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Pollen Characteristics of Egyptian Clover Honey

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Research Article

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ABSTRACT

The purpose of the present study was to investigate and chart pollen characteristics of Egyptian clover honey in order to evaluate its purity with respect to authentication. For this purpose, 8 clover honey samples were randomly collected from local products shops in the greater Cairo area and subjected to melissopalynological analysis, the official method for the determination of honey botanical origin, by providing exhaustive details on specific pollen grains that contribute to the overall pollen grains profile (PGP). Results showed that, the dominant pollen grains (>45%) in 7 of the 8 honey samples analyzed were *Trifolium alexandrinum* L. followed by lower pollen grain percentages of *Brassica* sp., *Melilotus sp*., *Umbeliferae*, and *Compositae*, along with others. Given that, research on PGP involving Egyptian clover honey is limited, the present study contributes to the characterization of clover honey that enters the domestic or international market

INTRODUCTION

Bee pollen is a raw, fine to coarse powdery substance, comprising pollen grains from which bees produce a tulip anther with many pollen grains, often called as ''bee bread'' [1]. Bees collect pollen from numerous plant anthers, mix it with secretions from their salivary glands or nectar, place it in specific baskets located at their hind legs, and transfer it to the hive [1]. Pollen may get to the nectar or honey by many different ways [2].

Honey is a highly concentrated water solution of two simple sugars, dextrose and levulose, followed by small amounts of at least 22 other more complex sugars. The principal physicochemical characteristics and behavior of honey are owed to its sugars, but the minor constituents, such as flavoring materials, pigments, organic acids, and minerals, are largely responsible for the considerable differences among individual honey types. It is produced by honey bees *Apis mellifera* and *Apis dorsata* through the collection of floral nectar, and less often with honeydew secretions, after the addition of enzymes (mainly invertase and glucose oxidase). Nectar can be characterized as a thin, easily spoiled sweet liquid that is ripened by the honey bees to a stable, high density, and high energy foodstuff [3].

Clover is a prolific source of honey, growing in temperate climates and irrigated sub-tropical regions around the world, including: USA, Canada, Western Europe, Australia, New Zealand, Argentina, China, Russia, Egypt, etc. Clover belongs to the genus *Trifolium,* Fabaceae family (ca. 250 species). The most important species for honey production are White or Dutch Clover, Alsike Clover, Red Clover, Crimson Clover, Egyptian or Berseem clover [4,5]. Most single flower clover honey is produced from agricultural crops although clover also grows wild and finds its way into many different honey types, through the action of honeybees. Furthermore, certain plants such as alfalfa, sweet clover, and sainfoin clover, are wrongly included in the “clover” family, because they originate from a different genus. In that sense, pure monofloral clover honey is somewhat rare, as it is often mixed with other honey types and called clover honey for market purposes [4].

Based on the above, the aim of the present study was to characterize Egyptian clover honey by means of pollen grain contents in terms of authenticity. Despite the limited honey samples analyzed, data on the pollen grain percentage of T*rifolium alexandrinum* in clover honey is very limited, this constituting the novelty of the present work.

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Finally, the practical application of the present study involves the correct labeling of clover honey that enters the domestic or international market.

MATERIALS AND METHODS

Honey Samples

Eight clover labeled honey samples, were purchased from local products shops from the greater Cairo area harvesting year 2014-2015. Samples were packaged in glass containers, shipped to the laboratory and maintained at 4°C ± 1°C until analysis.

Melissopalynological Analysis

In order to ensure the floral origin of honey samples, the melissopalynological method was applied according to a previous work [6]. The terms used for the frequency classes were: predominant pollen (more than 45% of the pollen grains counted), secondary pollen (16% to 45%), important minor pollen (3% to 15%), and minor pollen (less than 3%) [6]. Each honey sample was run in duplicate.

Statistical Analysis

The average values of the pollen grain percentages determined in the sediment of clover honey, were compared using t-test at the confidence level $p<0.05$. Statistical treatment was accomplished using the statistics software SPSS v.20 for Windows.

RESULTS

Full data regarding the pollen grain percentages are given in **(Table 1)**. The Egyptian clover honey samples were characterized by diverse contribution of minor pollen grains, whereas the most dominant pollen grains were those of *Trifolium alexandrinum*, recording an average value of ca. 68% **(Figure 1)**. The presence of *Trifolium alexandrinum, serving* as the dominant pollen grains in a high percentage, may be a qualitative criterion regarding the purity, and thus, authenticity of Egyptian clover honey [7,8].

Table 1. Pollinic profile (% pollen grains) of Egyptian clover honeys.

Honey sample	Trifolium alexandrinum	Melilotus sp.	Brassica sp.	Helianthus annuus	Umbeliferae	Compositae	Ononis sp.	Eucalyptus sp.	Vicia sp.	Pheonix sp.	Nectarless
1	41	22	16	6	6	3	2	nd	nd	nd	*Zea mays*
2*	56	3	26	6	3	nd	nd	3	nd	nd	*Zea mays*, Gramineae
3*	67	nd	13	6	6	3	nd	nd	nd	nd	*Zea mays*, Gramineae
4*	68	9	7	nd	9	3	nd	nd	nd	nd	*Zea mays*, Gramineae
5*	79	2	nd	nd	9	3	nd	nd	1	1	*Zea mays*, Gramineae
6*	51	nd	nd	nd	1	2	nd	45	nd	nd	*Zea mays*
7	89	nd	nd	nd	1	1	nd	5	nd	nd	*Zea mays*
8*	91	2	nd	nd	nd	nd	nd	2	nd	nd	*Zea mays*, Gramineae
Average values	68	5	8	2	4	2	-	7	-	-	

*plus<1% *Sesamum* sp., *Musa* sp., *Pheonix* sp., *Punica* sp., *Eucalyptus* sp., *Labiatea, Citrus* sp. nd: not determined, considered as zeros for comparison of pollen grains using t-test ($p<0.05$). Each value represents the average of two measurements.

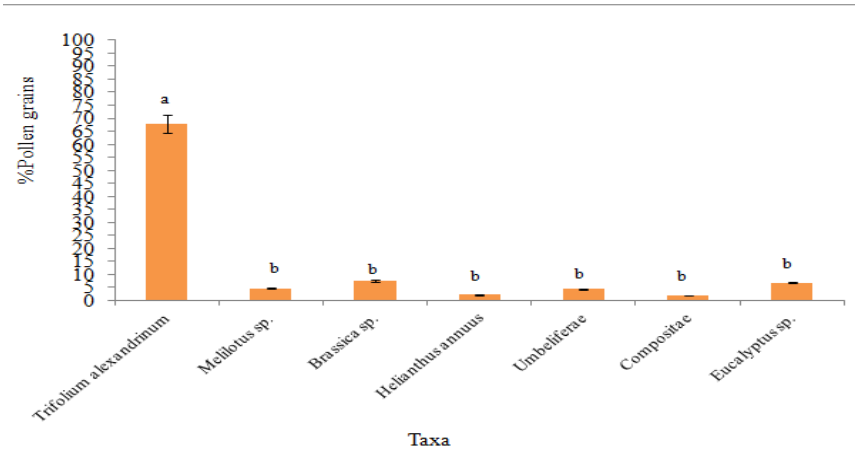

Figure 1. Type of taxa determined in Egyptian clover honeys and percentage contribution of characteristic pollen grains. Different lower-case letters indicate statistically significant differences at the confidence level $p<0.001$.

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DISCUSSION

In a previous study involving Croatian honeys, the presence of *Trifolium* sp. (*Trifolium pretense* L.) pollen grains were in lower percentages compared to those of the present study [7]. The same holds for the presence of *Brassica* sp. pollen grains. Therefore, the high pollen percentage of the aforementioned pollen grains in Egyptian clover honeys could be indicative of both: botanical and geographical origin. Argentinean clover honeys were characterized by the presence of *Trifolium* sp., *Trifolium* pratensis, *Lotus* sp., *Eucalyptus* sp., *Echium plantagineum*, *Helianthus annuus*, Poaceae, Brassicaceae, *Apium* sp., *Cichorium intybus*, *Carduus* sp., Mirtaceae, *Bidens* sp., and Chenopodiaceae [8]. As it can be observed, there are some considerable differences in the minor pollen grains between Argentinean vs. Egyptian clover honeys of the present study.

More recently, Jones and Bryant characterized 37 honeys produced in East Texas by means of melissopalynology. The authors reported that three taxa namely: *Berchemia scandens*, *Salix nigra* and *Toxicodendron radicans*, were found in >50% of the samples and were the most important for the overall characterization of East Texas honey. However, the presence of T*rifolium repens* and *Trifolium incarnatum* (Fabaceae family) in 17 and 16 honey samples, respectively, could also provide useful information regarding the geographical origin of East Texas honey, compared to Croatian, Argentinean, and Egyptian clover honeys of the present study.

CONCLUSION

Results of the present study showed that specific pollen grains may provide useful information about the purity and geographical origin of Egyptian clover honey. The mean value percentage of *Trifolium alexandrinum* pollen grains, serving as the dominant ones, was ca. 68%. The use of certain taxa called pollen coefficient values (PC) [2], may act as a good monitor of clover honey purity. In that sense, we propose the value of ≥ 50% for *Trifoliun alexandrinum* pollen grains in pure clover honey. Future perspectives, may involve the analysis of a larger number of clover honey samples from different markets. Thus, present results, preliminary in nature, will be further validated.

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