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Melissopalynological Study of Honey in some selected areas of Offa, Kwara State, North Central, Nigeria

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Abstract
Melissopalynology provides critical insights into the botanical and geographical origins of honey, supporting authentication, quality control, and ecological studies. This study examined thirty honey samples collected from twelve locations in Offa, Kwara State, Nigeria, between November 2012 and January 2013, using acetolysis, potassium hydroxide, and water-wash techniques. Microscopic analysis identified 108 plant species from 59 families, with a total of 32,539 pollen grains counted across all samples. All honeys were multifloral, with dominant contributions from Myrtaceae, Euphorbiaceae, Anacardiaceae, and Combretaceae. Notable species such as *Psidium guajava*, *Elaeis guineensis*, and *Carica papaya* were recurrently represented. Pollen counts varied markedly, ranging from 2,181 grains in Unilorin honey to 482 grains in Oja-Oba honey per five fields of view. Statistical analyses (ANOVA and Duncan's Multiple Range Test) revealed significant spatial variation in pollen frequencies, reflecting both regional floristic diversity and honeybee foraging preferences. The presence of pollen from both cultivated and wild savannah species underscores the ecological role of honeybees in linking natural and agricultural landscapes. These findings not only authenticate the multifloral nature of Offa honeys but also provide a baseline for detecting adulteration and monitoring honey quality in Nigeria. Overall, this study demonstrates the value of melissopalynological analysis as a robust tool for apicultural research, conservation of floral resources, and the promotion of honey as a high-value product for both local and international markets.

Keywords: Melissopalynology, Pollen analysis, Honey authentication, Multifloral Honey, Botanical origin, Offa, Nigeria,
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Introduction

Melissopalynology, the scientific analysis of pollen found in honey, is a fundamental technique for determining a honey's botanical and geographical provenance (Sahney *et al.*, 2018; Louveaux *et al*., 1978). By microscopically examining the sediment within honey samples, analysts can identify the plant species from which bees foraged, as each species produces pollen with unique morphological and genetic markers (Panseri *et al*., 2013; Chauhan & Trivedi, 2011). This method offers a more accurate assessment of nectar and pollen sources than field observations of bee activity, making it an indispensable tool for apicultural development (Begum *et al*., 2021). Based on its pollen composition, honey is categorized as either monofloral (derived mostly from one plant) or multifloral (originating from several species) (Louveaux *et al*., 1978; Addi & Bareke, 2021).

In Ethiopia, applying this knowledge of bee forage diversity and flowering cycles is vital

for enhancing honey production (Zekarias *et al*., 2016). This requires evaluating agroecological zones to identify available bee plants and create flowering calendars, which are essential for effective colony management. Factors like temperature and humidity directly impact nectar secretion, pollen production, and flowering times, thereby governing forage availability and ultimately determining honeybee colony productivity and development throughout the seasons (Tura & Admassu, 2018; Ambaw *et al*., 2021)

By analyzing the pollen in honey, it is possible to identify the geographical origin based on the presence of characteristic pollen combinations unique to specific locations (Louveaux *et al*., 1978). Furthermore, melissopalynology allows for taxonomic identification of the plant genera visited by honeybees, although honey may also contain airborne pollen from anemophilous plants, spores, and dust particles due to the electrostatic charge on the bee's body. The information derived from honey samples is invaluable for confirming the authenticity of honey sources, quality control, and detecting adulteration (Maurizio, 1951; Molan, 1998; Louveaux *et al*., 1978; Terrab *et al*., 2003).

The plant sources of honey are essential in determining its value. Monofloral honey, derived from a single plant species, is often more prized than bifloral or heterofloral honey, which comes from multiple bee-pollinated plants (Addi & Bareke, 2021). However, honey produced by free-flying bees is rarely entirely unifloral unless the bees are specifically exposed to a single plant species in apiaries. Beyond its economic and culinary importance, honey holds cultural and medicinal significance. In Nigeria, the three major ethnic groups—Yoruba, Igbo, and Hausa—use honey in various cultural practices such as naming ceremonies, paying bride price, and as gifts. Historically, honey has been utilized for over 2,700 years to treat ailments through topical application, with its antiseptic and antibacterial properties only recently being scientifically elucidated (Jusbin, 1996; Abdulla & Abdul-Aziz, 1998; Wahdan, 1998). In recent years, honey has shown effectiveness in combating drug-resistant biofilms, which are implicated in chronic rhinosinusitis.

In addition to its use as a food supplement, honey has gained global recognition for its medicinal properties, which are largely attributed to the integration of bioactive compounds from the pollen and nectar of medicinal plants that bees forage on (Molan, 2001). The growing interest in melissopalynology is reflective of honey's evolving role not only as a natural sweetener but also as a therapeutic agent in treating various health conditions.

The aim of this study is to conduct a melissopalynological analysis of honey samples collected from various locations in Offa, Kwara State, Nigeria, to identify the pollen types present and determine their botanical and geographical origins. The study seeks to provide evidence of the diversity of plant species visited by honeybees in the region, including the identification of key pollen types and families. The findings will contribute to understanding the floral sources of honey and support claims regarding honey authenticity and quality control.

Materials and Methods

Sample Collection

Honey samples were collected randomly from 12 locations in Offa, Kwara State, Nigeria: Owode-Offa, Oja-Oba, Isale-Ago, Eesa, Popo, Oyun-Offa Road, Ipee, Osunte, Poly Gate, Health Tech, Sanni-Abba, and Olorun O Shebi. These samples were gathered on October 1st, 2012 (first 5 samples) and December 23rd, 2012 (last 7 samples) for melissopalynological analysis.

Melissopalynological Analysis

Three methods were used for the melissopalynological analysis of honey samples. In the acetolysis method, five grams of honey from eight locations were mixed with distilled water, centrifuged, and treated with glacial acetic acid. Acetolysis mixture (acetic anhydride and sulfuric acid) was added, boiled in a water bath, and centrifuged again. The residues were washed with distilled water and mounted in glycerin for microscopic observation (Ahmad *et al*., 2025; Smith, 2024).

The second method was potassium hydroxide method followed similar steps with honey samples from three locations, where glacial acetic acid and 10% potassium hydroxide (KOH) were used. After centrifugation and washing, the samples were mounted in glycerin Jones (2024); Reis *et al.* (2023).
For the normal water washing method, honey from Sanni Abba was mixed with distilled water, centrifuged, and treated with glacial acetic acid. After several washes, the residue was stored in glycerin Jones (2024); Nunes *et al*. (2024).

Slide Preparation and Observation
Honey samples were smeared onto microscope slides. A micropipette was used to transfer a small portion of the sample, which was then mounted in glycerin. Cover slips were placed over the slides, sealed with nail varnish to prevent desiccation, and observed under a microscope. The pollen count was recorded, and identification was done using pollen atlases and floras Ahmad *et al*. (2025); Smith (2024). Photomicrographs were taken using a Kodak Easyshare C913 Digital camera.

Percentage pollen frequency
The percentage pollen frequency is determined using this formula:

$$\% \text{ frequency} = \frac{\text{total number of pollens of a particular species}}{\text{Total number of all observes pollen}} \times 100$$

Results and Discussion
Microscopic analysis of twelve honey samples collected from different locations in Offa, Kwara State, revealed a total of 32,539 pollen grains, representing 102 pollen populations, 20 pollen types, 39 families, and 59 plant species (Table 1; Figures 1–3). The pollen grains varied in shape, size, and aperture type, confirming the multifloral nature of all samples, a finding consistent with earlier melissopalynological studies in tropical Africa (Aina *et al*., 2015; Adeonipekun, 1989). Additional non-pollen particles such as dust and debris were also observed, highlighting the importance of microscopy in assessing honey purity (Molan, 1998).

Table 1: Percentage frequency of the plant species collected

Pollen Types	*Species*	Family	Frequency	ANOVA
Eesa				
Diporate	*Justicia americana*	Acanthaceae	6.9	12.400^{cd}
Tricolporate	*Terminalia cattappa*	Combretaceae	10	17.800bcd
Panporate	*Jatropha curcas*	Euphorbiaceae	10.2	18.000bcd
Monosulcate	*Eichhornia crassipes*	Pontederiaceae	1.5	2.600d
Triporate	*Morella cerifera*	Myricaceae	12.5	22.200abcd
Tricolporate	*Bacopa monnieri*	Schrophulariaceae	15.6	27.600abc
Periporate	*Ipomoea batata*	Convolvucaeae	18.4	32.600ab
Tricolpate	*Elaeis guneensis*	Arecaceae	22.5	39.800a
Tricolpate	*Alchonea cordifolia*	Euphorbiaceae	2.4	4.200d
Health Tech				
Triporate	*Betula pendula*	Betulaceae	5.2	10.000de
Tricolpate	*Alchornea cordifolia*	Euphorbiaceae	5.4	17.000cde
Panporate	*Xanthum italicum*	Asteraceae	1.2	17.400cde
Monoulcerate	*Typha latifoila*	Typhaceae	7.2	23.200bcde
Synocolprate	*Melaleuca quinqueneva*	Myrtaceae	8.3	26.800bcde
Tricolporate	*Viola occulia*	Violaceae	3	3.800e
Monoporate	*Setaria parviflora*	Poaceae	9.9	32.400bcd
Heterocolporate	*Heliotropium polyphyllum*	Boraginaceae	11.4	36.800abc
Triporate	*Morella cerifera*	Myricaceae	13.8	40.800abc
Tricolporate	*Eupatorium serotinum*	Asteraceae	14.9	48.200ab

Zonocolpate	*Melastoma polyanthum*	Melastomataceae	18.4	59.800a
Ipee sample				
Tricolporate	*Quercus laurifolia*	Fagaceae	5.8	12.400cd
Monoporate	*Zea mays*	Poaceae	5.9	12.800cd
Periporate	*Amaranthus spinosus*	Amaranthaceae	7.7	16.400bcd
Tricolporate	*Ricinus comunis*	Euphorbiaceae	1.2	2.600d
Triporate	*Casuarina equisetifolia*	Casuarinaceae	10.9	23.400abcd
Tetracolporate	*Tridax procumbens*	Asteraceae	13.5	28.800abc
Synocolporate	*Vitis rotundifolia*	Vitaceae	15.8	33.800abc
Tricolporate	*Psdium guajava*	Myrtaceae	18.4	39.200ab
Tricolporate	*Legucularia racemosa*	Combretaceae	20.7	44.200a
Isale Ago				
Tricolporate	*Triplochiton scleroxylon*	Steculiaceae	4.8	12.000bc
Triporate	*Cephatalus occidentale*	Rubiaceae	6.3	15.600bc
Ulcerate	*Taxa bacata*	Taxaceae	7.3	18.000abc
Tricolporate	*Schinus terebinthifolius*	Anacardiaceae	0.8	2.000c
Tricolporate	*Solanium americanum*	Solanaceae	9.2	22.800abc
Tricopate	*Sygzium guineese*	Myrtaceae	9.9	24.600abc
Triporate	*Vigna luteola*	Fabaceae	11.4	28.000abc
Zonoporate	*Genostoma spp.*	Loganiaceae	1.5	3.800c
Tricolporate	*Bacopa monnieri*	Schrophulariaceae	13.7	33.800ab
Periporate	*Ipomoea batata*	Convolvucaeae	14.3	35.2000ab
Zonocolpate	*Melastoma polyanthum*	Melastomataceae	18	44.4000a
Triporate	*Betula pendula*	Betulaceae	2.5	6.200c
Oja Oba				
Heterocolporate	*H.polyphyllum*	Boraginaceae	9.5	14.400abc
Inaperturate	*Peltandra virginica*	Araceae	11.9	18.200abc
Tricolporate	*Cassia occidentalis*	Fabaceae	15.5	23.400abc
Tricolporate	*Hydroctyle sp*	Apiaceae	16.2	23.800abc
Tricoporate	*Elaeis guineensis*	Arecaceae	17.3	26.000ab
Tricoporate	*Caspicum annuum*	Solanaceae	20.1	30.600a
Triporate	*Corlylus avellana*	Cannabaceae	2.7	4.200c
Tricolporate	*Terminalia cattappa*	Combretaceae	6.4	9.600b
Olorun O Shebi				
Ulcerate	*Cyperus haspen*	Cyperaceae	3.7	10.400d
Periporate	*Ipomoea batata*	Convolvulaceae	6.9	19.600cd
Tricolporate	*Vitis rotundifolia*	Vitaceae	8.2	23.400bcd
Stephanocolporate	*Waltheria indica*	Steculiaceae	11.3	32.000abcd
Tricolporate	*Rhizophora mangle*	Rhizophoraceae	14.3	40.600abc
Triporate	*Vigna luteola*	Fabaceae	16	45.600abc
Diporate	*Morus rubra*	Moraceae	18	52.400ab
Monosulcate	*Crinum americanum*	Liliaceae	21.7	56.000a
Osunte				
Diporate	*Morus rubra*	Moraceae	4	10.400e
Monoporate	*Eragrostic elliotti*	Poaceae	6	15.600de
Synocolporate	*Melaleuca quinquenervia*	Myrtaceae	7.8	20.200cde
Tricolporate	*Hydrocotyle sp*	Apiaceae	9.8	25.400bcde
Tricolporate	*Vitis rotundifolia*	Vitaceae	14.3	37.000abcd
Tricolpate	*Quercus laurifolia*	Fagaceae	16.3	42.200abc
Heterocolpate	*Lythum alatum*	Lythraceae	18.6	48.000ab

Panporate	*Jatropha curcas*	Euphorbiaceae	1.9	5.000e
Parasynocolporate	*Guio gracillus*	Sapindaceae	21.1	54.400a
Owode Sample				
Tricolpate	*Psidum guajava*	Myrtaceae	7.6	10.800abc
Zonocolpate	*Melastoma polyanthum*	Melastomataceae	8.8	12.400abc
Tricolporate	*Elaeis guineensis*	Arecaceae	12.9	18.200abc
Dicolpate	*Citrus* sp.	Rutaceae	1.8	2.600c
Tricolporate	*Terminalia cattappa*	Combretaceae	15.8	22.200abc
Triporate	*Rhus copallinum*	Anacardiaceae	21.1	29.600ab
Tricolporate	*Cephatalus occidentale*	Rubiaceae	2.7	3.800c
Parasyncolporate	*Guio gracilis*	Sapindaceae	24.5	34.400a
Tricolporate	*Caspicum annuum*	Solanaceae	4.5	8.400bc
Oyun				
Periporate	*Sida cordifolia*	Malvaceae	5.4	11.000bc
Diporate	*Justicia americana*	Acanthaceae	8.5	17.000abc
Triporate	*Casuarina equisetifolia*	Casuarinaceae	10.6	21.600abc
Tricolpate	*Psdium guajava*	Myrtaceae	15.2	31.000abc
Tricopate	*Quercus laurifolia*	Fagaceae	17.8	36.400ab
Tetracolporate	*Phylanthus tenellus*	Euphorbiaceae	2.3	4.800c
Tetracolporate	*Sonchus oleraceus*	Asteraceae	20	41.000ab
Inaperaturate	*Peltandra virginica*	Araceae	22.2	45.400a
Poly gate				
Heterocolporate	*Conocarpus erecta*	Combretaceae	5.7	10.400ab
Tricolporate	*Desmodium paniculatum*	Fabaceae	8.5	15.600ab
Triporate	*Casuarina equisetifolia*	Casuarinaceae	11	20.200ab
Tricolporate	*Ricinus comunis*	Euphorbiaceae	17.3	31.200ab
Stephanocolporate	*Waltheria indica*	Sterculiaceae	17.3	31.800ab
Tricolpate	*Psdium guajava*	Myrtaceae	17.4	32.000ab
Tricolporate	*Carica papaya*	Caricaceae	19.9	36.000a
Monosulcate	*Crinum americanum*	Liliaceae	3.2	5.8b
Popo				
Triporate	*Euroshinus elegans*	Anacardiaceae	7.8	12.000c
Heterocolporate	*Heliotropium polyphyllum*	Boraginaceae	14.7	22.400bc
Tricolpate	*Quercus laurifolia*	Fagaceae	17.3	26.400abc
Diporate	*Rhizophora mangle*	Rhizophoraceae	27.7	42.200ab
Tricolporate	*Baccharis spp.*	Astereaceae	32.2	49.200a
Sanni Abba				
Monosulcate	*Eichhornia crassipes*	Pontederiaceae	9.8	16.800b
Tricolporate	*Carica papaya*	Caricaceae	10.7	18.400b
Monoporate	*Andropogon virginicus*	Poaceae	16.9	29.000ab
Periporate	*Ipomoea batata*	Convolvucaceae	17.7	30.400ab
Tricolporate	*Ricinus communis*	Euphorbiaceae	20.7	35.600ab
Tricolpate	*Psdium guajava*	Myrtaceae	24	41.200a

Values carrying the same letters along the column are not significantly different at $p<0.05$

Pollen Types and Diversity

Twenty pollen types were identified, including dicolpate, diporate, heterocolpate, heterocolporate, inaperaturate, monoporate, monosulcate, monoulcerate, panporate, parasyncolporate, periporate, stephanocolporate, synocolporate, tetracolporate tricolpate, tricolporate, triporate, ulcerate, zonocolpate and zonoporate forms. Tricolporate and triporate

pollens were the most common, corroborating findings from Morocco (Terrab *et al*., 2003) and Cameroon (Tchuenguem Fohouo *et al*., 2004), where these apertures dominate bee-collected honeys. Some pollen types, such as dicolpate, monosulcate, inaperturate and zonoporate, were restricted to single species, reinforcing the heterogeneous nature of pollen deposition in honey (Barth *et al*., 2023; Alves & Santos, 2024).

Species diversity varied across sites: Eesa and Health Tech locations exhibited the highest diversity (7–9 pollen types; 9–11 species), while Popo and Sanni Abba had the lowest (five species each). This uneven distribution mirrors reports from Nigerian apiaries, where honey composition is shaped by the patchy distribution of flowering plants and seasonal savannah dynamics (Sowunmi, 1976; Adeonipekun, 1989). A total of 108 species across 59 families were identified in all samples. Myrtaceae emerged as the most frequent family, largely due to *Psidium guajava* and *Syzygium guineense* (Table 1, Figure 2). Similar dominance of Myrtaceae has been documented in Mediterranean and tropical honeys (Terrab *et al*., 2003; Louveaux *et al*., 1978). Other key contributors included *Elaeis guineensis* (Arecaceae), *Carica papaya* (Caricaceae), and *Baccharis* spp. (Asteraceae), reflecting the combined importance of cultivated crops and wild savannah species in honey production.

Pollen Counts and Bee Foraging

Pollen counts ranged from 2,181 grains in Unilorin honey to 482 grains in Oja-Oba honey per five fields of view (Figure 4, Table1). Such variation reflects differences in nectar flow, flowering intensity, and bee foraging behavior. Honeybees often show floral constancy, favoring plants that produce abundant or high-quality nectar, even when alternative resources are available (Waser, 1986; Adekanmbi & Ogundipe, 2009). Conversely, low pollen counts may indicate resource scarcity, seasonal transitions, or adulteration, as suggested in samples 9, 10, and 26. This aligns with observations that adulterated honeys often show reduced pollen density or disproportionate representation of specific taxa (Lieux, 1980; Jones *et al*., 1998).

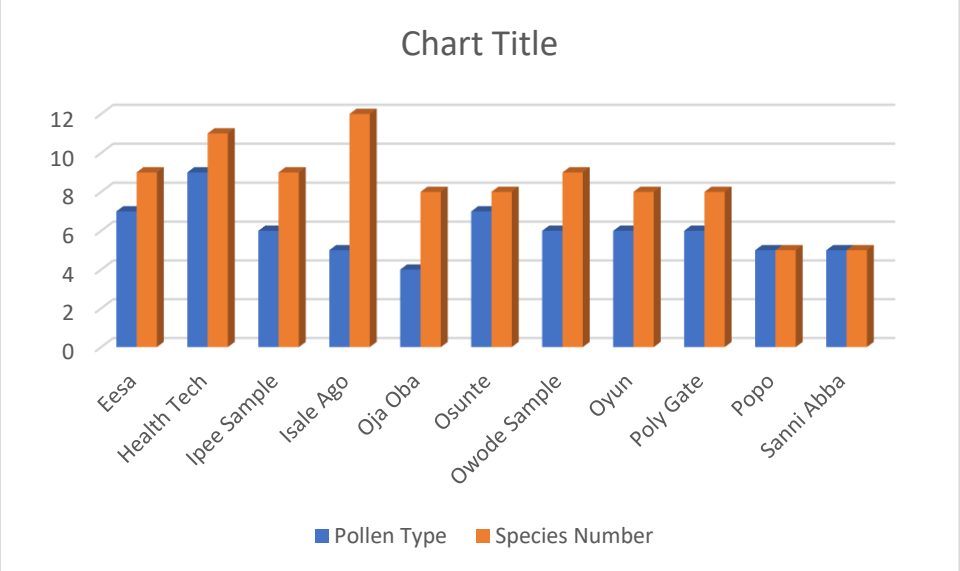
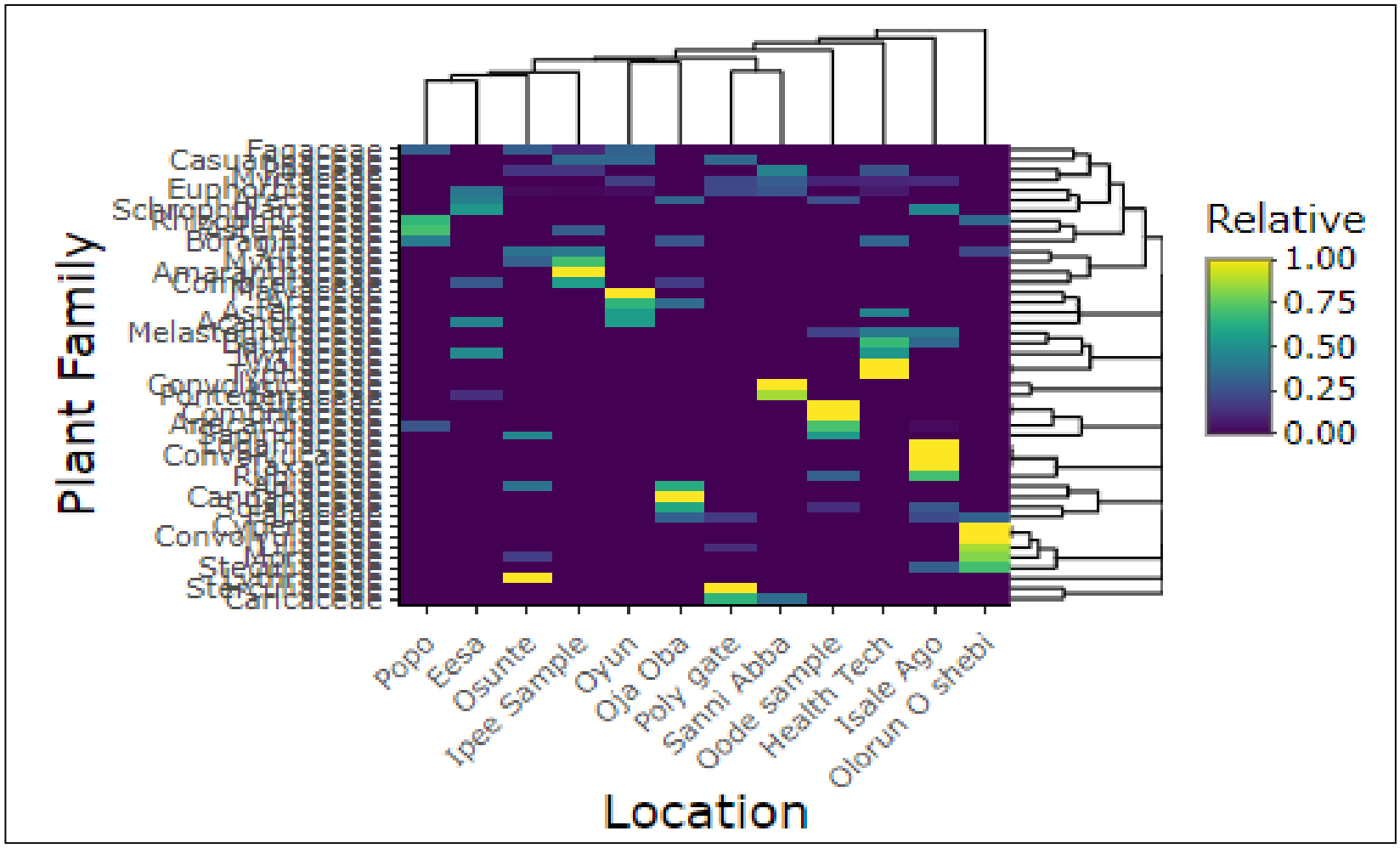


Figure 2: Chart showing the location against the pollen type and species number


Plant Family
Location
Relative
1.00
0.75
0.50
0.25
0.00
Popo
Eesa
Osunte
Ipee Sample
Oyun
Oja Oba
Poly gate
Sanni Abba
Oode sample
Health Tech
Isale Ago
Olorun O shebi

Spatial Variation and Statistical Patterns

Statistical analyses (ANOVA, Duncan's Multiple Range Test, and boxplots) demonstrated significant spatial variation ($p < 0.05$) in pollen frequencies across locations. Owode-Offa and Health Tech exhibited broad variability with outliers exceeding 20%, while Oja-Oba and Poly Gate showed narrower ranges and more consistent values. Outliers in Oyun and Ipee indicated occasional spikes in pollen frequency. Local dominance of *Baccharis* spp. in Popo (32.2% frequency; ANOVA 49.200a) and *Melastoma polyanthum* in Health Tech (18.4%; ANOVA 59.800a) illustrates how localized floral abundance strongly influences honey composition, as similarly reported in Ethiopian and Moroccan honeys (Fichtl & Adi, 1994; Terrab *et al*., 2003).

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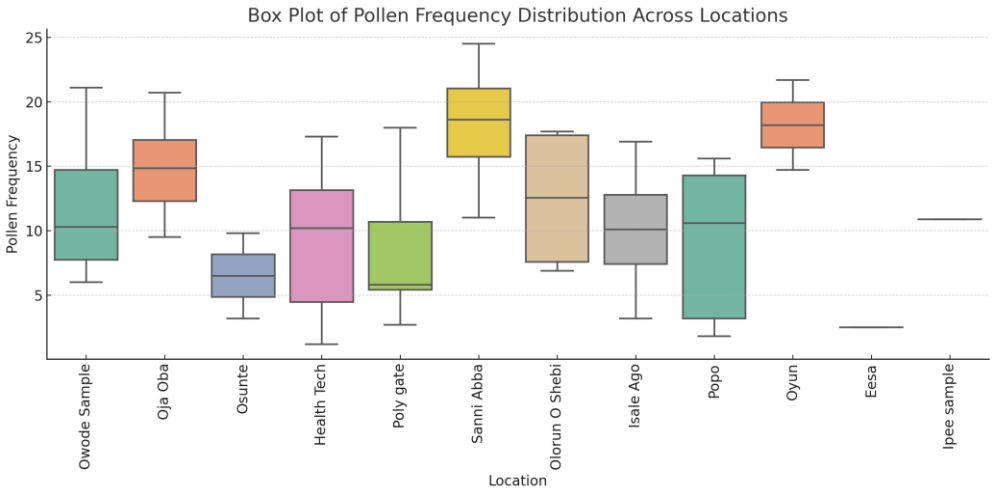

Figure 3: Box plot illustrates the distribution of pollen frequencies across several locations.

Ecological and Apicultural Implications

The dominance of families such as Myrtaceae, Euphorbiaceae, Asteraceae, and Fabaceae emphasizes their apicultural importance in the savannah landscape of North Central Nigeria (Figure 3). Seasonal vegetation dynamics marked by lush growth during rains and resource scarcity during the dry season likely shaped the observed patterns, consistent with studies on savannah apiculture (Agwu & Akanbi, 1985; Adeonipekun, 1989). The presence of both cultivated species (e.g., maize, papaya, sweet potato) and wild taxa underscores the role of bees as ecological connectors between agroecosystems and natural habitats.

Relevance for Honey Authentication

Melissopalynology is a widely recognized tool for honey authentication, quality assurance, and detection of adulteration (Louveaux *et al*., 1978; Molan, 2001). In this study, the combined application of acetolysis, KOH, and water-wash techniques enhanced pollen visibility, enabling identification to species level in many cases. While pollen analysis is not error-free, its integration with physicochemical and organoleptic methods provides a robust framework for determining honey origin and purity (Maurizio, 1951; Terrab *et al*., 2003).

Overall, this study establishes that honeys from Offa are distinctly multifloral, derived from both cultivated and wild savannah species. The variability in pollen frequency and diversity across sites reflects both ecological heterogeneity and bee foraging dynamics. These findings not only contribute to honey authentication in Nigeria but also highlight the broader significance of melissopalynology for apiculture, biodiversity conservation, and the promotion of Nigerian honey in international markets.

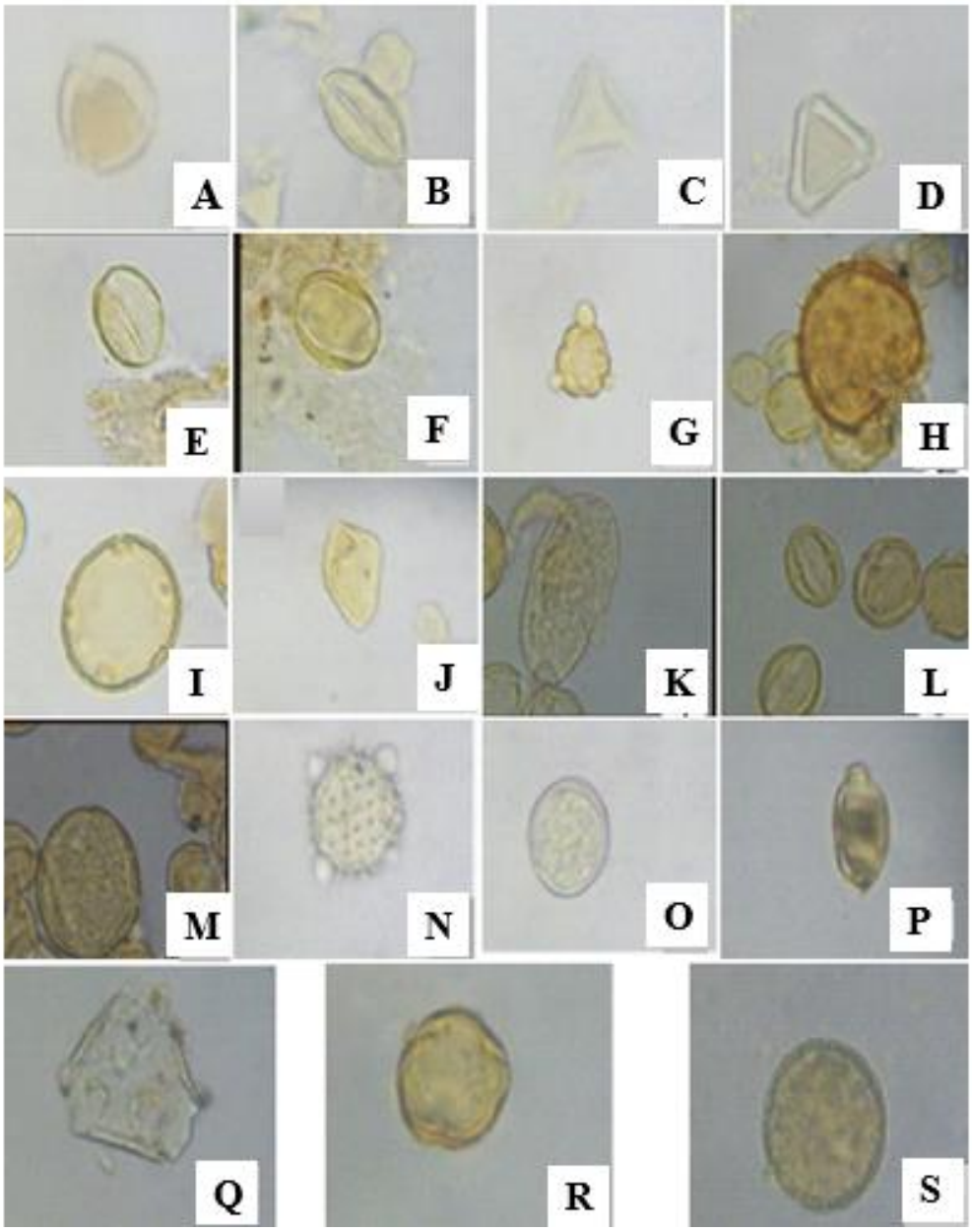

Figure 4: Pollen types in the twelve (12) locations in Ilorin North of Kwara State

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