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Melissopalynology of Honey from Sudan: Implication for the Honey Identity and Misdesignation

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Abstract: Due to the great nutritional and medicinal value of honey, there has been growing consumer's preference towards honey of a known identity. However, honey now is the third food in the world subjected to adulteration. Therefore, the current study was focused on judging the identity of Sudanese honeys and checking whether there is any misdesignation from originality. Melissopalynology was used as a tool for this purpose. A number of 60 honey samples were purchased from honey sellers. Results indicated that honey bees foraged on a bio-diversified number of plant species constituted of 11 major families [Fabaceae (43.3%), being the predominant family] and 8 minor families. Respectively, 18.3% & 2% of the honey samples were found to be misdesignated by the honey sellers from their botanical and geographical identities. Some samples were predicted by melissopalynology to be originated from Ethiopia by the presence of marker pollens such as Kniphofia foliosa, Guizotia abyssinica, and Acacia abyssinica an indigenous Ethiopian flora. Thus these findings proved that melissopalynology is an effective tool in judging the identity of honey and pro of being inexpensive.

Keywords: melissopalynology; honey identity; forage biodiversity

1. Introduction

Honey is the natural sweet substance that produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen. Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter [1].

The adulteration of honey have increased in recent years because of the higher prices of honey compared to adulterants, and the obsolescence of official methods to detect instances of fraud. The current version of the phenomenon has a tremendous magnitude and impacts both the price of honey and the viability of the beekeeping industry [2].

According to the US Pharmacopeia's Food Fraud Database, honey is now the third "favorite" food target for adulteration, ranking only behind milk and olive oil [3]. The overall result of this dishonesty from beekeepers and honey sellers who run after short-term gains and low prices on the shelves is a big threat to food safety, food security, and ecological sustainability [4]. Different forms of honey adulteration can currently be found [5]: 1. Intentional dilution with cheap syrups (corn, rice, beet, etc.). 2. Extracting immature honey and dehumidifying it by mechanical means. 3. Use of ion exchange resins to remove residues and lighten honey color. 4. Feeding hives during a nectar flow 5. Masking the geographical and/or botanical origin of honey.

Honey is one of the best-known bee products that is in very much need to be produced properly and prepared according to high-quality parameters. Due to the growing consumer's preference towards honey of a known identity. The current study was focused on judging the identity of Sudanese honeys and checking whether there is any misdesignation from originality using melissopalynology as a tool for this purpose.

Palynological information is widely used in different disciplines. In the field of geographical and archaeological investigations, pollen fossils are used to determine vegetation and climatic features of an era in the past and oil deposits in the earth. In criminology, palynology has been used in forensic evidence in confirming the season and scene of a crime [6,7]. Also it is important in the study of pollen spectra of honeys "melissopalynology", since honey carries within itself its own certificate of identity [8,9].

Market situation of world honey indicates an increasing trend of consumer's preferences towards honey types that are originated from one or majorly one plant source (mono-floral or unifloral honey concepts). This trend could attribute to the therapeutic value of each honey type [10].

According to the Codex Alimentarius Standards and the European Standards Directive, the use of a botanical designation of honey is allowed if it originates predominately from the indicated floral source. Honey may also be designated by the name of a geographical region if it was produced strictly within the area referred to in the label [11,12].

Honey bees normally forage within a circular distance of approximately 3 km from their hives. Therefore, its characterization as an authentic and natural foodstuff can be achieved by studying its botanical and geographical origin[13].This aspect is a requirement for the voluntary quality label of "Protected Designation of Origin" adopted by the European Union since 1992 [14].

It has been reported that identification of honey origin as well as the proof of its authenticity has become an important issue [15]. Therefore, the search for reliable chemical markers indicating its floral and/or geographical origin has been the focus of many studies". Among chemical markers reported are the volatile compounds [16] , amino acids [17], and trace elements [18]. However, melissopalynology stands as "gold standard" method because it is cheap and informative. Thus the analysis of pollen in honey can give information regarding the region, season of production, patterns and variations of the local flora [19].

Higher prices are paid for certain types of honey, therefore, beekeepers and honey packers often misdesignate the botanical and geographical source of the honey[13]. In Europe, honey imported from China or Latin America has lower price than locally produced one. Even differences persist between European countries. In Saudi Arabia, honey produced locally is 10-20 folds higher priced than imported honey[20].Thus there is a financial interest in misdesignation of the botanical and geographical source of honey[13].

In Sudan, there is consumer preference for honey from certain botanical origin as well as local honey that is produced in specific regions of the country. As for examples, consumers pay high price for Ziziphus and Acacia honey than other monofloral honeys for their ritual and therapeutic values. Also they are willing to purchase honey from Darfur region at higher price than honey produced in any other region [21]. This financial issue has led honey sellers for deliberately misdesignation of the botanical and geographical sources of honey. To address this issue we have performed a survey and melissopalynological investigation of the honey in Sudan. Aiming to prove and improve Sudanese honey identity and to check whether there is any misdesignation from originality.

2. Materials and Methods

Honey samples

Sixty honey samples were purchased from honey sellers of 10 regions in Sudan(Figure 1). Each sample of honey is stirred thoroughly to assure even distribution of any botanical elements. Samples were labeled (1-60) and stored under room temperature until used for the analysis. Primary data on sample location and botanical source were recorded.

Pollen analysis

The analysis of pollen of honey was carried according to melissopalynology method which was based on acetolysis technique [22,8]. Briefly, approximately 10 g of honey was weighted in a pointed glass centrifuge tube capacity (ca. 50ml) and dissolved with with 30-35 ml of warm(20 – 40C°), weak sulfuric acid (3 ml of concentrated acid to 1000 mi distilled water). The solution was centrifuged for 10 min and the supernatant was decanted. Distilled water (20 ml) was added to the completely dissolve remaining sugar crystals and centrifuged for 5 minutes and the supernatant was decanted. Taking sludge and placed in a Petri dish and stained with basic fuchsin and then distribute on glass slides. Then dried with a series of alcohols 50%, 70%, and 100% where it was put a drop of each focus on the slide and left for two minutes. One drop of glycerin jelly was applied to the cover slip and the sample was examined through the microscope using (X 400).

Pollen taxa were identified using pollen reference slides deposited in the Department of Bee Research, Environment and Natural Resources & Desertification Research Institute, Sudan. Other references such as the study on the pollen flora of Sudan [23] , website of Australasian pollen and Spore Atlas (APSA), [24] and the Atlas of pollen grains of major honey bee flora of Ethiopia [25] were also used for pollen identification.

3. Results

3.1. Pollen analysis

Analysis of pollen of honey from Sudan was carried out. Data on the number and distribution of the samples and sites of collection are given in Figure.1. Plant species constituting 11 families of major pollen sources were identified. Another 8 families of minor pollen sources were also identified. Table 1 shows sample frequencies as well as the major and minor pollen spectra of plant species normally foraged by honey bees in Sudan. Families and frequencies of major plants observed were: Fabaceae (43.3%), Rahmnaceae (21.6%), Boraginaceae (10%), Myrtaceae (8.3%), Meliaceae (3.3%), Astraceae (3.3%), Gramineae (3.3%), Apiaceae (1.6%), Amaranthaceae (1.6%), Malvaceae (1.6%), and Asphodelaceae (1.6%). On the other hand plant families like: Cucurbitaceae, Balanitaceae, Combretaceae, Caesalpinaceae, Convolvulaceae, Asteraceae, Pedaliaceae, and Fabaceae represented minor pollen sources in the investigated samples. Photomicrographs of some pollen morphs identified are depicted in Figure 2.

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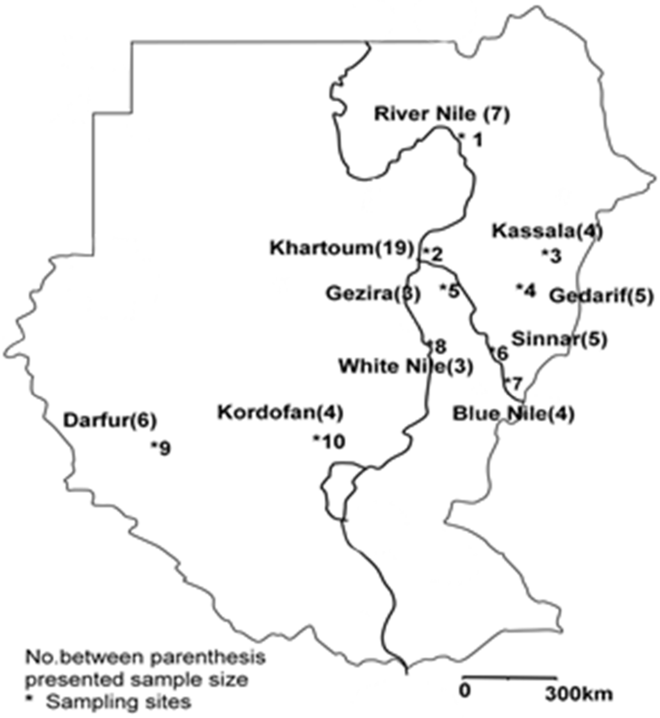

Figure 1. Map of honey samples distribution.

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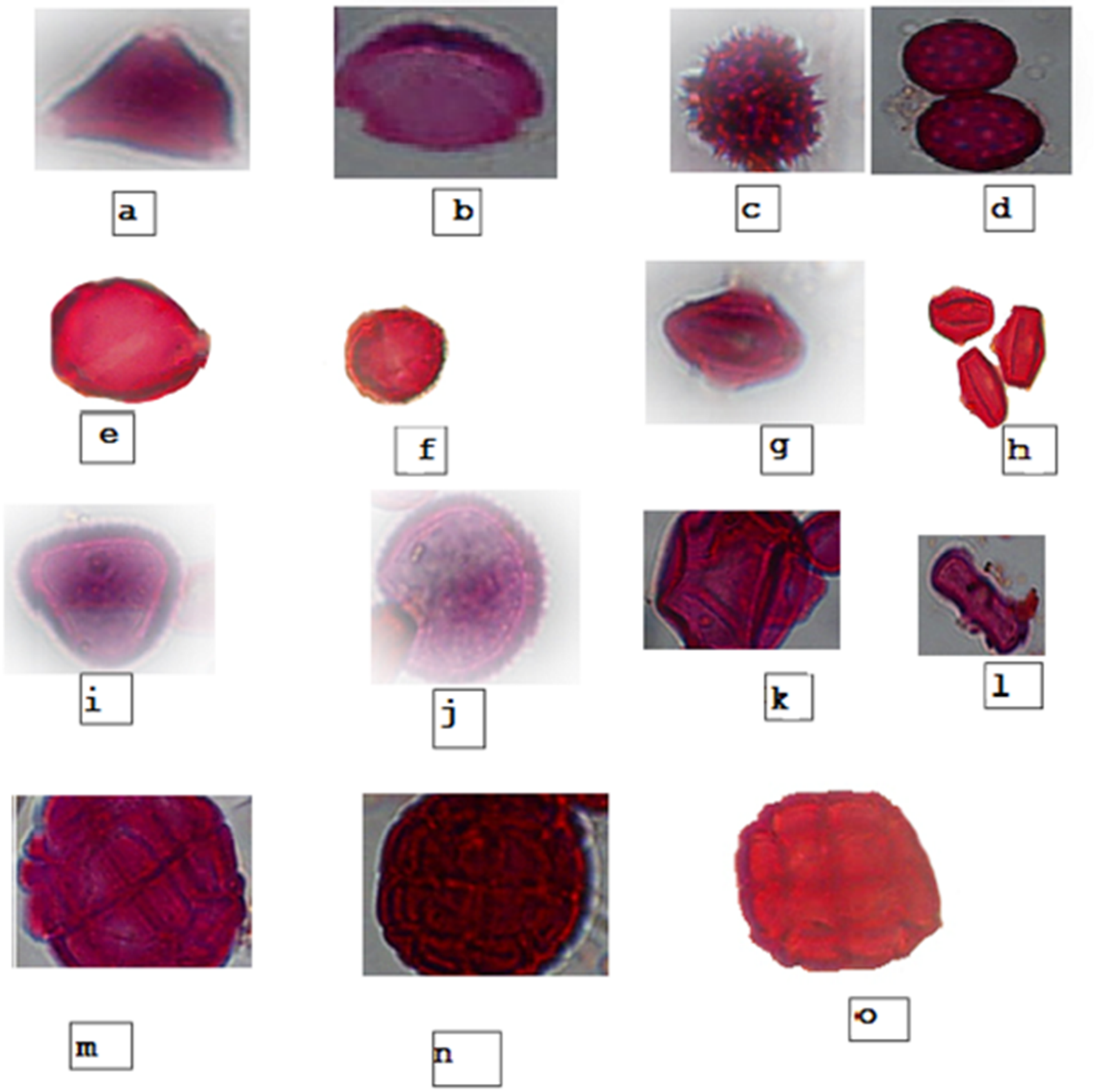

Figure 2: Photomicrographs of the identified pollen grain morphs: a - Myrtaceae (*Eucalyptus* spp.), b - Rhamnaceae (*Ziziphus* spp.) c- Astraceae (*Helianthus* spp.), d - Amaranthaceae (*Amaranthus* spp.), e- *Asteraceae (Guizotia abyssinica)*, f- *Pedaliaaceae (Seasamum indicum)*, g-h *Anacardiaceae* (*Kniphofia folios*), i-k Poaceae (*Gramineae* spp.) l- Apiaceae, *(Foeniculum* spp.) m-o Fabaceae (m- *Acacia nilotica*; n- *Faidherbia albida*; o- *Acacia abyssinica*) .

Table 1. Pollen spectra of the studied honey samples.

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Families of major pollen sources	**Sample frequency (%)**	**Plant species**	**Families of minor pollen sources**	**Plant species**
Rhamnaceae	21.6	*Ziziphus* spp.	Cucurbitaceae	*Cucuribta spp*
Fabaceae	43.3	*Acacia nilotica*	Balanitaceae	*Balanites aegyptiaca*
Meliaceae	3.3	*Azadirachta indica*	Combretaceae	*Terminalia brownie*
Myrtaceae	8.3	*Eucalyptus* spp.	Caesalpinaceae	*Cassia* spp.
Astraceae	3.3	*Helianthus annulus*	Convolvulaceae	*Ipomoea* spp.
Apiaceae	1.6	*Foeniculum* spp.	Asteraceae	*Guizotia abyssinica*
Amaranthaceae	1.6	*Amaranthus* spp.	Fabaceae	*Faidherbia albida*
Boraginaceae	10.0	*Anogeissus leiocarpus*	Fabaceae	*Acacia abyssinica*
Gramineae	3.3	*Poaceae* spp.	Pedaliaceae	*Sesamum indicum*
Malvaceae	1.6	*Hibiscus* spp.		

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Asphodelaceae	1.6	*Kinphofia foliosa*		

Note: the flora: Kinphofia foliosa, Guizotia abyssinica, and Acacia abyssinica are indigenous to Ethiopia and are not found in Sudan.

3.2. Misdesignation of honey's botanical and geographical identity

The results in table 2 showed that about 2% and 18.3% of the collected honey samples were, respectively geographically or botanically misdesignated by the honey sellers.

Table 2. Designation of the honey samples (n=60) by the honey seller and by the palynological analysis.

Code	**designation by**			**Code**	**designation by**		
	seller	**remark**	**palynology**		**seller**	**remark**	**palynology**
1	*Ziziphus* spp.	✓	*Ziziphus* sps.	31	*Ziziphus* spp.	✓	*Ziziphus* spp.
2	*A. seyal*	✓	*A. seyal*	32	*Ziziphus* spp.	✓	*Ziziphus* spp.
3	*A. nilotica*	✓	*A. nilotica*	33	*Ziziphus* spp.	✓	*Ziziphus* spp.
4	*A. seyal*	✓	*A. seyal*	34	*Ziziphus* spp.	✓	*Ziziphus* spp.
5	*Ziziphus* spp.	✓	*Ziziphus* spp.	35	*Ziziphus* spp.	✓	*Ziziphus* spp.
6	*A. nilotica*	✓	*A. nilotica*	36	*Ziziphus* spp.	✓	*Ziziphus* spp.
7	*A. seyal*	✓	*A. seyal*	37	*Ziziphus* spp.	✓	*Ziziphus* spp.
8	*Ziziphus* spp.	✓	*Ziziphus* spp.	38	*Ziziphus* spp.	✓	*Ziziphus* spp.
9	*A. nilotica*	✓	*A. nilotica*	39	*Ziziphus* spp.	✓	*Ziziphus* spp.
10	*Azadirachta* spp.	✓	*Azadirachta* spp.	40	*Ziziphus* spp.	✓	*Ziziphus* spp.

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11	NI		*Eucalyptus* spp.	41	NI		*Cordia* spp.
12	*Ziziphus* spp.	✓	*Ziziphus* spp.	42	NI		*Gramineae*
13	*Acacia seyal*	✓	*Acacia seyal*	43	NI		*Cordia* spp.
14	*Acacia nilotica*	✓	*Acacia nilotica*	44	*Ziziphus* spp.	✓	*Ziziphus* spp.
15	*Acacia seyal*	✓ √	*Acacia seyal*	45	NI		*Acacia nilotica*
16	*Eucalyptus* spp.	✓	*Eucalyptus* spp.	46	NI		*Acacia seyal*
17	*Helianthus annus*	✓	*Helianthus annus*	47	NI		*Acacia nilotica*
18	*Acacia nilotica*	✓	*Acacia nilotica*	48	NI		*Acacia seyal*
19	*Ziziphus* spp.	✓	*Ziziphus* spp.	49	*Acacia seyal*	✓	*Acacia seyal*
20	NI		Kinphofia foliosa	50	*Acacia nilotica*	✓	*Acacia nilotica*
21	*Eucalyptus* spp.	✓	*Eucalyptus* spp.	51	*Ziziphus* spp.	✓	*Ziziphus* spp.
22	*Eucalyptus* spp.	✓	*Eucalyptus sp.*	52	*Acacia seyal*	✓	*Acacia seyal*
23	*Eucalyptus* spp.	✓	*Eucalyptus* spp.	53	honeydew	✓	honeydew
24	NI		*Azadirachta* spp	54	honeydew	✓	honeydew
25	*Ziziphus* spp.	☒	*Amaranthus* spp.	55	*Helianthus annus*	✓	*Helianthus annus*
26	*Acacia seyal*	✓	*Acacia seyal*	56	*Acacia seyal*	✓	*Acacia seyal*
27	*Acacia seyal*	✓	*Acacia seyal*	57	*Acacia nilotica*	✓	*Acacia nilotica*
28	*Acacia seyal*	✓	*Acacia seyal*	58	*Ziziphus* spp.	✓	*Ziziphus* spp.
29	*Anogeissus leiocarpus*	✓	*Angeissus lieocarpus*	59	*Ziziphus* spp.	✓	*Ziziphus* spp.
30	NI	✓	*Acacia seyal*	60	*Eucalyptus* spp.	✓	*Eucalyptus* spp.

Key: Ni Not identified, ✓ the honey identity is correctly designated by the sellers, and ☒ the honey identity is incorrectly designated by the honey sellers.

4. Discussion

Our study demonstrated the photomicrographs and misdesignation of honey's botanical and geographical identity. The obtained result indicated predominance of pollen from forest trees (80%). This finding necessitated raising awareness about the value of forests biodiversity. Hence encourages beekeepers in conscious protection, conservation and sustainable management of forest resources. Moreover, in the marketing of honey it is very important to label the true floral sources of honey in which melissopalynology is useful tool in determining monofloral or multifloral honeys, dominant and predominant sources of honey "pollen frequencies" and detecting artificial and honeydew honeys "honey adulteration" [26].

The pollen photomicrographs depicted in figure 2 corresponded to some pollen morphs previously reported by other authors who have conducted mellisoplaynological analysis of Sudanese honeys as well as honeys from other regions [27, 28, 29].

Bogdanov and Martin [13] reported that in food control, pollen analysis is very efficient for the differentiation of honeys produced in distinctly different geographical and climatic areas. In the present study, sample coded number 20, which designated as from Sudan in table 2, after mellisoplaynological analysis this sample was proved to be originated from Ethiopia. Its pollen spectra revealed presence of pollens of Guizotia abyssinica, Kniphofia foliosa, and Acacia abyssinica an indigenous flora of Ethiopia (figure 2e, g-h, and o). The pollen morphs of these exotic flora observed in this study, were simulated by different reports of Ethiopian honeys [25, 30]. None of the three trees is reported to be grown in the Sudan. It is also necessary to note that some of the samples were purchased from Gadaref district of Sudan, which borders with Ethiopia. People from Gadaref State regularly exchange goods with traders from the boarders of Ethiopia. Therefore, this sample might have originated in Ethiopia and passed into Sudan through such trade.

Sample coded 25 (table 2), was sold by the sellers as ziziphus honey but after mellisopalynological investigation it was proved to be Amaranthus honey (figure 2d). It is normal phenomenon in Sudan that honey sellers allege that their honey is from ziziphus tree even though if it was from any else plant source to gain high price.

Also some honey samples were sold by the honey sellers as from anonymous botanical sources. However, after mellisopalynological analysis, their corresponding botanical sources were identified e. g samples coded 11, 20, 24, 30,41, 42, 43, 45, 46, 47, 48 (table 2) were respectively Eucalyptus spp., Kniphofia foliosa, Azadirachta spp., Acacia seyal, Cordia spp., Gramineae, Cordia spp., Acacia nilotica, Acacia seyal, Acacia nilotica, Acacia seyal (figure 2). Thus melissopalynology is informative technique. It was reported, "honey is a commodity that is closely linked to a territory" [31]. Thus pollen analysis of honey is a powerful tool in detecting honey identity.

5. Conclusions

From the results obtained here by pollen analysis of honey "millisopalynology" it can be concluded that palynology is informative for: (a) botanical and geographical identity of honey [18.3% and 2% of the studied samples showed geographical and botanical misdesignation, respectively], (b) honeybees forage biodiversity, and (c) requires inexpensive laboratory assets.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collectionand analysis were performed by [Mohammed A. El-Nebir], [Mogbel Ahmed Abdalla El Niweiri] and [Seif Eldin A. Mohammed]. The first draft of the manuscript was written by [[Mohammed A. El-Nebir] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare no conflict of interest

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