

Palynology of honeycomb and a honey sample from an apiary in Lagos, Southwest Nigeria

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ABSTRACT

*Most reported studies on honey samples from Nigeria have come from those samples bought in the open market. Though palynomorphs recovered from them have shown that they were produced in the derived savanna, savanna and rainforest vegetation ecozones, it is necessary to study honey samples from honeycomb to eliminate the adulteration allegation and provide more accurate palynomorph data as well as shed light on the habit of bees. To achieve these, a study of honeycomb and a honey sample from an apiary in Lagos State, southwest Nigeria was carried out. The middle and edge sections of the comb were sampled and studied along with a pressed honey sample. Comparison between the two sections of the comb was also made. Data from the pressed honey was also compared with the comb data. In all, a total of 36 species of plant have their pollen represented. The middle section of the comb has higher abundance with lower diversity while the comb edge sample has higher diversity but lower abundance. The pressed honey has higher proportion of small sized pollen with a relatively higher diversity than the middle section but lower diversity than the edge portion. Pollen of *Kigelia africana* was recovered in a higher proportion than found in the two comb samples most likely resulting from secondary contamination. Generated data from this comb has revealed the palynomorph components of honey produced in the area and insight into the habit of the bees is also provided. Most of the traditional pollen types of Nigeria honey were faintly recovered from the studied samples. The higher diversity and lower abundance of the comb Edge assemblage indicate that production most likely started from the centre and moved outward.*

Keywords: palynomorphs, honeycomb, honey sample, pollen, apiary.

INTRODUCTION

Melissopalynology- the study of pollen and spores in honey – started with the pioneering work of Pfister in 1895, but made popular by workers such as Maurizio, 1951 and Maurizio and Louveaux, 1965 (Sowunmi, 1976). Presently, much is known all over the world about botanical and ecological origins of honey samples, the honey plants and honey quality due to the pioneering works of these workers. Even in Nigeria, Sowunmi (1976), Agwu and Akanbi (1995), Ayodele *et al.* (2006), and Njokuocha and Ekweozor (2007), Adekanmbi and Ogundipe (2009), Adeonipekun (2010), Ige and Modupe (2010) and Aina and Owonibi (2011) have contributed immensely to our present knowledge on the origin (botanical and geographical) and biochemistry of honey as well as quality determination of honey across the vegetation zones of the country.

Adeonipekun (2010) studied an apiary in Ibadan southwest Nigeria so as to get insight as to what bees do with pollen in hives. In this work, Adeonipekun (2010) studied pollen pellets and honey sample from an apiary and found

out that: bees would not travel far as long as there are good quality pollen close by; size of pollen determines their recovery, but not presence and abundance in honey; pollen is used for food by bees and not for honey production and that pollen recovered from honey is only a partial representation of the plants foraged. Findings from this work shed light on some habit of bees. Aina and Owonibi (2011) also studied pollen pellets from four apiaries in four different locations in Anyigba, Kogi State of Nigeria. They were able to establish the fact that some pollen recovered abundantly from the pellets they worked on were sourced by bees for food only since their flowers of their plants do not produce nectar while they copiously produce pollen. Such plants are *Elaeis guineensis*, the Astaraceae family/Tubiflorae types and Poaceae.

To further shed light on the habit of bees in honey production, a honeycomb from an apiary was studied along with a pressed honey sample. This was done to compare the diversity of pollen in parts of the comb as well as comparing them with data from the pressed honey packaged for sale.

MATERIALS AND METHODS

Materials for this work were obtained from an apiary – Bee Conservation Project Farms, Ijaye Ogba, Lagos, Southwest Nigeria. A honeycomb (Figure-1) and a honey sample extracted from the compressed comb were used. Sub-samples of honey from the middle and marginal parts of the honeycomb and pressed honey were collected into labeled test tubes. Three milliliters of glacial acetic acid was added.

Acetolysis was carried out according to Erdtman's (1969) method with nine parts Acetic anhydride and one part concentrated Hydrogen tetra-oxo-sulphate six acid (H_2SO_4). After much rinsing with water, 50% glycerine was used to rinse further. The resulting sediment was later stored in 100% glycerine. For quantitative microscopic study, 1.0 ml of 100% glycerine was added to all the residues. Twenty microlitres each of the resulting 1.8 ml (comb Middle), 1.6 ml (comb Edge) and 2.0 ml (Pressed sample) sediments and 100% glycerine was pipetted onto the slides and 'cover-slipped'.



Figure-1: The studied honeycomb

RESULTS

Pollen grains of 36 species of plant were recovered (Table 1). Some of the recovered pollen were identified to species and family levels while others which could not be identified even to family level were morphologically described. Those that could not be categorized at all were regarded as undetermined.

Comparing the comb parts, the middle section has higher abundance with lower diversity while the edge sample has higher diversity but lower abundance. The Pressed honey has higher proportion of small sized pollen with a relatively higher diversity than the middle section but lower diversity than the edge portion (Table 2). Pollen of *Kigelia africana* was recovered in a higher proportion in the Pressed honey than found in the two comb samples.

Most of the traditional pollen types of Nigeria honey such as *Elaeis guineensis*, *Nymphaea lotus*, *Combretum/Melastomataceae*, and *Syzygium guineensis* etc were faintly recovered from the studied samples while some unpopular types like cf. *Olacaceae* type and *Meliaceae* type dominated.

Charred Gramineae cuticles were recorded only in the Edge sample and incidentally, this sample contains the only record of *Poaceae* pollen. A sizeable quantity of fungal elements was found in all the samples while *Aulacisiera* sp. (Diatom frustule) was recovered only from the Pressed sample.

Table- 1: PALYNOMORPHS RECOVERED FROM COMB MIDDLE, EDGE AND PRESSED HONEY SAMPLES

SN	SPECIES NAME	COMB MIDDLE	COMB EDGE	PRESSED HONEY
1.	cf. <i>Olacaceae</i>	7191	4390	9183
2.	<i>Pterocarpus santalinoides</i>	1168	1080	1330
3.	Myrtaceae	8	38	10
4.	Meliaceae	1533	86	917
5.	<i>Kigelia africana</i>	588	27	748
6.	Palmae 1	4	1	2
7.	Palmae 2	-	5	2
8.	Palmae 3	-	5	6
9.	Palmae 4	1	-	-
10.	Palmae 5	2	-	4
11.	Cf. <i>Uapaca</i> spp.	4	2	14
12.	" <i>Verrutricolporate gemmatus</i> "	9	15	17
13.	<i>Combretum/Melastomataceae</i>	-	2	-
14.	<i>Berlinia grandifolia</i>	1	8	-
15.	Euphorbiaceae	9	5	3
16.	<i>Alchornea cordifolia</i>	7	5	1
17.	<i>Elaeis guineensis</i>	1	4	1
18.	<i>Cyperus</i> sp.	1	4	-
19.	<i>Nymphaea lotus</i>	2	4	4
20.	<i>Gardenia imperialis</i>	-	5	3
21.	<i>Parinari</i> sp.	-	2	2
22.	Pteridophyte spore	-	7	-
23.	<i>Syzygium guineensis</i>	-	1	-
24.	Papilionaceae	2	8	-
25.	<i>Bombax</i> sp.	-	9	-
26.	Verrumonocolpate pollen	-	4	-
27.	Pteridaceae	-	1	-
28.	Verrutricolpate pollen	-	11	6
29.	Polyad1	-	2	-
30.	Retitricolpate pollen	-	4	-
31.	Psiladiporate pollen	-	1	-
32.	Chenopod/Amaranth	-	1	-
33.	Poaceae	-	6	-
34.	Cf Commelinaceae	-	1	-
35.	Apocynaceae	-	6	-
36.	Cf <i>Baphia</i> sp.	-	4	8
37.	Dicotyledon cuticle	-	1	-
38.	Charred Gramineae cuticles	-	3	-
39.	<i>Alternaria</i> type spore	2	3	2
40.	Fungal spore	20	20	15
41.	Fungal hyphae	11	8	3
42.	<i>Aulacisiera</i> sp. (Diatom)	-	-	2
43.	Undetermined pollen	5	2	4

Table -2: PALYNOMORPH STATISTICS OF THE THREE SAMPLES

SAMPLES	MIDDLE COMB	EDGE COMB	PRESSED HONEY
ABUNDANCE	10,569	5,795	12,287
DIVERSITY	21	29	24

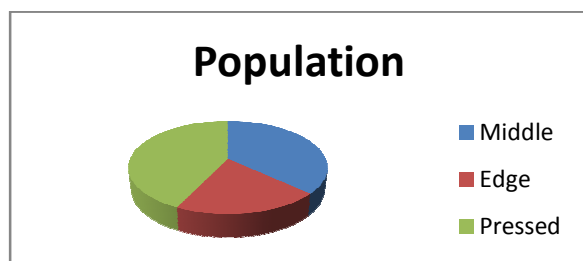


Fig.-2a: Pie Chart of Pollen Abundance in Honey Samples

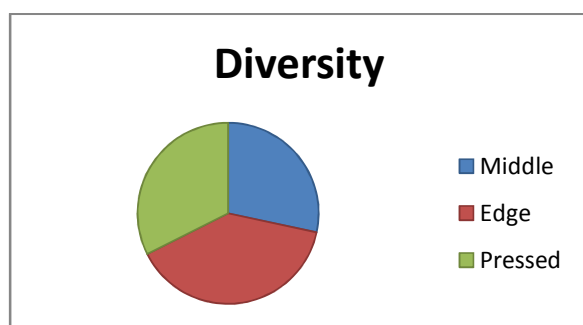


Fig.- 2b: Pie Chart of Pollen Diversity in Honey Samples

DISCUSSION

The total number of pollen of 36 species of plant found in this work is similar to previous findings from other works. Ige and Modupe (2009), Adeonipekun (2010) and Aina and Owonibi (2011) also recovered pollen of 36, 43 and 45 species of plants respectively from their Nigerian honey and pollen pellets/load samples. This high diversity is characteristic of the tropical climate vegetation where the apiary is sited. This is a major way to identify honey from outside the tropical areas.

Comparing the honeycomb parts, the middle section with higher abundance and lower diversity as well as the edge sample with higher diversity and lower abundance indicate that the bees most likely started filling the honeycomb from the center and later moved outwards. The high abundance and lower diversity resulted from the availability of abundant pollen of cf. Olacaceae, *Pterocarpus santalinoides* and Meliaceae. These three pollen types dominated the middle part. As the comb was being filled, other plants most likely started flowering and the bees occasionally visited those new pollen sources. This probably led to the reduction in abundance while diversity increased. In the Pressed honey, small sized pollen are common than in both samples from the comb. The pressing most likely reduced the chances of the large grains. Since the Pressed honey is a combination of both comb sections, it is expected that it should have highest diversity and highest abundance. It however has a higher diversity than the middle section sample while its abundance is highest overall.

The higher abundance of pollen of *Kigelia africana* in the Pressed honey than found in the two comb samples is an indication of secondary contamination. This is because being a large grain it should have been prevented by the pressing as inferred from the smaller sized pollen dominance of the Pressed honey. In support of secondary contamination, is the recovery of diatom frustule of *Aulacisiera* sp. only in the Pressed sample. Another reason in support of *Kigelia* recovery being due to secondary contamination is the fact that the comb honey samples recorded smaller proportions of this pollen that is abundant in the Pressed honey.

The abundant occurrence of cf. Olacaceae, Meliaceae and *Pterocarpus santalinoides* to the exclusion of some traditional honey pollen of Nigeria in this work is noteworthy. Even the few typical Nigerian honey pollen recovered were faintly present. Typical honey pollen of Nigeria include "*Lannea microcarpa*, *Senna spp*, *Daniellia oliveri*, *Parkia biglobosa*, *Hymenocardia acida*, *Lophira lanceolata*, *Syzygium guineensis*, *Parinari spp*, *Elaeis guineensis*,

Alchornea cordifolia and members of *Combretaceae/Melastomataceae*” Southeastern Nigeria Njokuocha and Ekweozor (2007); *Elaeis guineensis*, *Berlinia glandifolia*, *Tridax procumbens*, *Chromolaena odorata*, *Combretum* spp., *Nymphaea lotus* Southwest Nigeria (Adeonipekun, 2010) and *Parinari kerstingi*, *Lannea* spp., *Syzygium* sp. Poaceae, *Elaeis guineensis*, *Entada abyssinica*, *Butyrospermum paradoxum* North Central Nigeria Ige and Modupe (2009). Much of these were not recovered from this Lagos honey sample. The result from the present work shows that the Lagos honey has different characteristic pollen components compared to those of other areas of the country.

The dominating species in the present work are another addition to the list of pollen recovered from honey in Nigeria.

Recovery of Charred Gramineae cuticles only in the Edge sample with its incidental record of Poaceae pollen may mean that Poaceae is not a honey plant in the southern Nigeria except during the dry season or when other plants are not flowering. Ige and Modupe (2009) however recovered Poaceae in abundance in the North Central Nigerian honey they worked with. This must have been due to the fact that the area is mostly savanna vegetation dominated where grasses thrive more than any other plant group. Adeonipekun (1989) only recovered Poaceae pollen from pollen pellets and not from the produced honey during the wet season collection which could have been contributed by heavily cultivated *Zea maize* and by the grass - cf. *Panicum maximum*. During the dry season even when the grasses thrived, bees did not forage the grasses (Adeonipekun, 1989). Aina and Owonibi (2011) also recovered Poaceae in abundance from pollen pellets and ascribed their abundant recovery to its been collected by bees for food only.

A sizeable quantity of fungal elements was found in all the samples with the middle portion having highest proportion. This cannot be said to have resulted from secondary contamination since the least was found in the Pressed honey. It therefore got into the hives during honeycomb filling. However, the recovery of *Aulacasierra* sp. (Diatom frustule) from only the Pressed sample is an indication of secondary contamination during processing. This diatom is a constituent member of the aeroflora in Nigeria Adeonipekun and John (2011).

CONCLUSION

High diversity of the tropics is indicated in the honey produced from the Lagos area as found in honey samples of other parts of Nigeria.

Bee most likely started filling the honeycomb from the middle and later moved outwards to the frame due to the higher abundance and lower diversity of the middle portion honey from the comb compared to the edge.

Presence of higher proportion of *Kigelia africana* pollen and sole occurrence of diatom frustule in the Pressed Honey are indicative of secondary contamination during honey processing.

The characteristic honey plants of the Lagos area is different from other parts of Nigeria with the faintly occurrence of typical Nigeria honey pollen types while some unpopular ones such as cf. Olacaceae, Meliaceae and *Pterocarpus santalinoides* are over-represented. This new list of honey pollen from Lagos is a new addition to the Nigerian honey plant list.

Recovery of fungal spores from honey samples is found here not to be due to secondary contamination. Rather, the fungal materials find their way into honey during filling of the comb cells by bees.

MICROPHOTOGRAPHIC PLATES

PLATE-1:

Fig. 1: *Kigelia africana*, 2-4: *Pterocarpus santalanoides* x1000, 5 & 6: – Olacaceae, 7: “ Verrutricolporate pachydermatus”, 8 & 12 – “Psilatriscopolporate pachydermatus”, 10-11: Verrumonocolpate pollen, 13: Palmae 1, 14: *Nymphaea lotus*, 15: Chenopod/Amaranth, 16: Olacaceae x1000, 17: *Elaeis guineensis*, 18: *Pterocarpus santalinoides* x1000, 19: Pteridophyte spore. All x400, except otherwise indicated.

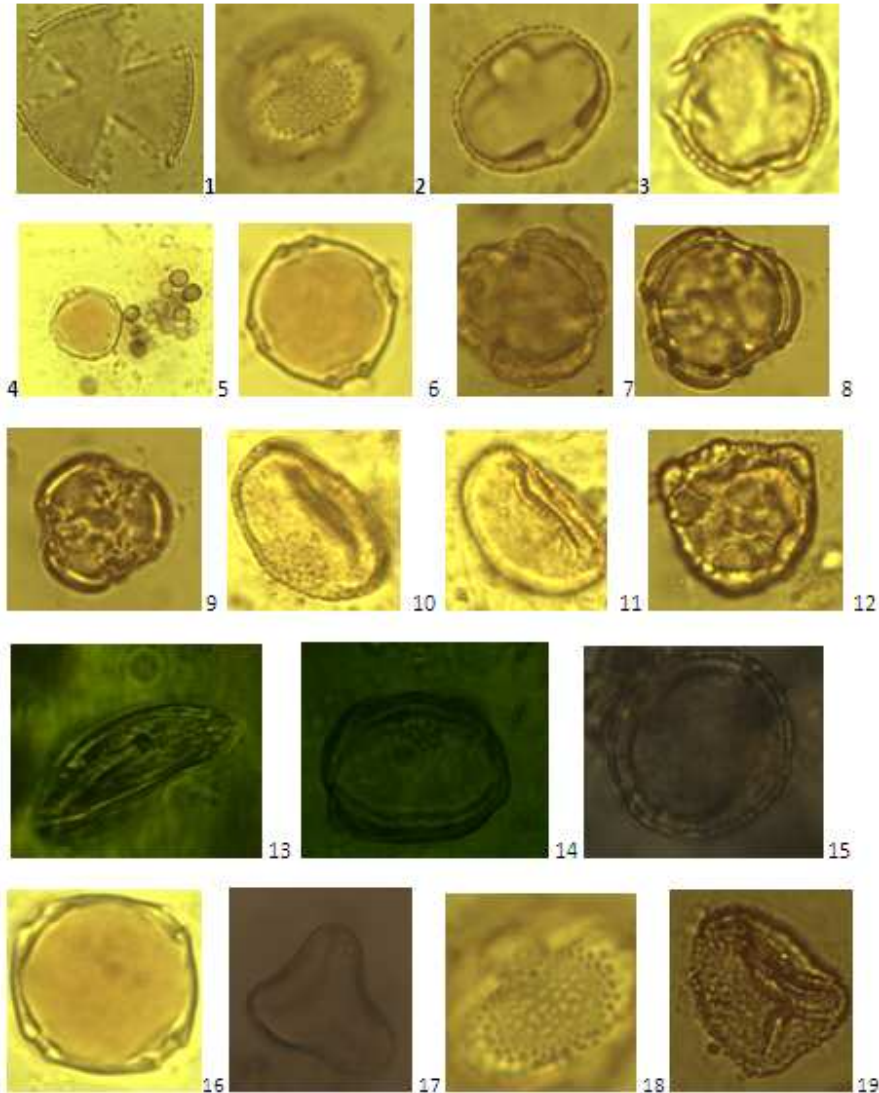


PLATE-2

Fig. 1: Verrumonocolpate pollen, 2-3 & 8: "Verrutricolporate pachydermtus", 4,5 & middle grain in 10: "Psilatriscopolporate pachydermtus", 6: Verrumonolete spore, 7: Meliaceae x1000, 8: "Verrutricolporate pachydermtus" x1000, 9: "Retitricolporate rugulatus" 2 grains and the lowermost pollen in 10, Uppermost pollen in 10 is Olacaceae, 11-12 & 14: *Nymphaea lotus*, 13: *Cyperus* sp. All x 400 except otherwise stated.

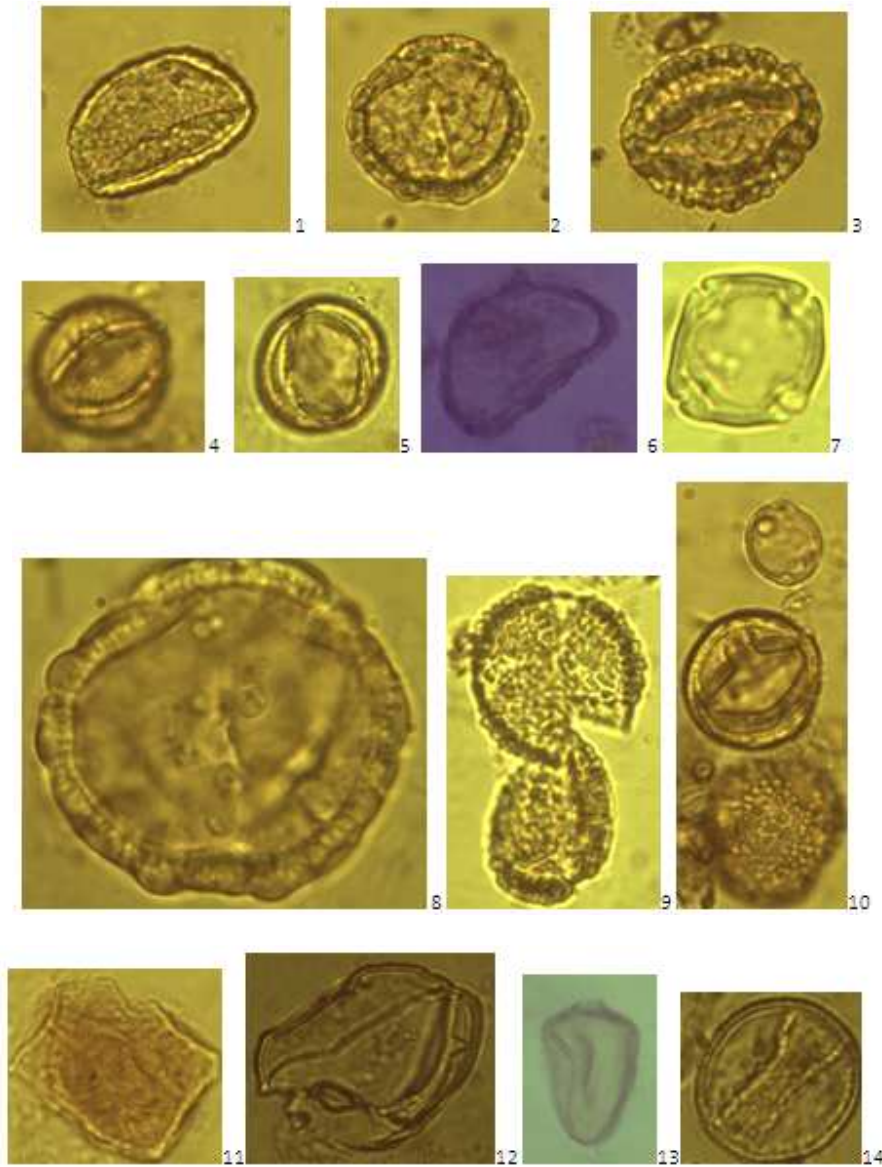


PLATE-3

Fig. 1: Verrutricolpate pachydermatus x1000, 2: *Gardenia imperialis* (one cell)x1000, 3: Retitricolporate pollen grains, 4: Three grains of Cyperus, 5: *Kigelia Africana*, 6: *Gardenia imperialis*, 7 & 8: *Psilatriscyncolporites pachydermatus* (2 pollen each) x400 except stated.

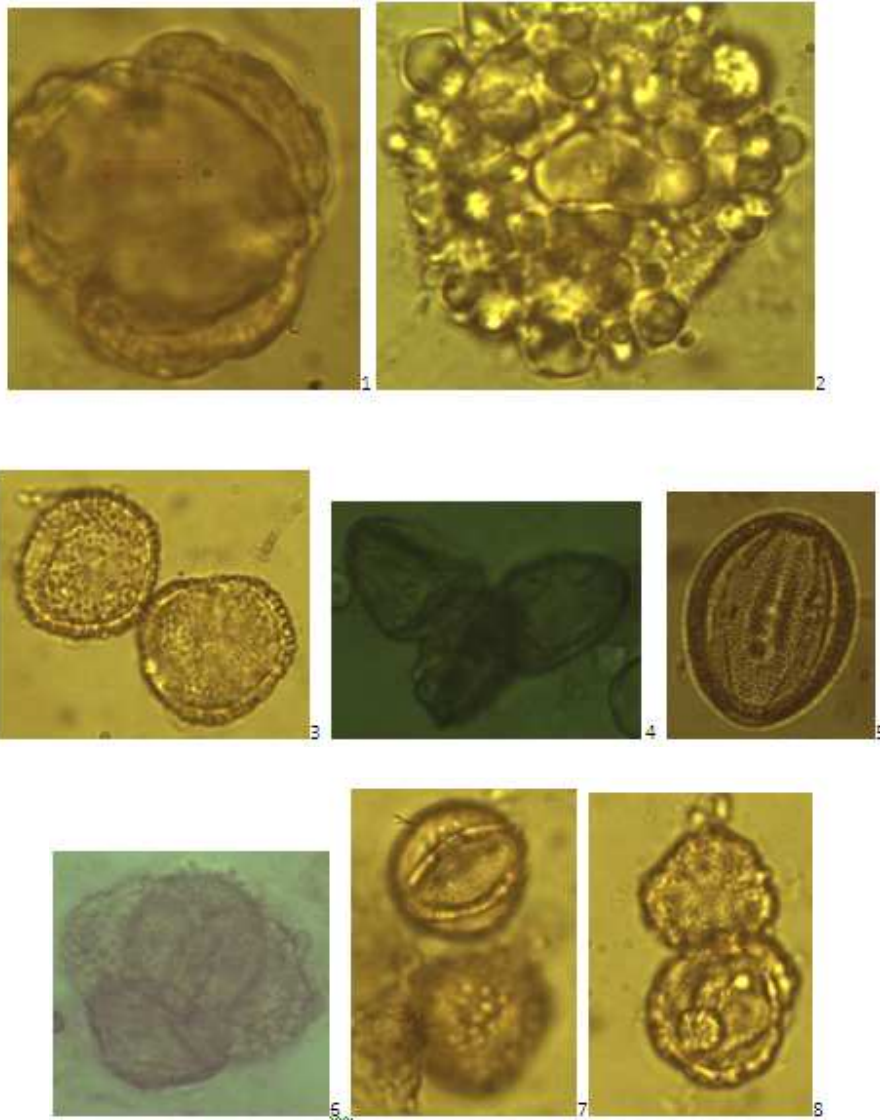


PLATE-4

Fig. 1& 2: Retitricolpate" irregularis", 3: Palmae -4 (1000), 4: Palmae-2 (1000), 5: Pteridophyte spore (1000), 6: *Gardenia imperialis*, 7: Fungal hyphae, 8: *Alchornia cordifolia*, 9: *Psilatriscyncolporites pachydermatus* (2 pollen), 10: Palmae – 3. All x400 except otherwise stated.

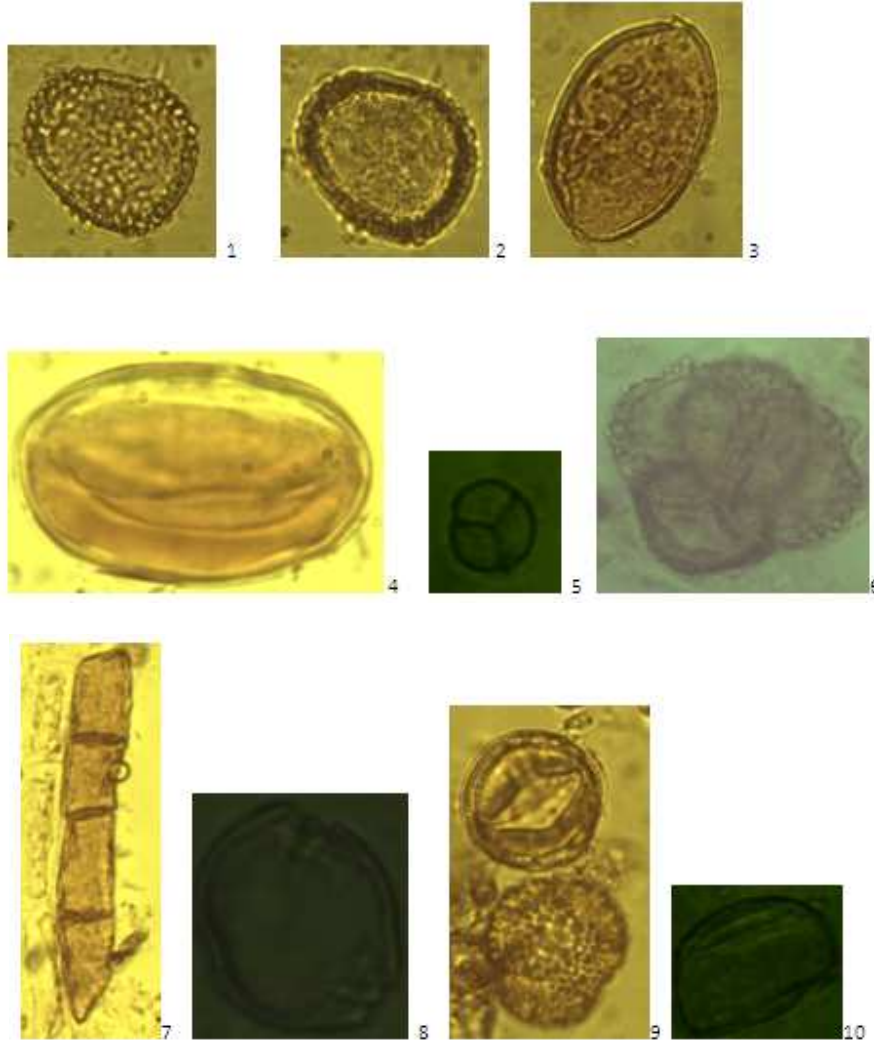
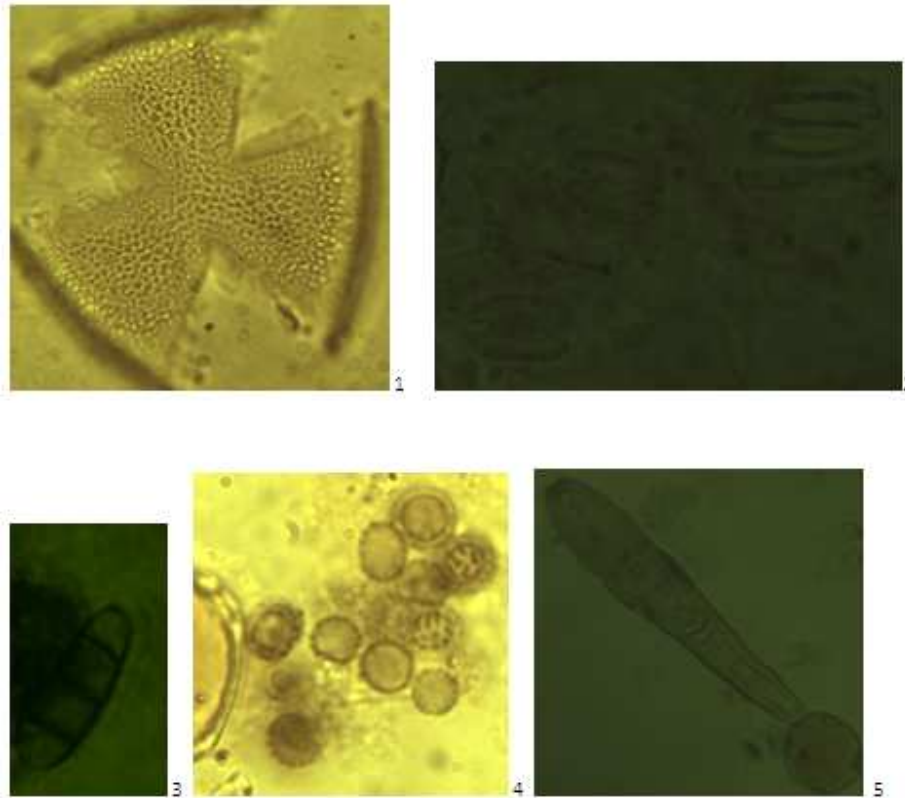


PLATE-5

Fig. 1: *Kigelia africana*, 2: Dicotyledonous cuticle with stomata, 3: Fungal spore, 4: Fungal spores, 5: ? Fungal spore
All x1000.

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