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Palynological Study of Honey Samples from Four Localities in Kogi East, Nigeria

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Abstract

Pollen-analytical examinations were made of four honey samples from four localities of Kogi State (Idah, Ajaka, Igalamela –Odolu and Inachalo-Oforachi). The control and check of honey for adulteration stimulated this study in honey pollen analysis. It sought to examine and ascertain the species of plants which were incorporated into honey. 10g of each thoroughly shaken honey sample were analyzed after acetolysis. Microscoscopic examinations were made using Carl Zeiss model of microscope. Pollen counts and fine morphological studies were made at x 400 magnification. Grain counts of 3,195, 2,274, 2,522 and 3,105 were recorded for Idah, Ajaka, Igalamela-Odolu and Inachalo-Oforachi respectively. A total of twenty one (21) pollen types were recorded, nineteen (19) of which were identified to family level while (2) remained unidentified. Identifications were carried out down to genetic level but sometimes to species or only to family levels. Some predominant pollen types included Leuceanea glauca, Parkia biglobosa, Elaeis guineensis, Phyllanthus sp and Bombax buonopozense. The four honey samples were unadulterated. This was deduced from the pollen weights and counts. From the pollen identifications, the botanical and geographical origin as well as the season of honey production of each sample were determined and also associated with definite vegetation

types which reflected the vegetation of low land rainforest/Guinea savanna.

Keywords: Honey, Pollen types, Acetolysis, Botanical origin, Geographical Origin, Guinea savanna

1. Introduction

Different flowering plants produce different quantities, qualities, colours and flavours of nectar. Honey bees in Nigeria (*Apis mellifera* var *adansonii*) forage these species and with them produce honey of different types (in quantity, quality, colour and flavour).

Melissopalynology is useful in finding out whether there are significant differences in the pollen composition throughout the year or if additional bee pasture may be needed at certain periods. It also facilitates the establishment of flowering periods of different species and helps in diagnosing the plants poisonous to bees (Lieux, 1972; Schulz, 1984). Microscopic analysis of honey is based on the fact that raw materials of Nigerian honey (nectar and honey dew) have certain constituents which remain identifiable in ripe honey.

Though the analytical examinations of pollen are usually in plant breeding, it is also involved in tracing the history of plant group, climatic history and pollen contents of honey (Agwu, 1997). Honey bees gather large quantities of pollen directly from entomophilous and anemophilous flowers (Chen et al., 1984).

Pollen analysis shows the possibility of determining the botanical, geographical and ecological origin of honey from the pollen grains they contain (Agwu, 1985). This study is based on the fact that honey bees collect their food from plants, especially those that offer higher concentration of high quality of nectar (Chen *et al*., 1984). Debut indigenous melissopalynological study in Nigeria was by Sowunmi (1976), consisting of six honey samples from wild bees and from the same numbers of source localities. Agwu and Akanbi (1985) carried out a palynological analysis of Nigerian honey from four vegetation zones of Nigeria. They ascertained the botanical and geographical origin and season of production of honey sample. Adulteration with hydrolyzed sugar syrup was detected. Ndu (1980) described the pollen types in honey samples from Nanka, Ohafia and Umuhia. Some other studies of honey samples from Nigeria have been carried out by Agwu and Abaeze (1992) who identified the botanical, geographical and season of production of the honey samples collected from various location in Anambra, Enugu, Ebonyi state of Nigeria. Agwu and Uwakwe, (1992) confirmed the botanical and geographical origin of honey sample collected in Abia and the possible application of the knowledge of Melissopalynology to palaeoecology and archeology (Zander; 1941; Sowunmi, 1976). Melissopalynological studies have also been carried out in other parts of Africa (Zander, 1941; Smith, 1956; Crane, 1986).

Honey is used commonly in different parts of Nigeria in ethno medical treatment of various ailments such as cough, chest pain, indigestion/constipation, sprains, burns, minor open injuries, etc. It is also used as food (source of sugar) and for cosmetics.

Due to unscientific cultural agricultural practices, the flora of several parts of Nigeria is threatened with increased uncontrolled and indiscriminate destruction. The knowledge of the

important honey plant of the various vegetation zones might in future lead to their legalized protection and or planned propagation in the development of bee farms. The key to the comprehensive knowledge of the plants which are in part dependent on bees for their pollination is possibly to be found in melissopalynological studies. The control and check of honey for adulteration stimulated this study in honey pollen analyses. This investigation seeks to examine and reveal the weight of the pollen in the honey samples which could be used to deduce adulteration and purity of honey.

2. Materials and Methods

2.1 Study Area

Igalamela-Odolu, Idah, Ajaka and Inachalo-Oforachi are localities of Kogi State. Kogi State lies in the middle-belt of Nigeria. The climatic conditions of these locations are similar with regard to rainfall, temperature and humidity. It is characterized by two seasons, the dry and the rainy season. The rainy season begins towards the end of March and ends towards the end of October. Peaks are usually between August and September. The dry season begins in November and lasts till late February. December and January are cold months due to the influence of the Northeast Trade wind otherwise known as the harmattan. The period of honey flow in these localities is between December and April. The vegetation of Kogi state is derived savanna/Guinea savanna which is characterized by the presence of forest species, fire sensitive and tolerant species with appreciable occurrence of grasses.

The four honey samples used for this study were obtained from wild honey collectors. The wild honey samples were from Idah, Ajaka, Igalamela-Odolu, and Inachalo-Oforachi. Each sample was filtered through a fine mesh copper gauze to eliminate debris (parts of bees, wax and plant materials). From each sample of filtered honey, 10g were carefully weighed using a sensitive electric weighing balance and the colour of each honey sample noted. They were acetolyzed, following the standard method. 5ml of glycerol/alcohol (2.1) was added to the sediment obtained after acetolysis and transferred into plastic vials for preservation (Erdtman, 1971; Louveaux, 1980).

The content of each vial was properly shaken and 10µm of the suspension was mounted on a slide and covered with a cover slip, sealed with a colourless nail varnish to prevent drying up of the mount. Pollen counts and fine morphological studies were made at x 400 magnification using Carl Zeiss light microscope. Identifications of pollen grains were based on comparison with descriptions and photomicrographs in books and journals (Raymonde Bonnefille el guy Riollet, 1980; Agwu and Akanbi, 1985; Sowunmi, 1973, 1976, 1995; Keay, 1980).

3. Results

Grain counts of 3,195, 2,274, 2,522 and 3,105 were observed for Idah, Ajaka, Igalamela-Odolu and Inachalo-Oforachi respectively. Twenty-one (21) pollen types were identified. Nineteen (19) were identified to family level while two remained unidentified. In most cases identification was made down to the generic level but sometimes even to the species or only to family levels.

The highest number of pollen types was recorded for Ajaka (17), while for Inachalo-Oforachi (14), Igalamela-Odolu (12) and Idah with the lowest number of pollen types, had (9). The predominant pollen types in the 4 samples were *Leuceana glauca, parkia biglobosa, Elaeis guineensis, Lannea acida, Bombax buonopozense, syzygium guineense and Euginea* sp. They were encountered in all the samples.

The classification recommended by Louveaux *et al.,* (1970) for expressing pollen grain frequencies have been adopted: very frequent (Over 45%), frequent (16-45%), rare (3-15%) and sporadic (less than 3%).

The detailed pollen spectrum of each sample is presented in Table I and II. After dilution, the colours of the honey samples were also observed and they ranged from dark-brown, light brown and yellowish brown (Table III). The weight of the pollen grains for the samples ranged from 0.25g to 0.40g per 10g of honey. The weights of the sediments recovered per sample were given in Table III. A few hyphae and fungal spores were encountered in the honey samples.

Table 1. The Pollen Count and Percentage Composition of the Honey Samples from Four Locations in Kogi East.

S/N	Pollen types	Idah		Ajaka		Igalamela-Odolu		Inachalo-Oforachi	
		Count	%	Count	%	Count	%	Count	%
1	Anacardaceae *Spondias mombin* *Lannea, acida* *Lannea welwithschii*	 - 575 -	 - 18.00 -	 37 70 -	 1.54 3.08 -	 - 242 -	 - 9.60 -	 - 113 39	 - 3.64 1.26
2	Arecaceae *Cocos nucifera* *Elaeis guineensis*	 170 550	 5.32 17.21	 20 311	 0.88 13.68	 26 289	 1.03 11.46	 51 547	 1.64 17.62
3	Asteraceae/tube type *Aspillia africana*	-	-	 40	 1.76	-	-	-	-
4	Bombacaceae *Bombax buonopozense*	 250	 0.78	 67	 2.95	-	-	 18	 0.58
5	Caesalpinaceae *Senna* sp.	-	-	 312	 13.72	-	-	-	-
6	Combrateceae/ melastometaceae	-	-	11	0.48	-	-	-	-
7	Euphorbiaceae *Alchornea cordifolia* *Hymenocardia acida* *Phyllanthus* sp.	 - 165 185	 - 5.16 5.79	 138 135	 6.07 5.94	 - 335 187	 - 13.28 7.41	 - 316 247	 - 10.18 7.95
8	Meliaceae/Sapotaceae	-	-	22	0.97	61	2.42	10	0.32
9	Mimosaceae *Leuceana glauca* *Parkia biglobosa*	 820 450	 25.67 14.08	 106 40	 4.66 1.76	 428 310	 16.97 12.29	 520 418	 16.75 13.46
10	Myrtaceae *Syzygium guineense* *Euginea* sp	 - -	 - -	 457 276	 20.10 12.14	 234 285	 9.28 11.30	 192 373	 6.18 12.01
11	Poaceae	-	-	116	5.10	-	-	189	6.09
12	Rubiaceae *Nauclea latifolia*	 -	 -	 -	 -	 117	 4.64	 72	 2.32
13	Verbanaceae	255	7.98	105	4.62	-	-	-	-
14	Indeterminata	-	-	22	0.97	8	0.30	10	0.32
TOTAL		3, 195		2, 274		2, 522		3, 105	

Table 2. Predominance of Pollen Types in Multifloral Honey Samples

Source Locality	Pollen types	Percentage	Frequency Class
Idah	*Leuceana glauca*	25.67	Frequent
	Lannea acida	18.00	Frequent
	Elaeis guineensis	17.21	Frequent
	Parkia biglobosa	14.08	Rare
	Phyllanthus sp.	5.79	Rare
	Cocos nucifera	5.32	Rare
	Hymenocardia acida	5.16	Rare
	Bombax buonopozense	0.78	Sporadic
Ajaka	*Syzygium guineense*	20.10	Frequent
	Euginea Sp.	12.14	Rare
	Elaeis guineensis	13.68	Rare
	Alchornia cordifolia	6.07	Rare
	Phyllanthus sp.	5.94	Rare
	Poaceae	510	Rare
	Leuceana glauca	4.66	Rare
	Senna sp.	3.72	Rare
	Lannea acida	3.08	Rare
	Bombax buonopozense	2.95	Sporadic
	Parkia biglobosa	1.76	Sporadic
	Aspillia Africana	1.76	Sporadic
	Spondias mombin	1.54	Sporadic
	Cocos nucifera	0.88	Sporadic
Igalamela-Odolu	*Leuceana glauca*	16.97	Frequent
	Hymenocardia acida	13.28	Rare
	Parkia biglobosa	12.28	Rare
	Elaeis guineensis	11.46	Rare
	Euginea sp.	11.30	Rare
	Lannea acida	9.60	Rare
	Syzygium guineense	9.28	Rare
	Phyllanthus sp	7.41	Rare
	Nauclea nodiflora	4.64	Rare
	Cocos nucifera	1.03	Sporadic
Inachalo-Oforachi	*Elaeis guineensis*	17.62	Frequent
	Leuceana glauca	16.75	Frequent
	Parkia biglobosa	13.46	Rare
	Euginea Sp.	12.01	Rare
	Hymenocardia acida	10.18	Rare
	Phyllanthus sp.	7.95	Rare
	Syzygium guineense	6.18	Rare
	Lannea acida	3.64	Rare
	Nauclea nodiflora	2.32	Sporadic
	Cocos nucifera	1.64	Sporadic
	Laenna welwithschii	1.26	Sporadic
	Bombax buonopozense	0.58	Sporadic

Table 3. Colour of Diluted Honey and Weight of Recovered Pollen from Samples of Four Localities in Kogi East.

Sample	Colour of diluted honey	Weight of honey in g.	Weight of pollen in g
Idah	Dark-brown	10	0.25
Ajaka	Yellowish-brown	10	0.40
Igalamela-Odolu	Light brown	10	0.27
Inachalo-Oforachi	Brown	10	0.30

4. Discussion

The honey samples have information of their botanical and geographical origins. They have also given clues as to the season of perhaps maximum honey production albeit without any confirmation that the indicator species were foraged only during the period of honey flow. Several of the identified honey plants are important ecological indicator types in the area of production. The weight of the recovered pollen indicated the pollen concentrations of the honey consequently the foraging activities of the bees that produce them.

On the basis of the results obtained in these examinations, the quantities of pollen in a given sample of honey have furnished the cue to determining the purity and genuineness of the honey samples. The four honey samples were undiluted. This was deduced from the weights of recovered pollen which were above 0.4g (Agwu and Akanbi, 1985).

Ten predominant pollen types (Table II) occured in Inachalo-Oforachi together with Elaeis guineensis. The male flowers of Elaeis guineensis are visited by bees for their pollen but the female flowers are not. Furthermore, oil palm tree is wind pollinated and neither of the flowers produce nectar (Agwu, 1985). Elaeis guineensis therefore serves the bees as a major source of pollen meal. It has not been possible to draw the same conclusion for other predominant pollen types which may also serve as other major sources of pollen meal. Ajaka with the highest number of pollen types had the lowest number of pollen counts. This could indicate less patronage i.e. less frequency of visits to a species in an environment of rich flora, or preference for diverse flora, leading to the production of multiflora honey.

The flowers of Poaceae which are anemophilous and the presence of their pollen in honey samples according to Agwu, (1985), are assumed to be accidental.

The percentage composition of important pollen types ranged from “frequent” to “sporadic”. Bees preferred the plants in the frequent class.

4.1Botanical Origin

Generally, entomophilous plants were more numerous in the pollen spectrum of each of the honey samples studied.

Certain species were common in some samples while some were found in all the samples despite the distinct source localities and the associated localized ecological characteristics. Ecological indicator species included *Leucenna glauca* which was frequent in all the samples except Ajaka where *Syzygium guineense* was frequent. Others included *Lannea acida*, *Elaeis guineensis, Parkia biglobosa, Hymenocardia acida, Bombax buonopozense, Euginea* sp, *Aspillia africana, Alchornia cordifolia, Nauclea nodiflora* and *Phyllanthus* sp.

4.2 Geographical Origin

According to Sowunmi (1976), most of the Nigerian honey comes from the savanna region (Mosaic of lowland rainforest and secondary grassland). Earlier investigations from different parts of the world (Maurizio, 1951, Agwu and Akanbi, 1976) have shown that the geographical origin of honey can be established through the pollen content.

The dominance of Leuceana glauca, Lannea Spp. Hymenocardia acida, Elaeis guineensis, Bombax buonopozense and Parkia biglobosa reflected the vegetation of (lowland rainforest and Guinea Savanna) White (1983).

The occurrence of *Elaeis guinnensis, Nauclea latifolia, Lannea* sp, and *Leuceana glauca* characterized farmlands and homesteads.

The general vegetation was typically a Guinea savanna as indicated by the pollen types which included: *Parkia biglobosa, Phyllanthus sp., Bombax buonopozense, Nauclea latifolia, Euginea* sp. *Syzygium guineensis* and *Lannea* sp.

4.3 Season of Honey Production

The season of major honey production can be deduced from the knowledge of flowering periods. Although, the honey samples were obtained at different months of the year, the flowering periods of the most important honey plants indicated that they were produced between October and May as seen in some of the pollen types. Elaeis guineensis (October - April), Hymenocardia acida (January - March), Nauclea Latifolia (May – June, November), Phyllanthus Sp. (January - October), Parkia biglobosa (December - March). (Sowunmi, 1976; Agwu and Akanbi, 1985).

5. Conclusion

This investigation has revealed that the pollen content of honey reflected to some extent, the floristic composition of the vegetation zone where it was produced. From the results obtained, the honey plants were typical ecological indicators of the source localities. This investigation has led to the identification of several honey plants in the source localities within Kogi State and has also shown that the predominant pollen types found within these source localities offer the preferred pollen meal for the bees.

The absence of pollen types belonging to other vegetation zones and therefore, varied floristic communities confirms the assertion that the origin of honey can be ascertained through pollen analysis.

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