INTERNATIONAL JOURNAL OF PHYTOFUELS AND ALLIED SCIENCES
(A Journal of the Society for the Conservation of Phytofuels and Sciences)

Melisopalynological Analysis of Honey Samples from *Jatropha* Plantation and Unilorin Apiary Farm

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ABSTRACT

Two honey samples produced by the University of Ilorin at the Jatropha Plantation and Unilorin Apiary Farm were collected and studied melissopalynologically to isolate and identify pollen types in the honey. The aim of the study thus is to determine whether the honey samples are uniflora and multiflora. Based on the pollen grain frequency, the pollens in the 2 honey samples could be categorized as "important minor or important isolated pollen'' and "rare or isolated pollen''. In estimation of PK (pollen grain) frequencies, therefore, the pollen grains of *Jatropha curcas*, *Guio gracilis* and *Capsicum annuum* are considered rich in the honey of Jatropha Plantation. In the honey of Unilorin Apiary Farm, *Tridax procumbense*, *Mangifera indica*, *Melastoma polyanthum* and *Psidium guajava* produced rich pollens. The presence of the pollen grains in the two honey samples is a clear indication that the honeys are not adulterated but pure and not unifloral but multifloral.

Keywords: Melisopalynology, honey samples, pollens, uniflora, multiflora, Jatropha Plantation, Unilorin Apiary Farm

INTRODUCTION

Beekeeping industry, one of the important agricultural and forest based rural industries in Nigeria, is mainly involved in the production of commercial quantities of honey. The industry is flourishing due to the medicinal importance of the honey. Recognition and initial screening of various bee plants representing potential sources of nectar and pollen for the honey bees throughout the year, is an important pre-requisite for launching apiary industry in any locality (Kalpana and Ramanjam, 1997). Beekeeping industry should be encouraged because it is environmental friendly and also provide employment opportunity. The study of pollen in honey is essential for many reasons.

Melissopalynology is the branch of palynology which deals with the study of the botanical and geographical distribution of honey by subjecting honey sediments, and therefore pollen, spores and other fungal spores contained therein to microscopic analysis. Melissopalynology is also an important tool in determining the floral sources upon which the bees foraged to produce honey. By extension, it also includes the study of pollen in the honey as well as the source of the pollen. Pollen grains are a product of the anthers by meiotic division and they are male gametophyte of flowers of vast vegetations (Brooks and Shaw, 1968). Pollen grains as palynomorphs possess an unusual compound known as sporopollenin, found in the outer wall (the exine), which makes them resistant to treatment by acetolysis during palynological studies.

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 typical 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). 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 plants that the bees foraged on.

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 group (Yoruba, Igbo and Hausa) use honey during occasions like naming ceremony of new born baby, as one of the requirement in paying bride price during a marriage ceremony, honey is also used as gift. For at least 2700 years, honey has been used by humans to treat a variety of ailments through topical application, but only recently have the antiseptic and antibacterial properties of honey been chemically explained (Jusbin, 1996; Abdulla & Abdul-Aziz, 1998; Wahdan, 1998).

This present study is a further contribution to the melissopalynological investigations of honey samples in Nigeria, focusing on the Jatropha Plantation and the Unilorin Apiary Farm at the University of Ilorin to provide information on the geographical and botanical origin of the two honey samples.

MATERIALS AND METHODS

Study area and samples collection

Ilorin is situated in the transitional zone between the forest and savannah region of Nigeria. The vegetation of Ilorin is characterized by scattered tall trees, shrubs of between the height of 10 and 12 feet. The main predominant crops grown in Ilorin are corn, cassava and soya. Some of the noticeable trees include butter trees, acacia,

locust beans, baobab, akee-apple etc. (Ajibade, 2008)

Honey samples were collected randomly from 2 different locations at the Jatropha Plantation Farm and Unilorin Apiary Farm where bee hives were placed at some strategic places on 6th November, 2012. Both farms are located in the University of Ilorin Campus in Ilorin, Kwara State, Nigeria. The samples were collected purposely for analysis of pollens therein.

Pollen isolation

Five grams of honey samples from each of the two locations were put in 20ml clean dry centrifuge tubes and 10 ml of distilled water was added to it. The honey samples and water were then mixed together by manual shaking until a uniform solution is properly formed. The solutions were then centrifuged at 3000 rpm for 5 minutes.

After centrifuging, the supernatant fluid and the residue were separated by decanting, with the supernatant fluid being discarded. Glacial acetic acid was added to the residue, stirred well and allowed to stand for five minutes before centrifuging and decanting. The acetolysis mixture (9ml of acetic anhydride mixed together with 1ml of sulfuric acid) is then added to the residue in a fume cupboard, after which it was boiled and stirred in a water bath at a temperature of 80 – 90 °C for 2 to 3 minutes. The acetolysis samples were then centrifuged and decanted. Further washing of the residues from the decanted samples was done at a minimum of three times using distilled water; centrifuging and decanting each time. The final residue was then centrifuged and placed in liquid glycerin, which is used as a mounting agent for observation (Erdtman, 1960).

Slide preparation

The residue obtained from the honey sample was smeared with spatula on the microscope slide and micropipettes were used to transfer a little portion of the isopropyl alcohol on the sample which was later mounted on glycerin. The cover slip was then placed on it; the already warmed liquid glycerin containing the sample was sealed with nail varnish used as sealants to prevent desiccation of the sample. Identification of pollen types was done with the aid of pollen atlases, and other published floras which gave brief descriptions of the observed pollen in the samples. Photomicrographs were taken with a Kodak Easyshare C913 Digital camera.

Pollen count and frequency

The pollens were identified and recorded using the view count method of Adeonipekun (1989). For the presentation of frequencies of pollen grains in honey, the system adopted by Louveaux (Louveaux *et al*., 1978) was used. Percentage frequency of each pollen type was determined using this formula: % Frequency

= Number of pollen of a Taxon x100

Total number of all pollen

Honey classification based on pollen grain frequency

The following terms have been used in estimation of PK (pollen grain) frequencies: "very frequent" for grains constituting more than 45% of the total, "spordic 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).

RESULTS

The honey analyzed in the study was found to contain pollen grains of 29 plant taxa. 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 and 2; Figs. 1 and 2).

Jatropha Plantation Honey

Sixteen plant species belonging to 13 families were observed in the honey samples collected from *Jatropha* Plantation with 9 pollen types. In the honey samples from *Jatropha* Plantation, pollen grains from *Guio gracilis* is more dominant (15.1%) followed closely by pollen grains from *Jatropha curcas* (14.1%) which is the dominant plant species in the area where beehives are located, and pollen grains from *Alchonea cordifolia* being the least with 0.6% frequency (Table 1; Fig. 1).

Unilorin Apiary Farm Honey

Thirteen plant species belonging to 13 families were seen in the honey samples collected from Unilorin Apiary Farm with 8 pollen types. Honey samples collected from the Unilorin Apiary Farm showed that pollen grain *Tridax procumbens* is dominant with 14.5% frequency and pollen grains from *Passiflora spp* is the least frequency with 2.3% (Table 2; Fig. 2).

DISCUSSION

The results of the microscopic analysis of the honey from the two studied areas demonstrated the 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, it 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 taxa that are non-nectariferous in nature e.g. Poaceae and Liliaceae whose pollens are also observed in the honey samples from the two farms.

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 samples from the two samples studied in this work are not blended but are polyfloral honey, also known as wildflower honey, which is 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.

In estimation of pollen grain frequencies, the two honey samples in this work fall within the category important minor pollen or important isolated pollen (4 – 15% pollen) and rare pollen or isolated pollen (less than 3% pollen) (Vergeron, 1964). Ten taxa in Jatropha Plantation honey are in important minor pollen or important isolated pollen ranging from (4.0% pollen frequency in Euphorbiaceae – *Phyllanthus casearoides* to 15.1% in

Sapindaceae – *Guio gracilis*). Remaining 6 taxa are in the category ‘’rare pollen or isolated pollen’’. Worthy of note is the percentage frequency of pollen (14.1%) of *Jatropha curcas* which is the plant species constituting the plantation where the beehives where located. Only 3 taxa namely Apiaceae – *Cicuta masulata* (3.2%), Acanthaceae – *Justicia americana* (1.2%) and Passifloraceae – *Passiflora spp.* (2.3%) in the Unilorin Apiary Farm are in ‘’rare pollen or isolated pollen’’ category.

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 pollen of the number of pollen grain per 1 gm of honey samples if 1200, 1000 and below 1000 pollen were counted. This is a modification to Maurizio (1975). Based on this, the pollen grains of *Jatropha curcas, Guio gracilis* and *Capsicum annuum* are considered rich in the honey of Jatropha Plantation. In the honey of Unilorin Apiary Farm, *Tridax procumbense, Mangifera indica, Melastoma polyanthum* and *Psidium guajava* produced rich pollens.

Conclusion

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 honey samples is a clear indication that the honeys are not adulterated but pure and they are not unifloral but multifloral.

Table 1: Frequency of the plant species in *Jatropha* Plantation honey sample

Plant species	Family	Pollen type	Frequency (%)
Alchonea cordifolia	Euphorbiaceae	Tricolpate	0.6
Anacardium occidentale	Anacardiaceae	Triporate	5.9
Apois americana	Fabaceae	Tricolporate	2.5
Astromytus spp.	Myrtaceae	Syncolprate	2.8
Canomyrica monticola	Myricaceae	Triporate	4.7
Capsicum annuum	Solanaceae	Tricolporate	13.5
Cephatalus occidentale	Rubiaceae	Tricolporate	5.2
Elaeis guineensis	Arecaceae	Tricolpate	4.5
Guio gracilis	Sapindaceae	Parasyncolporate	15.1
Jatropha curcas	Euphorbiaceae	Panporate	14.1
Pendiculous sibthorpii	Schnophulariaceae	Tricolporate	1.5
Phyllanthus casearoides	Euphorbiaceae	Triporate	4.0
Sabal palmetto	Araceae	Monosulcate	3.7
Syzigium guineense	Myrtaceae	Tricolpate	7.5
Taxa bacata	Taxaceae	Ulcerate	9.5
Zea mays	Poaceae	Monoporate	3.7

Table 2: Frequency of the plant species in Unilorin Apiary Honey sample

Plant species	Family	Pollen type	Frequency (%)
Bacopa monnieri	Schrophulariaceae	Tricolporate	8.7
Chamaesyce sp	Euphorbiaceae	Tricolporate	5.5
Cicuta masulata	Apiaceae	Tricolporate	3.2
Crinum americanum	Liliaceae	Monosulcate	4.4
Cunonia pterophylla	Cunoniaceae	Monocoporate	9.3
Justicia americana	Acanthaceae	Diporate	1.2
Mangifera indica	Anacardiaceae	Tricolporate	11.6
Melastoma polyanthum	Melastomataceae	Zonocopate	12.6
Passiflora spp.	Passifloraceae	Heterocolporate	2.3
Pontedia cordata	Pontederiaceae	Dicolpate	7.6
Psidum guajava	Myrtaceae	Tricolpate	10.6
Rhizophora mangle	Rhizophoraceae	Diporate	8.2
Tridax procumbens	Astereaceae	Tricolporate	14.5

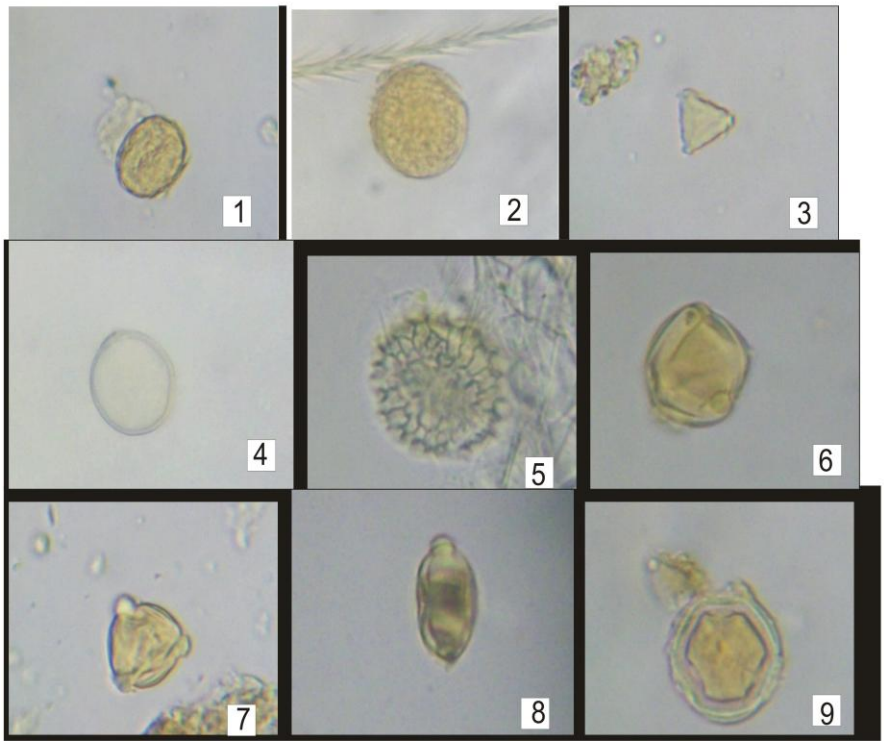

Plate 1: Pollens recovered from the honey of Jatropha Plantation Farm: 1 – monosulcate pollen (Arecaceae /*Sabal palmetto*), 2 – panporate pollen (Euphorbiaceae/*Jatropha curcas*), 3 – tricolpate (Myrtaceae/*Sygzium guineense*), 4 – monoporate (Poaceae/*Zea mays*), 5 – heterocolpate pollen (Passifloraceae/*Passiflora sp*), 6 – tricolporate (Solanaceae/*Caspicum annum*), 7 – tricolpate pollen (Euphorbiaceae/*Alchornea cordifolia*), 8 – Synocolprate (Myrtaceae/*Asteromytus sp*), and 9 – Tricolporate pollen (Rubiaceae/*Cephalathus occidentalis*)

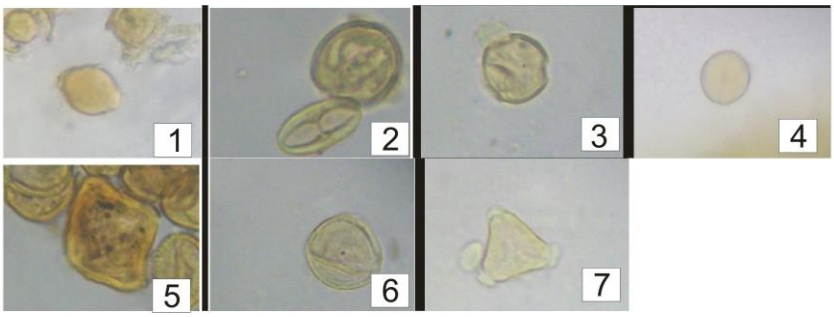

Plate 2: Pollens recovered from the honey of Unilorin Apiary Farm: 1 – diporate (Rhizophoraceae/*Rhizophora mangle*), 2 – diporate (Acanthaceae /*Justica americana*), 3 – tricolporate (Anacardiacae/*Mangifera indica*), 4 – monoporate (Cunoniaceae/ *Cunonia pterophylla*), 5 – diporate (Pontederiaceae/*Pontederia cordata*), 6 – monosulcate (Liliaceae/*Crinum americanum*) and 6 – tricolpate (Apiaceae/*Cicuta masulata*)

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