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Contribution to the Knowledge of Melliferous Plants: Pollen Analysis of Supposed Honeys of *Dialium guineensis* (FABACEAE), by the Beekeepers of Ziguinchor (Senegal)

Kady Diatta[1, *], Marie José Battesti[4], William Diatta[1], Alioune Dior Fall[1], Serigne Ibra Mbacké Dieng[1], Amadou Ibrahima Mbaye[1], Assane Goudiaby[2], Aliou Guisse[3], Emmanuel Bassene[1]

[1]Department of Pharmacy, Cheikh Anta DIOP University, Dakar, Senegal

[2]Institute of Sciences of the Environment, Cheikh Anta DIOP University, Dakar, Senegal

[3]Department of Plant Biology, Cheikh Anta DIOP University, Dakar, Senegal

[4]Faculty of Sciences, Pasquale PAOLI University, Corsica, France

Email address:

khadydiose@yahoo.fr (K. Diatta), mjbattesti@univ-corse.fr (M. J. Battesti), diattaw@yahoo.fr (W. Diatta), elieufall@yahoo.fr (A. D. Fall), simbdieng@yahoo.fr (S. I. M. Dieng), mbayeame85@gmail.com (A. I. Mbaye), assane.goudiaby@ucad.edu.sn (A. Goudiaby), aliou.guisse@ucad.edu.sn (A. Guisse), aynenut@gmail.com (E. Bassene)

[*]Corresponding author

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Abstract: Pollen analysis, a technique for identifying pollen grains and hence the plant taxa visited by bees, is a means of clarifying the floral origin of honey. The objective of this study is to determine the pollen composition of the supposed honey of *Dialium guineensis* Willd to verify the geographical and botanical origins given by the beekeepers. The pollen analysis of the supposed honeys of *Dialium guineensis* was carried out according by authors. The identification and enumeration of the pollen was done under the microscope. Pollens are usually classified according to the four categories proposed by authors according to the value of the relative frequency (R F): dominant pollen whose frequency > 45%; accompanying pollen; 16 ≤ frequency ≤ 45%; Important isolated pollen; 3 ≤ frequency ≤ 15% pollen isolated when frequency < to 3%. A total of 14 taxa divided into 7 families were identified. Depending on the maximum FR value reached, the dominant taxa are *Elaeis guineensis* with a RF of 74.04% and *Avicennia sp* with 46.67%. The largest RF found for *Dialium guineensis* is 14.56%. The nectariferous taxa represent 45.45%, the nectariferous and polliniferous taxa 36.36% and the polliniferous taxa 18.18%. The *Dialium guineensis* is nectariferous but is not dominant in any of the samples. As for *Elaeis guineensis*, it is dominant in a single sample but cannot determine the botanical origin because it is a polliniferous plant. The botanical origin is determined by the nectarifer taxon hence the name of honey. The honey name of *Dialium* is not appropriate because the taxon is either isolated or isolated important. However we have honey from *Avicennia sp* or mangrove honey whose taxon is nectariferous and dominant in one of the samples.

Keywords: Honey Plants, *Dialium guineensis*, Pollen Analysis, Honey, Ziguinchor, Senegal

1. Introduction

For a first study of the melliferous plants and the pollen spectrum of the honey of Senegal, Casamance was chosen for its high melliferous potentiality. Several types of honey are produced there and are identified by beekeepers as vegetable essence which has mainly served bees without scientific analysis. Thus, reference pollen blades cited by beekeepers were made to identify pollen grains in honey. Any honey produced under natural conditions always contains small amounts of pollen Battesti and

mellisopalynology or pollen analysis analyzes the pollen grain contained in the honey. It determines the link between honey and the nectar producing plant, which allows to know its botanical and geographical origin. To differenciate the productions and attribute them names conforming to the scientific data, samples supposed to be of "*Dialium guineensis"* were collected in lower Casamance more precisely in Ziguinchor In order to determine the pollen spectrum of these honeys and thus contribute to the knowledge of the melliferous plants of this area.

2. Material and Methods

The plant material consists of the pollen of the flowers of *Dialium guineensis* and the species in bloom around the apiaries in the study area. Honeys were collected in 2 areas of apiculture from Ziguinchor (Kasséle and Souloul to Kafountine) during the year 2014 between March and May during the harvest period. For this article the objective is to verify by pollen analysis the conformity of the name *"honey of Dialium guineensis"* given by the beekeepers.

2.1. Study Framework

The region of Ziguinchor is located at 12°33' North Latitude and 16°16' West Longitude, magnetic declination 13°05. Its Altitude, 19.30 m in the southwestern part of Senegal, covers an area of 7,339km^2, that is 3.73% of the national territory and is bounded in the North by the Republic of The Gambia, in the South by the Republic of Guinea Bissau, To the east by the regions of Sedhiou and to the west by the Atlantic Ocean. Its area is 28 340km^2, its population is now estimated at 1 827 683 inhabitants. See Figure 1 next:

Figure 1. *Map of the region of Ziguinchor.*

http://www.au-senegal.com/decoupage-administratif-de-la-region-de-ziguinchor,041.html

The region is influenced by the sub-Guinean climate, promoting a high rainfall of 6 months with rainfall 1500 mm on average compared to the centers and northern parts of the country. We note the formation of a forest area consisting of dense dry forests and gallery forests located mainly in the southern part. The mangrove and palm groves colonize the river-sea zone, also note the presence of roners. The sacred forests and wood, which exist in every village in Ziguinchor, are original places of animist worship and cultural events. Prohibitions meant to protect

natural resources are established there. Fire-making is strictly prohibited. Access to these forests is reserved for the initiated, therefore, apart from the loss of species related to the vagaries of the climate, these forests are among the sites least subject to degradation phenomena and have on the whole retained their original vegetation Goudiaby [9].

The hydrographic network of the region is mainly made up of the Casamance river (semi-permanent river flowing from June to March). This river receives the Sungrougrou, a tributary of 140 km, and the marshes of Guidel, Kamobeul, Bignona, etc. The basin drainage area is about 20,150 km² comprising major sub-basins (Baïla: 1 645 km², Bignona 750 km², Kamobeul: 700 km², Guidel 130 km² and Agnack: 133 km²) with very variable volumes from 60 to 280 million m^3 /per year. The Casamance River, 350 km long, is often lined with mangroves and invaded by sea water up to 200 km from its mouth (Diana Malari / Sédhiou) where flow in veryvariable volumes: 60 to 280 million m^3 of water per year. In addition to these favorable conditions, there is the high productivity of *Apis mellifera adansonii*, giving Casamance a place of choice among the honey production regions of West Africa. Figure 1 shows the location of Casamance in Senegal and Figure 2 shows the location of apiaries in all of our study areas, including the area studied in this article. The region of Ziguinchor originated from the administrative reform of July 1984 which split the former region of Casamance into two administrative entities: the region of Kolda and that of Ziguinchor. It is composed of 3 departments (Bignona, Oussouye and Ziguinchor), 8 districts, 5 communes, 25 rural communities and about 502 villages.

2.2. Methodology

2.2.1. Montage of the Reference Pollen Blades

The Wodehouse method, was used to make the reference pollen blades. The flowers of *Dialiumguinensis* and those of all flowering species were collected around the apiaries in the study areas (Figure 3). The fresh pollen of these different species is deposited in a drop of distilled water directly on the slide holder labeled with date of assembly, name of the species and degree of coloring of the gelatin. This slide is then placed on a histological plate to evaporate the water. Degreasing of the grains with ether is then carried out in order to remove the *"pollen mantle*", formed from lipid substances, so as to be able to correctly observe the characteristics of the envelope or sporoderm. On the dried and defatted pollen grains, one or two drops of gelatinized glycerol are deposited according to Kaiser, stained with Fuchsin of Ziehl, previously liquefied in a water bath at about 40°C. This inclusion liquid is immediately covered with a lamella. These blades are dried and varnished and then read under a microscope. The fresh pollen grain is the material seized as it is by the bee on the plant and enclosed in the honey which constitutes a privileged medium of conservation.

2.2.2. Honey Sampling

A total of 3 samples of fixed honey honeys from Kasséle and from Souloul to Kafountine in the Ziguinchor area were analyzed (Figure 2 and Table 1). All these honeys were harvested from framed hives. For each sample 250 g were taken. They were mostly extracted after desiccation with a knife, by centrifugal acceleration. The honeys presumed of *Dialium guineensis* noted (D1, D2, D3) are listed in Table 1 with their place and date of harvest.

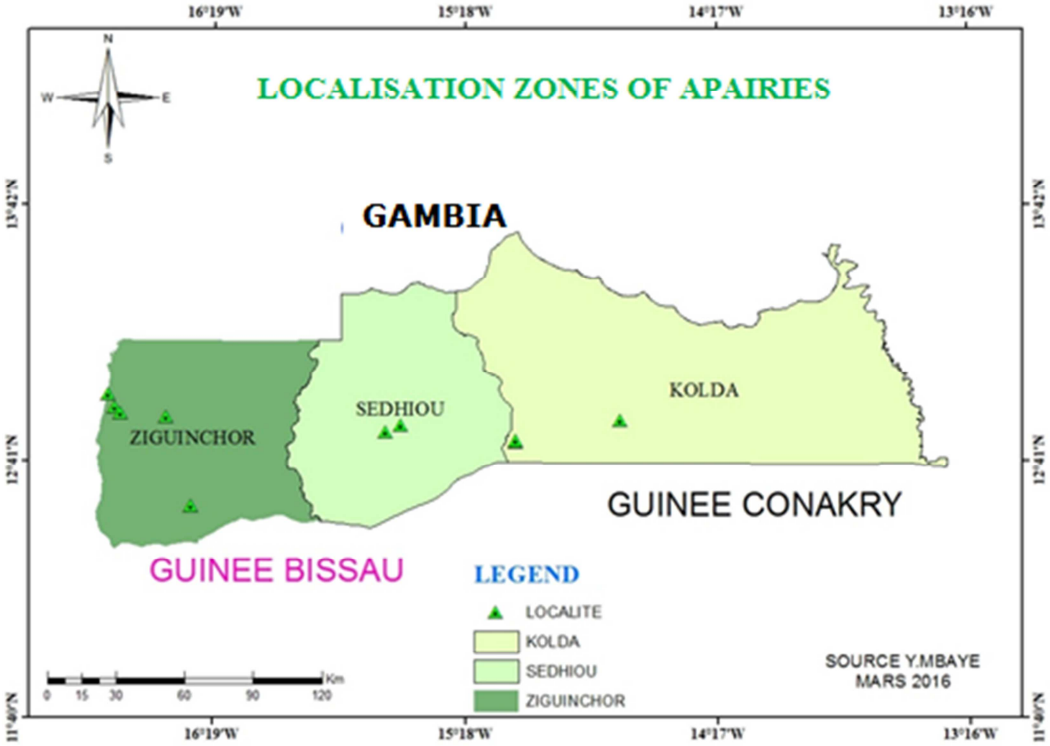

Figure 2. *Localisation Zones of the Apiaries in Casamance.*

Table 1. *List of harvested samples.*

Samples	Place of harvest	Date of harvest
D1	Kasséle	may-14
D2	Kasséle	march-14
D3	Souloul	march-14

2.2.3. Assembly of the Honeycombs

From a pot of 125g of honey, a test sample of 10 g is carried out. These 10 g are diluted in 40 ml of water acidulated with 5% of sulfuric acid and subjected to a first centrifugation for 12 minutes at 3000 rpm and then the pellet is taken up in distilled water for a second centrifugation. The whole of the pellet is collected; Depending on its volume, one or more deposits are made on the slide. After evaporation of the water, degreasing with ether precedes inclusion in gelatinized glycerin stained at an average gradient of 160 µl, 240 µl and 300 µl and enclose the preparation with a coverglass. The method is that described by the international commission of botanical apiculture, improved by Battesti, [4].

2.2.4. Identification of Pollen Grains

The pollen grains were identified by the Zeiss photon microscope at immersion x100 magnification. They were determined by comparison with the prepared reference preparations and those obtained at the Pollen and honey laboratory of Pasquale Paoli University of Corsica. The documentation used is composed of the Atlas of the Ivory Coast Ybert JP [22]. Pollen and spores of tropical Africa by Caritini et Guinet and allowed to determine the pollens generally at the level of the genus and sometimes at the level of the species and at the level of the family in the case where the pollinic characteristics are homogeneous. However, some pollens had to be classified as indeterminate.

2.2.5. Quantitative Pollen Analysis

First, the preparations were examined as a whole to establish a complete inventory of taxa. In a second step, the enumeration was carried out to establish the relative frequency (RF) of each of them. The pollen grains are counted using a gridded field eyepiece and an immersion x 50 lens. For each microscopic field observed, the number of all taxa present is recorded. The counting takes place on three lines of the blade: a line with ¼ upper, a middle line and a line with ¼ lower. Every 1 of 4 gridded fields are counted which makes 20 fields per line. Figure 3 illustrates the different counting lines.

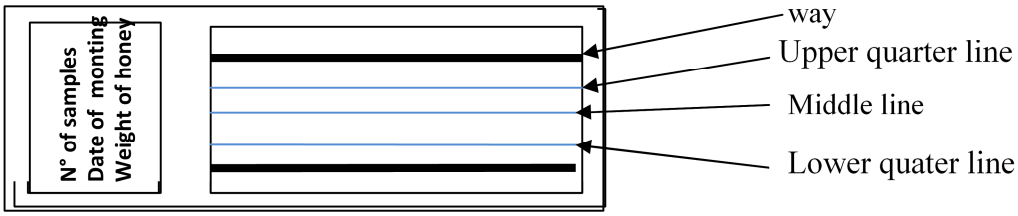

Figure 3. *The different counting lines.*

A sheet is prepared for each sample of honey. It carries in line the inventoried taxa and in column the number of grains counted per field. The International Commission on Botanical Beekeeping, referring to the works by Vergeron [21], authorizes the expression of the results in the form of percentages, provided that the total number of grains counted is at least 1200. An initial evaluation of the absolute content was carried out for all samples from the preparations used to establish the total raw quantified spectra. Each count sheet allows to define:

Ng, the number of grains counted,

NC, the number of fields done under the microscope,

NTC, the total number of fields of the studied deposit obtained by dividing the total area of this deposit by the surface of a field under the microscope at the magnification considered. When the whole pellet has been the subject of a single deposit, the formula for calculating the absolute content expressed in number of grains per 10 g (PK/10) is as follows:

$$PK/10 = Ng/NC \times NTC \times 10/Pm \quad (1)$$

Pm is the weight of the honey used. Depending on the value of PK / 10 g, the pollen diversity was expressed from the specific richness according to the different classes recommended by Maurizio 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 < 500000
- Class IV, PK / 10g> 1 000 000

According to Louveaux et al. [13, 14]:

Class I contains honey from flowers poor in Pollen;

Class II contains most of the flower honey and honey mixed with honeydew;

Class III includes honey rich in pollen and pure honeydew honeys;

Class IV corresponds to honey very rich in pollen and a part of press honeys;

Class V indicates the honeys from the flowers extremely rich in pollen or honeys of press.

2.2.6. Qualitative Pollen Analysis

The relative density is expressed by the quotient. In percentage of the pollen of the taxa encountered in relation to all the pollen grains counted. The result obtained makes it possible to determine the floral origin of the honey and it is not expressed in numerical form but using frequency classes, the Classes of Zander Louveaux et al. [13, 14] The

enumeration was carried out on at least 300 grains. The following terms are used for frequency classes:

Predominant pollen (more than 45% of the pollen grains counted);

Secondary pollen (16-45%);

Important minor pollen (3-15%);

Minor pollen (less than 3%).

The frequency of occurrence of a taxa with respect to all the samples studied was noted by the presence or absence of a taxa in all the samples studied. It shows a significant association of taxa inventoried in the honey concerned. This association makes it possible to recognize the geographical origin of honeys. It usually reflects the floristic composition of the vegetation of the environment. The frequency distribution is obtained by dividing the number of samples containing the taxa by the total number of samples studied Battesti [3]. According to Louveaux et al. [14], the distribution of the relative frequency of taxa present is classified into four categories:

- Class 1: very frequent for grains constituting more than 50% of the total;
- Class 2: frequent for grains constituting 20-50% of the total;
- Class 3: less frequent for grains constituting 10-20% of the total;
- Class 4: rare for grains constituting less than 10%.

3. Results

3.1. Qualitative Analysis

3.1.1. Pollen Spectra of Honeys Presumed of Dialium guineensis Willd

Table 2 shows the pollen spectra and incidence frequency of taxa in relation to the total number of samples of assumed honey from *Dialium guineensis*. The number of taxa identified varies from 6 to 10 per sample with their relative frequencies from 0.83% to 74.04%.

Table 2. *Pollen spectrum of Dialium honey according to RF and frequency of occurrence of taxa.*

Species	D1	D2	D3	Frequence of occurrence%	maximum RF%
Elaeis guineensis Jacq.	29.17	34.63	74.04	100	74.04
Avicennia sp	46.67	0	0	33.33	46.67
Daniellia oliveri (Rolfe) Hutch. & Dalz.	0	26.86	2.45	66.67	26.86
Dialium guineensis (Willd.)	8.33	14.56	2.13	100	14.56
Detarium senegalensis Gmel.	0	13.59	3.4	66.67	13.59
Type Graminae	5.83	0	0.11	66.67	5.83
Pterocarpus erinaceus (Poir.)	0	4.53	1.7	66.67	4.53
Type Myrtaceae	3.33	0	0.11	66.67	3.33
Afzelia africana (Smith ex Pers)	0	2.57	0	33.33	2.57
Ceiba pentandra (L.) Gaertn.	0	0.32	1.49	66.67	1.49
Rubiaceae	0.83	0	0.11	66.67	0.83

In all of these samples, 14 pollen types distributed in 7 families were encountered. The families encountered are recorded in the Histogram in Figure 4.

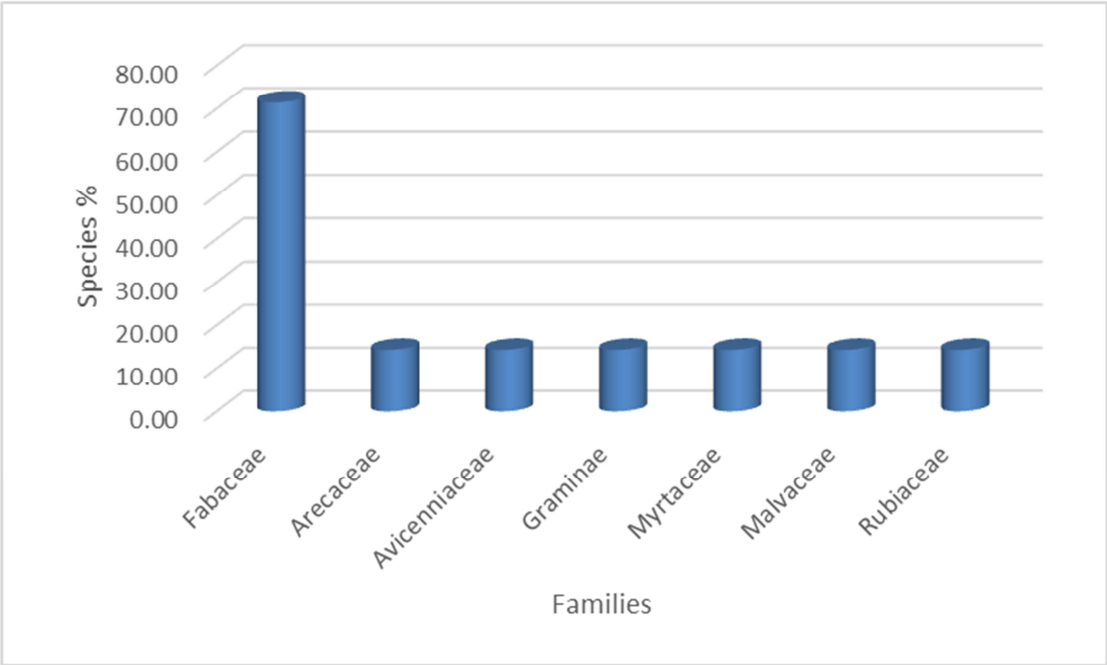

Figure 4. *Distribution of families according to species.*

The species identified at the systematic level species and genus are 8 (*Elaeis guineensis, Avicennia sp, Daniellia oliveri, Dialium guineensis, Detarium senegalensis, Pterocarpus erinaceus, Afzelia Africana, Ceiba pentandra,)* in 8 genera and 4 families (*Arecaceae, Avicenniaceae, Fabaceae and Malvaceae*). The taxa identified only at the systematic family level are 6 in 3 families ((*Graminae, Myrtaceae, Rubiaceae*). Depending on the maximum RF value, taxa above 45% (dominant) are: *Elaeis guinensis* and *Avicennia sp* with respectively 74.04% and 46.67%. The absence presence analysis shows that *Elaeis guineensis* is "very frequent" with 100% presence and *Avicennia sp* is frequent with 33.33%. For taxa that are at least once between 16 and 45% ("secondary pollen") we have: taxa of the *Daniellia oliveri* with a maximum FR of 26.86% and a presence rate of 66.67%. For taxa that are at least once between 3 and 16% ("important minor pollen") we have: *Dialium guineensis, Detarium senegalensis, Graminae type*, *Pterocarpus erinaceus, Myrtaceae type* with 14.56%, 13.59% , 5.83%, 4.53% and 3.33% respectively, all of which are "very frequent" with presence rates of 66.67 to 100%. For taxa always below 3%: *Afzelia Africana, with 2.57%, Ceiba pentandra with 1.49%, Rubiaceae* type with 0.83%; all are very common with presence rates of 33.33%, 66.67% and 100%. They are taxa isolated.

3.1.2. Distribution of Taxa According to Their Apicultural Interest

The nectariferous taxa represent 45.45% these are: *Avicennia sp*, *Dialium guineensis*, *Daniellia oliveri and Rubiaceae type with 2species*. The nectariferous and polliniferous taxa represent 36.36%: they are Detarium senegalensis, Pterocarpus erinaceus, *Myrtaceae* type with 2 species and Ceiba pentandra.

The Polliniferous taxa represent 18.18% they are: *Elaeis guineensis* and the *Graminae* type taxa with 2 species. See figure 5 next.

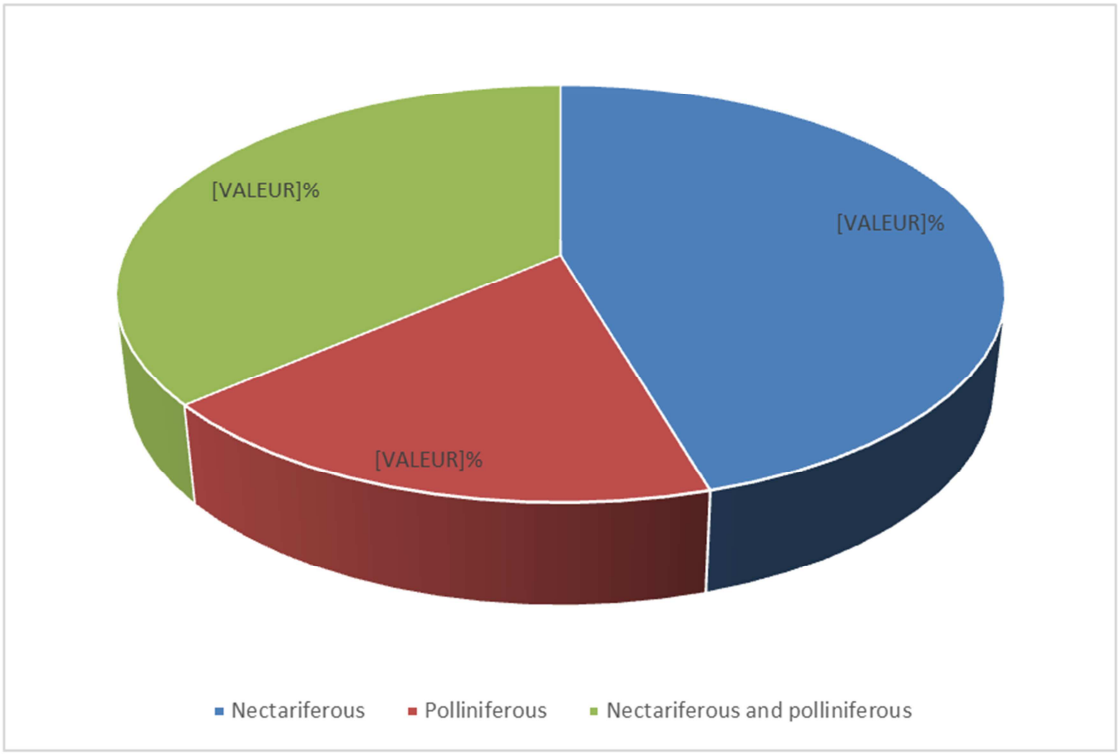

Figure 5. *Distribution of taxa according to their apicultural interest.*

3.2. Quantitative Pollen Analysis

Table 3 groups together the results of quantitative pollen analysis of samples of assumed honey from *Dialium guineensis*. This is the total number of pollen grains contained in 10 g of honeys. The honeys are classified according to their absolute pollen content. The amount of pollen varies between 16063.46 (D1) and 380700 (D3). According to the classes advocated by Maurizio [14], the presumed *Dialium guinensis* honeys are classifies I for D1 or classifies III for D2 and D3.

Table 3. *Results of the quantitative pollen analysis of presumed honeys of Dialium guinensis.*

Samples	Pollens content	Class
D1	16063.46	I
D2	125145	III
D3	380700	III

4. Discussion

This specific diversity is of 14 taxa divided into 7families. It is much lower than the 121 taxa identified by Tossou et al.

in honeys harvested from the Lama classified forest in southern Benin and the 43 taxa identified by Tossou et al. in honeys sold in Cotonou in Benin, from the 43 identified by Koudegnan et al. in honey from the Guinean zone of Togo, Dongock et al. which is of 41 species identified in the honeys of the Sudanese Guinean zone of Cameroon, Fohounfo [8], which is of 32 species for the honeys of Ewé-Kétou in the south east of Benin, to the 21 taxa found by Rasoloarijao [16], in the honeys of Madagascar. This specific diversity is however found by Lobreau-Callen et al. Boukounbé and Kandi, localities in Northern Benin. In agreement with Agwu et al. Tossou et al. this difference in taxonomic diversity can be explained by the number of samples analyzed, their harvest period and the floristic diversity of the types of formation used as sources of bee feeding**.**

Elaeis guineensis is found in all samples and is either accompanying or dominant in agreement with Koudegnan et al. [11]. Indeed, the high number of *Elaeis guineensis* pollen in the Azianfokopé-Takpla farm (51.15%) is due to the fact that in this locality the palm-tree is one of the main resources for the daily needs of the population. It is therefore the object of cultivation; which makes widely available this source of pollen supply for bees, Yedomonhan et al. [23].

And in addition Kasséle includes the mangrove and the forest which explains the presence of forest species and mangroves.

If we want to determine the botanical origin of these *Dialium* honey, the nectariferous taxa coming into the composition of these honeys will be examined from the indications of Ricciardelli D'Albore et al. [17], to specify their type of representation.

These supposed samples of *Dialium guineensis*, *Elaeis guinensis* is dominant in D3 and is accompanying in D1 and D2. It is a pollen taxon that has an anemophilous pollination. The presence of Elaeis in honeys is not related to the nectar collection necessary for their development, it is a taxon devoid of nectaries in both the male flower and the female flower. Its strong presence can only be due to contamination that takes place in the hive via bees themselves or during apiculture manipulations or by the incorporation of these pollen grains disseminated by the wind into droplets of Honeydew.

In D3, *Dialium guinensis* is isolated. *Dialium* is a nectarifer species that is visited by the bee for its nectar but according to Battesti reasoning it can be said that *Dialium* pollen is an under-represented pollen. And the over representation of *Elaeis* can mask its pollen marking. The other taxa associated with it are mostly isolated taxa.

In the D1 sample where *Elaeis* is the accompanying pollen, *Dialium* is isolated important and *Avicennia sp* which is a nectarifer species is dominant with 46.67%. All the others associated with it are either isolated or important isolated. If we take into account the absolute poverty in pollen grain of this sample, which is of class I according to Louveaux et al. [14], it is nectar honey. The dominant pollen of this D1 sample originates from a nectarifer species; therefore D1 is a honey of the mangrove and not of *Dialium*.

D2 and D3 are of Class III, according to Louveaux et al. these samples are mixtures of honeys rich In pure pollens and honeydews even though *Elaei*s is dominant in D3.

5. Conclusion

The pollen analysis of the 3 samples of supposed Dialium honey collected in Kasséle and Souloul in the Ziguinchor region identified 14 plant taxa forbidden by bees. The specific richness varies from 6 to 10 taxa per sample of honey. The name honeydew of Dialium is not consistent with the results of the pollen analysis. We found other names for these honeys, one of the samples of Kasséle D1 is a honey of Mangrove according to the results of the pollen analysis on the other hand the 2 samples of Kasséle and Souloul D2 and D3 are honeys Contaminated with *Elaeis guinensis*, rich in pure pollen and honeydew. The other species found then constitute a source abondante and permanent pollen fairly commonly forested by bee colonies.

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