**Pollen Analysis of Elaeis guineensis-Dominated Honeys in Lower Casamance: Floral Diversity and Honey Typology**


## ABSTRACT

**Background:**

A honey's pollen spectrum serves as an indicator for determining its place of origin in terms of vegetation resources. This data might be utilised to create pollen analytical standards, which would help certify honey. The melliferous plants are plant species on which the bee takes substances, including nectar, pollen and resin to feed and to develop its various products (honey, royal jelly...).

**Aim:**

The aim of this study was carried out to contribute to melliferous plants in Casamance (Senegal) : pollen spectrum of honeys with *Elaeis guineensis* dominant.

**Results:**

Pollen analysis of 14 samples of dominant *Elaeis guineensis* honey from Lower Casamance in the Ziguinchor region identified 25 plant taxa in 11 families foraging for bees. The species richness varied from 5 to 10 taxa per honey sample. The most representative families are Fabaceae with 7 species, followed by Malvaceae with 3 species, Combretaceae, Myrtaceae, Rubiaceae, Poaceae, Arecaceae, Lamiaceae with 2 species and Avicenniaceae, Méliaceae, Asteraceae with 1 species. Pterocarpus erinaceus is present in 11 spectra including 7 spectra where it is isolated and in 3 spectra where it is isolated. It is the only taxon that is a companion pollen in 1 of the spectra. The most common taxa associated with Elaeis are *Detarium senegalense*, *Daniellia oliveri* and Myrtaceae.

**Conclusion:**

The data reflects the floral situation of the place were particular honey was produced and the identification of geographical origin based on the presence of a combination of pollen types of that particular area.



**Keywords:** Pollen analysis, Honey, *Elaeis guineensis*, Casamance (Senegal)


## 1. INTRODUCTION

"Pollen analysis studied under the branch Melissopalynology, which is valuable tool for the identification of the botanical and geographical origin of honey sample. These pollen analytical studies provide information of resources of bee. During evolution, a special relationship has been developed between the plants and the bees" [1]. "The flower provides pollen and nectar as a food to visiting bees while the bees in the course of wandering from flower to flower provide a vehicle for pollen transfer leading to pollination. Pollen is a major source of proteins, fatty substances, minerals and vitamins for the honey bees. Bees deliberately collect the pollen grains to fulfill their protein requirement and store them in pollen chambers in the hive" [2]. "Furthermore, pollen provides proteins for bees required for building their body tissues especially during early embryonic growth" [3]. "Pollen grains contain number of metabolites which are essential for different physiological and metabolic activities during growth and development of the pollen" [4]. "Honey bees collect nectar and pollen from the flowers that provide the nutrients necessary for colony maintenance and development. Nectar is processed to form honey, the major energy source for the colony. Pollen is a source of protein and amino acids for the colonies. The quantity and quality of pollen collected by honey bees affects the reproduction, brood rearing and longevity, thus ultimately the productivity of the colony" [5]. The analysis of honey can help to determine changes in nectar and pollen sources and may help determine the causes of this decline [6]. Senegal is a country with varied climatic conditions. In Casamance, the flora is ideal, with a multitude of plant species ideal for beekeeping. It is essential to study relationship between regional flora and honey bees in order to obtain maximum production of a good quality of honey.

During the present investigation, 14 honeys samples were analyzed to determinate the pollinic spectrum of honeys with *Elaeis guineensis.*

## 2. Material and methods

### 2.1. Description of the study site

The natural region of Casamance is located in the south of Senegal between the Atlantic Ocean and the Tambacounda region; it is bordered to the north by Gambia and to the south by Guinea-Bissau and Guinea Conakry. It has a surface area of 28,340 km$^2$. The Gambia and the Gambia River separate Casamance from the rest of Senegal (see map below). Its current population is estimated at 2,121,632 [7]. Casamance covers the administrative regions of Ziguinchor (Lower Casamance), Kolda and Sedhiou (Middle and Upper Casamance). These two regions are located in the north-east and are drier from a climatic point of view, as they are influenced by the Sudanese climate. The plateaus are not very high and the soils are generally clayey-sandy. Flora is very similar throughout the region, which has a Sudano-Guinean climate with average annual rainfall of between 800 mm at the northern limit and 1300 mm at the southern limit, and a dry season lasting 7 to 8 months [8].The hydrographic network is dominated by the 300km long Casamance river. It rises in the area around Fafacourou, some fifty kilometres to the north-east of Kolda, where numerous small marigots meet. The surface area of classified forests is 607540 ha for 56 classified forests, including 30 in Lower Casamance (116776 ha), 12 in Middle Casamance (84453 ha) and 14 in Upper Casamance (396230 ha). This potential allows for the development of numerous, diverse botanical species that are particularly rich in honey. Added to these favourable conditions is the high productivity of the *Apis mellifera adansonii*, giving Casamance a prime position among the honey-producing regions of West Africa. Mangroves are specific plant formations. They have the status of classified forests. This ecosystem is also home to many species of mollusc, crustaceans, spiders and, above all, bees [9]. Fig. 1 shows the geographical location of Casamance in Senegal.


**Fig.1. Map of Casamance in Senegal**

### 2.2. Honey samples

Forty honey samples were provided by members of the Casamance Beekeepers Association. All samples were reported by the beekeepers as being from mixed floral sources.

### 2.3. Methodology

#### 2.3.1. Mounting the honey slides

From a 125 g pot of honey, a test sample of 10 g is taken. These 10 g are diluted in 40 ml of water acidified with 5% sulphuric acid and subjected to an initial centrifugation for 12 minutes at 3000 rpm, then the residue is transferred to distilled water for a second centrifugation. The entire residue is collected; depending on its volume, one or more deposits are made on the slide. After the water has evaporated, the slides are degreased with ether before inclusion in gelatinised glycerine stained at an average gradient of 160 µl, 240 µl and 300 µl. The preparations are then incubated to be read under the microscope. The method used is that described by the International Commission on Beekeeping Botany [10].

#### 2.3.2. Pollen identification

The pollen grains were identified using a Zeiss photonic microscope at x100 immersion magnification. They were determined by comparison with reference preparations made and those available at the Pollen and Honey Laboratory of the Pascal Paoli University of Corse. The documentation used consisted of the Atlas de la Cote d'ivoire [11], pollen and spores from tropical Africa and enabled pollens to be determined generally at genus level and sometimes at species level, and at family level in cases where the pollen characteristics were homogeneous. However, some pollens had to be classified as undetermined.

#### 2.3.3. Quantitative pollen analysis

Firstly, the preparations were examined in their entirety to establish a complete inventory of taxa. Secondly, counts were made to establish the relative frequency (RF) of each taxon. Pollen grains were counted using a checkered field eyepiece and a x50 immersion objective. For each microscopic field observed, the number of all the taxa present was noted. Counts are made on three lines of the slide: a line at ¼ superior, a middle line and a line at ¼ inferior. Every 1 in 4 checkered fields are counted which makes 20 fields per line. The following Fig. 3 illustrates the different Count lines.

A sheet is drawn up for each honey sample. The line shows the taxa inventoried and the columns show the number of grains counted per field. The International Commission of Apicultural Botany, referring to the work of [12] authorises the expression of results in the form of percentages, provided that the total number of grains counted is at least equal to 1200. An initial evaluation of the absolute content was carried out for all the samples on the basis of the preparations used to establish the total crude spectra. Each counting sheet is used to define :

Ng, the number of grains counted ; NC, the number of fields covered under the microscope ; NTC, the total number of fields of the deposit studied, obtained by dividing the total surface area of this deposit by the surface area of a field under the microscope at the magnification in question. When the entire sample has been the subject of a single deposit, the formula for calculating the absolute content expressed as the number of grains per 10 g is as follows:

$$\mathbf{PK/10 = Ng/NC \times NTC \times PK10/Pm}$$

Pm is the weight of the honey used. Depending on the PK/10 g value, the pollen diversity was expressed from the specific richness according to the different classes recommended by [13] and the types of honey.

- Class I PK/10g < 20 000

- Class II 20,000 < PK/10g < 100,000

- Class III 100,000 < PK/10g g < 500,000

- Class IV PK/10g > 1,000,000

According to Louveaux et al.,1970, 1978 [14,15] :

Class I includes flower honeys with a low pollen content;

Class II includes most flower honeys and flower honeys mixed with honeydew;

Class III includes pollen-rich honeys and pure honeydew honeys;

Class IV corresponds to very pollen-rich honeys and some press honeys;

Class V includes extremely pollen-rich flower honeys or press honeys.

### 2.3.4. Qualitative pollen analysis

The results concern the characteristics of the different honeys studied with their pollen composition. The relative density is expressed as the percentage quotient of the absolute density of all the pollen types in the sample. The percentage calculated is used to determine the floral origin of the honey. Their respective proportions are not expressed in figures but using frequency classes, the Zander classes [14,15]. The count was carried out on at least 300 grains, and the terminology is as follows:

- dominant pollen, frequency > 45% ;

- accompanying pollen, frequency ≥ 16 ≤ 45% ;

- important isolated pollen with frequency ≥ 3 ≤ 15% ;

- isolated pollen with frequency < 3%.

The frequency of appearance of a taxon in relation to all the samples studied was noted by the presence or absence of a taxon in all the samples studied. It highlights a significant association of taxa inventoried in the honey concerned. This association makes it possible to identify the geographical origin of the honeys. It usually reflects the floristic composition of the local vegetation. The frequency distribution is obtained by dividing the number of samples containing the taxon by the total number of samples studied [10].

According to Louveaux *et al.,* 1978 [15], the relative frequency distribution of the taxa present was classified into four categories:

- Class1: very frequent taxon >50% ;

- Class 2: frequent taxon ≥ 20 ≤ 50% ;

- Class 3: infrequent taxon ≥ 10 < 20% ;

- Class 4: rare taxon < 10%.

## 3. RESULTS AND DISCUSSION

### 3.1. RESULTS

The *Elaeis guineensis* studied in this article belongs to the Arecaceae family. The characteristics of its reference pollen are as follows:

- ➔ Symmetry and form: anisopolar pollen, monosulcate, triangular with concave sides in polar view. Size: L= 38.5 µm; l= 32 µm Aperture: 1 colpus, elliptical, diffuse margin, smooth membrane. Exine: endexine absent, tectum distally smooth to scabrous and proximally fossuleous, rough. Thickness 1 to 2 µm.
- ➔ The dimensions are within the range of the measurements of [16] who had found (28 to 43,5 µm) for the length and (19 to 39 µm) for the width. Fig.2. show *Elaeis guineensis* pollens


1 2

**Fig.2. *Elaeis guinensis* (1: 50X ; 2 : 100X )**

#### 3.1.1. Qualitative analysis

Pollen spectra of honeys where *Elaeis guineensis* is dominant Willd. In these samples 25 taxa in 12 families were identified. Their number varied from 5 to 10 per sample. To establish an approximate typology of honeys based on the distribution of taxa in each spectrum where Elaeis is dominant.

We began by carrying out a taxon-by-taxon analysis of the relative frequency (RF) distribution of each taxon in all samples. This approach allowed us to isolate the taxa with the highest maximum frequencies and to group together the spectrum in which they appear. Table 1 shows the pollen spectrum of *Elaeis guineensis* honeys according to the maximum FRs and their frequency of appearance.

Table 1: Pollen spectrum of *Elaeis guineensis* honeys according to maximum FR and frequency of occurrence of taxa

| **Species** | **Families** | **RF Max (%)** | **Attendance rate (%)** | **Zander Class** |
|---|---|---|---|---|
| *Elaeis guineensis* Jacq. | *Arecaceae* | 88.49 | 100 | **Dominant** |
| *Pterocarpus erinaceus* (Poir.) | *Fabaceae Faboïdae* | 22.32 | 78.5 | **Accompanying** |
| *Detarium senegalensis Gmel.* | *Fabaceae caesalpinioïdae* | 12.86 | 78.5 | Important isolated between 3% and 16 |
| *Daniellia oliveri* (Rolfe) Hutch. & Dalz. | *Fabaceae caesalpinioïdae* | 12.62 | 50 | |
| *Combretaceae* | *Combretaceae* | 9.79 | 14.28 | |
| *Vitex doniana Sweet* | *Verbenaceae* | 8.05 | 71.42 | |
| *Cocos nucifera L.* | *Areceae* | 6.45 | 7.14 | |
| *Parkia biglobosa* (Jacq.) R. Br. ex G. Don | *Fabaceae mimosoïdae* | 6.12 | 50 | |
| *Graminae* | *Graminae* | 6.06 | 21.43 | |
| *Myrtaceae* | *Myrtaceae* | 5.81 | 64.28 | |
| *Dialium guineensis* (Willd.) | *Fabaceae caesalpinioïdae* | 4 | 35.71 | |
| *Khaya senegalensis* (Desr.) A. Juss. | *Meliaceae* | 3.95 | 14.28 | |
| *Asteraceae* | *Asteraceae* | 2.63 | 14.28 | isolated always less than 3 |
| *Ceiba pentandra* (L.) Gaertn. | *Malvaceae* | 2.27 | 28.57 | |
| *Avicennia nitida* Jacq. | *Avicenniaceae* | 1.55 | 28.57 | |
| *Hyptis suaveolens Poit.* | *Lamiaceae* | 1.32 | 7.14 | |
| *Bombax costatum* Pellegr. & Vuillet | *Malvaceae* | 1.17 | 14.28 | |
| *Rubiaceae* | *Rubiaceae* | 0.95 | 14.28 | |
| *Afzelia africana* (Smith ex Pers) | *Fabaceae caesalpinioïdae* | 0.93 | 7.14 | |
| *Cassia sieberiana* DC. | *Fabaceae caesalpinioïdae* | 0.11 | 7.14 | |
| *Adansonia digitata* L. | *Malvaceae* | 0.1 | 7.14 | |

Among the families encountered, the Fabaceae dominate with 7 species, followed by the Malvaceae with 3 species, then the Combretaceae, Graminae, Rubiaceae, Arecaceae and Myrtaceae, each with 2 species, and finally the Asteraceae, Verbenaceae, Lamiaceae and Avicenniaceae, each with one species. The families encountered are shown in the following histogram (Fig. 3).

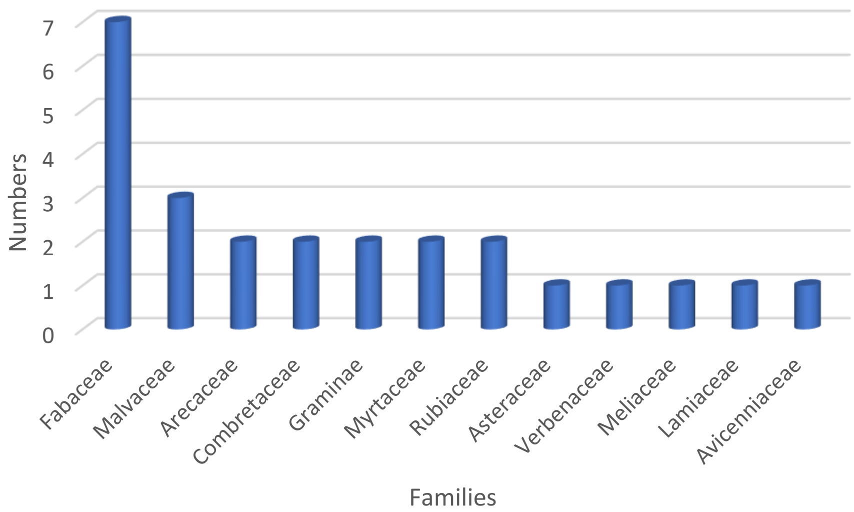


Fig.3. All families found in honeys

In terms of maximum frequency, there is no pollen associated with Elaeis that is dominant. The only companion pollen associated with *Elaeis* is *Pterocarpus erinaceeus*. The important Elaeis-associated isolates represent 40% of the 25 taxa. The Elaeis-associated isolates are 28% and are lower than those found by [17], which are 18.6% and 51.2% respectively. They are thus divided into three intervals:

- ➔ The taxon associated with Elaeis and which has at least one FR between 16 and 45 is :

Pterocarpus erinaceus with a frequency of 22.32% and a presence rate of 78.5%.

- ➔ Taxa associated with Elaeis and having at least one FR between 3 and 16 : 10 taxa in 7 families: *Detarium senegalensis* with a maximum FR of 12.86%, followed by *Daniellia oliveri* 12.62%, Combretaceae pollens with a maximum FR of 9.79%, Vitex doniana with 8.05%, *Cocos nucifera* with 6.45%, *Parkia biglobosa* with 6.12%, Graminae pollens with 6.06%, Myrtaceae pollens with 5.81%, *Diallium guineensis*, *Khaya senegalensis* with FR of 4%.
- ➔ Taxa associated with *Elaeis guineensis*, always less than 3%.

They comprise 8 species in 5 families. They are: Asteraceae pollen with 2.63%, *Ceiba pentandra* with 2.27%, *Avicennia sp* with 1.55%, *Hyptis suaveolens* with 1.32%, *Bombax costatum* with 1.17%, *Afzelia africana* with 0.93%, *Cassia sieberiana* and *Adansonia* with 0.11%. In relation to the rate of presence, the very frequent taxa with a rate of over 50% are *Pterocarpus erinaceus*, *Detarium senegalensis*, *Vitex doniana* and pollens of Myrtaceae types.

Frequent taxa with a rate between 20 and 50% are : *Daniellia oliveri*, *Parkia* taxa of the Graminae type, *Dialium guinensis*, *Ceiba pentandra* and *Avicennia sp*.

The less frequent taxa are present at a rate of between 10 and 20% and include

Combretaceae pollen, Khaya senegalensis, Asteraceae pollen, Bombax costatum and Rubiaceae pollen.

The most rare are *Cocos nucifera*, *Hyptis suaveolens*, *Afzelia africana* and *Cassia sieberiana.*

➔ *Distribution of taxa according to beekeeping interest*

*Nectariferous taxa associated with Elaeis guineensis represent 48% of the total: Avicennia sp, Daniellia oliveri, Dialium guineensis, Khaya senegalensis, Vitex doniana, Adansonia digitata Rubiaceae type with 2 species, Hyptis suaveolens , pollen of* Lamiaceae type*,* Rubiaceae type*.*

Nectariferous and polliniferous taxa represent 32%: they are Combretaceae type with 2 species and Myrtaceae type with 2 species*, Ceiba pentandra , Bombax costatum, Pterocarpus erinaceus, Parkia biglobosa.*

Pollinating taxa account for 20% of the total: : *Cocos nucifera*, *Cassia sieberiana* and taxa of the Graminae and Asteraceae types, each with 2 species. Fig.4 illustrates this distribution.

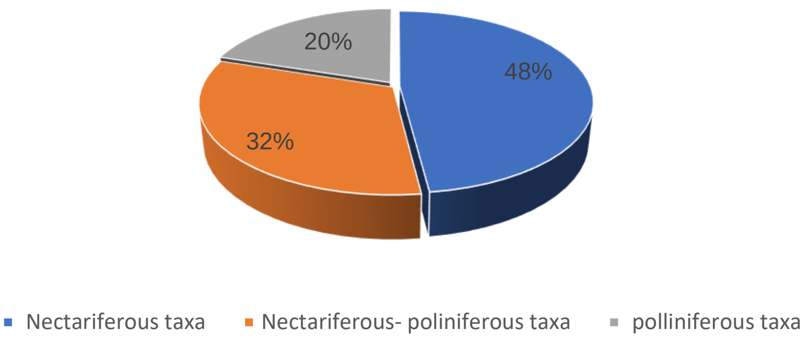


**Fig.4. Distribution of taxa according to beekeeping interest**

### 3.1.2. Quantitative analysis

Table 2 shows the results of the quantitative pollen analysis of the 14 samples of Elaeis dominant honeys. This is the total number of pollen grains contained in 10g of honey. The honeys are classified according to their absolute pollen content. The quantity of pollen varies between 17757.85 and 1422071.29.

**Table 2. Results of the quantitative pollen analysis of the 14 sample of Elaeis dominant honeys**

| N° | D A | Class |
|---|---|---|
| **E1 1402** | 17757,85 | I |
| **E2 1416** | 20458,62 | II |
| **E3 1611** | 96197,60 | II |
| **E4 1409** | 137288,13 | III |
| **E5 1403** | 350183,44 | III |

| | | |
|---|---|---|
| **E6 1408** | 453600 | III |
| **E7 1420** | 148432 ,5 | III |
| **E8 1604** | 344183,16 | III |
| **E9 1607** | 380700 | III |
| **E10 1405** | 722323,73 | IV |
| **E11 1410** | 713706,29 | IV |
| **E12 1404** | 1422071,71 | V |
| **E13 1411** | 2412251 | V |
| **E14 1412** | 1293626,37 | V |
| | | |

Of these samples, only one is class I, i.e. less than 20,000 grains per 10g of honey, representing 7.14%. Classes II, IV and V each represent 14.28%. Class III honeys are more important and represent 42.85% of the samples.

### 3.2. DISCUSSION

The specific diversity of these Elaeis-dominant honeys is 25 taxa in 11 families. This is much lower than the 121 taxa identified by [17] in honeys collected in the Lama classified forest in southern Benin and the 43 taxa identified by [18] in honeys sold in Cotonou in Benin, the 43 identified by [19] in honeys from the Guinean zone of Togo, 41 species identified by [20] in honeys from the Soudano Guinean zone of Cameroon and 32 species identified by [21] in honeys from Ewé-Kétou in south-east Benin. However, it is higher than the 21 taxa found by [22] in Madagascar honeys, and the 16 taxa found by [23] in Boukounbé and Kandi, localities located in northern Benin. In agreement with [18], this difference in taxonomic diversity can be explained by the number of samples analysed, their harvesting period and the floristic diversity of the types of formation used by the bees as foraging sources.

*Elaeis guineensis* was found in all samples and is dominant, in agreement with Koudegnan *et al.,*2012 [19]. The very high number of *Elaeis guineensis* pollens in the Azianfokopé-Takpla farm (51.15%) is due to the fact that in this locality oil palm is one of the main resources for the daily needs of the population [20,24]. In Basse Casamance, Elaeis is exploited for its oil, while palm wine is used to make brooms, house roofs, fences and so on. It is therefore cultivated because it is a plant with enormous economic potential, making this pollen source widely available to bees. The nectar-bearing pollens that accompanying it represent 48% and are either isolated or highly isolated, none of them being dominant.

*Elaeis guinensis* is a polliniferous taxa with anemophilous pollination. Its presence in honeys is not linked to the collection of nectar required to produce them, as it is a taxa with no nectaries on either the male or female flower [26]. Its strong presence can only be due to contamination in the hive by the bees themselves or during beekeeping or by the incorporation of these wind-dispersed pollen grains into honeydew droplets. After the wine has been harvested, Elaeis is visited by bees in search of sugar in its elaborate sap. In the case of nectariferous taxa such as vitex, *Dialium* and *Daniellia*, which are wooded savannah species, their high frequency and low abundance could be explained by their very low density in the area of distribution or the fact that their flowering period is short. In India, Singh AK *et al.,* 2023 [25] proved that the months of December to February were well favourable for good honey production due to the heavy flowering of several nectariferous and polliniferous species. In Togo, Koudégnan CM et al., 2013 [27] has identified Elaeis guineensis's pollens are the most abundant and the only one found in all the samples of honey analysed, confirming our studies. *Elaeis* can not determine the botanical origin of these honeys, even though it is dominant. And according to [15], only one of the samples comes from a nectariferous source because the number of grains is less than 20,000, i.e. sample 1402. In this sample the nectariferous and nectariferous polliniferous taxa represent 77.78%, so it is a mixture of nectar contaminated with Elaeis. The high presence of *Elaeis* in the honeys Class II and III honeys in our samples could be explained by beekeeping practices, as these are honeys that are either drained or centrifuged and not decanted. According to beekeepers, centrifuging causes a lot of pollen to be lost and stick to the sides. This pollen, separated from its honey, cannot be recuperated. However, dripping is done manually by turning the crank to extract the honey and pollen from the honeycombs. This technique has the advantage of containing more pollen. And according to Louveau, class II honeys are mixtures of flower honeys and nectar, while class III honeys are honeys rich in pollen and pure honeydew. And when the bee collects honeydew, it supplements its diet with a protein source, hence the high presence of Elaeis pollen. Class IV and V honeys are press honeys according to beekeeping practices.

## 4. CONCLUSION

Pollen analysis of 14 samples of dominant *Elaeis guineensis* honey from Lower Casamance in the Ziguinchor region identified 25 plant taxa in 11 families foraged by bees. The species richness varied from 5 to 10 taxa per honey sample. The most representative families are Fabaceae with 7 species, followed by Malvaceae with 3 species, Combretaceae, Myrtaceae, Rubiaceae, Graminae, Arecaceae, Lamiaceae with 2 species and Avicenniaceae, Meliaceae, Asteraceae with 1 species. Pterocarpus erinaceus is present in 11 spectra including 7 spectra where it is isolated and in 3 spectra where it is isolated. It is the only taxa that is a accompanying pollen in 1 of the spectra. The most common species associated with Elaeis are *detarium*, *daniellia* and Myrtaceae. All the species associated with Elaeis are either isolated or isolated, with the exception of *Pterocarpus*, which is a companion. Nectariferous taxa associated with *Elaeis* represent 4%, pollinating nectarifers 32% and pollinators 20%.

The mellissopalynological analysis shows that sample E1 is a nectar honey. The drained or centrifuged non-decanted honeys E2 to E8 are class II and III. Pressed honeys are class IV and V E9 to E14.

The region selected for the present study has good potential for sustaining beekeeping ventures because of the diversity of nectar and pollen taxa. The economically important plants constitute major part of the flora of this area.

## 5. DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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