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Diversity of pollen species in commercial honey samples collected from different governorates of Egypt

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Abstract

Analysis of pollen species in bee honey reflects the nectar sources of specific honey. It is a very important method to evaluate honey quality situation and frequency of adulteration. To determine dominant sources of Egyptian honeys, 16 commercial honey samples were collected from supermarkets during season of 2017. Using mellissopalynology technique, a total of 27 pollen species belonging to 18 families were identified in tested honey samples. The predominant pollen sources were clover, alfalfa, date palm, faba bean, coriander, eucalyptus, sunflower and citrus, while the other pollen species were present in honey samples as secondary pollen sources. Most of pollen types identified in honey samples were from necteriferous plants. The date palm was the only main polleneferous plant in the pollen spectrum of studied honeys. Pollen diversity was higher in summer honey samples in comparison with spring honey samples. The present results showed that all sixteen trademark tested honey samples had a natural source and unadulterated.

Keywords: pollen identification, qualitative pollen analysis, bee honey.

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1. Introduction

For many years, varietal identification of bee honeys has been a research subject in many scientific centers (Bogdanov, 1999). Using pollen analysis to assess quality and botanical origin of honey was discussed by Lukacs (1997). Quality control methods have been found to be able to classify honeys from different geographical regions. Bee honeys were considered monofloral honey whenever the dominant pollen type was found to be over 45% of total pollen grains in the tested honey (Amaral et al., 2003). Also, both pollen identification and count have been used to determine the authentication of honey according to the floral type (Serrano et al., 2004). Results of Wen et al. (1995) indicated that, about 30% of samples were adulterated with sugar syrup or other products. Due to its simplicity, pollen analysis was used extensively to identify different types of honey samples from different botanical origins in different countries (Devillers et al., 2004; Marini et al., 2004; Cordella et al., 2002; 2003). Pollen spectra of honey were influenced by time of the year and location (Ponnuchamy et al., 2014). In Egypt, the qualitative pollen analysis of honeys was done by many authors (El-Metwally, 2015; Rateb, 2005; Nour et al., 1991; Nour, 1988). Pollen analysis of sixty Egyptian honey samples was done by Nour (1988); he found that the main pollen sources of Egyptian honeys were clover (*Trifolium alexandrinum* L.), eucalyptus (*Eucalyptus* spp.), *Citrus* sp. date palm (*Phoenix dactylifera* L.), maize (*Zea mays* L.), sunflower (*Helianthus annus* L.) and faba bean (*Vicia fabae* L.). Cotton pollens were estimated at less than 1% of the total pollen found in market cotton honey samples. Rateb (2005) identified fifteen pollen types in honey samples from Assiut region, most of them (80%) from necteriferous plants. The main polleneferous plants of pollen spectrum in the studied honeys were *Zea mays*, *Phoenix dactylifera* and *Casuarina equistifolia* L. Also, the same author reported that pollen density of honey collected by Egyptian honey bee race was 9.6, 13.6, 5.1 and 16.1 times more than those collected by Italian, Caucaseca, Carniolan hybrid and Carniolan honey bee races, respectively. Furthermore, pollen density varied and depended on the collection locality. Morever, Egyptian bee honey samples were examined by El-Metwally (2015) and fourteen types of pollen were classified. Clover pollen was found in high percentage (30.2%) while date palm and umbliferus pollen were recorded in considerable percentages as 13.20 and 9.39%, respectively. The present work aims to evaluate and classify some commercial honeys (trademark) produced in different localities in Egypt according to their botanical origin.

2. Materials and methods

Laboratory works of the present investigation were carried out at Plant Protection Department, Faculty of Agriculture, Assiut University, Egypt during 2017 season.

2.1 Collection of honey samples

A total of 16 commercial honey samples with trademark were collected from markets in different regions of Egypt during 2017 season (Table 1) for evaluation and classification.

2.2 Pollen content determination

The method recommended by the International Commission of Bee Botany (ICBB) for pollen analysis was followed

to determine pollen grains in honey samples (Louveaux et al., 1978). Ten grams from each honey sample were mixed with 10 ml hot distilled water (40°C), then the solution was centrifuged for 20 min at 4500 rpm. The sediment was smeared in two slides, mounted in Fucsin-glycerin gel and examined microscopically for pollen analysis as described by Nair (1960). Pollen species were identified with the help of reference slides prepared from local flora during the present study, in addition to relevant literature (Rateb, 2005; Hussein, 1983).

Table 1: Commercial honey samples with trademark were collected from markets in different regions of Egypt for evaluation during 2017 season.

Code No.	Trademark	Locality of production
1	Isis	Different localities
2	Hero	Different localities
3	Shad Alaeyun	Different localities
4	Soud (1)	El-Manofia
5	Soud (2)	El-Manofia
6	Alryhan	El-Minia
7	Altemsah	Tanta, Cairo, Alex
8	Jana	Cairo
9	Aimtinan	Different localities
10	Khayer Zman (1)	Different localities
11	Khayer Zman (2)	Different localities
12	Dabur	El-Gharbia
13	Alhut	Alexandria
14	Alsafa	Different localities
15	Alassad	Cairo – Alexandria
16	Alwaha	El-Minia

2.3 Pollen diversity determination

Total number of pollen grains in 10 microscopic fields was counted separately as an indicator of pollen grain density. Abundance percentages of pollen species in each sample were calculated. After pollen analysis, examined honey samples can be defined as monofloral or polyfloral honeys. If the honey sample contains more than 45% of the total pollen count from one plant species; honey was considered as predominant class, secondary (16-45%), important (3-16%) and minor pollen types (less than 3%) as described by Maurizio (1975), Bryant and Jones (2001) and Amaral et al. (2003). However, classification of citrus honey was a special case. A minimum of 10% of citrus pollen grains in honey sample was enough to consider the honey as monofloral citrus honey as described by Serra and Ventura (1995) and Terrab et al. (2003). The diversity index (α) was calculated according to the following formula (Williams, 1947):

$$S = \alpha \log_e (1 + \frac{N}{\alpha})$$

Where S = number of species. N = number of individuals and α = diversity index.

3. Results and Discussion

Sixteen commercial honeys (trademark honeys) were collected from different Egyptian regions. After pollen analysis of honey samples, twenty-seven pollen species were identified. Classified pollen species are belonging to 18 families (Table 2). As shown in table (3), ten pollen species were represented by percentages ranged from 1 to 36%. Seventeen pollen species which everyone has less than 1% were represented 6.6% from the total pollen count. Clover (*T. alexandrinum*) pollen was the predominant one (36.2%) among the rest of other pollen types. Eucalyptus (*E. globulus*), date palm (*P. dactylifera*), alfalfa (*M. sativa*) and faba bean (*V. fabae*) pollen grains had the following percentages, 16.9, 12.6, 9.0 and 7.7%, respectively. Date palm is known as polleneferous sources only for honey bee colonies. However, other field crops which cultivated in large areas in

different regions of Egypt were the sources of both nectar and pollen. Thus, we considered that the commercial honey produced in Egypt were mainly collected from clover, alfalfa, faba bean, eucalyptus and partially from medicinal plants specially Umbelliferae, citrus and sunflower fields.

Table 2: Classification of pollen grain species which isolated from commercial honey samples.

No.	Common name	Scientific name	Family
1	Acacia	*Acacia arabica* L.	Leguminaceae
2	Alfalfa	*Medicagoi sativa* L.	Trifoliate
3	Apple	*Malus domestica* Borkh.	Rosaceae
4	Anise	*Pimpinella anisum* L.	Umbelliferae
5	Aster	*Aster tataricus* L.	Asteraceae
6	Basil	*Ocimum* sp.	Lamiaceae
7	Cabbage	*Brassica oleracea* var. *capitate* L.	Cruciferae
8	Eucalyptus	*Eucalyptus globulus* Labill.	Myrtaceae
9	Capparis	*Capparis spinosa* L.	Capparaceae
10	Casuarina	*Casuarina equistifolia* L.	Casuarinaceae
11	Citrus	*Citrus* spp. L.	Rotacoae
12	Coriander	*Coriandrum sativum* L.	Umbelliferae
13	Cotton	*Gossypium* spp.	Malvaceae
14	Dahlia	*Dahlia hybrid*	Compositae
15	Date palm	*Phoenix dactylifera* L.	Arecaceae
16	Egyptian clover	*Trifolium alexandrinum* L.	Leguminaceae
17	Faba beans	*Vicia faba* L.	Leguminoseae
18	Gazania	*Gazania joseph* Gaertner.	Compositae
19	Helichrysum	*Helichrysum stoechas* (L.) Moench	Gnaphalieae
20	Iberis	*Iberis gibraltarica* L.	Brassicaceae
21	Lettuce	*Lactuca sativa* L.	Asteraceae
22	Maize	*Zea mays* L.	Graminae
23	Onion	*Allium cepa* L.	Amarayllidaceae
24	Sesame	*Sesamum indicum* L.	Pedaliaceae
25	Sonchus	*Sonchus oleraceus* L.	Asteraceae
26	Sunflower	*Helianthus annuus* L.	Compositae
27	Zinnia	*Zinnia elegans* L.	Asteriudeae

To determine the nectar source of honey, pollen grain percentages in every sample were calculated and placed into one of the following four pollen frequency classes: predominant source (more than 45%); secondary source (16-45%); important minor source (3-16%) and minor source (less than 3%). As shown in Table (4). The predominant pollen more than 45% were recorded in 13 honey samples. Eight predominant pollen sources were identified in tested honey samples. Faba bean pollen was recorded in samples no. 8, 9 and 10 with 46, 67 and 84%, respectively. The sample no. 2, 4 and 13 contained clover pollen with 56, 57 and 97% respectively. Alfalfa pollen had predominant percentages in three samples no. 7, 12 and 15 with 54, 48 and 50%, respectively. Date palm, coriander, eucalyptus and sunflower pollens were recorded in samples no. 6, 11, 14 and 16, respectively, in predominant percentage > 45%. The sample no. 5 was the only one contained citrus pollen with 19% and it considered as citrus honey following Serra and Ventura (1995) and Terrab et al. (2003) as a special case; whereas, the pollen frequencies of 16–45% was noted for 9 taxa. Important minor sources of

pollen which had percentages from 3 to 16% were noted for 16 taxa. The lowest pollen frequencies <3% were noted for 12 taxa. The present data indicated that faba bean plants were considered a good source of nectar and pollen for honey bee colonies in the early spring period in many governorates in Egypt. Also, the forage crops clover and alfalfa cultivated in large areas are the important major source of honey bees forage in summer season.

Table 3: Representation % of different pollen species in supermarket honeys collected from different regions of Egypt during 2017 season.

Common name	Total pollen count	Representation (%)
Clover	452	36.2
Eucalyptus	211	16.9
Date palm	158	12.6
Alfalfa	113	9.0
Faba bean	96	7.7
Capparis	52	4.2
Coriander	43	3.4
Sunflower	15	1.2
Citrus	15	1.2
Cabbage	13	1.0
Other pollen types (17 species)	82	6.6
Total of count (27 species)	1250	100

Table 4: Pollen spectrum of sixteen honey samples (Trademark) collected from different Egyptian regions during 2017 season.

Code No.	Tradem-ark	Number of pollen Species	Predominant (>45%)	Secondary (16-45%)	Important (3-16%)	Minor (<3%)
1	Isis	9		Egyptian clover (32) Capparis (26)	Gazania (12) Helichrysum (12) Cabbage (4) Alfalfa (5) Sunflower (4) Basil (4)	Iberis (2)
2	Hero	6	Egyptian clover (56)		Alfalfa (13) Cabbage (9) Basil (9) Capparis (6) Helichrysum (6)	
3	Shhad Alaeyun	3		Alfalfa (42) Egyptian clover (32) Sunflower (25)		
4	Soud 1	9	Egyptian clover (57)	Cabbage (22)	Alfalfa (7) Iberis (6)	Zinnia (2) Sunflower (2) Basil (2) Dahlia (2) Aster (0.78)
5	Soud 2	8	Citrus (19)	Date palm (37)	Eucalyptus (13) Lettuce (11) Alfalfa (10) Cabbage (6)	Basil (3) Coriander (2)
6	Alryhan	3	Date palm (65)	Eucalyptus (29)	Acacia (6)	
7	Altemsah	4	Alfalfa (54)		Maize (15) Cotton (15) Sesame (15)	
8	Jana	6	Faba bean (46)	Eucalyptus (23) Apple (18)	Sonchus (8) Acacia (5)	Casuarina (3)
9	Aimtinan	3	Faba bean (67)	Eucalyptus (25)	Alfalfa (7)	
10	Khayer zman 1	4	Faba bean (84)		Cabbage (8) Acacia (4) Alfalfa (4)	
11	Khayer zman 2	2	Coriander (87)		Alfalfa (13)	
12	Dabur	6	Alfalfa (48)	Date palm (41)	Onion (4)	Eucalyptus (2) Citrus (2) Anise (2)
13	Alhut	4	Egyptian clover (97)			Capparis (1) Sunflower (1) Alfalfa (0.28)
14	Alsafa	2	Eucalyptus (72)	Date palm (28)		
15	Al'assad	4	Alfalfa (50)	Maize (21)	Sesame (14) Capparis (14)	
16	Alwaha	3	Sunflower (71)		Egyptian clover (14) Capparis (14)	

According to the present results alfalfa plants were the only pollen source which was identified in 12 samples from sixteen tested honey samples (75%) followed by clover plants and eucalyptus which everyone was found in six samples (37.5%). Only two honey samples (no. 1 and 3) were classified as polyfloral honey. However, fourteen samples were classified as monofloral honey. Generally, it could be summarized from the present results that commercial Egyptian honeys could be considered as monofloral honeys. The sixteen honey samples were divided into two groups (spring and summer honeys) after botanical classification of predominant pollen in every sample. The spring honeys included samples no. 5, 6, 8, 9, 10, 11, 12 and 14, while the summer honey included samples no. 1, 2, 3, 4, 7, 13, 15 and 16. The diversity index (α) of pollen species in spring and summer honey samples were represented in Tables (5 and 6).

Table 5: The diversity index (α) of pollen species in commercial spring honeys in Egypt collected in 2017season.

Code No. of sample	Predominant pollen	No. of pollen species	Total count of pollen grains (no/10 fields)	Diversity index (α)
5	Citrus	8	63	2.43
6	Date Palm	3	31	0.82
8	Faba bean	6	40	1.96
9	Faba bean	3	55	0.68
10	Faba bean	4	40	1.02
11	Coriander	2	46	0.43
12	Alfalfa	6	123	1.32
14	Eucalyptus	2	232	0.30
	Total	14	640	2.53

Table 6: The diversity index (α) of pollen species in commercial summer honeys in Egypt collected in 2017 season.

Code No. of sample	Predominant pollen	No. of pollen species	Total count of pollen grains (no/10 fields)	Diversity index (α)
1	Polyfloral	9	57	3.01
2	Clover	6	32	2.18
3	Polyfloral	3	11	1.36
4	Clover	9	72	2.72
7	Alfalfa	4	13	1.97
13	Clover	4	349	0.63
15	Alfalfa	4	14	1.87
16	Sunflower	3	7	1.99
Total		16	555	3.08

The results indicated that the values of the diversity (α) of spring honeys in pollen grains were relatively low in comparison with summer honey. The diversity index (α) of spring honeys ranged between 0.30 and 2.43. Eight honey samples contained pollen species from spring flowering plants and they were monofloral honeys according to the pollen classification. The results of summer honey represented in Table (6) showed that the values of α diversity

index ranged between 0.63 and 3.01. Two samples, no. 1 and 3, recorded as polyfloral honeys. The pollen analysis of summer honeys indicated that the diversity index (α) of pollen grain in tested honey samples were relatively higher than spring honeys. The present results provides ageneral picture on a nectar sources of Egyptian hones. From pollen analysis, all tested honey samples had a natural sources. Clover, Eucalyptus, Alfalfa were the main sources for honeybee colonies to produce honey. Pollen diversity in honey samples was higer in summer than in spring honeys.

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