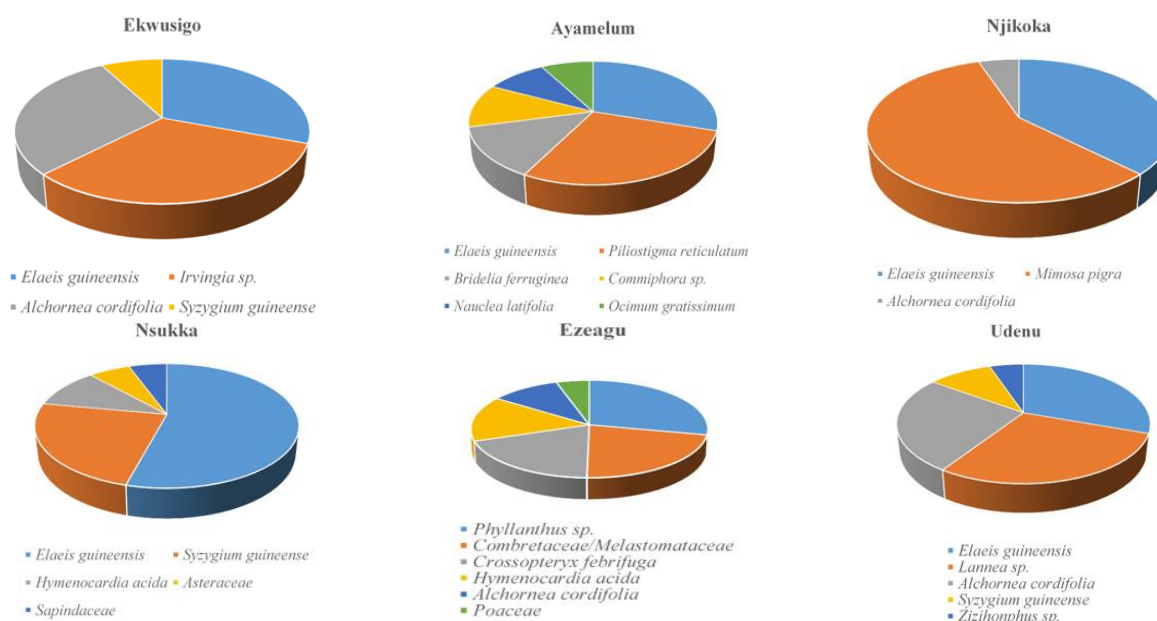


Figure 2. Spectra of dominant pollen types of honey samples collected from Anambra and Enugu States

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References

- Adekanmbi OH, Ogundipe OT. 2009. Nectar sources for the honey bee (*Apis mellifera adansonii*) revealed by pollen content. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 37(2): 211-217. <http://dx.doi.org/10.15835/nbha3723245>.
- Adekanmbi OH, Walter OJ, Ikegbunam NC. 2019. Pollen analysis and heavy metals detection in honey samples from Southern Nigeria. *World News of Natural Sciences*, 26: 176-190.
- Agwu COC, Obuekwe AI, Iwu MM. 1989. Pollen analytical and thin layer chromatographic examination of Nsukka (Nigeria) honey. *Pollen et Spores*, 31(1-2): 29-43.
- Agwu COC, Abaeze CC. 1991. Palynological studies of four honeys from Anambra, Enugu and Kogi States of Nigeria. *Journal of Agricultural Science and Technology*, 1(2): 126-131.
- Agwu COC, Akanbi TO. 1985. Palynological study of honey from four vegetation zones in Nigeria. *Pollen et Spores*, 27(3-4): 335 - 348.
- Agwu COC, Njokuocha RC. 2004. Pollen analysis of honey and the biological effects of honey as rooting medium. *Nigerian Journal of Botany*, 17: 74 - 82.
- Aino DO. 2016. Pollen and physicochemical characterization of honey samples from Ankpa Local Government Area of Kogi State, Nigeria. *Futo Journal Series*, 2(2): 160 - 172.
- Atanassova J, Bozilova E, Todorova S. 2004. Pollen analysis of honey from the region of three villages in West Bulgaria. *Phytologia Balcanica*, 10(2-3): 247 - 252.
- Bonnefille R, Rioulet G. 1980. *Pollen Des Savanes D'Afrique Orientale*. National Dela Recherche Scientifique, Paris.
- Bryant VMJ. 2001. Pollen content of honey. *CAP Newsletter*, 24(1): 10 - 24.
- Emuobosa AO. 2017. Comparative pollen analysis of honeys from apiary and open markets in Nigeria and Bénin Republic. *Ife Journal of Science*, 19(2): 217-225
- Erdtman G. 1969. *Handbook of Palynology. An introduction to the study of Pollen grains and Spores*. Hafnar Publishing Company, New York. 486 pp.
- Fagundez GA, Caccavari MA. 2006. Pollen analysis of honeys from the central zone of the Argentine Province of Entre Rios. *Grana*, 45: 305 -320. doi: 10.1080/00173130601086636
- Fallico B, Zappala M, Arena E, Verzera A. 2004. Effects of heating process on chemical composition and HMF levels in Sicilian monofloral honeys. *Journal of Food Chemistry*, 85: 305 - 313.
- Fasasi KA. 2012. Physicochemical attributes of Nigerian honey from honey bees (*Apis mellifera adansonii*) (Hymenoptera: Apidae) and its shelf life in storage at room temperature. *Pakistan Journal of Biological Science*, 15(21): 1027 - 1033.
- Forcone A. 2014. Pollen analysis of honey from Chubut (Argentinean, Patagonia). *Grana*, 47: 147 - 158. doi: 10.1080/10652460802106340.
- Gosling WD, Miller CS, Livingstone DA. 2013. Atlas of the tropical West African pollen flora. Review of Palaeobotany and Palynology, 199: 1-135. doi: 10.1016/j.revpalbo.2013.01.003.
- Ige OE, Apo KA. 2007. Pollen analysis of honey samples from two vegetation zones in Nigeria. *Science Focus*, 13: 36 - 43.
- Ige OE, Obasanmi OO. 2014. A Palynological assessment of honey samples from Delta State, Nigeria. *American International Journal of Biology*, 2(2): 47 - 59. doi: 10.1080/00173130410019497
- Jones GD, Bryant Jr VM. 2004. The use of ETOH (Ethyl alcohol) for the dilution of honey. *Grana*, 43: 174 - 182.
- Kayode J, Oyeyemi SD. 2014. Physicochemical investigation of honey samples from bee farmers in Ekiti State, Southwest Nigeria. *Journal of Plant Science*, 2(5): 246 - 249. doi: 10.11648/j.jps.20140205.26
- Liberato C, Moraes SM, Magalhães CEC, Magalhães IL, Cavalcanti DB, Silva MMO. 2013. Physicochemical properties, mineral and protein content of honey samples from Ceará State, Northeastern Brazil. *Food Science Technology Campinas*, 33(1): 38 - 46.
- Louveaux J, Maurizio A, Vorwohl G. 1978. International Commission for Bee Botany of IUBS. *Methods of Melissopalynology*. *Bee World*, 59: 139-157.
- Mbah CE, Amao AO. 2009. Natural foods and feeding habits of the African honey Bees *Apis mellifera adansonii* in Zaria, Northern Nigeria. *Science World Journal*, 4(1): 11 - 14.
- Moar NT. 2014. Pollen analysis of New Zealand honey. *New Zealand Journal of Agricultural Research* 28(1): 39 - 70. doi: 10.1080/00288233.1985.10426997
- Njokuocha RC, Ekweozor CC. 2007. Pollen contents of commercial honeys from Opi, Nsukka, and Enugu state, Nigeria. *Plants Products Research Journal*, 11: 5 - 11.
- Njokuocha RC, Nnamani NA. 2009. Palynological analysis of seven samples of honey from the rainforest-savannah vegetation of east-middle belts of Nigeria. *Nigerian Journal of Botany*, 22(1): 189 - 202.
- Njokuocha RC, Osayi EE. 2015. Physicochemical assessment and pollen analysis of honey from Nsukka, Nigeria. *Nigerian Journal of Botany*, 28(1): 95 - 107.
- Njokuocha RC, Dim KI, Onyejekwe OK, Nwokorie VU. 2019. Determination of the concentration of some mineral elements and pollen spectra of *Apis mellifera* L. honeys from different locations in Nigeria. *Animal Research International*, 16(1): 3186-3197.
- Nnamani VC, Uguru AN. 2013. Diversity Of honey producing plants of Southern Nigeria: Basic prerequisite for conservation. *Journal of Sustainability and Science Management*, 8(1): 103 - 112.
- Okereke NC, Mbaekwe EI, Nnabude PC, Ekwealor KU, Nwonumara GU, Iroka CF, Ukpaka CG. 2016. Comparative evaluation of the species richness and diversity of three parallel forest ecosystems in Southeastern Nigeria. *Journal of Agriculture and Ecology Research International*, 8(4): 1 - 12.
- Pfister R. 1895. Versuch einer Mikroskopie des Honigs. *Foschungsber. - Lebensmitt. Bez. Hyg. Chemical Pharmaceutical (München)*, 2: 29.
- Ramírez-Arriaga E, Navarro-Calvo LA, Díaz-Carbajal E. 2011. Botanical characterisation of Mexican honeys from a subtropical region (Oaxaca) based on pollen analysis. *Grana*, 50(1): 40 - 54.
- Ruoff K, Bogdanov S. 2004. Authenticity of honey and other bee products. *Apiacta*, 38: 317 - 327.
- Sadia B, Husan SZ, Malik RN. 2008. Pollen analysis and heavy metal detection in honey samples from seven selected countries. *Pakistan Journal of Botany*, 40(2): 507 - 516.
- Shah A, Sikandar F, Ullah I, Khan SU, Rana UA, McCoy T. 2014. Spectrophotometric determination of trace elements in various honey samples collected from different environments. *Journal of Food and Nutrition Research*, 2(9): 532 - 538.
- Y'bert JP. 1979. *Atlas Des pollen De cote D' Ivoire*. O.R.S.T.M, Paris.
- Yédomonhan H. 2009. *Plantes mellifères et potentialités de production de miel en zones guinéenne et soudano-guinéenne au Bénin*. Thèse de doctorat, université d'Abomey-Calavi, 366p.