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ORIGINAL RESEARCH ARTICLE

Authentication of the botanical and geographic origin of Egyptian honey using pollen analysis methods

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In the honey market, botanical and geographical mis-labeling has become common. Therefore, the aim of this study was to use pollen analyses to authenticate the botanical origin and labeling of seven kinds of Egyptian honey and to identify sources of pollen used by honey bees in Egypt. Honeys are labeled, based on sources of pollen, which are usually provided by Egyptian beekeepers are classified as; clover, sidr, citrus, banana, cotton, Brazilian peppertree, and sunflower honey. During 2016–2017, absolute and relative numbers of pollen types were collected synchronously from apiaries and recta of worker bees from which samples of honey were also collected. Pollen from 20 plant taxa, belonging to 14 families was observed. Results of pollen analysis confirmed identities of sources of honey and nectar, as indicated and labeled during collection for all honeys except cotton, sunflower, and Brazilian pepper honeys, where, the predominant types of pollen identified in honey and recta of bees were: *Zea mays* (69%) in cotton honey, *Cucurbitaceae* (79%) in sunflower honey, and *Eucalyptus* sp. (52%) in Brazilian pepper honey. These data suggest that these honey should be re-classification (labeling) as *Zea mays*, cucurbits, and *Eucalyptus* honey instead of cotton, sunflower, and Brazilian pepper honey, respectively. Most of the honeys screened contained pollen grains (PGs) of *Echium sp.* and *Trifolium alexandrinum,* which indicated their wide geographical distributions in Egypt. Quantitative data of PGs per 10 g of honey indicated unequal geographical distributions of appropriate plants for use by bees in Egypt.

Keywords: pollen analysis, Egyptian clover, unifloral honey, bee plants, sustainable beekeeping

Introduction

Honey can be derived from various species of plants as bees collect nectar from different flowers. Considering the growing global trade and due to the greater economic values of certain kinds of honey, connected with specific origins, these products are particularly susceptible to adulteration, mixing of honey, or inaccurate or fraudulent labeling of honey of lesser value. The authenticity of honey is, therefore, increasingly in question. Thus, in order to safeguard customers and encourage fair competition among manufacturers authentication is necessary, especially with respect to geographical and botanical origins (Bogdanov & Martin, 2002; Soares, Amaral, Oliveira, & Mafra, 2017).

Melissopalynology is a branch of palynology (the science of pollen and spores) dealing with microscopic investigations of bee honey and therefore represents one of the greatest discriminatory powers for classifying types of honey (Kilic, Kutlu, & Ozdemir, 2016; Ruoff & Bogdanov, 2004). It is used to determine sources of pollen utilized by honey bees to produce honey. Melissopalynology can be qualitative, by identifying species of pollen in honey or quantitative by quantifying absolute and relative numbers of pollen grains (PGs) in 10 g of honey (Song, Yao, & Yang, 2012; Von Der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004). It has been proposed that analyses of pollen in recta of bees can also be used as a complementary or/and confirmatory method to determine species of plants from which bees are collecting pollen in a specific area (Dimou & Thrasyvoulou, 2009). Melissopalynological analyses are useful not only for the determination of floral and geographical origins of honey but also in characterization/authenticity of types of honey, based on sources of pollen from plant species whose flowers are used to produce honey.

Among Arab nations as well as throughout Africa, Egypt is considered to be the most important country for beekeeping (Abou-Shaara, 2015; Al Naggar, Codling, Giesy, & Safer, 2018). Therefore, identifying flora used by bees to make honey and information on their relative importance to colonies of honey bees are essential for understanding preferences for foraging to facilitate the sustainable management of beekeeping in Egypt and worldwide (Abou-Shaara, 2015). This information about sources of nectar and pollen will help beekeepers to plan for managing their colonies and to move them to other areas during certain periods where preferred sources of nectar and pollen are available (Taha, 2015).

In Egypt, as well as worldwide, pollen traps have been used extensively as a method to determine

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Table 1. Sites of collection, periods of collection, and local vegetation in vicinities of apiaries, from which honey and bee workers were collected during 2016–2017 in Egypt.

Samples	Site of collection	Period	Common vegetation
Cotton honey	Desouk city, Kafr El Sheikh Governorate (31.3°N 30.93°E)	August 2016	*Gossypium* spp. (cotton), *Oryza sativa* (rice), and *Zea mays* (maize).
Sunflower honey	Abu El Matamir, Al Behira governorate (30.61°N 30.43°E)	August 2016	*Helianthus annuus* (sunflower), *Cucumis sativus* (cucumber), watermelon *Zea mays* (maize), and *Gossypium* spp. (cotton).
Banana honey	in Sadat city, Monufia Governorate (30.52°N 30.99°E)	September 2016	*Musa*spp. (banana), *Prunus* (peaches), and *Punicagranatum,* pomegranates
Brazilian pepper honey	Agricultural research center, Alexandria Governorate (31°10′N 29°53′E)	October 2016	*Schinus terebinthifolius* (Brazilian pepper trees)
Sidr honey	Qena Governorate (26.143°N 32.728°E)	January 2017	*Rhamnus* sp. (seder trees) *Solanumlycopersicum* (tomatoes) and *Arachis hypogaea* (peanuts)
Citrus honey	Nubaria city, Al Behira governorate (30.61°N 30.43°E)	April 2017	*Citrus sinensis* (orange), *Citrus reticulate* (tangerine), *Citrus aurantium* (bitter orange) and *Citrus limon* (lemon)
Clover honey	Aga city, Dakahlia governorate (31°03′N 31°23′E)	June 2017	*Trifoliumalexandrinum* (clover), *Oryza sativa* (rice), *Zea mays* (maize).

available pollen (Dimou & Thrasyvoulou, 2007; Hawkins et al., 2015; Ismail, Owayss, Mohanny, & Salem, 2013; Mesbah et al., 2017). However, most of these studies focused more on quantities of pollen collected in terms of (kg of pollen collected/colony/season) to monitor activities of bee colonies and richness of the foraging area with sources of pollen, regardless of the type of honey produced. In Egypt, these studies were also limited to specific regions or governorates. So, comprehensive melissopalynological studies of honey in Egypt were few (Ismail et al., 2013; Mesbah et al., 2017).

Based on the available literature, there is only one study that used melissopalynological analyses to differentiate between local and imported bee honey in Egypt (El Sohaimy, Masry, & Shehata, 2015). Consequently, the aim of this study was to authenticate the botanical and geographical origins of seven kinds of honeys collected during 2016–2017, from various geographical and botanical regions of Egypt by qualitative and quantitative melissopalynological methods. Moreover, the pollen spectrum of the recta of worker bees collected from the same apiaries was reported as well.

Materials and Methods

Collection of samples

A survey of geographical locations of honey production apiaries throughout Egyptian governorates was conducted during the harvesting seasons of 2016–2017. Locations for collections were selected to include various geographical and botanical regions (Table 1, Supplementary data, Figure S1) (El-Sofany, Al Naggar, Naiem, & Seif, 2018). Samples of fresh honey (1 kg each) were extracted from honeycombs of three randomly selected bee hives at each apiary into a disposable polyethylene container. Samples were stored at −4 °C until analysis. Workers bees were collected synchronously with samples of honey from the same hives. Workers bees were carefully brushed directly into disposable polyethylene bags after fuming by smoker to calm the bees after collection, all samples were frozen at −4 °C in the laboratory until analysis of pollen.

Analysis of pollen in honey

Qualitative melissopalynological analysis

The melissopalynological method recommended by the International Commission for Bee Botany (ICBB) (Louveaux, Maurizio, & Vorwohl, 1978; Von Der Ohe et al., 2004); was used to identify species of pollen in honey. Ten-gram samples of each honey were dissolved in 20 ml of water (20–40 °C) in 50 ml Falcon tubes (El-Gomhouria Company, Cairo, Egypt). The diluted honey was centrifuged for 10 min at 3500 rpm. The supernatant liquid was decanted, and 20 ml of distilled water was added to completely dissolve the remaining sugar crystals and a micro-spatula or Pasteur pipette was used to get into the tip of the centrifuge tube. Samples were centrifuged again for 5 min at 3500 rpm. The supernatant was then decanted, and the entire sediment was then placed on two slides and spread over an area about 20 × 20 mm, after drying by slight heating at 40 °C, sediment was covered with glycerin gelatin liquefied by heating in a water bath at 40 °C and photographed under a Leica DM2500 light microscope. Types of pollen were identified by comparison with reference slides of pollen collected directly from the plants cultivated in Egypt. Relative abundances (%) of each species of pollen were calculated (Equation 1) (Rosdi, Selvaraju, Vikram, Thevan, & Arifullah, 2016).

$$\textbf{Abundance}\ (\%) = \frac{\textbf{Total number of a particular species x } 100}{\textbf{Total number of pollen types observed}} \quad (1)$$

At least 300 PGs were counted, and their relative frequency classes were determined using the international melissopalynological nomenclature (Louveaux et al., 1978); predominant pollen: for pollen occurring for more than 45% of the total pollen count, accompanying pollen or secondary pollen (16–45%), important minor pollen (3–15%) and minor pollen, occurring <3%. Honey was considered mono-floral if the representation of pollen was predominant. Otherwise, it was considered multi-floral.

Quantitative melissopalynological analyses

The aim of quantitative melissopalynological analysis is to determine absolute counts of pollens in honey; to determine if the honey is rich or poor with PGs. Pollen content per 10 g of each honey was quantified according to Von Der Ohe et al. (2004). Ten grams of each honey was dissolved with 40 ml of tepid distilled water (20–40 °C) into a beaker. A vacuum filtration apparatus, using a membrane filter of mixed cellulose esters, with a pore size of 3 µm and diameter 25 mm was used. Filters were dampened with a small amount of water after which the honey solution was passed through the filter. Filters were then placed on microscope classes to which a few drops of immersion oil had been added and then covered with a cover slip. At least 500 PGs were counted using an appropriate magnification of a microscope. In order to calculate the absolute number of PG (***N***), it was necessary to calculate the surface area of the part of the filter containing sediment (***S***) and the area of the microscope fields at the magnification used (***s***). The latter was measured using a stage micrometer. The absolute number of PG in 10 g of honey (PG/10 g) was calculated (Equation 2).

$$\textbf{PG}/10\textbf{g} = \frac{\textbf{S x npg x } 10}{\textbf{s x a x p}} \quad (2)$$

where ***S*** is the surface area of the part of the filter containing sediment (mm^2) ***s*** is the area of one microscopic field at the magnification used (mm^2) $\boldsymbol{n_{PG}}$ is the total number of PG counted ***a*** is the number of fields counted, ***p*** is the mass of honey (g)

Results were expressed in thousands (10^3), rounding to the nearest thousand (e.g., $N/10\,g = 26{,}342$ is expressed as 26×10^3). Honey was then classified according to Maurizio's (1939) pollen representatives classes: Class I, honeys poor in pollen (PG/10 g; $<20 \times 10^3$), Class II, honeys with normal pollen representation (PG/10 g; $20–100 \times 10^3$), Class III, honeys with overrepresented pollen (PG/10 g; $100–500 \times 10^3$), Class IV, honeys with strongly overrepresented pollen (PG/10 g; $500 \times 10^3–10^6$), and Class V, pressed honeys (PG/10 g $> 10^6$).

Ecological parameters were calculated using a diversity index (Shannon & Weaver, 1949) and Pielou (1977). The diversity index of Shannon and Weaver (1949) was calculated (Equation 3).

$$\boldsymbol{H} = -\sum\nolimits_i^n \boldsymbol{pi}\ .\ln\boldsymbol{pi} \quad (3)$$

where *H* is the Shannon–Weaver diversity index (here measured for pollen diversity), ***pi*** is the proportion of each pollen type ***I*** encountered in the sample, and ln is the natural logarithm.

The Pielou (1977) indicates when heterogeneous utilization of resources occurred and values approach zero; if the resources were exploited homogeneously, then values approach 1.0 (Equation 4).

$$\boldsymbol{F} = \frac{\boldsymbol{H}}{\boldsymbol{H\ max}} \quad (4)$$

where *F* is evenness, *H* is the Shannon–Weaver diversity index, and *H* max is the natural logarithm of the total number of plant species found in every honey studied in this study.

Pollen analysis of honey bee recta

Alimentary canals of honey bee workers collected at each apiary were removed and recta were isolated and 5–10 individual recta were mixed by vortex with 1 ml distilled water in a test tube. A fraction of 50 µl of the solution was spread onto a 22 × 22 mm area on a slide. Ten fields of view distributed uniformly over the area were analyzed at a magnification of 400× by use of light microscopy. On average 650 ± 150 (mean ± SD) PGs were identified and counted per slide (Dimou, Thrasyvoulou, & Tsirakoglou, 2006). Photomicrographs of some types of PGs recovered from honey and bee recta investigated in this study are shown (Figure 1).

Results

Qualitative pollen analyses of honey

Qualitative melissopalynological analyses identified pollen of 20 species of plant in various honeys, with percentages ranging from ≥1% to 79% and belonging to 14 families and 1 fungal spore were identified from the seven types of honey studied (Table 2). Cotton and Brazilian pepper honey contained the minimum ($n = 3$) and maximum ($n = 9$) number of plant taxa, respectively. Unidentified PGs were found in two kinds of honey (citrus and sunflower) with relative frequencies of (6%) and (6.5%), respectively (Table 2). Two types of PGs were found in ≥43% of natural honey studied. These types of pollen included *Echium* sp. (family: Boraginaceae) that have been found in four (57%) of

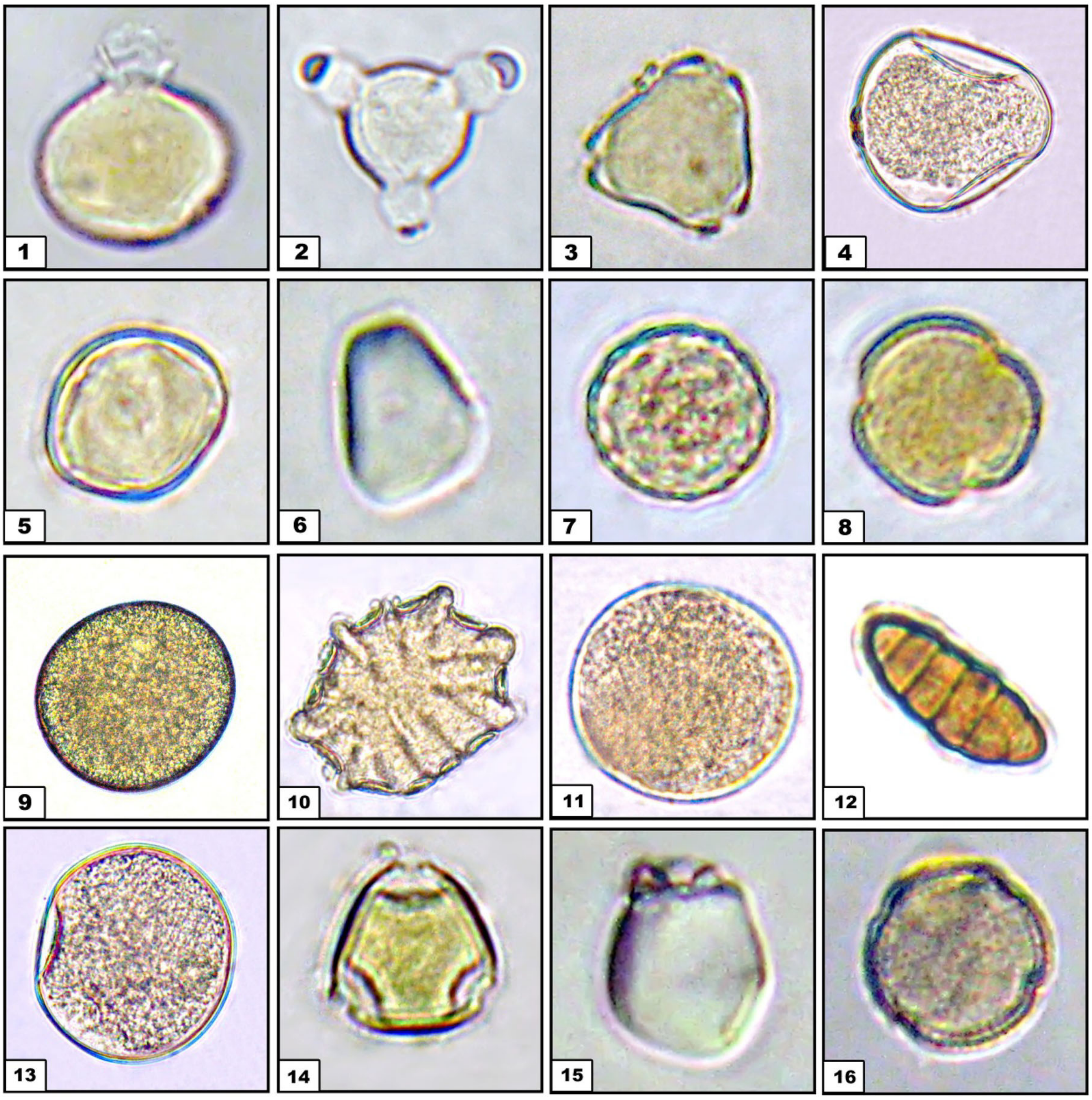

Figure 1. Photomicrographs of selected types of pollen grains recovered from natural honey collected from different botanical and geographical origins in Egypt during 2016–2017. (1) *Trifoliumalexandrinum*, (2) *Medicago* sp., (3) *Eucalyptus* sp., (4) *Phoenix dactylifera*, (5) *Umbellifera* sp., (6) *Echium* sp., (7) *Chenopodiaceae*, (8) *Citrus* sp., (9) *Cucurbitaceae*, (10) *Sesamum* sp., (11) *Zea mays*, (12) *Sporangia*, (13) *Trifolium sp*., (14) *Rhamnus* sp., (15) Unknown, and (16) *Papaver* sp.

types of honey (citrus, cotton honey, clover, and Brazilian pepper), and *Trifolium alexandrinum* (family: Fabaceae) that have been identified in three (43%) of honeys studied (cotton, clover, and Brazilian pepper) (Table 2).

Banana honey was free of PGs, however, all other investigated honeys were classified as unifloral, represented by six predominant pollen types *Citrus* sp.(50%) in citrus honey, *Trifolium alexandrinum* (49%) in clover honey, *Zea mays* (69%) in cotton honey, *Eucalyptus* sp. (52%) in Brazilian pepper honey, *Rhamnus* sp. (49%) in sidr honey, and *Cucurbitaceae* (79%) in sunflower honey. Results of both the Shannon–Weaver pollen diversity index and Pielou index indicated that the resources of PG in honey were homogeneously exploited from available sources of pollen (Table 2).

Quantitative melissopalynological analyses of honey

The absolute count of PGs in honeys studied showed that 33.3% ($n = 2$) of the honeys studied; cotton honey (9×10^3) and sunflower honey (16×10^3) were classified as Maurizio's class I (poor in PGs representation) (Table 3). While 33.3% ($n = 2$) of honeys investigated: citrus honey (21×10^3) and Brazilian pepper honey (25×10^3) were placed into Maurizio's class II (normal in PGs representation). Both clover (102×10^3) and sider (105×10^3) honeys were Maurizio's class III (overrepresented with PGs).

Pollen analysis in recta of honey bee workers

Qualitative analyses of PGs recovered from recta of worker bees showed that a total of 19 pollens,

Table 2. Qualitative melissopalynologic analyses of honey collected from different botanical, seasonal, and geographical origins in Egypt during 2016–2017.

Pollen species	Family name	Citrus honey	Cotton honey	Sunflower honey	Clover honey	Banana honey	B. pepper honey	Sidr honey
Echium sp.	Boraginaceae	3%	22%	–	5%	–	10%	–
Papaver sp.	Papaveraceae	26%	–	–	–	–	–	–
Plantago sp.	Plantaginaceae	3%	–	–	–	–	–	–
Trifolium alexandrinum	Fabaceae		9%		46%		3%	
Medicago sp.		–	–	–	33%	–	–	–
Prosopis sp.		–	–	–	11%	–	2.5%	
Trifolium sp.		–	–	–	–	–		5%
Chenopodiaceae	Chenopodiaceae	5%	–	–	–	–	15%	–
Eucalyptus gunnii	Myrtaceae	–	–	–	5%		–	–
Eucalyptus sp.		–	–	–	–	–	52%	19%
Umbellifera	Nyctaginaceae	–	–	–	–	–	5%	4%
Sporangia	Sporangia	7%	–	–	–	–	2.5%	
Phoenix dactylifera	Arecaceae		–	–	–	–	2.5%	15%
Citrus sp.	Rutaceae	50%	–	–	–	–	7.5%	
Cucurbitaceae	Cucurbitaceae	–	–	79%	–	–	–	3%
Sesamum sp.	Pedaliaceae	–	–	2.5%	–	–	–	–
Zea mays	Poaceae	–	69%	12%	–	–	–	–
Casuarina sp.	Casuarinaceae	–			–	–	–	5%
Rhamnus sp.	Rhamnaceae	–			–	–	–	49%
Unknown[a]	Unknown	6%		6.5%	–	–	–	
Total number of pollen grains found		490	440	475	550		515	580
Total number of pollen types		6	3	4	5	–	9	7
Number of unknown pollen types		1	–	1	–	–	–	–
Unknown pollen frequency (%)		6%	–	6.5%	–	–	–	–
No. of plant families in each sample		6	3	4	3	–	8	7
H		1.75	0.81	0.71	1.26	0	1.56	1.47
F		0.899	0.74	0.51	0.78	0	0.71	0.75

[a]Unknown = two different types of pollen.

Table 3. Quantitative melissopalynological analyses of different investigated honeys collected from different seasonal, botanical, and geographical origins in Egypt during 2016–2017.

Honey sample	APC/10 g honey	Maurizio's classes	Botanical source (Pollen species)
Sunflower honey	16×10^3	I	*Cucurbitaceae* (79%), *Chenopodiaceae (*12%) *Sesamum* sp. (2.5%)
Cotton honey	9×10^3	I	*Zea mays* (69%), *Echium* sp. (22%), *Trifolium alexandrinum* (9%)
Citrus honey	21×10^3	II	*Citrus* sp. (50%), *Papaver sp.* (26%), *Chenopodiaceae* (5%), *Plantago* sp. (3%), *Echium* sp. (3%)
Brazilian pepper honey	25×10^3	II	*Eucalyptus* sp. (52%), *Chenopodiaceae* (15%), *Echium* sp. (10%), *Citrus* sp. (7.5%)
Clover honey	102×10^3	III	*Trifolium alexandrinum* (46%), *Medicago* sp. (33%) *Prosopis* sp. (11%)
Sidr honey	105×10^3	III	*Rhamnus* sp. (49%), *Eucalyptus* sp. (19%), *Phoenix dactylifera* (15%)

belonging to 14 families and 1 fungal spore were identified (Table 4). Similar to pollen in honeys, recta of honey bee workers in hives producing honey classified as cotton's or Brazilian pepper, contained pollen from the minimum ($n = 4$) and maximum ($n = 8$) number of plant taxa, respectively. Unidentified PGs were found in recta of worker honey bees in hives producing citrus, sunflower or banana types of honey, with relative frequencies of (1%), (10%), and (12%), respectively (Table 4).

PGs of *Echium* sp. (family: Boraginaceae) were identified in recta of five (71.42%) workers bees producing citrus, cotton, clover, Brazilian pepper, and sidr honeys, respectively (Table 4). PGs in recta of six worker bees

Table 4. Species of pollen recovered from recta of honey bee workers collected simultaneously from the same hive as honey in different botanical, seasonal and geographical origins in Egypt during 2016–2017.

Pollen species	Family	Rectum of citrus bee's honey	Rectum of cotton bee's honey	Rectum of sunflower bee's honey	Rectum of clover bee's honey	Rectum of banana bee's honey	Rectum of B. pepper bee's honey	Rectum of sidr bee's honey
Echium sp.	Boraginaceae	1%	15%	–	3%		7%	3%
Papaver sp.	Papaveraceae	17%	–	–	–	–	–	–
Plantago sp.	Plantaginaceae	12%	–	–	–	–	–	–
Trifolium alexandrinum	Fabaceae	–	14%	–	51%	–	–	–
Medicago sp.		–		–	25%	–	–	–
Prosopis sp.		–		–	16%	–	5%	
Trifolium sp.		–		–	–	–		5%
Chenopodiaceae	*Chenopodiaceae*	7%		15%	–	–	10%	
Umbellifera	Nyctaginaceae	–	–	–	–	–	3%	7%
Sporangia	Sporangia	5%	–	–	–	12%	5%	
Eucalyptus sp.	Myrtaceae	–	–	–	–	34%	60%	20%
Phoenix dactylifera	Arecaceae	–	–	–	–	17%	5%	10%
Citrus sp.	Rutaceae	57%	–	–	–	–	5%	
Cucurbitaceae	Cucurbitaceae	–	12%	47%	5%	–	–	–
Sesamum sp.	Pedaliaceae	–		10%	–	–	–	–
Zea mays	Poaceae	–	59%	18%	–	–	–	–
Casuarina sp.	Casuarinaceae	–	–	–	–	25%	–	–
Rhamnus sp.	Rhamnaceae	–	–	–	–	–	–	55%
Unknown[a]	Unknown	1%		10%		12%	–	–
Total number of pollen grains found		1250	1150	1225	1275	1300	1100	1375
Total number of pollen types		7	4	5	5	5	8	6
Number of unknown pollen types		1	–	1	–	1	–	–
Unknown pollen frequency (%)		1%	–	10%	–	12%	–	–
No. of plant families in each sample		7	7	5	3	5	8	6

[a]Unknown = two different types of pollen.

confirmed the uni-floral classification of their produced honey. In recta of citrus, clover, cotton, B. pepper, sider, and sunflower worker bees, the predominant pollen types were *Citrus* sp.(57%) and *Trifolium alexandrinum* (51%), *Zea mays* (59%), *Eucalyptus* sp. (60%), *Rhamnus* sp. (55%), and *Cucurbitaceae* (47%), respectively (Table 4, Figure 2). While in the case of banana honey where no PGs were observed, PGs recovered from the rectum of its related worker bees resulted in its classification as multi-floral honey (Table 4).

PGs observed in various kinds of honey and recta of related worker bees were consistent and six of the seven honeys investigated contained the same PGs identified in recta of corresponding honey bee worker's (Figure 2). Total numbers of PGs observed in recta of worker bees were more than 2-fold greater than the total number of PGs recovered from their associated honeys (Tables 2 and 4).

Discussion

Honey bee workers collect PGs from entomophilous and anemophilous plants to obtain protein for their survival and reproduction (Barth, Munhoz, & Luz, 2009). They frequently collect a wide variety of PGs, but generally concentrate on a few species (Baum, Rubink, Coulson, & Bryant, 2011). This study represents the first of its kind to report the results of melissopalynological analyses of different types of honey produced in Egypt. Moreover, taxa of pollen recovered from the honeys studied revealed important information about flora essential for the production of honey by bees in Egypt. Additionally, it holds the potential to be a rationale for a significant action (correct labeling; authenticity standards) in Egypt beekeeping sector.

The results of melissopalynological analyses suggest that PGs found in recta of worker bees and in different kinds of honey originated from several botanical sources: cultivated crops, garden plants, and wild plants. Twenty species of pollen belonging to 14 plant families were identified in all types of honey investigated and in recta of their corresponding honey bee workers, respectively. Observations made during this study were consistent with results of pollen traps employed during previous studies in Egypt, which revealed that there were 39 species of pollen, belonging to 15 families in the Kafr El-Shiekh region (Taha, 2005), 26 species of pollen, belonging to 15 families in Dakahlia (Fathy, 2008), and 65 belonging to 25 plant families in plants from which bees collected pollen in Alexandria and El-Beheira provinces (Esmael, Salem, Mahgoub, & El-Barbary, 2016). This data represent an indicator of the richness of Egyptian plants suitable as sources of pollen or nectar or both for honey bees (Abou-Shaara, 2015).

In 57% of the honeys studied, the plant families Fabaceae and Boraginaceae were the most frequently represented, indicating that honey bees frequently visit plants belonged to both families. Plants in these families are important for the production of honey, especially, Egyptian clover ("berseem"; *Trifolium alexandrinum*),

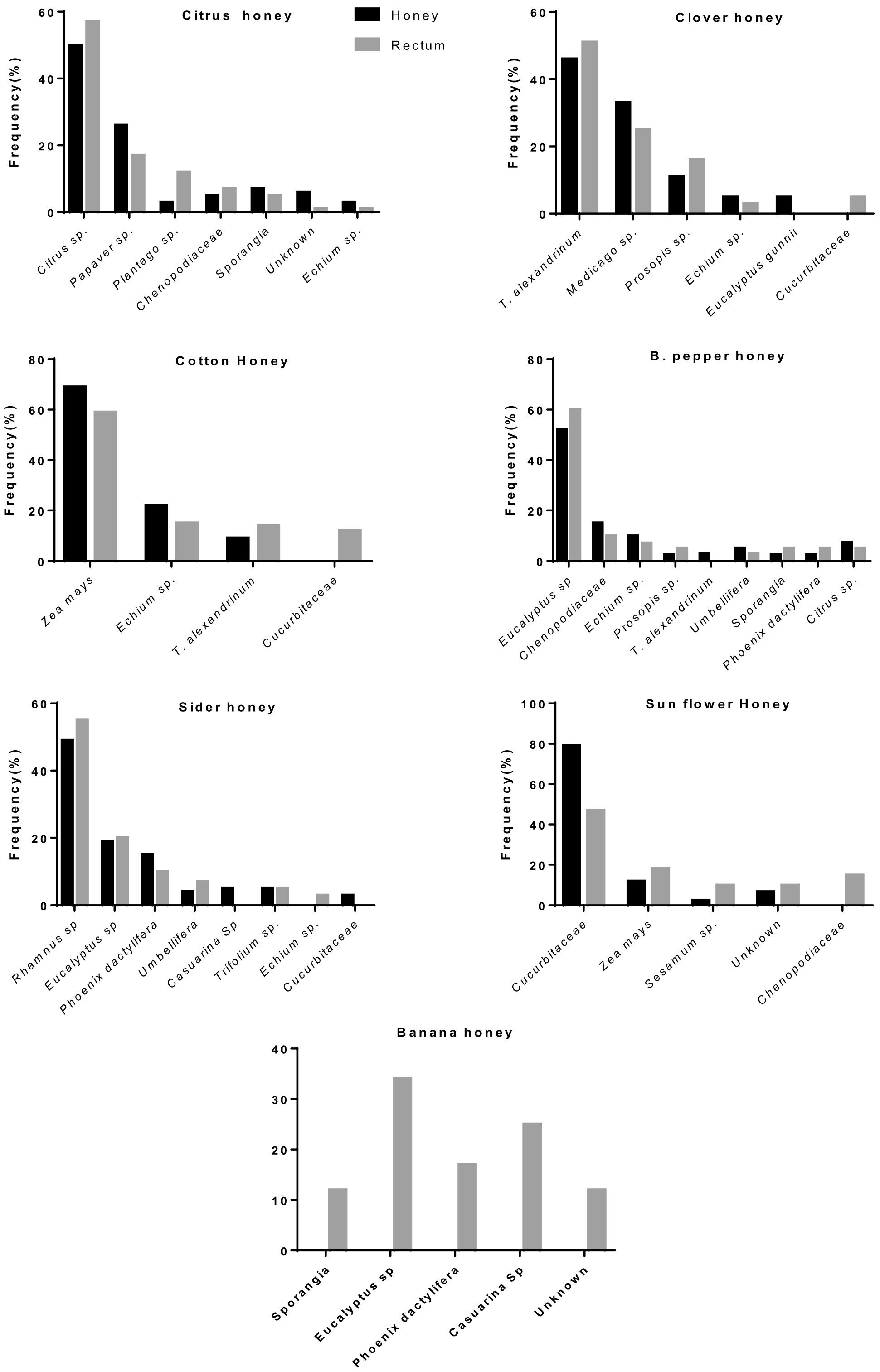

Figure 2. Pollen in various types of honey and recta of worker bees making each type of honey from hives, from different botanical and geographical origins in Egypt during 2016–2017.

which is known as the "king of forages". Pollen of berseem was found in 43% of the honeys investigated with the greatest relative frequency of 46% in clover honey and the least of 3% in Brazilian pepper honey. These results are in agreement with the findings of Karabournioti and Karabagias (2017), who randomly collected eight samples of honey from local shops in the greater Cairo area and found PGs of *T. alexandrinum* predominate (>45%) in seven of the eight honeys analyzed. In this context Hussein (1982) previously reported that most PGs found in honey (65%) were collected from the plants belonging to the family Fabaceae, which is the third-largest family of angiosperms, comprising more than 800 genera and 20,000 species

(Lewis, Schrire, Mackinder, & Lock, 2005) and possessing the greatest number of domesticated crops of any plant family (41 species) (Harlan, 1992).

Similarly, *Echium* sp., which is known as viper's bugloss and blueweed (Dickinson, Metsger, Bull, & Dickinson, 2004), is a species of flowering plant in the borage (Boraginaceae) family, its PGs were recovered in (57%) of the honeys studied. It represents an important source of nectar or/and pollen for honey bees because it provides nectar for bees all day long and flourishes in dry conditions and poor soil. Moreover, honey bees visit this biennial plant mostly in humid weather, when there is enough nectar per flower for their relatively short tongues to reach (Corbet, 1978). These findings also confirm its wide geographical distribution in Egypt.

Pollen observed in honeys investigated, and in recta of worker bees from the same hives, confirmed identities of sources of honey and nectar, as indicated by the Egyptian beekeepers during collection for all honeys except cotton, sunflower, and Brazilian pepper honey. For cotton honey and recta of bees in those hives, the predominant pollen was *Zea mays* with relative frequencies of (69%) and (59%), respectively. This data suggest the re-classification (labeling) of this honey as *Zea mays* honey, instead of cotton honey, which is well known in Egypt. However, it has been found that honey bees cannot collect pollen pellets from cotton flowers, due to the specific dislodging of cotton PGs (Owayss, 1996), even when 32 bee workers were collected immediately after they had been observed collecting nectar of cotton flowers (*Gossypium hirsutum*) (Todd and Vansell, 1942). To their amazement, they could not find even one PG in the full honey stomach. The ICBB also does not include cotton honey in its list as being under-represented in pollen of the plant origin (Louveaux et al., 1978). Therefore, the observable activity of bee foragers during cotton blooming is for collecting cotton nectar and gathering pollen from other coincided plants, such as maize (Ismail et al., 2013).

In this study, in sunflower honey as proposed and recta of associated worker bees, the predominant pollen belonged to the family Cucurbitaceae with relative frequencies of (79%) and (47%), respectively. These results suggest reclassification and subsequent labeling of this type of honey as "cucurbits honey" instead of sunflower honey. In contrast, Thrasyvoulou and Manikis (1995) found a range of 21.1–81.17% PGs of Helianthus in sunflower honey collected in Greece. This discrepancy in the botanical composition of sunflower honey indicated the importance of frequent authentication of botanical and geographical origin of honey in honey markets. Similar observations have been made for Brazilian pepper honey and recta of associated bees, where the predominant pollen was *Eucalyptus* sp. with relative frequencies of 52% and 60%, respectively. According to Louveaux et al. (1978) nomenclature of honey, these results support reclassification and subsequent labeling as "*Eucalyptus* honey" instead of Brazilian pepper honey. In the other hand, the palynological differences in the pollen content of the relabeled honey as "*Eucalyptus* honey" in this study greatly matched with pollen analyses results of 75 eucalyptus honey samples collected from different countries which revealed important differences in their pollen spectra (Carmen Seijo, Jesús Aira, & Méndez, 2003).

Differences in the pollen content of honey might be generally attributed to 1) honey bees foraging on different plants and for a wide range (several kilometers); thus honey is always made from a mixture of nectar from different sources (Estevinho, Rodrigues, Pereira, & Fea's, 2012, p. 2) resources used by honey bees vary due to ecological distributions and in periods of availability according to their flowering times (Chemas & Rico-Gray, 1991) and seasonal shortages of bee forage is critical. Therefore, Egyptian beekeepers usually move colonies of bees according to availability of forage for bees (Taha, 2005, p. 3) the foraging activities of honey bees for pollen are greatly influenced by the weather conditions and availability of pollen (Neupane & Thapa, 2005, p. 4) several species of plant offer both pollen and nectar but some do not provide sufficient pollen. For instance, in Egypt foraging bees mainly depend on cotton as a source of nectar and maize as a source of pollen. Therefore, in some cases it is difficult to discriminate between kinds of honey by use of melissopalynological analyses and these methods could be more useful for the differentiation between honeys produced in distinctly different geographical and climatic areas (Přidal & Vorlová, 2002).

In banana honey, there was a complete absence of PGs. In contrast, pollen of three species and some unknown pollen species have been recovered with a greater frequency (34%) for *Eucalyptus* sp. in recta of worker bees making banana honey. While no PGs for banana (*Musa* sp.) have been found in recta of bees making banana honey, the absence of (*Musa* sp.) PGs are due to the fact that this plant is a rich source of nectar, but in Egypt never produces pollen (Taha, 2007). Also, the complete absence of PGs in honey has been previously reported by, Demianowicz (1964) who found *Asclepiassyriaca* honey during 1962 contained zero PGs per 10 g of honey.

Total numbers of PGs observed in recta of worker bees making various honeys were 2-fold greater than the total number in associated honeys. This might indicate the importance of analysis of pollen in recta of bees as a complementary or/and a confirmatory tool for pollen taxa in honey (Dimou & Thrasyvoulou, 2009).

In this study, the Shannon–Weaver and Pielou indices of pollen indicated that resources of PGs in all investigated honeys were exploited homogeneously except in sunflower honey in which 79% of PGs were from cucurbits. These findings confirm the diversity of plants used by bees and the lack of huge monocultural fields around bee colonies in Egypt (Tammet, 2007). Based on absolute numbers of PGs per 10 g of honey, 33.3% of honeys fell in Maurizio's class I (poor in pollen), 33.3% fell in class II (normal pollen representation), and 33.3% fell in class III (overrepresented pollen). There are many factors for honeys to be rich with PGs (Chauvin, 1968). Production of pollen by visited plants is one factor, which depends greatly upon climatic conditions can be considered as the main factor (Battesti & Goeury, 1992). Moreover, the relationship between plants and bees is also important. In some plants, PGs are not available for honey bees and also diameters of PGs influence the amount of pollen in honey. Additionally, Von der Ohe (1994) reported that the larger types of pollen are preferentially blocked by the bee's proventriculus relative to smaller PGs. Other factors such as the distance of the beehive to visited flowers and fitness of colonies of bees, are also significant factors. Quantitative pollen analysis showed that both sidr and clover honey were very rich with PGs. This data indicate the wide availability of Egyptian clover and sidr trees and their wide geographical distribution in Egypt (Bakheit, 2013; El Sohaimy, Masry, & Shehata, 2015).

Conclusions

Six of the seven honeys investigated were mono-floral. Analyses of pollen in recta of bees could be more useful for discrimination between honeys produced in distinctly different geographical and climatic areas. The information obtained in this study provided information on the diversity of plants used by bees in Egypt and emphasized the great potentiality for beekeeping activity in Egypt around the year. Melissopalynological studies should be carried out annually to authenticate the botanical and geographical origins of honey and to track the availability and geographical distribution of appropriate bee plants in Egypt.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Supplementary material

Supplementary Figure S1 (map of Egypt showing sampling locations of different honeys) is available via the 'Supplementary' tab on the article's online page (http://dx.doi.org/10.1080/00218839.2020.1720950).

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