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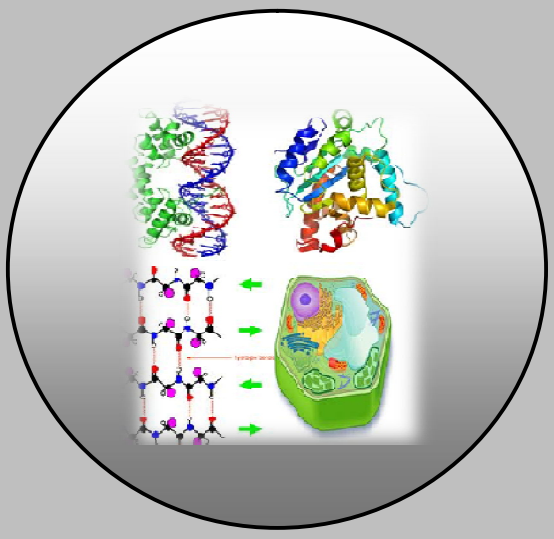

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RESEARCH PAPER

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Melissopalynological Study of Honey Samples from four localities in Dekina Local Government Area of Kogi State, Nigeria

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ABSTRACT

Honey samples from four different localities in Dekina Local Government Area of Kogi State (Olowa, Ajogoni, Itama, and Ojowu), Nigeria were studied and analyzed palynologically to determine their floral sources, ecological origin and season of production. This investigation seeks to examine and ascertains the phytoecological indicator species of plants which were utilized in the honey production. Pollen counts and fine morphological studies were made at x400 and x1000 magnification. Grains counts of 532, 589, 1033 and 720 were recorded for Olowa, Ajogoni, Itama and Ojowu respectively. Out of the thirty- two (32) pollen types encountered, 23 (twenty- three) were identified to family level and 2 (two) were unidentified. The predominant pollen types include those of Acanthus spp., Alchornea cordifolia, Anacardium occidentale, Cassia mimosoides, Elaeis guineensis, Hymenocardia acida, Phyllanthus niruri, Mangifera indica, Tridax procumbens, and Zea mays. The pollen weights were between 0.25- 0.5gram indicating that the four honey samples were unadulterated. The botanical and geographical origins as well as the season of honey production were also determined. Results suggested vegetation types reflecting the lowland rainforest and secondary grassland. This study is anticipated to provide useful information on the conservation and sustainable exploitation of these indicator species through appropriate biotechnological intervention. Identification of forage plants and their propagation could also help in proving the bees' forage which in turn improves the efficiency of beekeeping and commercial honey production.

Keywords: Melissopalynology, Honey Samples, Phytoecological Indicator, Kogi State and Nigeria.

INTRODUCTION

Melissopalynology is the study of pollen grains and spores found in honey. Honey is a sweet, sticky, yellowish and brownish viscous liquid produced by bees from nectar. Different flowers produced different quantities, qualities, colour, consistencies and flavor of nectar. It is these varieties that are responsible for the many different types of honey. Beekeeping is entirely depending on the types of flowering plants available in any given area (Shubharani *et al*., 2013). There is a need to understand honeybee – plant relationship to study food preferences of honey bees and pollination requirement. Honey is used commonly in different parts of Nigeria in ethnomedical treatment of various ailments (e.g. cough, chest pain, indigestion/ constipation, sprains, burns, ulcer and minor open injuries).

Pollen analysis of honey shows the possibility of determining the botanical, geographical and ecological origin of honey from the pollen grains they contain (Agwu and Akanbi, 1985). This is based on the fact that honey bees (*Apis mellifera*) collect their food from plants, especially those that offer high concentration of high quality of nectar (Agwu and Uwakwe, 1992). The importance of bees in pollination ecology of the tropics is tremendous and consequently of great relevance to agriculture, forestry and generally in the maintenance of genetic flow and balance among cultivated and wild economic plants (Norr *et al*., 2004).

Pollen of various plants representing potential sources of nectar and pollen for the honey bees is an important pre- requisite for the developing apiary (Kalpana and Ramanujam, 1997).

Microscopical analysis of pollen of plants forged by bees is an established method to determine the source of honey in an area. The information on bee plants, pollen morphology and types of pollen in this locality is limited.

Due to unscientific, cultural and agricultural practices, the flora of this part of Nigeria is threatened with increase and indiscriminate destruction. The knowledge of the important honey plants of the various vegetation zones might in the future lead to their legalized protection and /or planned propagation in the development of apiculture. The control and check of honey for adulteration stimulated this study in honey pollen analysis.

The study is aimed at identifying the different pollen types and therefore the species of plants which participated in the honey production. It will also examine the weight of the pollen grains which could be used in deducing adulterated and pure honey.

MATERIAL AND METHODS

The four honey samples used in this study were obtained from honey collectors who gathered them from the various wild sources within the four localities in Dekina Local Government Area of Kogi State, Nigeria. The honey was extracted by pressing and squeezing the entire comb without cutting off the pollen (storage) cells. Each sample was filtered through fine-mesh copper gauze to eliminate debris (parts of bees, wax, and plant bits). From each sample of filtered honey, 10gm were carefully weighed using a weighing balance and the colour noted. The sample was then diluted with 35ml of warm (40- 50^{0}C) dilute sulphuric acid solutions (3ml in 1000 ml of water) (Agwu and Akanbi, 1985).

Table 1. The Pollen Spectrum of the Honey Samples from Four Localities in Kogi State.

Pollen Types	17 Olowa	19 Ajogoni	26 Itama	22 Ojowu
ACANTHACEAE				
Acanthus spp	50	60	40	5
AMARANTHACEAE				
Amaranthus spp.	-	3	5	40
ANACARDIACEAE				
Mangifera indica	5	15	5	5
Anacardium occidentale	75	105	125	100
Lannea acida	50	70	65	25
ARECACEAE				
Cocos nucifera	-	5	-	5
Elaeis guineensis	45	85	125	175
ASTERACEAE TUBILIFLORAE				
Tridax procumbens	10	35	10	10
BIGNONIACEAE				
Stereospermum kunthianum	-	-	10	-
BOMBACACEAE				
Ceiba pentandra	-	35	50	5
BURSERACEAE				
Canarium schweinfurthii	-	-	6	-
CAESALPINOIDEAE				
Berlinia grandiflora	-	-	5	-
COMBRETACEAE/				
MELAST. *Combretum spp*	-	-	50	10
CONVOLVULACEAE				
Ipomoea aquatic	-	3	-	5
CYPERACEAE	-	-	-	20
EUPHORBIACEAE				
Alchornea cordiflora	65	40	130	80
Phyllanthus niruri	55	50	150	55
Hymenocardia acida	15	5	55	5
FABACEAE				
Cassia occidentalis	20	3	1	-
Cassia mimisoides	7	10	10	5
MIMOSACEAE				
Parkia biglobosa	15	30	-	20
Neptunia oleracea	40	-	30	-
MYRTACEAE				
Psidium guajava	-	-	10	-
Syzygium guineense	35	-	10	50
OLACACEAE	-	-	5	-
ONAGARACEAE	-	-	-	5
POACEAE				
Zea mays	10	20	30	35
RUBIACEAE				
Nauclea latifolia	15	-	25	5
RUTACEAE				
Citrus spp	-	10	20	-
SAPINDACEAE				
Vitellaria paradoxa	-	-	1	-
Manilkara obovata	-	5	-	-
SOLANACEAE	20	-	60	55
TOTAL POLLEN COUNT	532	589	1033	720

After a thorough shaking of the honey- acid solution, it was centrifuged for 5 minutes at 2000 revolution per minute and the supernatant decanted. The weight of the recovered pollen was determined for each honey sample (Table II).

The recovered sediments were treated with 10ml glacial acetic acid to remove water before acetolysis. Acetolysis mixture was freshly prepared in a ratio of 9:1 from acetic anhydride and concentrated sulphuric acid. Acetolysis was carried out using the method of Erdtman, 1969; Agwu and Akanbi, 1985. The mixture was placed in water–bath at 100˚C for 5 minutes, stirred and then centrifuged for 5 minutes and supernatant decanted. The recovered precipitates were finally washed twice with distilled water, centrifuged each time and decanted. The recovered residue were stored in a plastic vials in glycerin and ethanol solution (2:1).

Two drop of thoroughly shaken suspension was mounted on microscope slide and covered with an 18×18mm cover slip. The mount was sealed off with colour less nail varnish to prevent drying up of the mount. The prepared slide was then examined microscopically with Olympus microscope at x400 magnification for counting and Leica microscope at x1000 magnification for detailed morphological studies. Identification, counting and classification was done with the help of reference descriptions and photomicrographsin books and journals: Agwu and Akanbi (1985); Bonnefille and Riollet (1980); Sowunmi (1978); Sowunmi (1995) and Ybert (1979).

RESULTS

The four honey samples were examined microscopically to identify the plant species in form of pollen components. About 32 pollen types were identified to the family level while out of the thirty-two, four were not identified to genera level. Grain count of 532, 589, 1033 and 720 were recorded for Olowa, Ajogoni, Itama and Ojowu respectively.

In most cases, identification was made down to the generic level but sometimes even to the species or only to family levels. The identified species belong to varying genera of native herbs, shrubs, grass and trees. There were pollen of varying shapes, sizes and morphological features suggesting that the honey samples are multi and unifloral.

Table 2. Colour of Diluted Honey and Weight of Pollen in Honey Samples from Four Localities in Dekina Local Area of Kogi State.

Sample	Colour of Diluted Honey	Weight of Honey (Gram)	Weight of Pollen (Gram)
Olowa	Light yellow	10	0.27
Ajogoni	Yellowish-brown	10	0.40
Itama	Dark-brown	10	0.25
Ojowu	Light-brown	10	0.30

The highest number of pollen types (26) was recorded for Itama, while Ojowu (22), Ajogoni (19) and Olowa (17) had fewer pollen types. The predominant pollen types in the four samples were those of *Acanthus spp*, *Mangifera indica*, *Anacardium occidentale*, *Lannea acida*, *Elaeis guineensis*,

Tridax procumbens, Alchornea cordifolia, *Phyllanthus niruri*, *Hymenocardia acida*, *Cassia mimosoides* and *Zea mays*. Photomicrograph of some important indicator species are given in Fig. 1.

The classification recommended by Louveaux et al., (1970) for expressing pollen grains 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 1. After dilution, the colour of the honey samples were also observed and ranged from light yellow, brown, light brown and dark brown and the result are given in Table II. The weight of the pollen grains for the samples ranged from 0.25g to 0.40g per 10g of honey. The weight of the sediment recovered per sample and the color of the honey after dilution are given in Table 2.

PHOTOMICROGRAPH OF IMPORTANT INDICATOR SPECIES

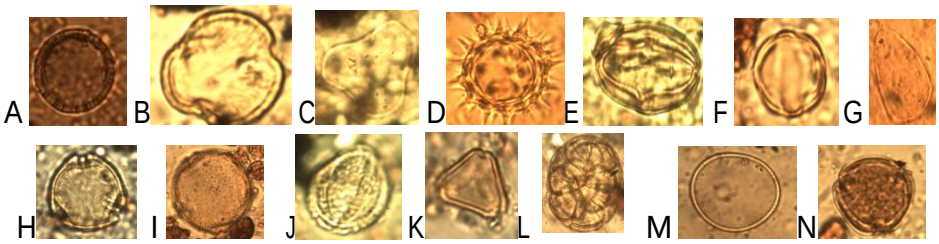

Figure 1.A -*Amaranthaceae/ Chenopodiaceae*; B- *Lannea acida*; C-*Elaeis guineensis*; D-*Asteraceae*; E-*Senna sp.*; F- *Combretaceae/ Melastomataceae*; G- *Cyperaceae*; H- *Alchornea cordifolia*; I- *Hymenocardia acida*; J- *Phyllanthus sp.*; K- *Syzygium guineense*; L- *Parkia biglobosa*; M- *Poaceae*; V- Y- *Solanaceae*.

DISCUSSION

The honey samples have complete information on the botanical and geographical origin. They have also given clues as to the probable season of maximum honey production. The most common plant species in pollen in the sample were *Sygyzium guineense* (occurring in Olowa, Itama and Ojowu), a representative of the Acanthaceae family occurred in all the samples. *Alchornea cordiflora* (occurring in all the samples). The highest pollen frequently was found in the Itama sample and the lowest in the Olowa sample. Several of the identified honey plants are important ecological indicator species in the area of production. The weight of the recovered pollen indicates the pollen concentration of the honey and consequently the forage activities of the bees that produce them.
The quantity of pollen in a given sample of honey will furnish the clue to determining the purity and genuineness of the honey samples. Results of this study indicate that the four honey samples were undiluted. This was deduced from the pollen weight which was above 0.4g when compared to the report of Agwu and Akanbi (1985).

Average undiluted Nigerian honey contains 4.0-4.5g of pollen in 100g of honey (0.025-05 indicates unadulteration from oral communication with Agwu, 2001).

Twenty- six (26) predominant species (Table 1) occur in Itama including *Elaeis guineensis* which are visited by bees for their pollen. *Elaeis guineensis* (oil palm) tree is wind pollinated and neither of the flowers (male of female) produces nectar (Agwu and Akanbi, 1985). *Elaeis guineensis* therefore serve 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.

The flowers of Poaceae which are also wind pollinated and the presence of their pollen in honey sample according to Agwu and Akanbi (1985) is assumed to be accidental. The percentage composition of important pollen types ranged from "frequent to sporadic", bees prefer the very frequent plant. The moderate occurrence of pollen of the Poaceae family is an indication of the proximity of Kogi State to the fringe of the savanna belt of the North, as reported by Agwu and Abaeze (1991).

This study has shown the indicator species that honey bees visit most for their pollen and nectar sources. Such plants could be properly conserved and their sustainable exploitation managed, to enhance economic production of honey from this part of the country.

BOTANICAL ORIGIN

Generally, entomorphilous plants were numerous in the pollen spectrum of each honey sample studied and the honeys from the source localities were fairly rich in pollen types. Certain species are common to some while some are found in all the samples despite the distinct source localities and the associated localized ecological characteristics. They include those of *Lannea acida, Elaeis guineensis, Anacardium occidentale, Phyllanthus niruri, Parkia biglobosa,* and *Cassia mimosoides*.

GEOGRAPHICAL ORIGIN

According to Sowunmi (1976), most Nigerian honey comes from the savanna regions (Mosaic a-lowland rainforest and secondary grassland). Earlier investigation from different parts of the world (Maurizio, 1951) has shown that the geographical origin of honey can be established through the pollen content.

The dominance of *Anacardium occidentale*, *Lannea acida*, *Hymenocardia acida*, *Elaeis guineensis*, *Parkia biglobosa* reflects the vegetation of lowland rainforest and guinea savanna (White, 1983).The occurrence of *Elaeis guineensis*, *Nauclea latifolia*, *Lannea acida* and *Anacardium occidentale* characterized farmland and homesteads. The occurrence of all the above listed pollen types in different proportions confirms their geographical origin as reflecting Guinea savanna.

SEASON OF HONEY PRODUCTION

On the basis of ethno-cultural knowledge, information and market survey results, most of the Nigerian honey is produced during the season of little or no rainfall: September to April. This season coincides with the flowering period of the most important honey plants.

The season of major honey production can be deduced from the knowledge of flowering periods. Although, the honey samples were collected or obtained at different months of the year, the flowering periods of the most important honey plants indicates 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-July, November), *Phyllanthus sp*. (January-October), *Parkia biglobosa* (December-March) (Sowunmi, 1976; Agwu and Akanbi, 1985).

On the account of the given flowering periods of major nectar plants, honey in Nigeria is produced mainly between the months of October and April.

CONCLUSION

The results have identified indicator species that honey bees visit most for their pollen and nectar sources. Such plants should be properly conserved and their sustainable exploitation managed to enhance large production of honey from this part of the country. The present investigation examines palynologically the pollen contents of four honey samples from four localities in Dekina Local Government Area of Kogi State, Nigeria. This study is anticipated to provide useful information on the conservation and sustainable exploitation of these indicator species through appropriate biotechnological intervention.

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