

Pollen Analysis of Honey Samples from 13 Local Government Areas of Ebonyi State, Nigeria

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Abstract

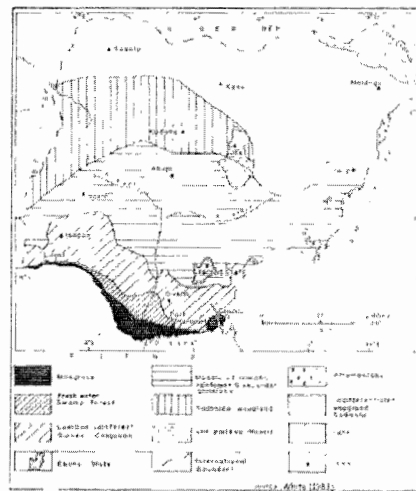
Twenty- six honey samples from 13 Local Government Areas in Ebonyi State of Nigeria were acetolysed and analyzed palynologically to determine their pollen and spore content. Ninety- one pollen types belonging to fifty plant families were identified. Forty- three of these pollen types were identified to specific, forty-two to generic and three to family levels. The predominant honey plants were *Elaeis guineensis* Jacq, *Anacardium occidentale* Linn., *Citrus* sp, *Crossopteryx febrifuga* Afzel ex G. Don., *Nauclea latifolia* S.M., *Hymenocardia acida* Tull, *Lannea acida* A. Rich, *Phyllanthus discoideus* (Baill) Mull. Arg., *Olax viridis*, *Irvingia wombolu* Vermeesen ex Baill, *Bombax buonopozense* P. Beauv, *Brachystegia eurycoma* Harms, *Parkia biglobosa* (Jacq.) Benth, *Berlinia grandifolia* S. M., *Ceiba pentandra* Linn and members of the *Poaceae* and *Combretaceae*/Melastomataceae families. All these plants are characteristic species of the Forest-Savanna ecozone. The analysis also reflected some non-nectariferous taxa such as *Elaeis guineensis*, *Alchornea cordifolia* and members of the *Poaceae* family, that honey bees visit for their pollen meal. Other pollen contributors were some socio-cultural plants, which are selectively protected and propagated for their economic and aesthetic values.

Keywords: Pollen analysis, Honey samples, Ebonyi State, Nigeria

Introduction

In more recent times the use of honey has gained widespread interest and popularity within the society. It has become an integral part in the menu of most Nigerians, sweetener in bakeries, base for jam making, an active component in ethnomedical industries and appreciable sources of income to both the honey gatherers and apiculturists (Agwu and Njokuocha, 2004). Their quality and genuineness are mostly determined by the biological elements contained in them, particularly pollen and spores. Pollen analysis is made possible because of some important and basic characteristic features of pollen and spores. Such diagnostic features include the presence of sporopollenin in their outer wall layer, known as exine and which makes them resistant to biodegradation. This together with their structural and sculptural patterns, size, as well as their production in large numbers make them extremely useful for palynologists.

Ebonyi State is located within the southeastern zone of Nigeria in the Mosaic of Lowland Rainforest and Secondary Grassland, Guinean-Congolian type (White, 1983) (Fig. 1). It has a land area of approximately 5935 sq km, occupying the middle Cross River plain within Latitude 7°30'N and 8°30'N and Longitude 5°40'E and 6°45'E (Ofomata, 1975). It is bounded to the east by Cross River State, to the west by Enugu State, to the north by Benue State and to the south by Abia State of Nigeria. The vegetation constitutes an important ecosystem of both economic and ecological significance. The pollen and spore content of honey samples from different parts of Nigeria have been studied by Sowunmi (1976); Agwu and Akanbi (1985); Agwu *et al.*, (1989); Agwu



and Abaeze (1991); Agwu and Okeke (1997); Sowunmi (2001); Agwu and Njokuocha (2004). Ebonyi State has so far not been sampled. The aim of this work was to investigate palynologically the pollen and spore contents of 26 honey samples from 13 Local Government Areas of Ebonyi State and to establish the botanical and geographical origins of the 26 honey samples and to identify from these, the most important predominant honey plants visited by bees for the synthesis of honey. Knowledge of these could lead to the development of apicultural farm in this State where such practice is not in existence.

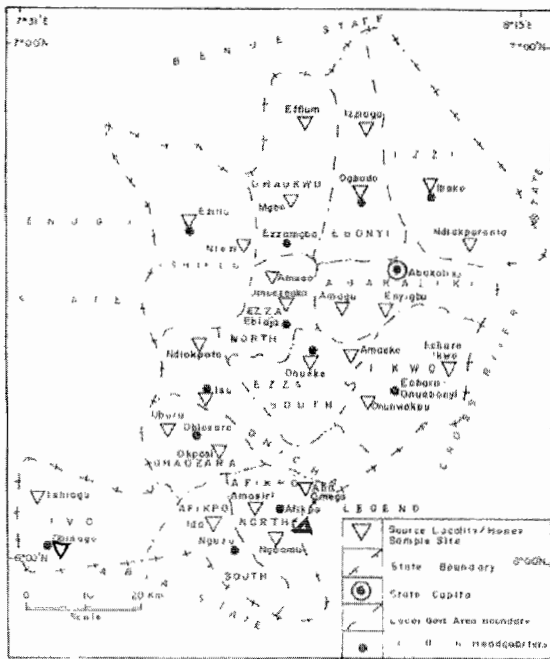


Fig.2 Map of Ebonyi State showing the Local Government Areas and source localities of honey samples

Materials and Methods

Two honey samples were collected from each of the 13 Local Government Areas in Ebonyi State of Nigeria. These L.G.As were Abakaliki, Afikpo North, Afikpo South, Ebonyi, Ezza North, Ezza South, Izzi, Ishielu, Ikwo, Ivo, Ohaukwu, Ohaozara and Onicha (Fig. 2). Samples were sourced during the major honey production periods of January-May in 2002 and 2003 and were tagged I and II.

Extraction of pollen: The 26 honey samples were vigorously shaken to ensure an even distribution of the biological components contained in them. Ten grams of the honey samples were weighed out with the aid of Microwa Swiss 7720 sensitive weighing balance and washed with 35 ml of warm (50°C) dilute sulphuric acid solution (3 ml conc. Sulphuric acid to 997 ml of water) (Lieux, 1972; Agwu and Akanbi, 1985). This process removes the superfluous matters like the sugar content and colloidal materials contained in the honey. The acidified honey solution was centrifuged for 5 minutes at 2000 r.p.m. The precipitates were recovered by decanting the supernatant. The recovered precipitates were washed twice with distilled water, followed by centrifugation and decanting. The recovered precipitates were acetolysed according to Erdtmans (1971) acetolysis method of 9:1 ratio (9 ml of acetic anhydride to 1 ml of 2H₂SO₄). The recovered pollen grains were then washed twice with distilled water and stored in plastic vials in 2:1 glycerol and ethanol.

Analysis: Microscopic analyses of prepared temporary slides were carried out using x 400 magnification. Pollen grains were identified, counted and classified as far as family, generic and specific levels while the predominant honey plants were

classified using Louveaux *et al.*, (1970) recommendation, for expressing pollen frequencies i. e. Very frequent >45%, Frequent >16-45%, Rare > 3-15% and sporadic <3%. Photomicrographs of some of the important honey plants were made (Plate 1).

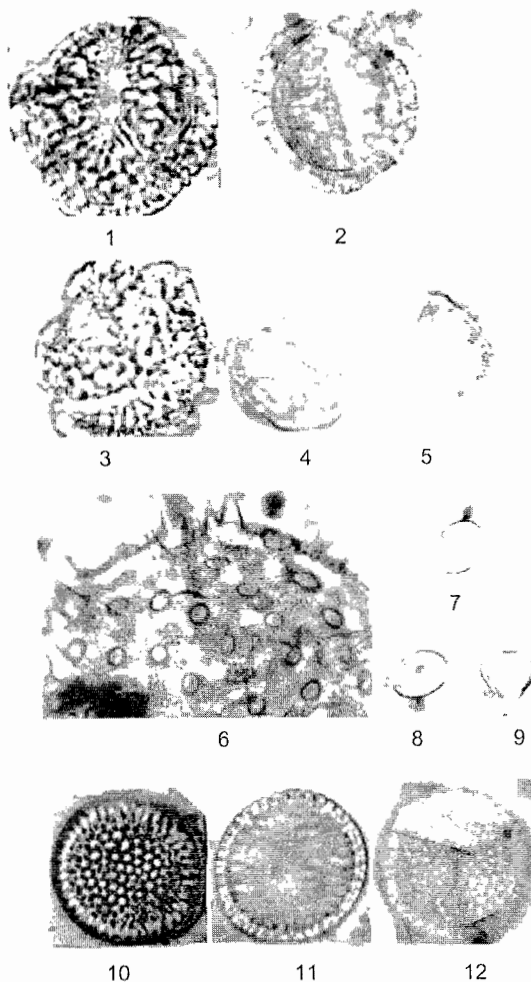


Plate 1: Predominant pollen: Caesalpinaceae: 1 – 2, *Azelia africana* SM, 3 – 5 *Brachystegia eurycoma*, Convolvulaceae: 6 *Ipomoea involucrata* Planck ex Benth, Irvingiaceae: 7 -9 *Irvingia wombolu* Baill, Euphorbiaceae: 10 - 12 *Manihot exculenta* Linn. All x 1000

Results

Ninety one pollen types belonging to fifty plant families were identified. Forty-three of the pollen types were identified to species, forty-two to generic and three to family levels. Five monocotyledonous families and forty-one dicotyledonous families were identified, while three remained unidentified. The highest quantity of pollen was recorded from Ikwo I while the least pollen came from Abakaliki I. Pollen quantities from the various localities are shown in Table 1.

Table 1: Number of Plant Families and Pollen Quantity Identified from the 26 Honey Samples

Localities	Pollen Families/Quantity (Grains/)			
	(1)	grains	(11)	grains
Abakaliki	11	328	12	585
Afikpo North	26	2589	28	3039
Afikpo South	28	3227	22	1395
Ebonyi	33	3576	22	772
Ezza North	19	1403	14	1411
Ezza south	23	2988	27	2962
Ikwo	23	7586	25	5053
Ishielu	10	1057	22	1249
Izzi	31	2853	28	1633
Ohaozara	17	674	18	792
Ohaukwu	18	753	16	3438
Onicha	31	6140	28	2806
Ivo	20	2806	22	1989

Table 2: Predominant honey plants and their percentage

Locality	Pollen Types	Pollen Quantity	Pollen %	Frequency Class
Abakaliki	I <i>Elaeis guineensis</i>	206	63.0	V. Frequent
	<i>Trichilia lanata</i>	36	11.01	Rare
ii	<i>Irvingia wombolu</i>	156	26.8	Frequent
	<i>Elaeis guineensis</i>	308	52.8	V. Frequent
Afikpo North	I <i>Anacardium occidentale</i>	356	14.5	Rare
	<i>Crossopteryx febrifuga</i>	298	12.1	"
	<i>Nauclea latifolia</i>	240	9.75	"
	Combre/Melastomataceae	160	6.5	"
	<i>Elaeis guineensis</i>	318	12.3	Rare
	<i>Carica papaya</i>	120	4.87	"
	ii <i>Phyllanthus sp</i>	450	15	Rare
Afikpo North	I <i>Anacardium occidentale</i>	530	17.85	Frequent
	Combre/Melastomataceae	140	4.67	Rare
	<i>Crossopteryx febrifuga</i>	305	12.9	"
	<i>Elaeis guineensis</i>	406	22.6	Frequent
	<i>Crossopteryx febrifuga</i>	508	34.4	Frequent
	Combre/Melastomataceae	150	6.9	Rare
	ii <i>Elaeis guineensis</i>	458	21.1	Frequent
Afikpo South	I <i>Trichilia lanata</i>	1052	48.8	V. Frequent
	<i>Elaeis guineensis</i>	450	32.7	Frequent
	<i>Trichilia lanata</i>	436	31.7	Frequent
	ii <i>Irvingia wombolu</i>	645	18.4	Frequent
	<i>Elaeis guineensis</i>	615	17.3	Frequent
	<i>Trichilia lanata</i>	385	10.8	Rare
	<i>Nauclea latifolia</i>	602	16.8	Frequent
Ebonyi	I <i>Irvingia wombolu</i>	135	17.5	Frequent
	<i>Phyllanthus sp</i>	103	13.3	Rare
	<i>Elaeis guineensis</i>	58	7.5	Rare
	<i>Brachystegia eurycoma</i>	43	5.6	Rare
	ii <i>Elaeis guineensis</i>	701	50	V. Frequent
	<i>Crossopteryx febrifuga</i>	380	27.1	Frequent
	<i>Elaeis guineensis</i>	408	29.0	Frequent
Ezza North	I <i>Crossopteryx febrifuga</i>	385	27.3	Frequent
	<i>Lannea sp</i>	508	36.1	Frequent
	ii <i>Crossopteryx febrifuga</i>	306	10.1	Rare
	<i>Trichilia lanata</i>	436	14.4	Rare
	<i>Lannea sp</i>	956	31.5	Frequent
	<i>Elaeis guineensis</i>	775	25.5	Frequent
	<i>Crossopteryx febrifuga</i>	1025	35	Frequent
Ezza South	I <i>Elaeis guineensis</i>	408	13.8	Rare
	<i>Lannea acida</i>	388	13.1	"
	<i>Elaeis guineensis</i>	825	10.2	"
	ii <i>Trichilia lanata</i>	3835	47.2	V. Frequent
	<i>Lannea sp</i>	2250	27.7	Frequent
	<i>Elaeis guineensis</i>	858	17	Rare
	<i>Nauclea latifolia</i>	440	9	"
Ikwo	I <i>Trichilia lanata</i>	2031	40.13	Frequent
	<i>Irvingia wombolu</i>	375	7.4	Rare
	<i>Lannea sp</i>	751	14.8	"
	ii <i>Elaeis guineensis</i>	109	10.3	"
	<i>Crossopteryx febrifuga</i>	755	71.1	V. Frequent
	<i>Elaeis guineensis</i>	232	19.4	Frequent
	<i>Crossopteryx febrifuga</i>	102	8.5	Rare

		<i>Olax viridis</i>	209	17.5	Frequent
		<i>Trichilia lanata</i>	205	17.1	Frequent
Izzi	I	<i>Elaeis guineensis</i>	550	19.3	Frequent
		Poaceae	135	4.74	Rare
		<i>Crossopteryx febrifuga</i>	574	20.0	Frequent
		<i>Phyllanthus</i> sp	504	17.7	Frequent
		<i>Irvingia wombolu</i>	103	3.6	Rare
	ii	<i>Elaeis guineensis</i>	340	11.9	"
		Poaceae	75	2.6	Sporadic
		<i>Irvingia wombolu</i>	380	13.3	Rare
Ohaozara	I	<i>Elaeis guineensis</i>	340	45.6	V. Frequent
		<i>Irvingia wombolu</i>	380	50.9	V. Frequent
	ii	<i>Elaeis guineensis</i>	200	15.3	Frequent
		<i>Crossopteryx febrifuga</i>	125	15.8	Frequent
		<i>Irvingia wombolu</i>	168	21.2	Frequent
Onicha	I	<i>Trichilia lanata</i>	2367	38.5	Frequent
		<i>Elaeis guineensis</i>	1708	27.8	Frequent
		<i>Lannea</i> sp	1285	20.9	Frequent
	ii	<i>Trichilia lanata</i>	1150	30.2	Frequent
		<i>Irvingia wombolu</i>	1698	44.6	Very freq
		<i>Lannea</i> sp	260	6.8	Rare
Ivo	I	<i>Crossopteryx febrifuga</i>	355	12.6	Rare
		<i>Citrus</i> sp.	233	8.29	Rare
		<i>Parinari curatellifolia</i>	259	9.22	Rare
		<i>Elaeis guineensis</i>	436	15.5	Rare
		<i>Anacardium occidentale</i>	576	20.5	Frequent
	ii	<i>Nauclea latifolia</i>	320	16.1	Frequent
		<i>Crossopteryx febrifuga</i>	170	8.55	Rare
		<i>Lannea</i> sp	320	16.1	Frequent
		<i>Citrus</i> sp	189	9.50	Rare

Discussion

The 26 honey samples from Ebonyi State of Nigeria have furnished information on their botanical and geographical origins and important predominant honey plants as well as some non nectariferous plants preferred by honey bees for the synthesis of honey.

The pollen analysis revealed very distinct floral composition for each local government area, even though these localities generally belong to the same geographical zone. These differences could be attributed to major diversities in the flora of some of the localities, which are controlled by the influence of local edaphic and micro-climatic factors as well as anthropogenic activities; this is in line with the work of Sowunmi (1976). The edaphic factor is related to the soil type and the moisture content of the soil in the various localities, which to some extent, determine the type of vegetation cover of the place. Micro-climatic factors such as relative humidity and the intensity of the rainfall have great impact on the flora of the various localities. The proximity of Ikwo, Onicha, Afikpo, Ebonyi and Ivo L.G.As to water bodies like Western Ebonyi, Asu, Eze Aku and Cross River is reflected by the high number of forest species encountered from those areas, while areas under the influence of intensive human activities such as Ishielu, Izzi and Ezza North showed more of savanna species.

Botanical origin: The honey samples were rich in pollen types as illustrated from their pollen contents. This probably is a reflection of the high species diversity characteristic of the mosaic of lowland rainforest and secondary grassland. The ninety-one pollen types identified from the 26 honey samples compared favourably with the 50 pollen types in six samples reported by Sowunmi (1976),

56 pollen types identified from seven honey samples by Agwu and Akanbi (1985) and 42 pollen types from four honey samples reported by Agwu and Njokuocha (2004). The following species were considered to be the most common honey plants encountered during the study: *Elaeis guineensis*, *Parkia biglobosa*, *Lannea acida*, *Irvingia wombolu*, *Trichilia* sp, *Azelia africana*, *Berlinia grandifolia*, *Dialium guineense*, *Diospyros* sp, *Mangifera indica*, *Phyllanthus* sp, *Syzygium guineense*, *Lophira lanceolata*, *Parinari curatellifolia*, *Canthium* sp, *Khaya* sp, *Senna* sp, *Pycnanthus* sp, *Olax viridis*, *Brachystegia eurycoma*, *Crossopteryx febrifuga*, *Nauclea latifolia*, *Anacardium occidentale*, *Citrus* sp, *Hymenocardia acida* and members of the Combretaceae/Melastomataceae and Poaceae families.

Geographical origin: The geographical origin of the above honey samples, as reflected by their pollen composition is a composite of derived savanna with relics of lowland rainforest species (Ofomata 1975). Ebonyi State is located within the Mosaic of Lowland Rainforest and Secondary Grassland, which is derived from the northern limits of the Tropical Rain Forest belt of Cross River State. It is characterized by the following plants: *Elaeis guineensis*, *Parkia biglobosa*, *Lannea acida*, *Syzygium guineense*, *Irvingia wombolu*, *Trichilia* sp, *Parinari curatellifolia*, *Azelia africana*, *Berlinia grandifolia*, *Dialium guineense*, *Diospyros* sp, *Mangifera indica*, *Phyllanthus* sp, *Lophira lanceolata*, *Canthium* sp, *Pycnanthus* sp, *Olax viridis*, *Brachystegia eurycoma*, *Crossopteryx febrifuga*, *Nauclea latifolia*, *Anacardium occidentale*, *Hymenocardia acida*, members of the Combretaceae/Melastomataceae and Poaceae families. The relative occurrence of members of the

Poaceae family in all the honey samples indicates savanna origin (Table 2).

Some of the samples were actually low in pollen grains such as recorded in Abakaliki 1 with 328 grains. This low pollen quantity may be attributed to adulteration of honey by addition of sugar-cane (*Saccharum officinarum*) syrup (cf. Sowunmi, 1976).

Predominant honey plants: The pollen analysis also showed great variations in the total number of pollen types and percentage composition of the predominant honey plants, visited by bees for their nectar and pollen sources. The regular occurrence of these plants may be attributed to their abundance and / or preference of these plants to others by the bees. It could further be linked to the prolific production of pollen of such species within the vicinity of the bee hives or their proximity to the bee hives. Some of these plants include : *Elaeis guineensis* 63%, *Irvingia wombolu* 50%, *Trichilia lanata* 48.8 %, *Nauclea latifolia* 16.%, *Parinari curatellifolia* 9.2 %, *Phyllanthus* sp 13.3 %, *Crossopteryx febrifuga* 71.1%, *Anacardium occidentale* 20.5%, *Olax viridis* 17.5%, *Lannea* sp 36.1 %, *Carica papaya* 4.8 %, *Brachystegia eurycoma* 5.6%, *Citrus* sp 9.5% and members of the Combretaceae/Melastomataceae family 6.9%.

Some of these plants are indigenous, socio-cultural plants that are selectively protected and preserved for their economic values, such plants as *Elaeis guineensis*, *Irvingia wombolu*, *Anacardium occidentale*, *Carica papaya* and *Citrus* sp, are used as sources of food and fruits. *Brachystegia eurycoma* and Combretaceae/Melastomataceae family are used as raw materials for building and constructions while some are used as medicine and for their aesthetic values as reported by Agwu and Okeke (1997). The above knowledge can help the individual honey gatherers and apiculturists to know where best to locate their bee hives, having in mind those plants that honey bees prefer in their production of honey.

Non-nectariferous taxa: Of great significance are the regular occurrence of *Elaeis guineensis*, *Achornea cordifolia* and members of the Poaceae family in most of the honey samples. The abundance of these agrees with the report of Gary (1975), on bees visiting some anemophilous plants for the sole purpose of collecting pollen for their "beebread". These pollens serve as the major sources of protein, fatty acids, mineral salts and vitamins available in honey. Pollen of *Elaeis guineensis* and grasses have sweet scents that are attractive to honey bees (Agwu, Personal

communication). It further shows that bees collect pollen of grasses actively, and that the grass pollen found in honey were not accidental occurrence, introduced by wind as reported by Lieux (1972).

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