MELLISOPALYNOLOGICAL ANALYSIS OF SOME COMMERCIAL HONEYS

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Abstract
Cases of adulterated honey sold in the markets is a crucial national concern as a source of food and health remedies. As part of efforts to monitor the level of adulteration in the neighbourhood, the present study is aimed at identifying the origin of the honey samples and ascertaining their authenticity. Laboratory and microscopic studies on 40 honey samples collected from 13 localities in Abeokuta (Majekodunmi, Itoku, Lafenwa, Somaarin, Odo-eran, Randa, Crescent University, Obantoko, Sanni, Iyana-cele, Osiele, Sabo and Atinsola), Ogun State and 15 places in Ibadan (University of Ibadan, Moor Plantation, Molete High School, Alegongo, Olegede, Agara, Ring Road, Aleshinloye, Basorun, Kasumu, Oremeji, Mapo, Apata, Oluyole, and Adelabu areas), Oyo State, South-West, Nigeria revealed that out of the 40 honey samples, 16 samples were adulterated with no pollen grains and the remaining 24 samples contained 12 pollen grain types from 81 plant taxa. In total, 168 pollen grains were extracted and identified. Some impurities were also observed in some of the honey samples. The frequency of the bee's visit to each identified plant species was also determined based on the pollen grain frequency in each honey samples. The presence of the pollen grains in the 24 honey samples is a clear indication that those honeys are not adulterated but pure and multifloral.

Key words: Adulterated honey, bee foraging, honey, pollen, mellisopalynology, pure honey

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1. INTRODUCTION

Melissopalynology (i.e. the study of pollen in honey) is an important tool in determining the floral sources upon which the bees foraged to produce honey. Each flower species has a unique pollen grain, which using proper techniques, may be studied to determine the geographical origin and major floral sources of the honey. There are set procedures and laboratory guidelines for the study of pollen in honey and their relationship to the floral resources (Bryant and Jones, 2001).

The study of pollen in a sample of honey makes it possible to gain evidence of the geographical location by observing the honey samples for the presence of varying combination of pollen that is typically only to that particular location (Louveaux *et al*., 1978). It is also possible to identify taxonomically the genera of the plants the honey bees visited, although honey may also contain airborne pollen from anemophilous plant species, spore and dust due to electrostatic charge of the worker bee. Information gained from a given honey sample is useful when substantiating claims of a particular honey source and is also of great importance for quality control and helps to ascertain whether honey is adulterated or not (Maurizio, 1951; Molan, 1998; Louveaux *et al*., 1978; Terrab *et al*., 2003). Honey samples may be mono-floral, bi-floral and hetero-floral or pluri-floral. Mono-floral honey may be more valuable than bi-floral honey and hetero-floral honeys (plurifloral) (Zet-Sche, 1932). There is no honey produced by bees flying free is entirely unifloral, unless the bees were reared in apiaries and exposed to a particular species of bee pollinated plant.

In recent years, melisopalynology has attained a topic of global status. This is borne out of the fact that not only is honey useful as a food supplement; it is now increasingly being used in the treatment of various diseases (Molan, 2001). These healing properties of honey are as a result of the integration of pollen and nectar containing bio active ingredients from medicinal Honey is a natural sweet substance

and is produced by honeybees from the nectar of blossoms, from secretion of living parts of plants. Although honey contains many chemical components such as carbohydrates i.e. glucose, maltose, sucrose, fructose and other complex carbohydrates, vitamins and minerals, the specific composition of any batch of honey depends on the flowers available to the bees that produced the honey.

In many cultures, honey has associations that go beyond its use as a food. Honey is frequently a talisman and symbol of sweetness. In Nigeria, the 3 most common ethnic groups (Yoruba, Igbo and Hausa) use honey during occasions like naming ceremony of new born baby, as one of the requirements in paying bride price during a marriage ceremony, honey is also used as a gift. For at least 2700 years, honey has been used by humans to treat a variety of ailments through topical application, but only recently has the antiseptic and antibacterial properties of honey chemically explained (Jusbin, 1996; Abdulla and Abdul-Aziz, 1998; Wahdan, 1998).

This present study is a further contribution to the melissopalynological investigations of honey samples in Nigeria, focusing on the honey samples collected from 40 localities in Ibadan and Abeokuta, Nigeria. Therefore, the study is aimed at identifying the origin of the honey samples and ascertaining their authenticity.

2. MATERIALS AND METHODS

Forty honey samples were collected from twenty areas, each in Ibadan, Oyo State and Abeokuta, Ogun State, South-West, Nigeria. The 15 samples from Ibadan include those collected from University of Ibadan, Moor Plantation, Molete High School, Alegongo, Olegede, Agara, Ring Road, Aleshinloye, Basorun, Kasumu, Oremeji, Mapo, Apata, Oluyole, and Adelabu areas, and 13 samples from Abeokuta included honeys from Majekodunmi, Itoku, Lafenwa, Somaarin, Odo-eran, Randa, Crescent University, Obantoko, Sanni, Iyana-cele, Osiele, Sabo and Atinsola. Melissopalynological studies were performed on the 28 samples.

Distilled water was warmed on a Bunsen burner. Five grams (5g) of each honey sample was weighed using the weighing balance into a beaker. 10mls of the hot distilled water was added and stirred by manual shaking till the honey samples dissolved totally. The solution was poured into the centrifuge tube and placed in the centrifuge. It was allowed to spin at 3000rpm for 5 minutes. The supernatant fluid was discarded. To the residue, 3-5ml glacial Acetic acid was added, stirred and allowed to stand for 5 minutes. The solution was centrifuged again, with the supernatant fluid being discarded. Acetolysis mixture was added to the residue in a fume cupboard, boiled in the water bath at 80-90^{0}c for 2-3 minutes. The mixture was centrifuged and decanted. To the residue, distilled water was added thrice to rinse the residue, centrifuging and decanting at each time. The final residue was mounted on a slide with the aid of dropper or micropipette and allowed to dry. Few drops of Isopropyl alcohol were added to the dried residue, and allowed to dry. Then a few drops of already warmed liquid glycerine were added to the slide as a mounting agent for observation (Erdtman, 1960). The slide was covered using a coverslip. The slide was made permanent by using nail varnish as sealant on the edges of the coverslip. The slide was taken for microscopic examination, identification, and frequency of the pollen species. Micrographs were taken using the Amscope camera and pollen images were cropped out for identification. The slides were labeled accordingly.

Acetolysis mixture: this was prepared by addition of concentrated sulphuric acid to acetic anhydride in the ratio 9:1. Liquid glycerin: This was prepared by the addition of distilled water to liquid glycerol. Pollen observed in the honey samples were identified using some pollen atlas such as Dieter *et al*., 2013; and William *et al*., 2013.

Using 35 snaps of field observation at 40x magnification as quadrat, the number of pores on pollens was noted to determine the frequency of the different pollen types present.

The frequency of each pollen type was expressed as the percentage occurrence of such pollen type based on all occurrences. For the presentation of frequencies of pollen grains in honey, the system adopted by Louveaux *et al*. (1978) was used.
The percentage frequency of each pollen type was determined using this formula:

$$\%\text{Frequency} = \frac{\text{Number of pollen of a taxon}}{\text{Total number of all pollens}} \times 100$$

The following terms have been used in the estimation of PK (pollen grain) frequencies: "very frequent" for grains constituting more than 45% of the total, "sporadic or rare for grain constituting less than 3% and the following terms used for frequency classes. The term „"predominant pollen" (more than 45% of the pollen grains counted), "secondary or accompanying pollen"" (16-45%), "important minor or important isolated pollen'' (3-15%), "minor or isolated pollen'' (less than 3%) (Vergeron, 1964). Necessary precautions were taken to avoid contamination of one pollen source from other pollen sources.

3. RESULTS AND DISCUSSION

3.1. RESULTS

Of the 40 honey samples collected, 16 samples were adulterated with no pollen grains and the remaining 24 samples analyzed in the study were found to contain pollen grains of 81 plant taxa and 12 pollen types. The identified taxa belong to varying genera of native herbs, shrubs, grass and trees.
These pollens are of varying shapes, sizes and morphological features, suggesting that the honey samples are multifloral (Tables 1-24; Plates 1-24).

Apata honey

Five pollen types of eight plant species belonging to seven families were observed in the honey samples collected from Apata area. Pollen grains from *Gerradima foliosa* is more dominant (28.3%) followed by pollen grains from *Mangifera indica* (18.9%) and pollen grains from *Amaranthus* spp. being the least with 0.3% frequency (Table 1; Plate 1).

Kasumu honey

Six pollen types from eight plant species belonging to six families were observed in the honey samples collected from Kasumu area. Pollen grains from *Tilia americana* is more dominant (26.8%) followed by pollen grains from *Combretum guienzii* (17.1%) and pollen grains from *Acidanthera brevicollis* being the least with 4.2% frequency (Table 2; Plate 2).

Plate 1: Pollen samples (a- *Amaranthus sp,* b- *Zea mays,* c- *Gerradina foliosa* d- *Mangifera indica,* e- *Tetrochidium didymostemon,* g- *Chaemacyparis nootkatensis,* h- *Lannea acida,* i- *Monotes kerstingii*) and impurity (f- leaf-like) obtained from the honey collected in Apata, Ibadan, Nigeria.

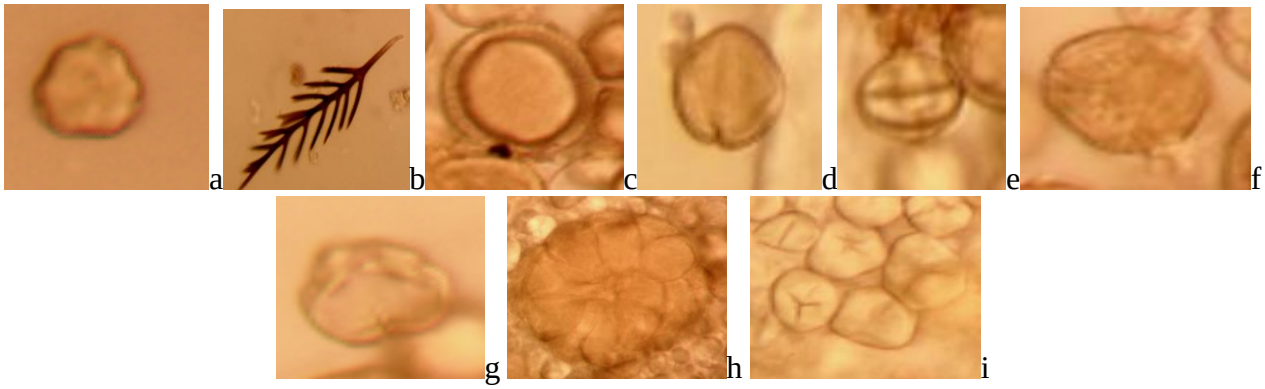

Plate 2: Pollen samples (a- *Combretum guienzii,* c- *Trema guineensis,* d- *Gunnera chilensis,* e- *Acidanthera brevicollis,* f- *Entada abyssinica,* g- *Tilia Americana,* h- *Parkia inundabilis,* i- *Piptadenia Africana*) and impurity (b- a leaf-like), obtained from the honey collected in Kasumu, Ibadan, Nigeria.

Table 1: Plant sources, types and frequency of pollens obtained from Apata honey

Plant species	Family	Pollen type	Frequency (%)
Amaranthus spp.	Amaranthaceae	Pantoporate	0.3
Zea mays	Poaceae	Monoporate	14.6
Gerradina foliosa	Gerrardinaceae	Tricolporate	28.3
Mangifera indica	Anacardiaceae	Tricolporate	18.9
Tetrochidium didymostemon	Euphorbiaceae	Tricolporate	15.6
Chaemacyparis nootkatensis	Cupressaceae	Monocolpate	7.3
Lannea acida	Anacardiaceae	Tricolporate	9.2
Monotes kerstingii	Dipterocarpaceae	Tricolpate	5.8

Table 2: Plant sources, types and frequency of pollens obtained from Kasumu honey

Plant species	Family	Pollen type	Frequency (%)
Combretum guienzii	Combretaceae	Heterocolporate	17.1
Trema guineensis	Cannabaceae	Diporate	15.8
Gunnera chilensis	Gunneraceae	Tricolpate	13.1
Acidanthera brevicollis	Iridaceae	Monocolpate	4.2
Entada abyssinica	Fabaceae	Triporate	6.6
Tilia americana	Tiliaceae	Tricolpate	26.8
Parkia inundabilis	Fabaceae	Tricolporate	7.3
Piptadenia africana	Fabaceae	Tricolporate	9.1

Lafenwa honey

Three pollen types of five plant species belonging to three families were observed in the honey samples collected from Lafenwa area. Pollen grains from *Acidanthera brevicollis* is more dominant (35.4%) followed by pollen grains from *Piptadenia africana* (21.0%) and pollen grains from *Clifftortia nitidula* being the least with 9.8% frequency (Table 3; Plate 3).

Molete High School honey

Two pollen types of six plant species belonging to five families were observed in the honey samples collected from Molete high school area. Pollen grains from *Typha capensis* is more dominant (25.7%) followed by pollen grains from *Typha augustifolia* (21.2%) and pollen grains from *Gerradima foliosa* being the least with 3.5% frequency (Table 4; Plate 4).

Moor plantation honey

Eight pollen types of nine plant species belonging to seven families were observed in the honey samples collected from Moor Plantation area. Pollen grains from *Clausena anisata* is more dominant (21.8%) followed by pollen grains from *Rhizophora mangle* (15.3%)

and pollen grains from *Clifftortia nitidula* being the least with 3.5% frequency (Table 5; Plate 5).

Ologede honey

Five pollen types of eight plant species belonging to eight families were observed in the honey samples collected from Ologede area.

Pollen grains from *Berlinia bifoliata* is more dominant (24.1%) followed by pollen grains from *Hymenocardia acida* (19.3%) and pollen grains from *Gunnera chilenis* being the least with 3.6% frequency (Table 6; Plate 6).

Oremeji honey

Four pollen types of six plant species belonging to six families were observed in the honey samples collected from Oremeji area.

Pollen grains from *Acidanthera brevicollis* is more dominant (29.5%) followed by pollen grains from *Lannea acida* (21.2%) and pollen grains from *Elaeis guineensis* being the least with 3.6% frequency (Table 7; Plate 7).

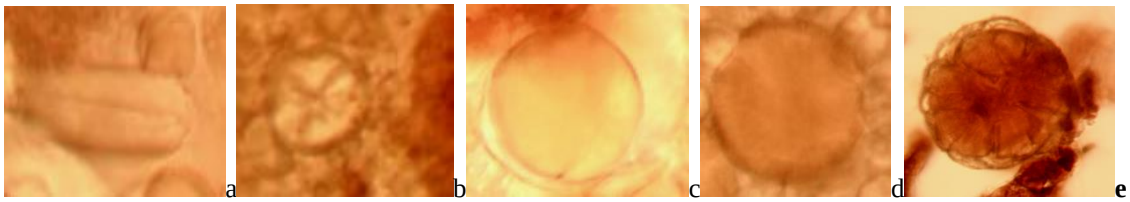

Plate 3: Pollen samples (a- *Acidanthera brevicollis,* b- *Piptadenia africana* c- *Clifftortia nitidula* d- *Poliostigma reticulatum* e- *Parkia bussei*) obtained from the honey collected in Lafenwa, Abeokuta, Nigeria

Plate 4: Pollen samples (a- *Typha capensis,* b- *Lannea acida,* d-*Typha augustifolia,* e- *Gerradina foliosa,* f- *Grewia glandulosa,* g- *Piptadenia africana*) and impurity (c- leaf-like) obtained from the honey collected in Molete High School, Ibadan, Nigeria

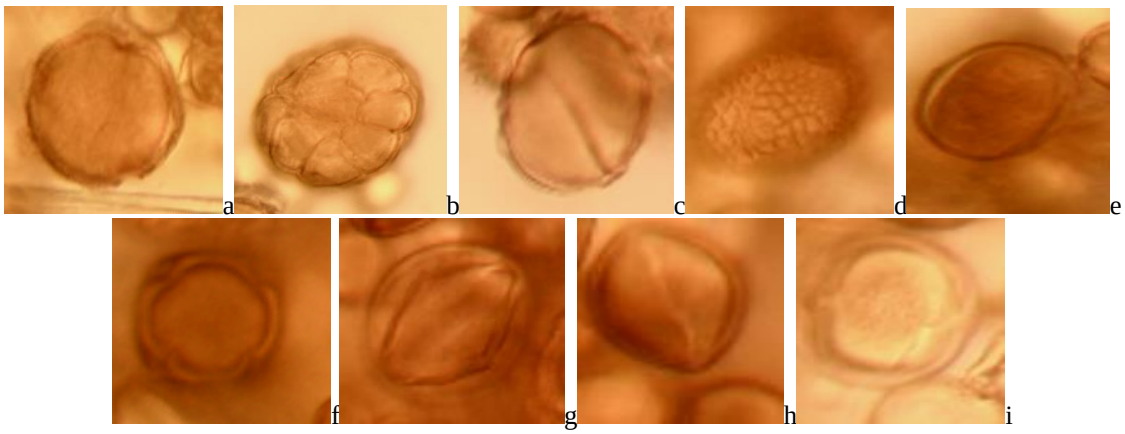

Plate 5: Pollen samples (a- *Hymenocardia acida,* b- *Parkia bussei,* c- *Clifftortia nitidula,* d- *Jatropha curcas,* e- *Combretum* spp, f- *Rumex acetosella,* g- *Clausena anisata,* h- *Rhizophora mangle,* i- *Terminalia aemula*) obtained from the honey collected in Moor plantation, Ibadan, Nigeria.

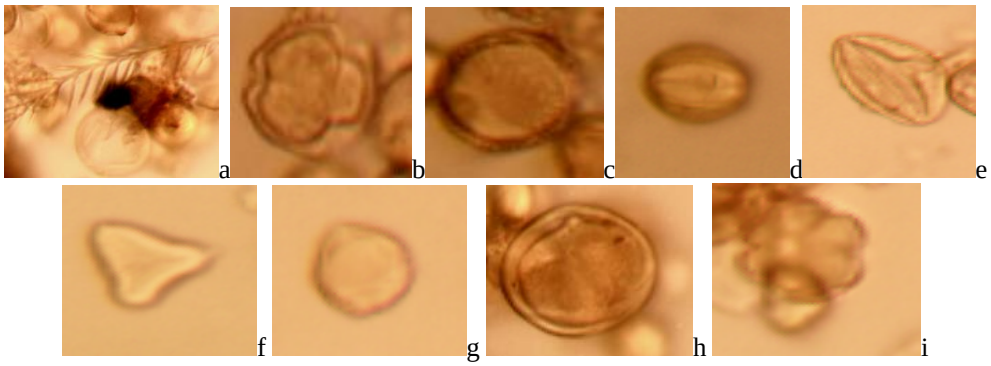

Plate 6: Pollen samples (b- *Lannea acida,* c- *Hymenocardia acida,* d- *Gunnera chilensis,* e- *Berlinia bifoliolata,* f- *Syzygium guineense,* g- *Typha capensis,* h- *Aesculus carnifornica,* i- *Securidaca longependuculata*) and impurity (a- leaf-like) obtained from the honey collected in Ologede, Ibadan, Nigeria.

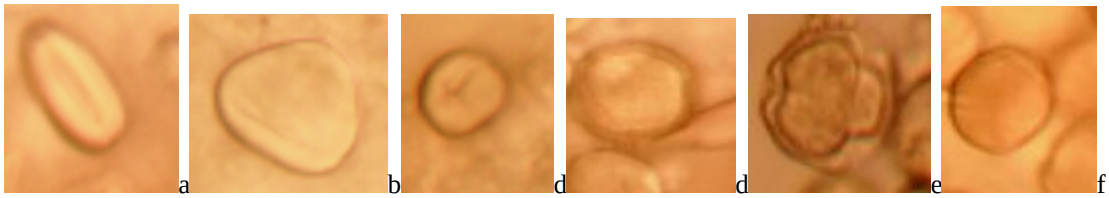

Plate 7: Pollen samples (a- *Acidanthera brevicollis,* b- *Elaeis guineensis,* c- *Piptadenia Africana,* d- *Hymenocardia acida,* e- *Lannea acida,* f- *Typha capensis*) obtained from the honey collected in Oremeji, Ibadan, Nigeria.

Table 3: Plant sources, types and frequency of pollens obtained from Lafenwa honey

Plant species	Family	Pollen type	Frequency (%)
Acidanthera brevicollis	Iridaceae	Monocolpate	35.4
Piptadenia africana	Fabaceae	Tricolporate	21.0
Clifftortia nitidula	Rosaceae	Tricolporate	9.8
Poliostigma reticulatum	Fabaceae	Triporate	15.6
Parkia bussei	Fabaceae	Tricolporate	18.2

Table 4: Plant sources, types and frequency of pollens obtained from Molete High school honey

Plant species	Family	Pollen type	Frequency (%)
Typha capensis	Typhaceae	Monoporate	25.7
Lannea acida	Anacardiaceae	Tricolporate	13.8
Typha augustifolia	Typhaceae	Monoporate	21.2
Gerradina foliosa	Gerrardinaceae	Tricolporate	3.5
Grewia glandulosa	Malvaceae	Tricolporate	15.9
Piptadenia africana	Fabaceae	Tricolporate	19.9

Table 5: Plant sources, types and frequency of pollens obtained from Moor Plantation honey

Plant species	Family	Pollen type	Frequency (%)
Hymenocardia acida	Euphorbiaceae	Triporate	7.3
Parkia bussei	Fabaceae	Heterocolpate	12.1
Clifftortia nitidula	Rosaceae	Tricolporate	4.3
Jatropha curcas	Euphorbiaceae	Panporate	11.8
Combretum spp.	Combretaceae	Heterocolporate	5.9
Rumex acetosella	Polygonaceae	Paniculate	15.3
Clausena anisata	Rutaceae	Tricolporate	21.8
Rhizophora mangle	Rhizophoraceae	Diporate	15.3
Terminalia aemula	Combretaceae	Monocolpate	6.2

Table 6: Plant sources, types and frequency of pollens obtained from Ologede honey

Plant species	Family	Pollen type	Frequency (%)
Lannea acida	Anacardiaceae	Tricolporate	9.5
Hymenocardia acida	Phyllanthaceae	Triporate	19.3
Gunnera chilensis	Gunneraceae	Tricolpate	3.6
Berlinia bifoliolata	Fabaceae	Tricolporate	24.1
Syzygium guineense	Myrtaceae	Tricolpate	12.3
Typha capensis	Typhaceae	Monoporate	11.4
Aesculus carnifornica	Sapindaceae	Tricolporate	15.6
Securidaca longependuculata	Polygalaceae	Stephanocolporate	4.2

Table 7: Plant sources, types and frequency of pollens obtained from Oremeji honey

Plant species	Family	Pollen type	Frequency (%)
Acidanthera brevicollis	Iridaceae	Monocolpate	29.5
Elaeis guineensis	Arecaceae	Monocolpate	8.3
Piptadenia africana	Fabaceae	Tricolporate	11.6
Hymenocardia acida	Phyllanthaceae	Triporate	19.8
Lannea acida	Anacardiaceae	Tricolporate	21.2
Typha capensis	Typhaceae	Monoporate	9.6

University of Ibadan honey

Four pollen types of seven plant species belonging to six families were observed in the honey samples collected from University of Ibadan area. Pollen grains from *Turraeanthus africana* is more dominant (26.7%) followed by pollen grains from *Typha capensis* (21.2%) and pollen grains from *Passiflora* spp. being the least with 2.7% frequency (Table 8; Plate 8).

Alegongo honey

Four pollen types of seven plant species belonging to five families were observed in the honey samples collected from Alegongo area. Pollen grains from *Elaeis guineensis* is more dominant (24.5%) followed by pollen grains from *Milletia oblata* (18.9%), and pollen grains from *Ginkgo biloba* being the least with 7.4% frequency (Table 9; Plate 9).

Atinsola honey

Three pollen types of seven plant species belonging to six families were observed in the honey samples collected from Atinsola area. Pollen grains from *Alchornea cordifolia* is more dominant (28.2%) followed by pollen grains from *Hymenocardia acida* (23.6%) and pollen grains from *Adenia nicobarrica* being the least with 2.4% frequency (Table 10; Plate 10).

Crescent University honey

Seven pollen types of twelve plant species belonging to twelve families were observed in the honey samples collected from Crescent University area. Pollen grains from *Caryolus cornuta* is more dominant (15.6%) followed by pollen grains from *Protea sussane* (13.7%) and pollen grains from *Acridocarpus macrocalyx* being the least with 1.2% frequency (Table 11; Plate 11).

Itoku honey

Three pollen types of five plant species belonging to four families were observed in the honey samples collected from Itoku area. Pollen grains from *Piptadenia africana* is more dominant (38.8%) followed by pollen grains from *Typha augustifolia* (19.3%) and pollen grains from *Entada abyssinica* being the least with 7.9% frequency (Table 12; Plate 12).

Iyana cele honey

Four pollen types of five plant species belonging to five families were observed in the honey samples collected from Iyana-cele area. Pollen grains from *Acridocarpus macrocalyx* is

more dominant (33.3%) followed by pollen grains from *Vitex doniana* (23.8%) and pollen grains from *Parkia velutina* being the least with 5.3% frequency (Table 13; Plate 13).

Majekodunmi honey

One pollen type of six plant species belonging to three families was observed in the honey samples collected from Majekodunmi area. Pollen grains from *Prosopis africana* is more dominant (39.3%) followed by pollen grains from *Zanthoxylum procerum* (18.8%) and pollen grains from *Xylla evansii* is the least with 3.8% frequency (Table 14; Plate 14).

Basorun honey

Four pollen types of six plant species belonging to six families were observed in the honey samples collected from Basorun area. Pollen grains from *Cunnonia pterophylla* is more dominant (33.3%) followed by pollen grains from *Guiera senegalensis* (19.8%) with pollen grains from *Sterculia tragacantha* being the least with 6.9% frequency (Table 15; Plate 15).

Table 8: Plant sources, types and frequency of pollens obtained from University of Ibadan honey

Plant species	Family	Pollen type	Frequency (%)
Passiflora spp.	Passifloraceae	Heterocolpate	2.7
Typha augustifolia	Typhaceae	Monoporate	15.9
Typha capensis	Typhaceae	Monoporate	21.2
Amaranthus spp.	Amaranthaceae	Pantoporate	7.5
Turraeanthus africana	Meliaceae	Triporate	26.7
Prunus africana	Rosaceae	Tricolporate	17.1
Vitex ambionensis	Lamiaceae	Tricolpate	8.9

Table 9: Plant sources, types and frequency of pollens obtained from Alegongo honey

Plant species	Family	Pollen type	Frequency (%)
Piliostigma reticulatum	Fabaceae	Triporate	12.3
Rhynchosia spp.	Fabaceae	Tricolporate	13.8
Ginkgo biloba	Ginkgoaceae	Dicolporate	7.4
Elaeis guineensis	Arecaceae	Monocolpate	24.5
Elaeodendron buchananii	Celestraceae	Tricolporate	14.2
Protea susannae	Proteaceae	Triporate	8.9
Milletia oblata	Fabaceae	Tricolporate	18.9

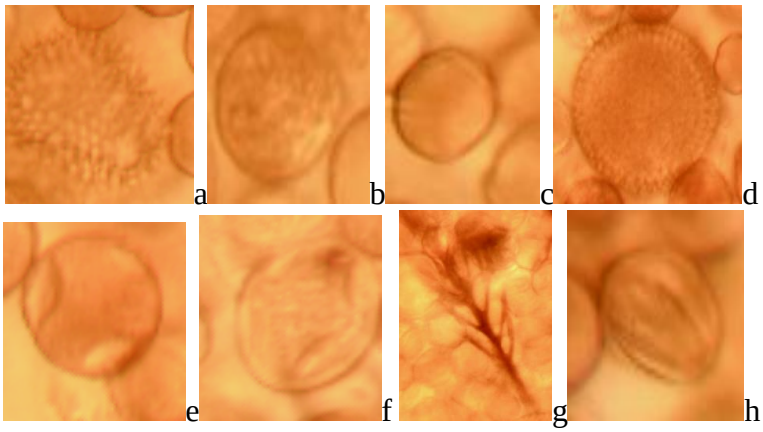

Plate 8: Pollen samples (a- *Passiflora* spp., b- *Typha augustifolia,* c- *Typha capensis,* d-*Amaranthus* spp., e- *Turraeanthus africana,* f- *Prunus africana,* h-*Vitex ambionensis*) and impurity (g-leaf-like) obtained from the honey collected in University of Ibadan, Ibadan, Nigeria.

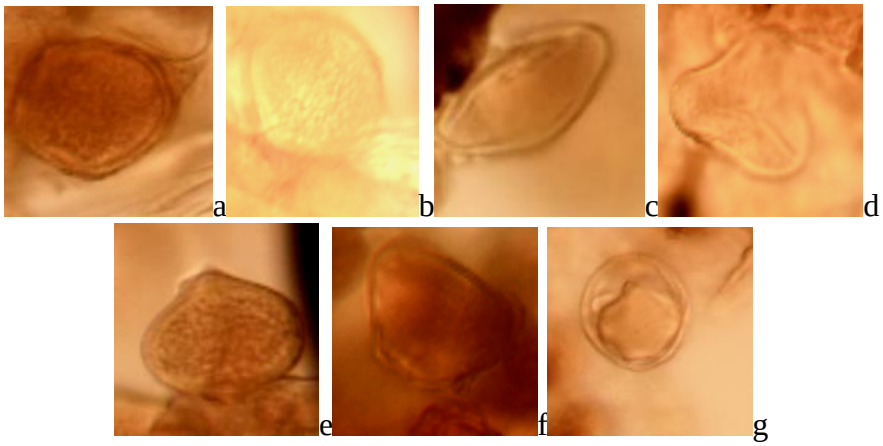

Plate 9: Pollen samples (a-*Piliostigma reticulatum*, b-*Rhynchosia* spp., c-*Ginkgo biloba*, d-*Elaeis guineensis*, e-*Elaeodendron buchananii*, f-*Protea susannae*, g-*Milletia oblata*) obtained from the honey collected in Alegongo, Ibadan, Nigeria.

Table 10: Plant sources, types and frequency of pollens obtained from Atinsola honey

Plant species	Family	Pollen type	Frequency (%)
Adenia nicobarrica	Passifloraceae	Tricolporate	2.4
Parkia inundabillis	Fabaceae	Tricolpate	7.3
Actinidia palmatum	Actinidiaceae	Tricolporate	15.7
Alchornea cordifolia	Euphorbiaceae	Tricolpate	28.2
Entada umbonata	Fabaceae	Triporate	11.3
Acridocarpus macrocalyx	Malpighiaceae	Tricolporate	11.5
Hymenocardia acida	Phyllanthaceae	Triporate	23.6

Table 11: Plant sources, types and frequency of pollens obtained from Crescent University honey

Plant species	Family	Pollen type	Frequency (%)
Huperzia spp.	Huperziaceae	Tricolpate	2.2
Parinari curatellifolia	Chrysobalanaceae	Tricolporate	7.6
Jatropha curcas	Euphorbiaceae	Panporate	10.8
Typha augustifolia	Typhaceae	Monoporate	5.3
Elaeis guineensis	Arecaceae	Monocolpate	8.5
Premna maxima	Lamiaceae	Tricolpate	4.2
Acidocarpus macrocalyx	Malpighiaceae	Tricolporate	1.2
Alcea rosea	Malvaceae	Monocolpate	3.0
Hippocratea affinis	Celastraceae	Tricolporate	11.9
Agelaea heterophylla	Connaraceae	Tricolporate	6.2
Caryolus cornuta	Betulaceae	Tricolpate	15.6
Protea susannae	Proteaceae	Triporate	13.7

Table 12: Plant sources, types and frequency of pollens obtained from Itoku honey

Plant species	Family	Pollen type	Frequency (%)
Entada abyssinica	Fabaceae	Tricolporate	7.9
Gerradina foliosa	Gerrardinaceae	Tricolporate	17.3
Piptadenia africana	Fabaceae	Tricolporate	38.8
Hymenocardia acida	Phyllanthaceae	Triporate	16.7
Typha augustifolia	Typhaceae	Monoporate	19.3

Table 13: Plant sources, types and frequency of pollens obtained from Iyana cele honey

Plant species	Family	Pollen type	Frequency (%)
Vitex doniana	Lamiaceae	Tricolpate	23.8
Celtis integrifolia	Cannabaceae	Monoporate	19.2
Parkia velutina	Fabaceae	Tricolpate	5.3
Acridocarpus macrocalyx	Malpighiaceae	Tricolporate	33.3
Jatropha curcas	Euphorbiaceae	Panporate	18.4

Table 14: Plant sources, types and frequency of pollens obtained from Majekodunmi honey

Plant species	Family	Pollen type	Frequency (%)
Xylla evansii	Fabaceae	Tricolporate	3.8
Entada abyssinica	Fabaceae	Tricolporate	9.6
Zanthoxylum procerum	Rutaceae	Tricolporate	18.8
Prosopis africana	Fabaceae	Tricolporate	39.3
Hippocratea affinis	Celastraceae	Tricolporate	17.2
Rhynchosia spp.	Fabaceae	Tricolporate	11.3

Table 15: Plant sources, types and frequency of pollens obtained from Basorun honey

Plant species	Family	Pollen type	Frequency (%)
Sterculia tragacantha	Malvaceae	Tricolporate	6.9
Typha augustifolia	Typhaceae	Monoporate	11.8
Mangifera indica	Anacardiaceae	Tricolporate	16.7
Cunonia pterophylla	Cunoniaceae	Monoporate	33.3
Guiera senegalensis	Combretaceae	Heterocolporate	19.8
Rhizophora mangle	Rhizophoraceae	Diporate	11.5

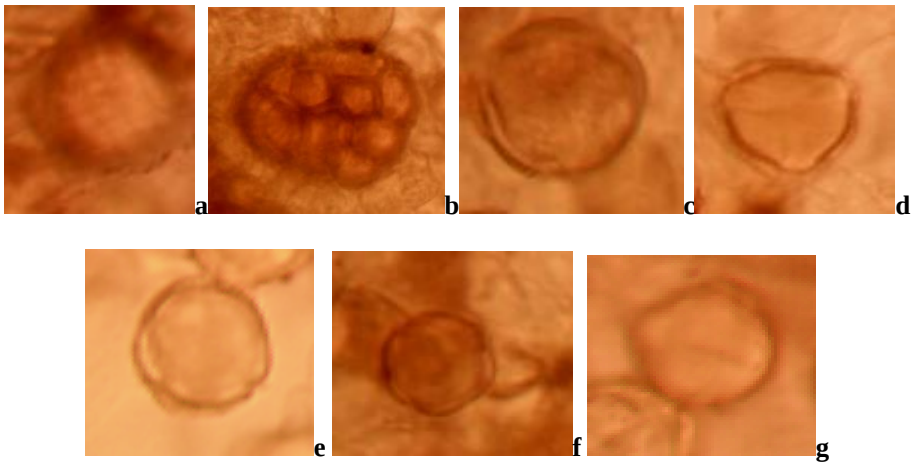

Plate 10: Pollen samples (a-*Adenia nicobarrica,* b-*Parkia inundabillis,* c-*Actinidia palmatum,* d-*Alchornea cordifolia,* e-*Entada umbonata,* f-*Acridocarpus macrocalyx,* g-*Hymenocardia acida*) obtained from the honey collected in Atinsola, Abeokuta, Nigeria.

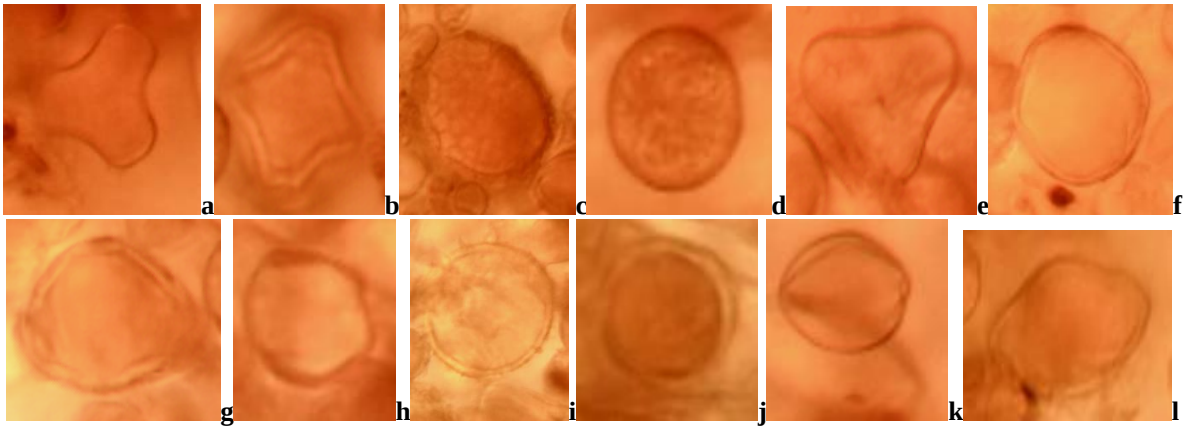

Plate 11: Pollen samples (a-*Huperzia* spp., b-*Parinari curatellifolia,* c-*Jatropha curcas,* d-*Typha augustifolia,* e-*Elaeis guineensis,* f-*Premna maxima,* g-*Acidocarpus macrocalyx,* h-*Alcea rosea,* i-*Hippocratea affinis,* j-*Agelaea heterophylla,* k-*Caryolus cornuta,* l-*Protea susannae*) obtaiined from the honey collected in Crescent University, Abeokuta, Nigeria.

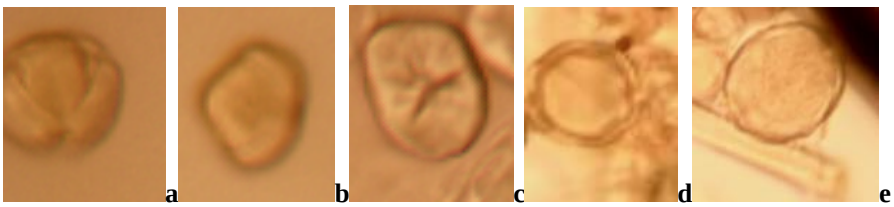

Plate 12: Pollen samples (a-*Entada abyssinica,* b-*Gerradina foliosa,* c-*Piptadenia africana,* d-*Hymenocardia acida,* e-*Typha augustifolia*) obtained from the honey collected in Itoku, Abeokuta, Nigeria.

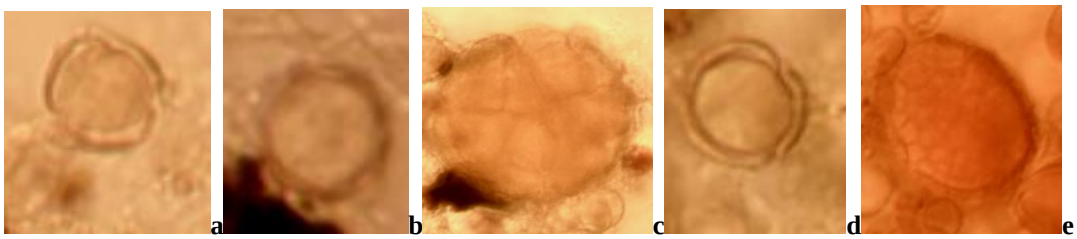

Plate 13: Pollen samples (a-*Vitex doniana,* b-*Celtis integrifolia,* c-*Parkia velutina,* d-*Acridocarpus macrocalyx,* e-*Jatropha curcas*) obtained from the honey collected in Iyana cele, Abeokuta, Nigeria.

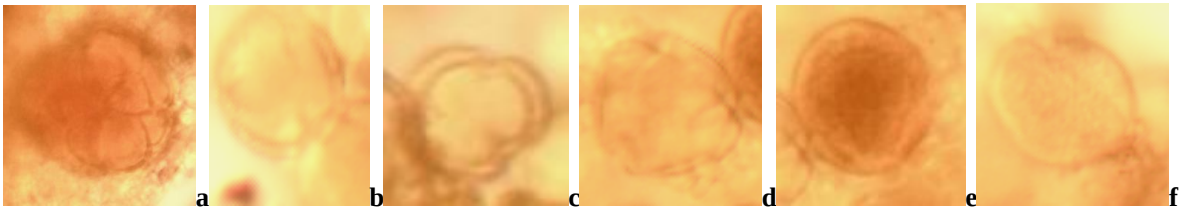

Plate 14: Pollen samples (a-*Xylla evansii,* b-*Entada abyssinica,* c-*Zanthoxylum procerum,* d-*Prosopis africana,* e-*Hippocratea affinis,* f-*Rhynchosia* spp.) obtained from the honey collected in Majekodunmi, Abeokuta, Nigeria.

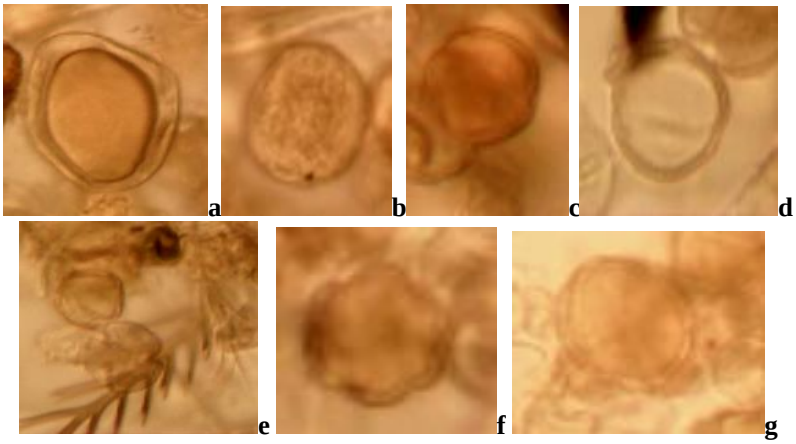

Plate 15: Pollen samples (a-*Sterculia tragacantha,* b-*Typha augustifolia,* c-*Mangifera indica,* d-*Cunonia pterophylla,* f-*Guiera senegalensis,* g-*Rhizophora mangle*) and impurity (e- leaf-like) obtained from the honey collected in Basorun, Ibadan, Nigeria.

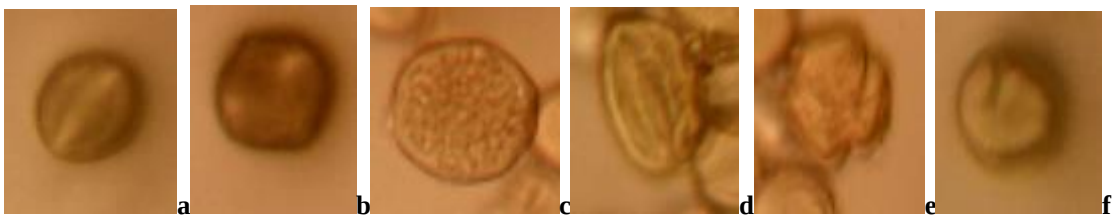

Plate 16: Pollen samples (a-*Phoenix reclinata,* b-*Indigofera leptoclada,* c-*Typha augustifolia,* d-*Scytopetalum tieghemii,* e-*Alchornea chordifolia,* f-*Aesculus indica*) obtained from the honey collected in Obantoko, Abeokuta, Nigeria.

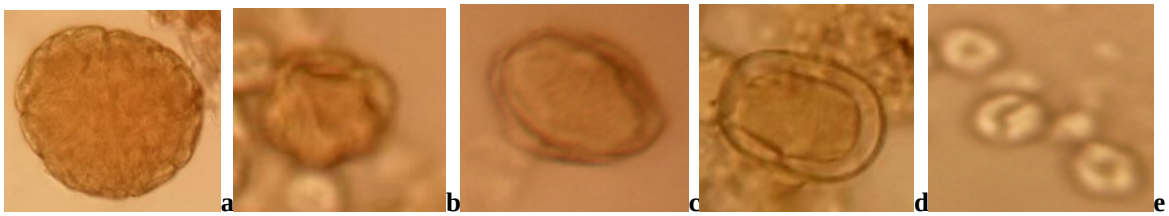

Plate 17: Pollen samples (a-*Parkia velutina,* b-*Terminalia brownii,* c-*Agrimonia eupatoria,* d-*Terminalia aemula,* e-*Piptadenia africana*) obtained from the honey collected in Oluyole, Ibadan, Nigeria.

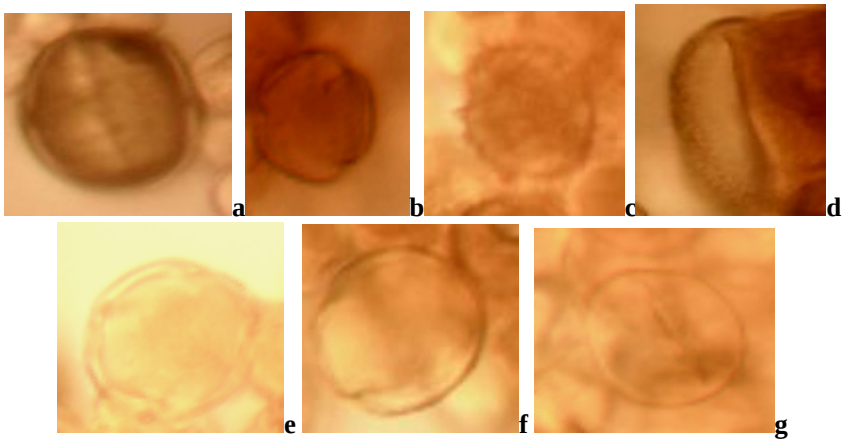

Plate 18: Pollen samples (a-*Combretum aculeatum,* b-*Vitex doniana,* c-*Gomphrena celosioides,* d-*Raphia ruffia,* e-*Acridocarpus macrocalyx,* f-*Lotus arabicus,* g-*Grewia glandulosa*) obtained from the honey collected in Osiele, Abeokuta, Nigeria.

Table 16: Plant sources, types and frequency of pollens obtained from Obantoko honey

Plant species	Family	Pollen type	Frequency (%)
Phoenix reclinata	Arecaceae	Monocolpate	16.7
Indigofera leptoclada	Fabaceae	Tricolporate	23.6
Typha augustifolia	Typhaceae	Monoparate	8.3
Scytopetalum tieghemii	Lecythidaceae	Tricolporate	11.3
Alchornea chordifolia	Euphorbiaceae	Tricolporate	29.2
Aesculus indica	Sapindaceae	Monocolpate	10.9

Table 17: Plant sources, types and frequency of pollens obtained from Oluyole honey

Plant species	Family	Pollen type	Frequency (%)
Parkia velutina	Fabaceae	Tricolpate	6.9
Terminalia brownii	Combreataceae	Heterocolporate	12.7
Agrimonia eupatoria	Rosaceae	Tricolporate	25.8
Terminalia aemula	Combretaceae	Monocolpate	13.4
Piptadenia africana	Fabaceae	Tricolporate	41.2

Table 18: Plant sources, types and frequency of pollens obtained from Osiele honey

Plant species	Family	Pollen type	Frequency (%)
Combretum aculeatum	Combretaceae	Heterocolparate	5.2
Vitex doniana	Lamiaceae	Tricolporate	13.3
Gomphrena celosioides	Amaranthaceae	Pantoporate	16.4
Raphia ruffia	Aracaceae	Monocolpate	7.3
Acridocarpus macrocalyx	Malpighiaceae	Tricolporate	9.5
Lotus arabicus	Fabaceae	Tricolporate	11.1
Grewia glandulosa	Malvaceae	Tricolporate	37.2

Obantoko honey

Three pollen types of six plant species belonging to six families were observed in the honey samples collected from Obantoko area. Pollen grains from *Alchornea cordifolia* is more dominant (29.2%) followed by pollen grains from *Indigofera leptoclada* (23.6%) with pollen grains from *Typha augustifolia* being the least with 8.3% frequency (Table 16; Plate 16).

Oluyole honey

Four pollen types of five plant species belonging to three families were observed in the honey samples collected from Oluyole area. Pollen grains from *Piptadenia africana* is more dominant (41.2%) followed by pollen grains from *Agrimonia eupatoria* (25.8%) with pollen grains from *Parkia velutina* being the least with 6.9% frequency (Table 17; Plate 17)

Osiele honey

Four pollen types of seven plant species belonging to seven families were observed in the honey samples collected from Osiele area. Pollen grains from *Grewia glandulosa* is more dominant (37.2%) followed by pollen grains from *Gomphrena celosioides* (16.4%) with pollen grains from *Combretum aculeatum* being the least with 5.2% frequency (Table 18; Plate 18).

Randa honey

Five pollen types of nine plant species belonging to nine families were observed in the honey samples collected from Randa area. Pollen grains from *Entada abyssinica* is more dominant (24.0%) followed by pollen grains from *Celtis integrifolia* (18.3%) with pollen grains from *Vitex doniana* being the least with 5.4% frequency (Table 19; Plate 19).

Somaarin honey
Four pollen types of seven plant species belonging to six families were observed in the honey samples collected from Somaarin area. Pollen grains from *Typha augustifolia* is more dominant (23.6%) followed by pollen grains from *Typha capensis* (18.1%) with pollen grains from *Pteleopsis diptera* being the least with 8.3% frequency (Table 20; Plate 20).

Odo-eran honey
Five pollen types of seven plant species belonging to seven families were observed in the honey samples collected from Odo-eran area. Pollen grains from *Typha augustifolia* is more dominant (26.3%) followed by pollen grains from *Acidanthera brevicollis* (20.7%) with pollen grains from *Lannea acida* being the least with 4.9% frequency (Table 21; Plate 21).

Sanni honey
Five pollen types of seven plant species belonging to six families were observed in the honey samples collected from Sanni area. Pollen grains from *Acridocarpus macrocalyx* is more dominant (24.6%) followed by pollen grains from *Entada abyssinica* (19.8%) with pollen grains from *Phaeoceros laevis* being the least with 3.2% frequency (Table 22; Plate 22).

Aleshinloye honey
Six pollen types of six plant species belonging to six families were observed in the honey samples collected from Aleshinloye area. Pollen grains from *Trema guineensis* is more dominant (27.6%) followed by pollen grains from *Parkia inundabilis* (23.9%) with pollen grains from *Tylophora sylvatica* being the least with 7.4% frequency (Table 23; Plate 23).

Agara honey
Seven pollen types of nine plant species belonging to nine families were observed in the honey samples collected from Agara area. Pollen grains from *Ipomoea orchracea* is more dominant (17.1%) followed closely by pollen grains from *Justica cordata* (15.4%) with pollen grains from *Combretum guienzi* being the least with 3.5% frequency (Table 24; Plate 24).

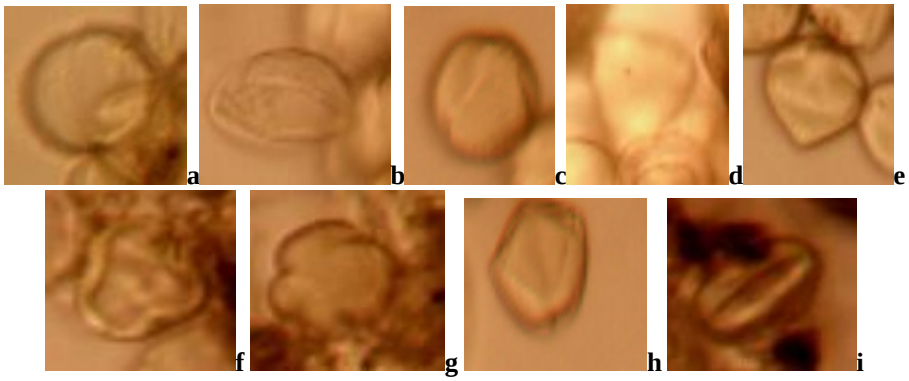

Plate 19: Pollen samples (a- *Celtis integrifolia,* b-*Xyris Montana,* c-*Entada abyssinica,* d-*Syzigium guineense,* e-*Phaeceros laevis,* f-*Erica arborea,* g-*Vitex doniana,* h-*Gerradina foliosa,* i-*Acidanthera brevicollis*) obtained from the honey collected in Randa, Abeokuta, Nigeria.

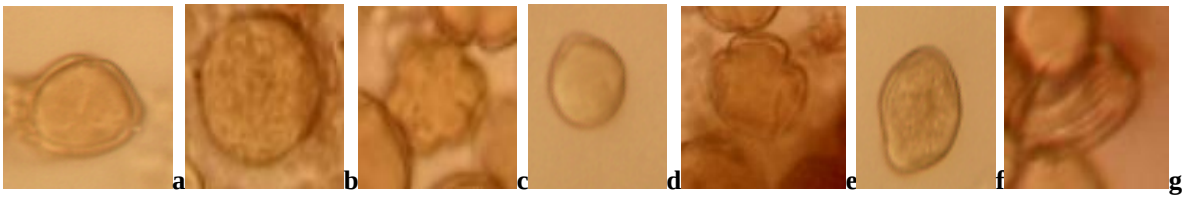

Plate 20: Pollen samples (a-*Acidocarpus macrocalyx,* b-*Typha augustifolia,* c-*Pteleopsis diptera,* d-*Typha capensis,* e-*Lannea acida,* f- *Indigofera leptoclada,* g-*Xyris Montana*) bgobtained from the honey collected in Somaarin, Abeokuta, Nigeria

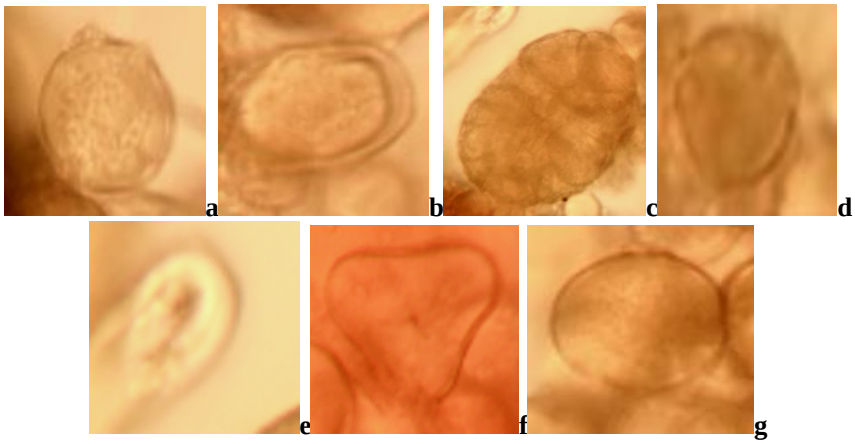

Plate 21: Pollen samples (a-*Typha augustifolia,* b-*Terminalia aemula,* c-*Acacia eglengii,* d-*Lannea acida,* e-*Acidanthera brevicollis,* f-*Elaeis guineensis,* g-*Celtis integrifolia*) obtained from the honey sample collected in Odo eran, Abeokuta, Nigeria

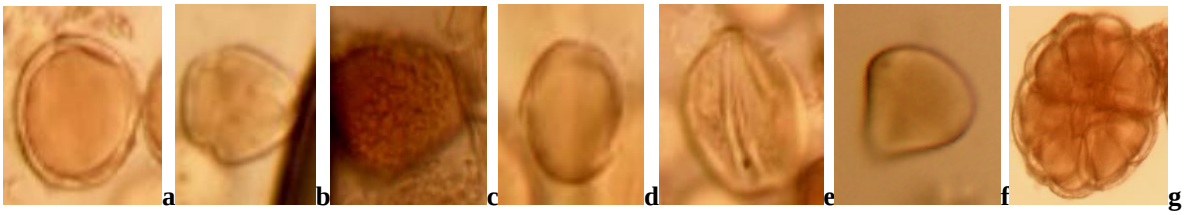

Plate 22: pollen samples (a-*Acridocarpus macrocalyx,* b-*Entada abyssinica,* c-*Polygonum lapathifolum,* d-*Anthosthema senegalense,* e-*Xyris Montana,* f-*Phaeoceros laevis,* g- *Parkia inundabilis*) obtained from the honey collected in Sanni, Abeokuta, Nigeria.

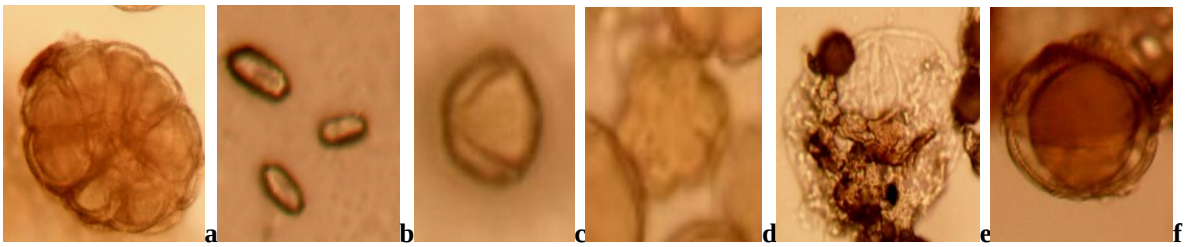

Plate 23: Pollen samples (a-*Parkia inundabilis,* b-*Acidanthera brevicollis,* c-*Terminalia brownii,* d-*Gerradina foliosa,* e-*Tylophora sylvatica,* f-*Trema guineesis*) obtained from the honey collected in Aleshinloye, Ibadan, Nigeria

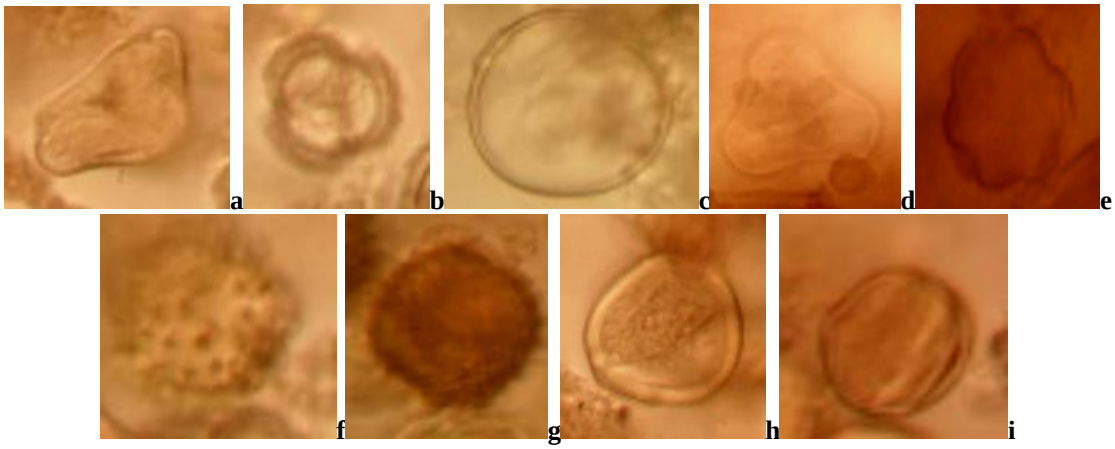

Plate 24: Pollen samples (a-*Tilia americana,* b-*Erica arborea,* c-*Celtis integrifolia,* d-*Elaeis guineensis,* e-*Combretum gueinzii,* f-*Ipomoea ochracea,* g-*Gomphrena celosioides,* h-*Carya sp,* i-*Justica cordata*) obtained from the honey collected in Agara, ibadan, Nigeria.

Table 19: Plant sources, types and frequency of pollens obtained from Randa honey

Plant species	Family	Pollen type	Frequency (%)
Celtis integrifolia	Cannabaceae	Triporate	18.3
Xyris Montana	Xyridaceae	Monocolpate	10.8
Entada abyssinica	Fabaceae	Tricolporate	24.0
Syzigium guineense	Myrtaceae	Tricolpate	6.7
Phaeceros laevis	Notothyladaceae	Inaperturate	8.5
Erica arborea	Ericaceae	Tricolpate	6.3
Vitex doniana	Lamiaceae	Tricolpate	5.4
Gerradina foliosa	Gerrardinaceae	Tricolporate	12.8
Acidanthera brevicollis	Iridacaeae	Monocolpate	7.2

Table 20: Plant sources, types and frequency of pollens obtained from Somaarin honey

Plant species	Family	Pollen type	Frequency (%)
Acidocarpus macrocalyx	Malpighiaceae	Tricolporate	13.8
Typha augustifolia	Typhaceae	Monoporate	23.6
Pteleopsis diptera	Combretaceae	Heterocolporate	8.3
Typha capensis	Typhaceae	Monoporate	18.1
Lannea acida	Anacardiaceae	Tricolporate	17.3
Indigofera leptoclada	Fabaceae	Tricolporate	8.7
Xyris Montana	Xyridaceae	Monocolpate	10.2

Table 21: Plant sources, types and frequency of pollens obtained from Odo eran honey

Plant species	Family	Pollen type	Frequency (%)
Typha augustifolia	Typhacae	Monoporate	26.3
Terminalia aemula	Combretaceae	Heterocolporate	13.4
Acacia eglengii	Fabaceae	Tricolporate	6.7
Lannea acida	Anacardiaceae	Tricolporate	4.9
Acidanthera brevicollis	Iridaceae	Monocolpate	20.7
Elaeis guineensis	Arecaceae	Monocolpate	19.8
Celtis integrifolia	Cannabaceae	Triporate	8.2

Table 22: Plant sources, types and frequency of pollens obtained from Sanni honey

Plant species	Family	Pollen type	Frequency (%)
Acridocarpus macrocalyx	Malpighiaceae	Tricolporate	24.6
Entada abyssinica	Fabaceae	Tricolporate	19.8
Polygonum lapathifolum	Polygonaceae	Pantoporate	13.2
Anthosthema senegalense	Euphorbiaceae	Tricolporate	7.9
Xyris Montana	Xyridaceae	Monocolpate	14.5
Phaeoceros laevis	Notothyladaceae	Inaperturate	3.2
Parkia inundabilis	Fabaceae	Tricolpate	16.8

Table 23: Plant sources, types and frequency of pollens obtained from Aleshinloye honey

Plant species	Family	Pollen type	Frequency (%)
Parkia inundabilis	Fabaceae	Tricolpate	23.9
Acidanthera brevicollis	Iridaceae	Monocolpate	16.6
Terminalia brownii	Combreataceae	Heterocolporate	10.3
Gerradina foliosa	Gerrardinaceae	Tricolporate	14.2
Tylophora sylvatica	Apocyanaceae	Inaperturate	7.4
Trema guineesis	Cannabaceae	Diporate	27.6

Table 24: Plant sources, types and frequency of pollens obtained from Agara honey

Plant species	Family	Pollen type	Frequency (%)
Tilia Americana	Tiliaceae	Tricolpate	8.7
Erica arborea	Ericaceae	Tricolpate	13.5
Celtis integrifolia	Cannabaceae	Triporate	9.6
Elaeis guineensis	Arecaceae	Monocolpate	12.4
Combretum gueinzii	Combretaceae	Heterocolporate	3.5
Ipomoea ochracea	Convolvulaceae	Periporate	17.1
Gomphrena celosioides	Amaranthaceae	Pantoporate	14.5
Carya spp.	Juglandaceae	Dicolporate	5.3
Justica cordata	Acanthaceae	Dicolporate	15.4

3.2. DISCUSSION

The results of the microscopic analysis of the honey from the 24 studied areas demonstrated abundant and diversified pollen composition of the honey samples examined. The excess of bees visiting or foraging many plants may be connected to the fact that pollen is the only proteic food within the beehive; as a consequence, the pollen plays an important role in feeding the colony. In fact, pollen is used for feeding the larvae and the young bees. It contributes to body growth in general and is a determining factor in the development and the functionality of certain organs such as the adipose body, ovaries and in particular the hypopharingeal glands; these glands play an important role in Royal jelly secretion; royal jelly is used for feeding the larvae for the first three days of their life and provides the queen bee with necessary nourishment. In line with this, most of the families of plants recorded in this study have been reported to be visited by bees (Shubharani *et al*., 2013). It is thus shown in this study that bees visit plants regardless of their habits and habitat. The same notion was earlier reported by Adeonipekun (1989). In addition, bees prefer to visit plants with good nectar and attractive flora (Ige and Apo, 2007); though there are taxa that are non-nectariferous in nature e.g. Poaceae whose pollens are also observed in the honey samples from Apata area.

Most commercially available honey is blended, meaning it is a mixture of two or more honeys differing in floral source, colour, flavour, density or geographic origin. Meanwhile, honey sample from the 16 samples without pollens are adulterated and honey samples from the 24 pure samples are not blended but are polyfloral honey or wildflower honey i.e. derived from the nectar of many types of flowers. Generally, honey is classified by the floral source of the nectar from which it was made. Honeys can be from specific types of flower nectars or can be blended after collection. The pollen in honey is traceable to floral source and therefore region of origin (AbdulRahaman *et al*., 2013).

In the estimation of pollen grain frequencies, the 24 pure honey samples in this work fall within the category: "secondary or accompanying pollen" (16-45%), "important minor pollen or important isolated pollen" (4 – 15% pollen) and "rare pollen or isolated pollen" (less than 3% pollen) (Vergeron, 1964). Three taxa in Apata area honey are in "secondary or accompanying pollen" ranging from (28.3% pollen frequency in Gerradinaceae -*Gerradima foliosa* to 15.6% in Euphorbiaceae- *Tetrochidium didymostemon*), four taxa are in the category "important minor pollen" and the last taxa falls to the category "minor or isolated pollen". The pollen grain of a honey sample of Kasumu area has three taxa belonging to the category "secondary or accompanying pollen" and five taxa in the category "important minor pollen or important isolated pollen". Pollen grains from Lafenwa honey have five taxa in "secondary or accompanying pollen" and the remaining taxa falls in the category of the "important minor pollen". Pollen grain of honey samples from areas of Molete high school, Moor plantation, Ologede, Oremeji, Alegongo, Itoku, Iyana-cele, Majekodunmi, Basorun, Obantoko, Oluyole,

Osiele, Randa, Somaarin, Odo-eran, Sanni, Aleshinloye, and Agara has their pollen grains in the category of "secondary or accompanying pollen", and "important minor pollen or important isolated pollen". Pollen grains from areas of the University of Ibadan, Atinsola, and Crescent University have their pollen grains in the category: "secondary or accompanying pollen", "important minor pollen or important isolated pollen" and "minor or isolated pollen". The number of granules examined depends on the degree of precision required when estimating the PK (pollen grain) frequencies. For an indicative sample evaluation, the computation of about 100 PK (PK = pollen grain) should be sufficient. It is necessary to count 200-300 PK to determine the frequency classes. For a precise percentage calculation 1000-1200 PK have to be counted (Vergeron, 1964). Thus the honey samples were considered rich, poor and extremely poor in number of pollen grain per 1gm of honey samples if 1200, 1000 and below 1000 pollens were counted. This is a modification to Maurizio (1975). Based on this, the pollen grains of *Gerradina foliosa* in Apata honey, *Tilia americana* in Kasumu honey, *Acidanthera brevicollis* and *Piptadenia africana* in Lafenwa honey, *Typha capensis* and *Typha augustifolia* in Molete High School honey, *Clausena anisata* in Moor plantation honey, *Berlinia bifoliata Hymenocardia acida* in Ologede honey, *Acidanthera brevicollis*, *Hymenocardia acida*, and *Lannea acida* in Oremeji honey, *Typha capensis* and *Turraeanthus africana* in University of Ibadan honey, *Elaeis guineensis* in Alegongo honey, *Alchornea cordifolia* and *Hymenocardia acida* in Atinsola honey, *Piptadenia africana* in Itoku honey, *Vitex doniana and Acridocarpus macrocalyx* in Iyana-cele honey, *Alchornea cordifolia* and *Indigofera leptoclada* in Obantoko honey, *Agrimonia eupatoria* and *Piptadenia africana* in Oluyole honey, *Celtis integrifolia* in Randa honey, *Acridocarpus macrocalyx* in Sanni honey and *Parkia inundabilis Trema guineensis* in Aleshinloye honey are considered rich and produced rich pollens.

4. CONCLUSIONS

The pollen composition of the honey samples in this study has shown that honeybees travel a considerable distance in search of suitable food materials (nectar) for their survival and production of honey. The presence of the pollen grains in the 24 honey samples is a clear indication that the honeys are not adulterated but pure and they are not unifloral but multifloral, and the absence of pollen grains in the remaining 16 honey samples is an indication that the honey samples are adulterated and not pure.

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