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MELISSOPALYNOLOGICAL ANALYSIS OF HONEY TO DETECT MELLIFEROUS PLANT SPECIES VISITED BY *Apismellifera adansonii latereille* (West African Honeybee) IN GUINEA SAVANNA ZONE OF NIGERIA

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Abstract
Honey collection is a long aged traditional activity throughout most parts of Africa. Melissopalynological analysis is widely used to verify geobotanical origin of honeys. Although continuous destruction of forest and different vegetation as a result of incessant demand for land is affecting the population of honey bee, pollination, honey yield, and loss of gene pool. Investigations were carried out on melissopalynological analysis of honey produced by Apis mellifera Adansoniilatereille (West African honeybee) in guinea savanna zone of Nigeria. Fresh honey samples from two sites were analyzed for pollen spectra and composition (abundance of pollen). The result showed that pollen grains of syzygium guinense recorded the most abundant pollen grains in honey samples collected from Nassarawa State (40.06) and Plateau State (16.55). The most frequent pollen grains of different plant species identified in Nassarawa include Parkia biglobosa, Dicranolepsis usambarica, Syzygium guinense, Bridelia ferruginea, Eucalyptus spp, Nauclea latifolia and Pterocarpus spp while Dicranolepsis usambarica, Ixora spp and Syzygium guinense were most frequent in plateau State. Relative abundance of pollens of these plant species is an indication of the plants foraged on by the bees and it is therefore important that planting and regeneration of these forest species be intensified, protected and conserved within apiaries or farms in the Guinea Savanna zone of Nigeria.

Key Words: *Melissopalynological, Geobotanical, Pollination, Pollen grains*

Introduction

Honey collection is an age-long traditional activity in many parts of Africa. Traditional beekeepers use simple hives made from calabash, clay pot or hollow wood logs, closed at both ends with a hole as an entrance for the bees. The empty hives are then placed on trees and become occupied by-passing swarms and in due course are plundered by bee keepers. This methods of harvesting or beekeeping practice have led to a poor yield of low quality honey. However, modern beekeepers make use of bee hives like the Kenyan-top bar hives and the langstroth hives which are placed within apiaries to maximize the goods and services delivered by the Honey bees (Amulen *et al*., 2019).

The benefit of keeping bees are numerous, most people think of the rewards of harvesting their own honey or the positive impact the increased pollination will have on their garden. While these are great reasons to have a beehive, it does not count out the many other bee products such as bee bread and wax can be harvested from a hive. The management skills and concept of every bee keeper should therefore be focused on the interactions of the honey bees and its immediate environment (vegetation belt). This is because honeybees visit various pasture plants during foraging serving the purpose of pollinating the plant.

Pollen is a fine to coarse powdery substance comprising pollen gains which are made of microgametophytes of seed plants that produces male gametes (sperm cells). The pollen involuntarily collected by bees at nectar collection time is an important indicator of its botanical and especially geographical origin (Borth, 1989). The theory is that since bees collect pollen from around us, eating the small amounts of pollen present in local honey can actually immunize the body against the pollen so there is minimal irritation during allergy season (Chang, 2015).

Melissopalynology or pollen analysis is a branch of palynology and is the study of pollen and spores in honey. It analyses the pollen content of honey and provides reliable information on the floral component of honey and thereby identify the source of plants used by bees in producing honey. (Jones *et al*., 2004). Pollen analysis of honey is of great importance for quality control and helps to ascertain whether honey is adulterated or not (Terrab *et al*, 2003; Ohe *et al*., 2004). This study therefore, was aimed at determining the composition and frequency of occurrence of pollen grains in various honey samples and to determine the pollen spectra of the study area.

Methodology

Study Area

This study was carried out within the Guinea Savannah zone of Nigeria in Plateau and Nasarawa States. Plateau State (Longitude 8º 40′ and 9º50′E and latitude 9º and 10º 45′N) which is located in the Northern Guinea savanna zone consist of seventeen local government areas while Nasarawa State (latitude 7° 45′ and 9° 25'N and longitude 7° and 9° 37'E) has thirteen Local Government Areas and is located in the southern guinea savanna zone. Clearance of vegetation for farming, fuel wood extraction for domestic and cottage industries has led to the development of regrowth vegetation at various levels of serial development leading to dense forests that are few and far apart. Such forests are found in lowland areas, particularly where population pressure is less on the land. Gallery forests are common along major streams and pronounced depressions. Forest reserves are being developed mostly near major urban centres like Lafia, Nassarawa, Keffi, Akwanga and Wamba.

The vegetation on the hilly parts of the states is composed mainly of grasses and isolated trees. Trees of economic value, including locust bean (*Parkia biglobosa)*, shea butter (*Vitellaria paradoxa}*, mango, citrus are scattered across the states, particularly the lowland areas.

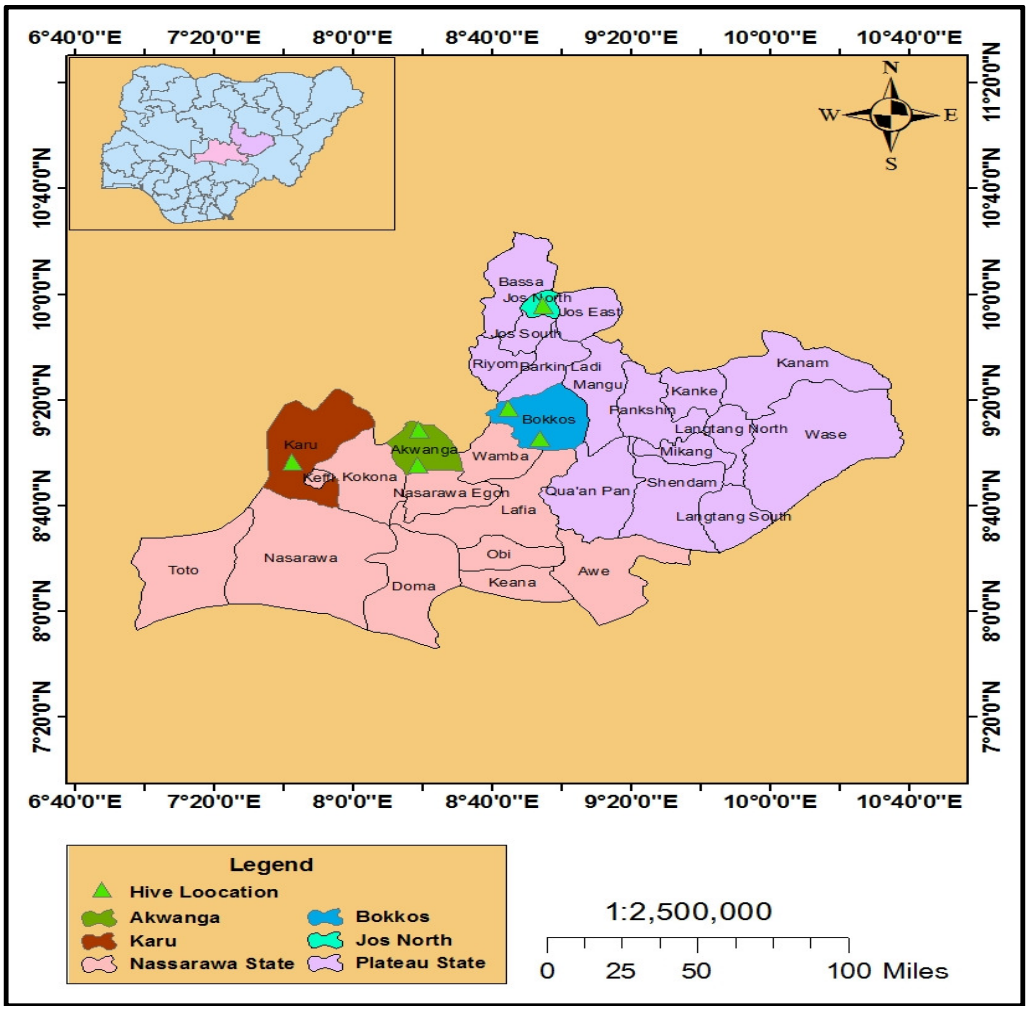


Fig. 1: Map of Plateau and Nasarawa States

Inventory and Field Survey

Inventory (record) of beekeepers was collected from Beekeepers Association of Nigeria, (North Central Chapter). A field Survey was carried out to identify the beekeepers. Three (3) beekeepers were randomly selected from each of the states studied giving a total of six (6) beekeepers. The number of hives owned by the beekeepers ranges from 10-40 hives. Based on the average number of beehives owned by the beekeepers; four (4) hives were selected from each of the beekeepers giving a total of twenty four (24) beehives comprising twelve (12) from each state.

Extraction of Pollen from Samples of Honey

Each of the honey samples were thoroughly shaken to ensure even distribution of the botanical elements. 10g of each honey sample was weighed out using a digital sensitive weighing balance and washed with 35ml of warm (35 - 40°C) diluted tetraoxosulphate (VI) acid (1.5ml of concentration sulphuric acid to 498.5ml of distilled water). Sulphuric acid dissolves most of the colloids, sugar and other constituents associated with the honey (Louveaux, 1970). The colour of each diluted honey sample was noted. The honey-acid solution was stirred and centrifuged for 5 minutes at 2000

revolution per minute (r.p.m) (Osayi *et al.*, 2012).

The precipitates were washed twice with distilled water centrifuged for five minutes at 2000 r.p.m. and decanted each time. The precipitate (ppt) was acetolysed according to Erdtman's (1969) procedure.

Acetolysis

The acetolysis solution was prepared from nine parts of acetic anhydride and one part of tetraoxosulphate (vi) acid. 2 - 3ml of the acetolysis solution was added to each of the samples. The centrifuge tubes containing the acetolysis mixture (palynomorphs and acetolysis solution) were placed in a water bath at 100°C and allowed to stand for 3 - 5 minutes. The tubes were removed from the water bath, centrifuged for 3 minutes at 1500 r.p.m. and then decanted. The precipitate (pollen and spores) recovered were then treated once with 2-3ml glacial acetic acid, centrifuge and decanted. The covered sediment was further washed twice with water; centrifuging and decanting followed each wash. 10ml of glycerol/alcohol solution in the ratio of (2:1) was added to each precipitate and transferred into plastic vials for storage.

Temporary Slides

The content of each vial was properly shaken and 2 drops (0.2ml) of the suspension was mounted on a slide and covered with 22mm x 22mm cover slip. Each mount was sealed with colourless nail varnish to prevent drying up. In this state, the specimen can stay for as long as 4 weeks without completely drying up.

Microscopic examinations were made with Leica model light microscope. Pollen counts and fine morphological studies were made at x400 and xl000 magnifications respectively. The species frequency was based on a count of at least 1200 pollen per honey sample. At least two temporary slides were studied per airborne sample in other to get more than a thousand pollen grains. The classification used for expressing pollen grain frequency in honey were that recommended by Louveax *et al.* (1970): very frequent (over 45%), frequent (15 - 45%), rare (3-15%) and sporadic (<3%).

Pollen Identification and Composition

Identification of pollen grains were based on comparison with reference collection of pollen slides in the Department of Archeology and Anthropology, University of Ibadan and with descriptions and photomicrographs in books and journals; Van, *et al*., (1965), Sowunmi (1975) and the collection of identified plant species within the study area. Composition of pollens within the samples was identified through the microscope by counting the number of occurrences per slide (Orijieme, 2017).

Result and Discussion

Composition and Abundance of Pollen Grains in Honey Samples

Identified species from the pollen analysis were distributed among Thirty-nine (39) different plant species and two (2) fungal spores (Table 1). The plants species whose pollens where identified were distributed among 19 different families with Fabaceae (9), Malvaceae (5), Asteraceae (4) and Myrtaceae (3) being the most dominant. Large presence of the Fabaceae in the study area supports Aguoru *et al.* (2014) and Wakawa *et al.* (2016) from different studies in North Central and North Western part of Northern Nigeria.

Pollen grains of *syzygium* spp was most abundant in honey samples collected from both Nassarawa state (40.06) and Plateau State (16.55) and was significantly different from all other pollen grains in the honey samples from the study sites. This suggests that *Syzygium* spp is dominant in

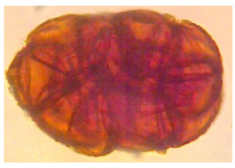
the study areas and most likely comprise a major forage for bees. This agrees with the work of Keay (1989) noting that the presence of *Syzigium guinensis* are found by fringing forests by streams and rivers in savanna region and the plants from the family of Myrtaceae was most dominant and had the highest sucrose content in its study this might be the reason bees foraged on the plant so much.

Table 1: Percentage Composition and abundance of pollen grains Identified in Honey Samples Collected from the Study Area

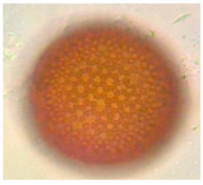
S/N	Pollen grains	Family	Nasarawa	Frequency	Plateau	Frequency
1	5-celledfungal spore		0.00	nil	1.60	sporadic
2	*Adansonia digitata*	Malvaceae	0.60	sporadic	0.60	Sporadic
3	*Afzelia Africana*	Fabaceae	0.00	Nil	0.30	Sporadic
4	*Bombax buonopozense*	Malvaceae	3.00	Rare	0.00	Nil
5	*Borassus aethiopicum*	Arecaceae	0.30	Sporadic	0.00	Nil
6	*Bridelia ferruginea*	Phyllanthaceae	7.60	Rare	0.00	Nil
7	*Ceiba pentandra*	Malvaceae	0.30	Sporadic	0.30	Sporadic
8	*Chromolaena odorata*	Asteraceae	1.00	Rare	0.00	Nil
9	*Delonix regia*	Fabaceae	6.30	Rare	0.30	Sporadic
10	*Dicranolepis usambarica*	Thymelaeaceae	12.00	Rare	9.00	Rare
11	*Diospyros* spp	Ebenaceae	0.00	Nil	1.30	Sporadic
12	*Elaeis guineensis*	Arecaceae	0.00	Nil	0.30	Sporadic
13	*Entada* spp	Fabaceae	0.30	Sporadic	0.00	Nil
14	*Eucalyptus* spp	Myrtaceae	16.30	Frequent	0.00	Nil
15	*Ficus* spp	Moraceae	2.30	Sporadic	0.00	Nil
16	Fungal spore		0.30	sporadic	0.00	Nil
17	*Grewia* spp	Malvaceae	0.30	Sporadic	0.00	Nil
18	*Helianthus annuus*	Asteraceae	1.60	Sporadic	0.60	Sporadic
19	*Hygrophylia* spp	Acanthaceae	0.30	Sporadic	0.00	Nil
20	*Ixora* spp	Rubiaceae	0.00	Nil	7.60	rare
21	*Jatropha curcus*	Euphorbiaceae	0.00	Nil	0.30	Sporadic
22	*Khaya senegalensis*	Meliaceae	0.00	Nil	1.60	Sporadic
23	*Lactuca* spp	Asteraceae	0.00	Nil	0.30	Spoilradic
24	*Ludwigiarepens*	Onagraceae	0.30	Sporadic	0.00	Nil
25	*Mangifera indica*	Anacardiaceae	0.00	Nil	1.60	Sporadic
26	*Syzygium guinense*	Myrtaceae	40.06	Frequent	16.55	Frequent
27	*Nauclea latifolia*	Rubiaceae	9.30	Rare	0.00	Nil
28	*Parinari* spp	Chrysobalanaceae	0.30	Sporadic	0.00	Nil
29	*Parkia bicolor*	Fabaceae	0.60	Sporadic	0.00	Nil
30	*Parkia biglobosa*	Fabaceae	9.30	rare	0.30	Sporadic
31	*Peltophorum pterocarpum*	Fabaceae	1.00	sporadic	0.00	Nil
32	*Phyllanthus* spp	Phyllanthaceae	0.60	sporadic	0.00	Nil
33	*Piptadenia Africana*	Fabaceae	1.00	sporadic	0.00	Nil
34	*Psidium guajava*	Myrtaceae	0.00	Nil	1.00	sporadic
35	*Pterocarpus* spp	Fabaceae	32.00	frequent	0.00	nil
36	*Senna* spp	Fabaceae	2.30 c	Sporadic	3.00	Rare
37	*Sida acuta*	Malvaceae	0.00	Nil	0.30	Sporadic
38	*Solanum* spp	Solanaceae	0.30	Sporadic	0.30	Sporadic
39	*Vernonia frondosa*	Asteraceae	0.30	Sporadic	0.00	Nil
40	*Vitex doniana*	Lamiaceae	0.00	Nil	0.30	Sporadic
41	*Zea mays*	Poaceae	0.00	Nil	0.30	Sporadic

Very frequent (over 45%), frequent (15-45%), rare (3-15%) and sporadic (<3%), Louveax *et al.* (1970)

The result from pollen spectra captured eleven different plant species in Nasarawa State (Plate 1) and ten species including eight plant and two fungal spores in Plateau State (Plate 2). The species identified in the spectra were completely different, indicating a difference in species composition and vegetation cover in the two locations and supports (Ribbands, 1951) that bees would forage within a range of two miles in radii.

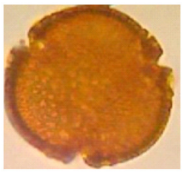


(i) *Parkia biglobosa*

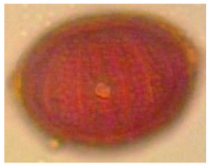


(ii) *Dicranolepis usambarica*

(iii) *Ceiba pentandra*



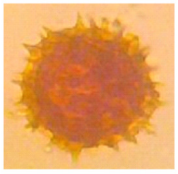
(iv) *Bombax buonopozense*



(v) *Hygrophylia spp*

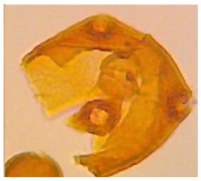
(vi) *Grewia spp*

(vii) *Azadirachta indica*

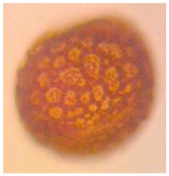


(viii) *Vernonia spp*

(ix) *Parkia bicolor*



(x) *Ludwigia repens*



(xi) *Peltophoru pterocarpum*

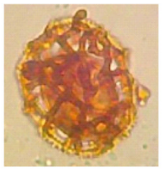
Plate 1(i-xi): Identified pollen spectra of honey samples from study sites (Nassarawa State)


(i) *Syzgium guiniense*


(ii) *Eucalyptus* spp


(iii) *Diospyros* spp


(iv) *Khaya senegalensis*


(v) *Lactuca* spp


(vi) *Mangifera indica*


(vii) *Khaya* spp


(viii) Fungal spore


(ix) *Sporaschisma* spp

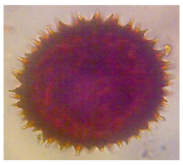

(x) *Sida acuta*

Plate 2(i-x): Identified pollen spectra of honey samples from study sites (Plateau State)

Conclusion

This study has revealed from pollen types identified that the floristic composition of the vegetation zones were good ecological indicators from their forage sources with predominant pollen types found, offered preferred forage for the honey bees. The numerous pollen types and their diversity showed that honey bees travel considerable distance to collect nectar and pollen which are suitable forage materials for their survival and production of honey.

The relative abundance of pollens of S*yzygium guineense, Eucalyptus species* and other identified pollens in the two study locations indicates that these plants are the most visited by the bees. It is therefore important to grow, protect and conserve the plant species identified in this study within apiaries or farms. The occurrence of the species could also be a reflection of the vegetation of apiaries within the savannah zone/region of Nigeria.

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