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Pollen analysis of *Apis mellifera* honey collected from Nigeria

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Abstract: Pollen analyses of twelve honey samples collected in two successive years (2011-2012) from different towns and villages in Ekiti State, South western Nigeria were carried out in order to ascertain their pollen compositions. The results from the analysis revealed eighty-five taxa belong to thirty-three botanical families. Of these, thirty-two were identified to generic level and forty-five plants were identified to the specific level. The number of pollen grains in the honey samples varied significantly (between 4,818 - 85,087 pollen grains), indicating their richness in pollen grains. Also in this study, some important honey plants such as *Spondias mombin, Alchornea cordifolia, Lannea sp*, Asteraceae, *Alchornea sp*, Rubiaceae, *Elaeis guineensis, Pavetta sp, Oldenlandia corymbosa, Triplochiton scleroxylon, Mimosops warneckii, Blighia sapinda, Piptadeniastrum africanum, Entada gigas*, *Tithonia diversifolia*, and Combretaceae/Melastomataceae have been identified to be characteristics of vegetation typical of Ekiti State and reflection of common pollen load and nectar sources.

Keywords: Honey Sample, Pollen Composition, Nectar Source, Ekiti State

1. Introduction

Honey is defined as the natural sweet substance produced by honey bees from the nectar of plants or from secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants [1,2]. Honeybees collect this material, transform and combine it with specific substance of their own, store and leave to ripen and mature inside the honey combs. Honey contains pollen grains and other microscopic particles such as fungi and spores originating from the plants from which the nectar has been collected by the bee. Therefore the pollen composition of a honey sample reflects the vegetation type where the honey has been produced and is useful for the determination of the geographical as well as botanical origin of honey [3, 4]. Honey composition, flavor, and color varies considerably depending on the floral sources [5]. Other external factors such as seasonal and environmental factors and processing methods play an important role in honey composition. Honey is unique in its compositions and uses. Because of this unique and complex nature, honey is proved to be useful in the treatments of burns, wounds, skin ulcers, skin rashes, as an antioxidant as well as in treatment of external eye diseases [6, 7]. Increasing interest in the therapeutic uses of certain honey varieties may contribute to the demand of a reliable determination of their botanical origin. The various variety of honey may be grouped into unifloral or multifloral depending basically on whether a dominant pollen grain originated from one particular plant or no dominant pollen type in the honey sample [8, 9]. Pollen analysis is an indispensable method to authenticate honey origin and honey characteristics. Assessment of honey botanical source is of great importance in food analysis, since authenticity guarantee the quality of honey [10]. From economic point of view, the assessment of floral origin and other parameters usually add to quality and commercial value of honey. Palynological examination of honey provides some important information about honey extraction methods, filtration, fermentation [11] and some kind of adulteration [12].

In Southwestern Nigeria, the geographical and prevailing climatic conditions provide a suitable environment for beekeeping and honey production. In Ekiti State, beekeeping activities have undergone a noticeable development with the formation of Bee Farmers Association, training and financial assistances from

government to the beekeepers. All these activities have boosted honey production in the state. Recently, [13, 14] reported that there is dearth of information on palynological investigations of honey produced in the area. Hence, this study is to establish the botanical origin of honey in the area and to identify the pollen and nectar sources important for the bees.

2. Materials and Methods

2.1. Area and Material of the Study

Ekiti state is situated entirely within the tropics. It lies between longitudes 7^0 25 North and 5^0 00 and 6^0 00 East in the rain forest belt of Nigeria. It lies South of Kwara and Kogi State, East of Osun State and bounded by Ondo State in the East and in the South. The state enjoys tropical climate with two distinct seasons; rainy season (April-October) and dry season (November-March). Temperature ranged between 21 ^{0}C and 28 ^{0}C with high humidity. Tropical forest exists in the South while Guinea savanna occupies the Northern peripheries of the State. The land enjoys luxuriant vegetation as a result of the favourable climatic condition thus, has abundant resources of different species of timber such as *Daniellia oliverii* (Rolfe), *Vitex doniana* (Linn.), *Parkia biglobosa* (Jacq.), *Nauclea diderrichii* (Merill), *Mimosops warneckii*, *Terminalia sp*. Food crops like yam, cassava and also grains like rice and maize are grown in large quantities in the area [15, 16, 17].

The experimental materials were twelve samples of honeys collected in the year 2011-2012 from different towns and villages in Ekiti State, South Western Nigeria.

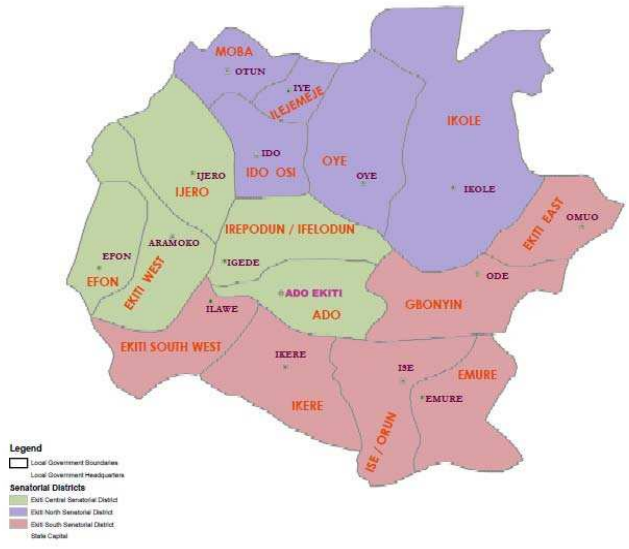

Fig. 1. *Map of Ekiti State.*

2.2. Collection of Honey Sample

The twelve honey samples were collected randomly from three Senatorial District of Ekiti State. The honey samples were collected from various Apiaries of *Apis melifera* at different locations in the State. The locations were: EK-I (IKERE), EK-2 (IJAN), EK-3 (ILAWE), EK-4 (EFON), EK-5 (IGBEMO), EK-6 (ARAMOKO I), EK-7 (ESURE), EK-8(ADO), EK-9 (IKOLE),EK-10 (IDO), EK-11 (OYE) and EK-12 (ARAMOKO II). The honey samples were collected from domesticated bee-hives and were extracted through pressing, without separating the pollen and honey cells of the combs. The honey samples were later stored in airtight containers at room temperature. The analyses were done at the Palynological Laboratory of the Department of Archeology, University of Ibadan, Oyo-State, Nigeria. Samples were subjected to acetolysis following standard method [18]. Five milliliter of honey per sample was taken from a well reserved stocks of each sample and dissolved in 10ml of distilled water and centrifuged for 10 minutes at 2,500rpm and the supernatant liquid was drawn out. The sediment was treated with 5ml of glacial acetic acid and centrifuged at 2,500rpm for 10minutes. After decanting acetic acid, the sediment was acetolyzed [18]. One part of concentrated Sulphuric acid was added drop by drop to nine parts of Acetic Anhydride acid and warmed in water bath till the liquid turned chest brown color. After cooling, it was again centrifuged at 2,500rpm for 10minutes. The supernatant liquid was decanted off. The sediment was then treated with glacial Acetic acid, later centrifuged at 2,500rpm for 10minutes, followed by two or three rising with distilled water. After each rinsing, it was again centrifuged at 2,500rpm for 10minutes. The 50% aqueous glycerin prepared in distilled water was added and centrifuged for 10minutes at 3,500rpm. The supernatant liquid was decanted off. The pollen sediment was taken on a pellet of glycerin jelly and transferred to the slides, covered with cover slips and sealed with paraffin was [19]. In the microscopic analysis, three slides were prepared for each of the twelve samples and examined under the light microscope. The pollen count (that is the frequency of the pollen of the different representative plants or families in each sample) was taken and recorded [20]. In the evaluation of botanical source of the honeys, specific percentage like: (>45%) for dominant, (16-44%) for secondary, (3-15%) for important minor and <3% for minor pollen were applied in the study. Honey samples containing more than 45% of a single type of pollen were considered as unifloral honey. Plant species with percentage below 1% were not considered in the tables.

3. Results

The types of pollen, the percentage pollen frequency class as well as the quantitative pollen spectra of the honey samples from Ekiti State have been summarized in table 1-3.

Among the different honey samples analyzed, three samples were found to be unifloral while nine samples were multifloral (Table 1). In the group of honeys examined, the contribution of pollen from *Alchornea sp* was found to be the dominant type (>45%) in one sample EK-1 (IKERE) and secondary type (16-44%) in two

samples EK-2 (IJAN) and EK-10 (IDO). Other predominant pollen grains identified were *Lannea sp*, Asteraceae, *Elaeis guineesis* and *Pavetta sp* with the *Elaeis guineesis* as the most dominant having dominated in three samples (EK 6, 7 and 8) and secondary in two samples. Secondary pollen types identified from the samples include, Papilionaceae, *Tithonia diversifolia*, *Triplochiton sclerexylon*, *Triumfetta sp*, *Adenodolichas paniculata*, *Oldenlandia corymbosa*, *Mimosops warnekii*, *Spondias mombin*, *Blighia sapinda* and *Euphorbia hirta.* The rest of the pollen types were categorized as important minor and minor pollen types.

Table 1. *Pollen types found in the honey from Ekiti State*

Locality	Type of honey	Pollen types			
		Predominant(>45%)	**Secondary(16-44%)**	**Important minor(3-15%)**	**Minor Pollen(<3%)**
IKERE (EK-1)	Multifloral	*Alchornea sp* (63.3%)	*Elaeis guineensis* (19.2%)	Papilionaceae (12.4%)	*Adenia sp,Zea mays, Solanium sp, Justica flava, Asystasia gangetica,* Poaceae
IJAN (EK-2)	Unifloral	*Lannea sp* (50.2%)	*Alchornea cordifolia* (18.6%)	*Spondias mombis (5.7%)*, *Uapaca sp (3.2%)*, *Entada gigas (8.8%)*, *Blighia sapinda (3.3%), Mimusops warneckii (3.3%*)	Verbenaceae, *Elaeis gueneensis*, *Syzygium guineensis*
ILAWE (EK-3)	Unifloral	Asteraceae (58.5%)	Papilionaceae (32.4%)	*Mollotus sp* (5.4%)	*Asystasia gangetica, Mesozerum sp*, *Tridax procumbens*
EFON (EK-4)	Multifloral	-	*Elaeis guineensis* (43.8%), *Tithonia diversifolia* (39.6%)	Combretaceae/ melastomataceae (7.1*%), Asystasia gangetica (*4.3%)	*Nauclea sp*, Asteraceae, *Cardiospermum halicacabum,Zea mays,* Poaceae,*Asystasia gangatica.*
IGBEMO (EK-5)	Multifloral	-	*Triplochiton scleroxylon (39.3%)*, *Triumfetta sp (26.2%)*, *Adenodolichas paniculata (21.0%)*	*Venonia amygdalina (6.6%)*	*Asystasia sp,Ceiba pentandra,Cadiospermum sp,Spondias mombin*
ARAMOKO(I) (EK-6)	Multifloral	*Elaeis guineensis* (68.6%)	-	*Adenia sp (6.3%)*, *Alchornea sp (8.3%)*, *Mansonia sp (4.7%)*	*Choriosa sp,Lannea sp,Morus sp,Spondis mombin*
ESURE (EK-7)	Multifloral	*Elaeis guineensis (46.7%)*	*Oldenlandia corymbosa* (34.0%)	*Adenia sp (13.3%)*	*Alchornea sp*,Asteraceae*, Lantana camara,Spodias mombin,*Rubiaceae.
ADO (EK-8)	Muitifloral	*Elaeis guineesis (61.1%)*	Asteraceae (17.48%)	Rubiaceae (11.46%)	*Justicia flava*, *Asystasia gangetica, Adenia sp*,Poaceae, *Alchornea sp*
IKOLE (EK-9)	Multifloral	-	*Mimosops warnekii(34.5%),*Rubiaceae (34.5%)	*Piptadenisatrum africanium (23.0%)*	*Lannae welwitschii*, *Elaeis guineensis* Poaceae,*Chromoleana odorata*
IDO (EK-10)	Multifloral	-	*Elaeis guineesis(37.1%), Alchornea cordifolia (38.9%)*	*Cassia sp (5.7%), Uapaca sp (3.7%), Adenodolichas paniculata (3.3%) Vernonia amygdalina (10.4%), Pavetta owariensis (3.5%),*	Asterasceae, *Chromoleana odoranta,Vernonia amygdalina, Triumfetta sp*, *Erythrinia sp*
OYE (EK-11)	Multifloral	-	*Spondias mombin (16.6%), Alchornea sp (16.6%), Blighia sapida (16.3%)*	*Brachystegia eurycoma (3.4%)*, *Choris sp (4.2%)*, *Morus sp (3.6%)*, *Celtis sp (3.4%)*, *Phyllanthus discoides (3.7%)*	*Elaeis guineensis*, Asteraceae, *Bombax buonopozense*
ARAMOKO II (EK-12)	Unifloral	*Pavetta sp (62.4%)*	*Euphorbia hirta (25.7%)*	Anacardiaceae *(8.4%)*, Combretaceae *(5.3%) Spondias mombin (12.2%).*	*Ceiba pentandra*, *Daniellia ogea*, *Trilepisium nadagascariensis*

Table 2.*The percentage pollen frequency class of honey samples from Ekiti State.*

Locality/Sample	Pollen type	Percentage frequency	Frequency class
IKERE	*Asystasia gangetica*	1.2	Sporadic
	Elaeis guineensis	19.2	Frequent
	Papilionaceae	12.4	Rare
	Alchornea sp	63.4	Very frequent
IJAN	*Spondias mombin*	5.7	Rare
	Lannea sp	50.2	Rare
	Uapaca sp	3.2	Rare
	Alchornea cordifolia	18.6	Frequent
	Entada gigas	8.8	Rare
	Blighia sapinda	3.3	Rare
	Mimusops warneckii	3.3	Rare
	Verbenaceae	2.1	Sporadic
ILAWE	*Spondias mombin*	27.0	Frequent
	Lannea sp	3.8	Rare
	Elaeis guineensis	6.0	Rare
	Combretaceae/Melastomataceae	5.4	Rare
	Blighia sapinda	24.2	Frequent
	Rubiaceae	23.0	Frequent
	Pavetta owariensis	2.0	Sporadic
EFON	*Elaeis guineensis*	43.8	Frequent
	Asystasia gangetia	1.3	Sporadic
	Combretaceae/Melastomataceae	7.1	Rare
	Nauclea sp	2.9	Sporadic
	Tithonia diversifolia	39.3	Frequent
IGBEMO	*Triumfetta sp*	26.2	Frequent
	Venonia amygdalina	6.6	Rare
	Triplochiton scleroxylon	39.3	Frequent
	Adenodolichas paniculata	21.0	Frequent
	Ceiba pentandra	2.4	Sporadic
	Cadiospermum sp	1.2	Sporadic
	Vernonia amygdalina	6.6	Rare
ARAMOKO (I)	*Adenia sp*	6.3	Rare
	Alchornea sp	8.3	Rare
	Mansonia sp	4.7	Rare
	Elaeis guineensis	68.6	Very frequent
ESURE	*Oldenlandia corymbosa*	34.0	Frequent
	Adenia sp	13.3	Rare
	Elaeis guineensis	46.7	Very frequent
	Spondias mombin	1.6	Sporadic
ADO	*Elaeis guineensis*	61.0	Very frequent
	Asteraceae	17.5	Frequent
	Rubiaceae	11.5	Rare
IKOLE	*Mimusops warneckii*	34.5	Frequent
	Rubiaceae	34.5	Frequent
	Piptadeniastrum africanum	23.0	Frequent
	Lannea welwetschii	2.3	Sporadic
	Chromoleana odoranta	1.8	Sporadic
IDO	*Cassia sp*	5.7	Rare
	Uapaca sp	3.7	Rare
	Elaeis guineensis	37.1	Frequent
	Alchornea cordifolia	38.9	Frequent
	Adenodolichas paniculata	3.3	Rare
OYE	*Vernonia amygdalina*	10.4	Rare
	Pavetta owariensis	3.5	Rare
	Brachystegia eurycoma	3.4	Rare
	Chorisa sp	4.2	Rare
	Spondias mombin	16.6	Frequent
	Alchornea sp	16.6	Frequent
	Blighia sapinda	16.3	Frequent
	Morus sp	3.6	Rare
	Celtis sp	3.4	Rare
	Phyllanthus discoides	3.7	Rare
ARAMOKO (II)	*Pavetta sp*	62.4	Very frequent
	Euphorbia hirta	25.7	Frequent
	Anacardiaceae	8.4	Rare
	Spondias mombin	12.2	Rare
	Ceiba pentadra	1.4	Sporadic

Table 3. *Quantitative summary of pollen spectra identified in the honey samples*

Sample	Number of families	Number of pollen types	Quantity of pollen grains/sample
IKERE	23	27	65,589
IJAN	13	20	4,818
ILAWE	12	17	4,818
EFON	20	29	28,086
IGBEMO	18	24	85,087
ARAMOKO (I)	24	28	7,582
ESURE	16	20	8,285
ADO	17	23	14,902
IKOLE	13	15	9,912
IDO	16	27	57,375
OYE	18	24	50,664
ARAMOKO II	21	28	7,582

The range in frequency percentage pollen occurrences of the honey samples varied widely (Table 2). The number of pollen types that occurred as "very frequent" and "frequent" were below 30% while those that occurred as "rare" and "sporadic" were above 70%. *Alchornea sp*, *Elaeis guineensis* and *Pavetta sp* occurred as "very frequent" in six honey samples while *Alchornea cordifolia*, *Spondias mombins*, Rubiaceae, *Tithonia diversifolia*, *Oldenlandia corymbosa*, Asteraceae, *Piptadeniastrum africanum*, *Euphorbia hirta* and *Mimosops warnerkii* occurred frequently in the honey samples. The result of this analysis (Table 3) showed that a range of pollen types varying from a minimum of 15 to a maximum of 29 different plant taxa was identified. Ikole presented the lowest while Efon showed the highest value of pollens. The results show that some of the honey investigated revealed high variety of pollen grains from nectarless plants such as, Amaranthaceae/Chenopodiaceae, *Alchornea sp*, *Alchornea cordifolia*, Combretaceae/Melastomataceae and Cyperaceae which serve as good source of pollen load for the bees. This agreed with the earlier works of [21, 22, 14]. The pollen grains of anemophilous plants of Poaceae were also identified in seven honey samples in the area studied.

4. Discussion

The pollen in Ekiti honeys presented very rich and heterogenous spectra of plant taxa. Pollen count revealed a total of eighty-five plant taxa belonging to thirty-three families among which are Rubiaceae, Fabaceae, Euphorbiaceae, Combretaceae, Asteraceae, Sapindaceae, Arecaceae, Anacadiaceae, Sapotaceae, Poaceae, Passifloraceae and Acanthaceae. In the analyzed honeys, *Elaeis guineesis, Pavetta sp* and *Alchornea sp,* are generally very frequent in the honey samples from the areas, an indication that the pollen and nectar of these botanicals are important bee foods. The abundance and regular occurrence of pollen grains of *Elaeis guineensis* may be attributed to the fact that the indigenous palm trees are not discriminately fell in the area. This finding agreed with the report of [24] who stated that *Elaeis guineensis* pollens are produced abundantly in honey investigated from two vegetation zones of Nigeria. Results point out to a great variety of forest resources and generalized habits of pollen harvesting by the bees, *Apis mellifera*. This study has shown that bees do not respect plant habit or plant stratification. This confirmed the earlier work of [25] in a study carried out in Southwest Nigeria.

5. Conclusion

The areas selected for this study have good potential for sustaining beekeeping activities because of the diversity of nectar and pollen taxa. The honey samples are rich in pollen contents an indication that they are not adulterated. Since *Elaeis guineensis* are major sources of forage for wild honey bees (*Apis mellifera*) efforts should be intensified in increasing their cultivation. More so, plants in the families Rubiaceae, Asteraceae, Euphorbiaceae, Sapotaceae and Acanthaceae which are characteristics of Ekiti vegetation and also reflecting common pollen load and nectar sources should be cultivated the more.

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