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Pollen Characterization of Polyfloral Honeys from Laghouat Region (Algeria)

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ABSTRACT

Honey is naturally produced by honeybees from the nectar of plants. It is widely consumed as a health food product all over the world. Melissopalynology plays an important role in ascertaining the botanical and geographical origins of honey by studying the pollen contained in the honey. The pollen content of 19 honey samples of *Apis mellifera L.* from the Laghouat region of Algeria was analyzed. 100 pollen types belong to 49 families were identified, of which the most diverse were Asteraceae (12 pollen types), Fabaceae (9), Apiaceae, Boraginaceae and Lamiaceae (5 pollen types each). Asteraceae and Fabaceae were present in all the samples, while, Apiaceae Nitrariaceae, Euphorbiaceae, Brassicaceae, and Oleaceae were identified in more than 84% of analysed honey samples. The number of pollen types identified per sample ranged between 13 and 27 (mean of 19.1). All honey samples were classified as a polyfloral honey. The best represented secondary pollen types were *Peganum harmala, Centaurea sp., Trifolium sp., Eucalyptus sp., Euphorbia sp., Olea europaea, Echium sp., Onopordum arenarium, Lotus* type and *Cistus sp.* For the quantitative analysis, the pollen content of the studied honey samples ranged from medium (class II, 57.9% of the samples) to high (class III, 42.1 % of the samples), where the pollen density ranged from 23 400 to 351 250 in 10 g of honey, with an average of 108 002 grains /10 g.

Key words: Melissopalynology, *Apis mellifera*, Botanical origin, Honey, Laghouat.

INTRODUCTION

Honey is the natural viscous sugar solution produced by honey bees from the nectar of the flowers and also from the honeydew. Its composition varies depending on the type of flowers visited by the bees, the climatic and the environmental conditions, and the beekeeping practices (Zerrouk *et al*. 2018).

The melissopalynological examination is the identification of pollens in honey by microscopic examination, for which pollen reference collections are needed (Bodor *et al*. 2021). Pollen analysis in honey is a way to carry out searches of floral sources used by bees in the search for food resources. The pollen present in this bee product is a natural marker that can indicate which flowers are parts of their diet (Vieira *et al*. 2020). The honey bee (*Apis mellifera*) uses plant pollen as an important nutritional source for amino acids, fats, minerals, proteins, starch, sterol and vitamins; where the nutritional quality of pollen pellets is dependent on the botanical origin of the pollen (Hornby *et al*. 2022). The botanical origin covers the dominant plant source from which the honey is produced, while the geographical origin refers to the region from which the product is collected (da Luz *et al*. 2020).

As a result of the climatic and floral diversity in Algeria, there are a large number of regions that produce honey. Consequently, there are many different types of honey with a multitude of tastes, aromas and colours, which are well received on the market. Where we can find in Eastern the honeys of *Eucalyptus sp*, *Hedysarum coronarium*, *Echium vulgare*, *Erica sp*., *Myrtus communis*, *Rubus sp* ., *Capparis spp*., *Erica arborea*, (Laouar, 2017, Ouchemoukh *et al*. 2007), in Western, *Thymus vulgaris*, *Citrus sp*., *Eucalyptus sp*., *Lavandula Angustifolia*, *Lavandula stoechas* and *Hedysarum coronarium* (Nair, 2014) and in Southern region of Algeria we can find honeys from *Ziziphus lotus*, *Peganum harmala* and *Euphorbia sp*. (Zerrouk *et al*. 2014).

The botanical origin is an important characteristic mainly in the evaluation of honey quality and for consumer confidence. Depending on the botanical origin, honey can be classified as monofloral (unifloral) or multifloral (polyfloral) depending on whether it is coming from a single or from several plant species, respectively

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(Balkanska *et al*. 2020). Usually, a honey is considered mainly from one plant (unifloral) if the pollen frequency of that plant is >45%. Unifloral honey does not denote a honey containing exclusively a single type of pollen, but rather the dominance of pollen collected from a certain plant (Bodor *et al*. 2021). While a polyfloral honey is classified as one in which there is no dominance of any pollen type in the sample.

The Laghouat Region is located in the steppe region between two mountain ranges, the Tell Atlas to the north and the Saharan Atlas to the south. It's one of the important arid and semi-arid areas for beekeeping in Algeria. It is considered as a transition zone between the Mediterranean region and the Sahara region.

There has been a limited research on pollen analyses of Laghouat honeys and more are needed to determine the pollen and plant sources in different regions. One of our research-lines is to analyse the pollen characteristics of honeys from Laghouat, with the aim of improving their quality, and commercialising them and at the same time to avoid fraudulent adulteration with foreign honeys. Within the scope of a more extensive research programme this paper gives the results of the melissopalynological analysis of nineteen honeys that were typified as polyfloral. The goal of this work is, on one hand, the knowledge of the melliferous plants (pollen and nectar sources) of this region, with the contribution of new data about the produced honeys and, on the others hand, to establish geographical markers that may help to distinguish the polyfloral honeys of this region from those with a different geographical origin.

MATERIALS AND METHODS

Honey sampling

The present work was carried out on 19 honey samples produced by *Apis mellifera* L. and provided directly by beekeepers in the Laghouat region during 2017 and 2019 (Table 1). The honey was obtained by centrifugation of combs and stored in hermetically closed glass jars at room temperature until analysis.

Melissopalynological analysis

The method without acetolysis described by Louveaux *et al*. (1978) was followed. Ten grams of honey were weighed and dissolved in 20 mL of hot distilled water not above 40 °C. The solution was centrifuged for 10 min (RCF about 1900G) and the supernatant was drawn off. The supernatant was again removed after the second centrifugation for 5 min following the same conditions. Finally, the residue transferred using a Pasteur pipette onto a microscope slide for analysis.

Quantitative analysis

Based on the total number of plant elements, the samples were classified into one of the five classes as proposed by Maurizio (1939): class I with less than 20 000 pollen grains per 10 grams of honey; class II with 20 000–100 000 pollen grains; class III with 100 000–500 000 pollen grains; class IV with between 500 000–1000 000; and class V with more than 100 000 pollen grains per 10 grams of honey.

Table 1: Origin of the honey samples

Samples	Region	Harvest
1	Aflou	2018
2	Aflou	2018
3	Sebgag	2018
4	Nasser Ben Chohra	2018
5	Laghouat	2018
6	Kasr El-Hiran	2019
7	El-Assafiya	2019
8	Ain Madi	2019
9	Tadjrouna	2019
10	Oued Touil	2018
11	Brida	2019
12	Brida	2019
13	Laghout	2019
14	El-Assafiya	2019
15	Khat El Oued	2017
16	Hamda	2017
17	El-Bourdj	2017
18	Sidi Makhlouf	2017
19	Gueltat Sidi Saad	2017

Qualitative analysis

The pollen types present in the honey samples were identified, counted, and classified, according to their frequency classes as follows: dominant pollen (≥ 45%), secondary pollen (15–45%), important minor pollen (3–15%), minor pollen (1–3%) and present pollen (< 1%) (Louveaux *et al*. 1978).

The pollen slides were examined with a light microscope (400 × or 1000×, as appropriate), in order to improve the identification of the pollen types.

Pollen types were identified by comparison with reference slides of pollen collected directly from the plants in the study area, different pollen morphology guides (Reille 1992, 1995, 1998) and information from several Websites. For quantification of the pollen types, at least 600 pollen grains were counted on two slides from each sample.

RESULTS AND DISCUSSION

Quantitative analysis

The concentration of pollen grains per 10 gram of honey varies from 23 400 to 351 250, with a mean value of 108 002 grains /10 g (Figure 1); which can be considered lower than those found in honeys from north-east Algeria (Azzedine *et al*. 2007). Quantitative analysis of honeys revealed that eleven samples (57.9%) had a moderate amount of pollen grains per 10 gram of honey (class II) and eight samples (42.1 %) were rich in pollen (class III). Honeydew elements were practically absent.

The pollen content varied highly with the type of honey but depend on others factors as the procedure used by the beekeeper to obtain the honey including the honey extraction from the combs, the filtering system, or the type of hive used (Zerrouk and Bahloul, 2020). In the samples studied, additionally to the botanical origin of the honey, some management practices as the harvest of honey combs with bee bread could contribute to increase the pollen content.

Qualitative analysis

Honeybees collect pollen grains from plants to obtain protein for their survival and reproduction (Pernal and Currie, 2000). The bees frequently collect a wide variety of pollen types, but they generally concentrate on a few

Table 2: Principal pollen types and their frequency classes in the honey samples*.

Family	Pollen types	Rep.	P	R	I	A	D	Max.
Amaranthaceae	Amaranthaceae	52.6	10.5	21.1	21.1	-	-	3.9
Apiaceae	Apiaceae	68.4	36.8	26.3	5.3	-	-	6.2
	Eryngium Type	57.9	31.6	5.3	10.5	10.5	-	18.4
Asteraceae	*Centaurea sp*	89.5	42.1	15.8	26.3	5.3	-	39.7
	Onopordum arenarium	68.4	21.1	21.1	26.3	-	-	13.5
	Asteraceae	57.9	36.8	15.8	5.3	-	-	6.5
	Launaea sp	52.6	31.6	15.8	5.3	-	-	7.4
	Carthamus sp	47.4	47.4	-	-	-	-	0.8
Boraginaceae	*Echium sp*	68.4	31.6	15.8	10.5	10.5	-	22.8
Brassicaceae	Brassicaceae	84.2	26.3	21.1	31.6	5.3	-	22.8
	Eruca vesicaria type	57.9	26.3	15.8	10.5	5.3	-	39.1
Cistaceae	*Cistus sp*	63.2	26.3	5.3	31.6	-	-	14.3
Euphorbiaceae	*Euphorbia sp*	78.9	36.8	21.1	15.8	5.3	-	27.5
Fabaceae	*Trifolium sp*	89.5	42.1	21.1	21.1	5.3	-	2-
	Fabaceae	84.2	21.1	21.1	36.8	5.3	-	18.9
	Lotus type	63.2	15.8	10.5	31.6	5.3	-	28.7
Fagaceae	*Quercus sp*	57.9	36.8	21.1	-	-	-	2.4
Myrtaceae	*Eucalyptus sp*	78.9	21.1	10.5	21.1	15.8	10.5	46.9
Oleaceae	*Olea europaea*	73.7	36.8	15.8	15.8	5.3	-	40.7
Poaceae	Poaceae	47.4	42.1	5.3	-	-	-	2.0
Polygonaceae	*Rumex sp*	57.9	52.6	5.3	-	-	-	2.4
Rhamnaceae	*Ziziphus lotus*	57.9	15.8	15.8	10.5	15.8	-	40.6
Salicaceae	*Salix sp*	57.9	15.8	31.6	10.5	-	-	4.7
Urticaceae	Urticaceae	47.4	26.3	10.5	10.5	-	-	4.5
Zygophylaceae	*Peganum harmala*	94.7	-	5.3	42.1	47.4	-	35.9

* Pollen that present below 47% in the honey samples was not including in table: Rep: presence in samples (%). P: present pollen (< 1% of pollen spectra). R: minor pollen (1–3%). I: important minor pollen (3–15%). A: secondary pollen (15–45%). D: predominant pollen (≥ 45%). Max: maximum value (%). t.: The pollen grain had similar morphology for some genus and species.

species (Song *et al*. 2012). The present study provides new insights into the pollen composition of polyfloral honey samples from Laghouat region of Algeria.

The qualitative pollen analysis of the 19 honey samples showed a great variety of pollen grain types. The pollen spectrum of the honey samples from Laghouat demonstrated 100 morphologically distinct pollen types related to 49 plant families (Table 2). The number of pollen types found in our study agrees with those found in honeys produced in Algeria, reported by Ghorab *et al*. (2021) (with 96 pollen types), and different than those from others regions of Algeria cited by Nair *et al*. (2013) and Makhloufi (2010) (with 36 and 124 pollen types, respectively). According to Herrero *et al*. (2002), the pollen content varied highly with the type of honey but also depends on other factors such as the procedure used by the beekeeper to obtain the honey including the honey extraction from the combs, the filtering system or the type of hive used.

In terms of the number of identified pollen types, the most represented were as follows: Asteraceae (12 types) Fabaceae (9 types), Apiaceae, Lamiaceae and Boraginaceae (5 types each); Liliaceae (4 types); Arecaceae, Cistaceae, Euphorbiaceae, Salicaceae and Zygophyllaceae (three types each); and Anacardiaceae, Brassicaceae, Cupressaceae, Oleaceae, Polygonaceae and Rosaceae (two types each). The others families (31) each exhibited a single pollen type. The results of this study are in agreement with those obtained by Terrab *et al*. (2001, 2003), La-Serna Ramos and Gomez-Ferreras (2006), Sajwani *et al*. (2007) and Costa *et al*. (2013).

For the percentage of occurrence in the honey samples, the following pollen types were frequent: *Peganum harmala* (recorded in 94.7% of the samples) *Centaurea sp.* and *Trifolium sp*. (each in 89.5 % of the samples) Brassicaceae (others) Fabaceae (others) (each in 84.2 % of the samples), *Eucalyptus sp.* and *Euphorbia sp.* (each in 78.9 % of the samples) *Olea europaea* (in 73.7% of the samples) Echium sp., *Onopordum arenarium* and Apiaceae (others) (each in 68.4 % of the samples), *Lotus* type and *Cistus sp*. (each in 63.2 % of the samples); *Ziziphus lotus*. *Eruca vesicaria* type, *Eryngium* type, Asteraceae (others), *Salix sp., Quercus sp., Rumex sp., Launaea sp.* and Amaranthaceae (others) present in more than 50% of the honey samples.

The region selected for the present study has good potential for sustaining beekeeping ventures because of the diversity of nectar and pollen taxa. The quantitative pollen analysis of the honeys demonstrated the important participation of *Peganum harmala, Centaurea sp, Trifolium sp Eucalyptus sp* and *Euphorbia sp*. in the makeup of honeys from Laghouat. In addition, pollen grains from *Eucalyptus sp., Plantago sp., Olea europaea, Ziziphus lotus, Centaurea sp.* and *Eruca vesicaria* type found at high percentages in some samples (> 39%), could indicate that a honey came from the Laghouat region.

75 pollen types were present below 40% in the honey samples (Table 3). Some of them *Ononis natrix, Carex sp, Hedera helix* and *Echinops sp*. that is present in more than 36% (each) of the honey samples, *Plantago sp., Gleditsia sp., Schinus molle, Citrus sp*. and *Elaeagnus angustifolia* in more than 29% (each) of the samples, *Casuarina sp., Polygonum sp., Prunus* Type, *Phoenix dactylifera, Populus sp*. and *Daucus carota* (>10%), and twenty-eight of the 100 identified pollen types are present in less than 6% of the samples.

The number of pollen types found in each honey sample was varied from 13 to 27 (average number of 19.1). 6 samples contained a great diversity (more than 20 pollen types). The highest incidence is in the interval 15- 20 with 8 samples (Figure 2). Most of the honey samples (52.63%)

Table 3: Pollen types presented with a percentage below 1% in samples.

Pollen types	Rep.
Rosaceae (others), *Ononis natrix*, *Carex sp*., *Hedera helix*, *Echinops sp*., *Chamaerops sp*., *Taraxacum* Type, *Vicia sp*., *Ligustrum vulgare* and *Matricaria sp*.	30-40%
Euphorbiaceae (others), *Plantago sp*., *Gleditsia sp*., Silybum eburneum, *Solanum tuberosum*, *Schinus molle*, Boraginaceae (others), *Citrus sp*., Cupressaceae (others), *Convolvulus sp*., *Cupressus sp*., Iridaceae and *Elaeagnus angustifolia*.	20-30%
Casuarina sp., *Polygonum sp*., *Prunus* Type, *Phoenix dactylifera*, *Populus sp*., *Daucus carota*, *Acacia sp*., *Malva sylvestris*, *Artemisia sp*., *Vitis sp*., *Medicago sativa*, *Helianthemum sp*., *Erica arborea*, *Mentha sp*., *Ammi majus* Type, *Punica granatum*, *Erodium sp*., *Limonium bonduelli*, *Thymus sp*., *Tribulus terrestris*, *Cucumus sativus* and *Allium sp*.	10-20%
Zygophyllaceae (others), *Reseda sp*., Cistaceae (others), *Tamarix sp*.,*Viburnum tinus*, Scropholariaceae, Salicaceae (others), *Thapsia garganica*, *Opuntia ficus-indica*, Simaroubaceae, Anacardiaceae (others), *Marrubium vulgare*, *Chrozophora tinctoria*, *Borago officinalis*, *Ambrosia sp*., *Helianthus* Type, Liliaceae (others), *Cerinthe major*, *Pinus halepensis*, *Anchusa sp*., *Asphodelus sp*., *Retama raetam*, Lamiaceae (others), *Muscari comosum*, *Scabiosa sp*., *Lavandula sp*., Arecaceae (others) and Acanthaceae Ephedra sp.	< 6%

Rep: percentage of representation in samples.

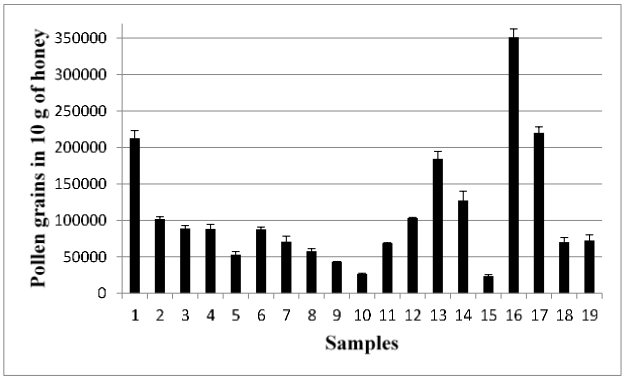

Fig. 1: Quantitative pollen analysis of the honey samples

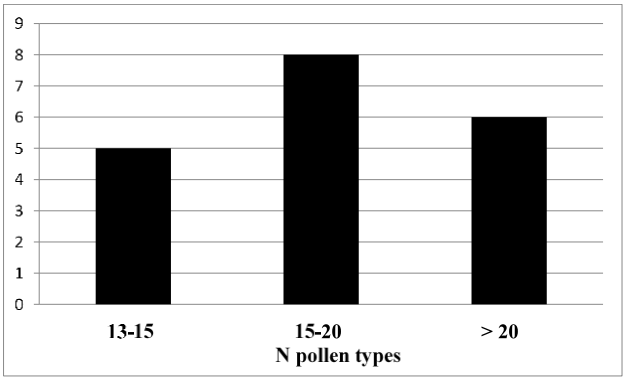

Fig. 2: Number of pollen types found in honey samples.

had more than 17 pollen types, indicates the abundance of pollen and/or nectar sources in the study area and the generalist behaviour of *Apis mellifera* when searching for resources (Bosco and Luz, 2018). Flower availability in the Laghouat is brief as generally occurs in arid and semi-arid ecosystems. It begins continues through spring until early summer, what makes the bees take advantage of this period to visit and collect the greatest amount of pollen and nectar, which explains the presence of a variety of plant species.

Eucalyptus sp. was identified as predominant pollen (D) in one samples (Table 2), (46.9%) but was discarded from the monofloral honey because this species are considered to be over-represented pollen (which must contain not less than 70% of the same pollen); the same specie was identified as secondary pollen also in one samples (43.2%). Louveaux and Abed (1984) mentioned the presence of *Eucalyptus* honey in the laghouat region. As secondary pollen (A) were identified: *Plantago sp., Olea europaea, Ziziphus lotus, Centaurea sp., Eruca vesicaria* type, *Gleditsia sp., Peganum harmala, Lotus* type, *Euphorbia sp., Echium sp., Trifolium sp.,* Brassicaceae (others) Fabaceae (others), Rosaceae (others) and *Eryngium* Type. With regard to minor pollen or important minor pollen, their presence was observed in almost all samples studied (Tables 2 and 3). This pollen type has little importance with regard to the amount of nectar supplied; however, it provides information with regard to the source and geographic origin of the sample (Sodré *et al*. 2007).

Pollen spectra of polyfloral honeys revealed a variety of not only nectariferous but also nectarless sources available to bees and included taxa with varying percentages, such as *Urticaceae, Pinus halepensis, Carex sp., Populus sp., Plantago sp., Olea europaea, Ligustrum vulgare, Quercus sp., Rumex sp.* and Poaceae but generally with lower percentages. According to Louveaux (1958) the pollen spectrum of many honeys shows the presence in sometimes high proportion of pollen grains belonging to plants devoid of nectaries, and bees only visit them to collect pollen.

Pollen of native plants of the study area is intensely utilised by honey bees and, in general, is a nutritional food for them because it combines high protein content and nectar. Beekeepers should take these results into account in apiculture exploitation of Laghouat.

Conclusion

Honey analysis indicates a good potential for the development of bee colonies in this locality. Bees used pollen for brood rearing, growth in colony strength, and nectar for their carbohydrate requirement. The identification of pollen and nectar sources in honey would help beekeepers in maintaining their colonies.

Typical polyfloral honeys from the Laghouat region contain mostly *Peganum harmala, Ziziphus lotus, Centaurea sp., Eruca vesicaria* type, *Olea europaea, Lotus* type, *Euphorbia sp., Echium sp., Plantago sp., Gleditsia sp., Trifolium sp.* and *Eucalyptus sp.* These pollen types may be mentioned among the characteristic accompanying species of the polyfloral honey produced in this region of Algeria.

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