PHYSICOCHEMICAL PROPERTIES AND POLLEN SPECTRA OF HONEYS PRODUCED IN TUNISIA (SOUTHWEST OF KEF)

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Abstract:

Interested in floristic biodiversity of the Southwest of Kef, we sought to identify the principal melliferous plants and know the chemical composition of the honeys, natural local products. The melissopalynological analysis and the study of the physicochemical properties (humidity, diastase activity, acidity, H.M.F., sugars, amino acids, electrical conductivity) of seven different honeys collected throughout the area, have allowed us to check their natural state**.** The sugar spectrum, the electrical conductivity and the acidity are those of blossom honeys. Five samples for which the controlled parameters met the norms required by the legislation and were in line with what was expected from a high quality honey are investigated. The pollen spectrum shows that the flora found within the composition of honeys is wealthy, diversified and mainly spontaneous. Indeed, 47 pollen forms, corresponding to different taxonomic levels, have been identified. The representation and the abundance of *Peganum harmala* L. are striking features of the studied honeys.

Keywords: Pollen/ Honey/ Physicochemical properties/ Tunisia/ Southwest of kef

Introduction:

Due to its relief and great variety of climates and soils, Tunisia is endowed with wealthy and diversified natural flora offering bees an anthology of a concern numerous plant species, which are visited for the nectar, pollen or even both of them. The Southwest of Kef (Northwest of Tunisia) constitutes also a favorable shelter to honeybees. The forest and preforest ecosystems seem to play a crucial socioeconomic role for rural people ensuring for them an important melliferous potential. Nevertheless, the honeys produced in the region remain relatively unknown, in point of view of their pollen spectrum, and commercially under promoted. It is important to emphasize that the quality of honey becomes a concern topical issue notably with the globalization, which has given new dimensions to the beekeeping activity. This one undergoes not only the vagaries of climate but also those of agricultural policy, which is evolving according to the economic necessities and the rules of market [1]. In this research work, we focused on determining the physicochemical properties of some honeys produced in the southwest of Kef and identifying their pollen spectrum for better promotion of the floral

varieties. Indeed, for imperatives of technology or marketing, such as a classification in "origin appellation", this accurate data becomes of an utmost importance [2] increasing our honeys market, since origin and quality are often linked.

Materials and methods :

The study was carried out on seven different honeys produced and originating from the Southwest of Kef. As it is shown in table I and figure 1, the samples were chosen in order to represent the different geographical localities of the region. These honeys were harvested at the end of June/beginning of July (2004), when the beekeepers, who were using traditional methods, did their unique harvest.

Table I: Provenances of the honeys produced in Southwest of Kef

Honey reference	S042213	S042214	S042215	S042216	S042217	S042218	S042219
Provenance of honeys	Houdh-Tajerouine	Kalaât Khasba	Garn Halfaya-Tajerouine	Ouled Messoud-Kalaât Khasba	Jbel Slata-Tajerouine	Felta-Kalaât Snen	Saddine-Sakiat Sidi Youssef

The study area:

The region concerned by our investigation is in the Southwest of Kef, situated in the Northwest of the Tunisian territory (Figure 1).

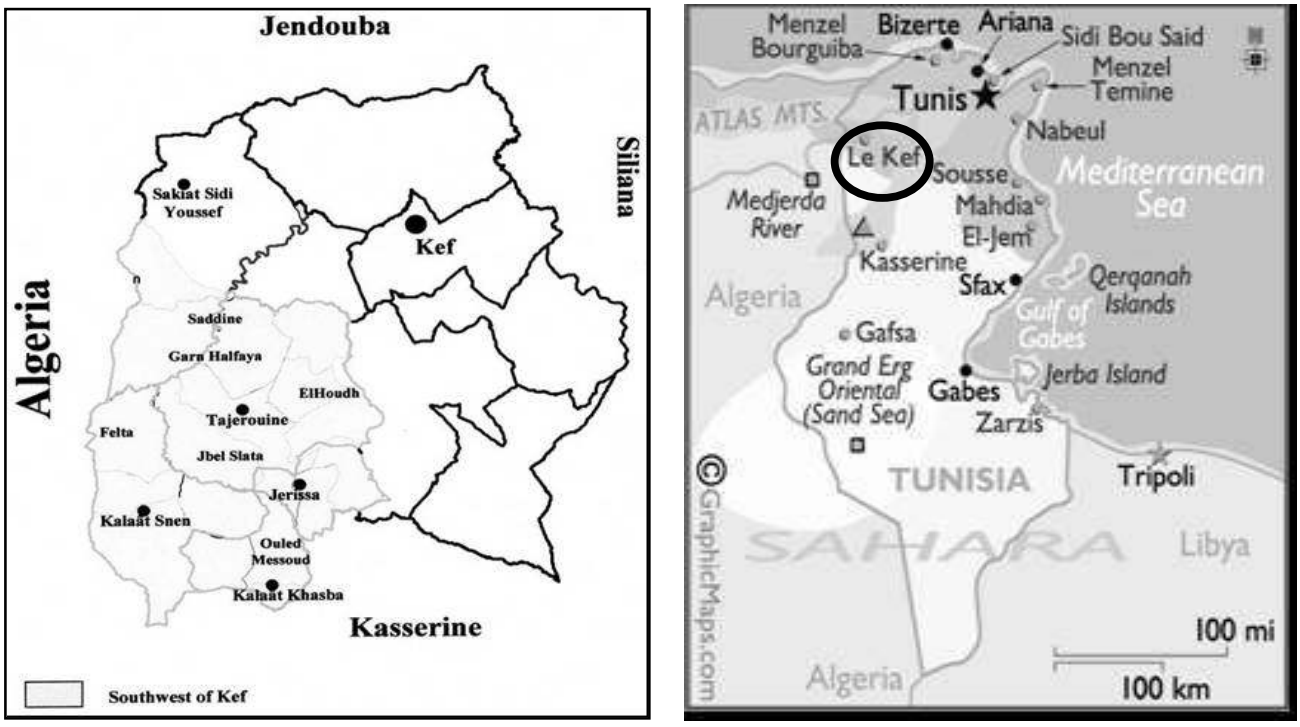

Figure 1: Regions from where samples were collected.

It is a mountainous region covering some 194036 ha, marked by its semi-arid and continental climate. The average annual precipitations vary from 300 to 500 mm. The temperatures are moderate and clement having generally an average of 7 to 9°C and 25 to 27°C for the most cold and the most hot months of the year, respectively. On the summits, it snows regularly. Moreover, we should point out that the diversity of soils, landscapes and relief (mountains and plains) in the region has favored the growth of a wide variety of natural vascular flora [3].

Physical and chemical analysis of honeys:

The analysis of honeys was performed at CETAM (Centre d'Etudes Techniques Apicole de Moselle, Lorraine, France). To determine the different physicochemical properties of honeys such as moisture, electrical conductivity, diastase activity, acidity, H.M.F. (5-(Hydroxymethyl)-2-Furfural), sugars, amino acids, we have applied the harmonized methods of the European Honey Commission which are officially recognized by the EU and by the *Codex Alimentarius* [4]. The honey moisture (water content of honey) was determined using refractometric method. The electrical conductivity was measured in a solution at 20% dry weight and 20°C temperature using continuous flow immersion cells. The determination of diastase activity followed Phadebas method where an insoluble blue dyed cross-linked type of starch was used as the substrate. This was hydrolyzed by the enzyme, yielding blue water-soluble fragments, determined photometrically at 620 nm. The free and lactones acidity were evaluated and the pH was measured on a 10% honey solution using an Expandable ion Analyzer EA 920 (Crion Research) pH-meter.The concentration of hydroxymethylfurfural was determined following the original method of Winkler where a spectrophotometer for measuring absorbance at 550 nm with 1-cm cells, was used. The proportion of the predominant sugars in the studied honeys was quantified by HPLC with pulsed amperometric detection and using a HPLC chromatograph of type Dionex Bio LC 40001. Finally, to verify the ripeness and the quality of honey samples, we have measured also the proline content according to the original method of Cough.

Melissopalynological analysis:

The pollen analysis is based essentially on the identification and counting of pollen grains contained in a determined quantity of honey [5-6]. That is why we have treated our samples in accordance with the standard methods of the International Commission of Bee Botany (ICBB) of the International Union of Biological Sciences (IUBS) [6]. The slides prepared were observed under an immersion triocular microscope at different magnification factors of 100 and 400. First, we have examined the whole slides and marked all the pollen forms met. Subsequently, we counted 300 pollen grains taken at random but exhausting completely all the fields of view. The identification of pollen types has been done at magnification factors of 400 and 1000 at immersion according to the pollen size and the need of visualizing some particular details of the exine or the apertures. Some microphotographies have been established using a digital imagery. This identification has been made easier by consulting palynological literature [7-8-9-10-11-12-13-14-15-16-17-18] and the numerical data bank of CETAM – Lorraine.

Moreover, the reference slides that we prepared by acetolysis technique [14] from 57 plant species belonging to 50 genera and 20 botanical families growing in Southwest of Kef were very helpful. A voucher specimen for each taxon has been collected in the field and identified using some references relating to the Tunisian flora [3-19-20-21] as well as the Herbarium of National Agronomic Institute of Tunisia (INAT). The pollen grains identified were classified, after ZANDER, depending on their frequencies as follow: predominant pollen (more than 45% of the pollen grains counted), secondary pollen (16 – 45 %), important minor pollen (3 – 16 %) and minor pollen (less than 3 %). Pollens of wind-pollinated or nectarless plants are noted separately and are subtracted from the total before calculating the frequencies of pollens of nectariferous plants [6].

Results :

The values of physical and chemical parameters of the honeys collected throughout the Southwest of Kef are given in table II providing estimates of their variability.

Table II: Physicochemical properties of the honeys produced in Southwest of Kef

		Honey samples						
Physico-chemical properties	**Norms required by the *Codex Alimentarius***	**S042213**	**S042214**	**S042215**	**S042216**	**S042217**	**S042218**	**S042219**
Moisture	≤ 20%	18,3	17,9	16	17,5	21,8	17,9	16,4
HMF	≤ 80 mg/Kg	14	8,4	11,9	16,9	45,3	45,3	7,5
Diastase activity	≥ 8 (Units of Schade)	27,4	39,6	16,4	21,3	3	7,5	8,5
Free acidity	≤ 50 mEq/Kg	14,2	16,2	9,4	12,5	16,6	14,7	9,9
Electrical conductivity	≤ 800 µS/cm but more if honeydew	314	477	618	319	334	382	586
Proline		740,6	597,3	483	465,3	827,6	536,2	529,7
Sugar composition (%)								
Glucose		31,7	33,4	25,7	34,8	32,5	30,8	26,9
Fructose		40,8	34,4	33,3	34,6	39,6	37,1	33,4
Sucrose	General ≤ 5% (except *Lavandula*,..)	ND	2,1	14,4	2	0,2	1,1	4,5
Maltose		2	1,8	2,9	2,9	1,9	2,4	6,8
Erlose		0,8	ND	4,8	0,6	0,1	0,3	3
Turanose		1,2	1,4	1,4	2,2	1,1	1,9	2,2
Isomaltose		1,4	1,5	0,8	1,8	0,9	1,3	1,1
Melezitose		0,1	0,03	0,1	0,2	0,1	0,01	0,2
Trehalose		ND	0,01	0,04	ND	ND	ND	ND
Raffinose		ND	ND	ND	0,5	ND	ND	0,2
Total sugars		78,1	74,6	83,5	79,7	76,4	75	78,4
Glucose + fructose	≥ 60% (honeydew ≥ 45%)	72,5	67,8	59	69,4	72,1	67,9	60,3

ND : not detected

Figure 2 illustrates data obtained from the pollen analysis. Forty-seven morphological pollen types corresponding to different taxonomic levels were identified. Only 13 taxa were refined to a species level. In many cases, the identification was limited to a genus level (22 pollen forms) or even family level (12 pollen forms).

The different taxa met belong to 28 botanical families that one is monocotyledonous and 27 are dicotyledonous. Asteraceae and Lamiaceae are the most represented families having each six pollen forms, followed by Papilionaceae (4 pollen forms). The number of pollen forms is between 11 (S042214) and 25 (S042215) with an average of 18 per honey sample.

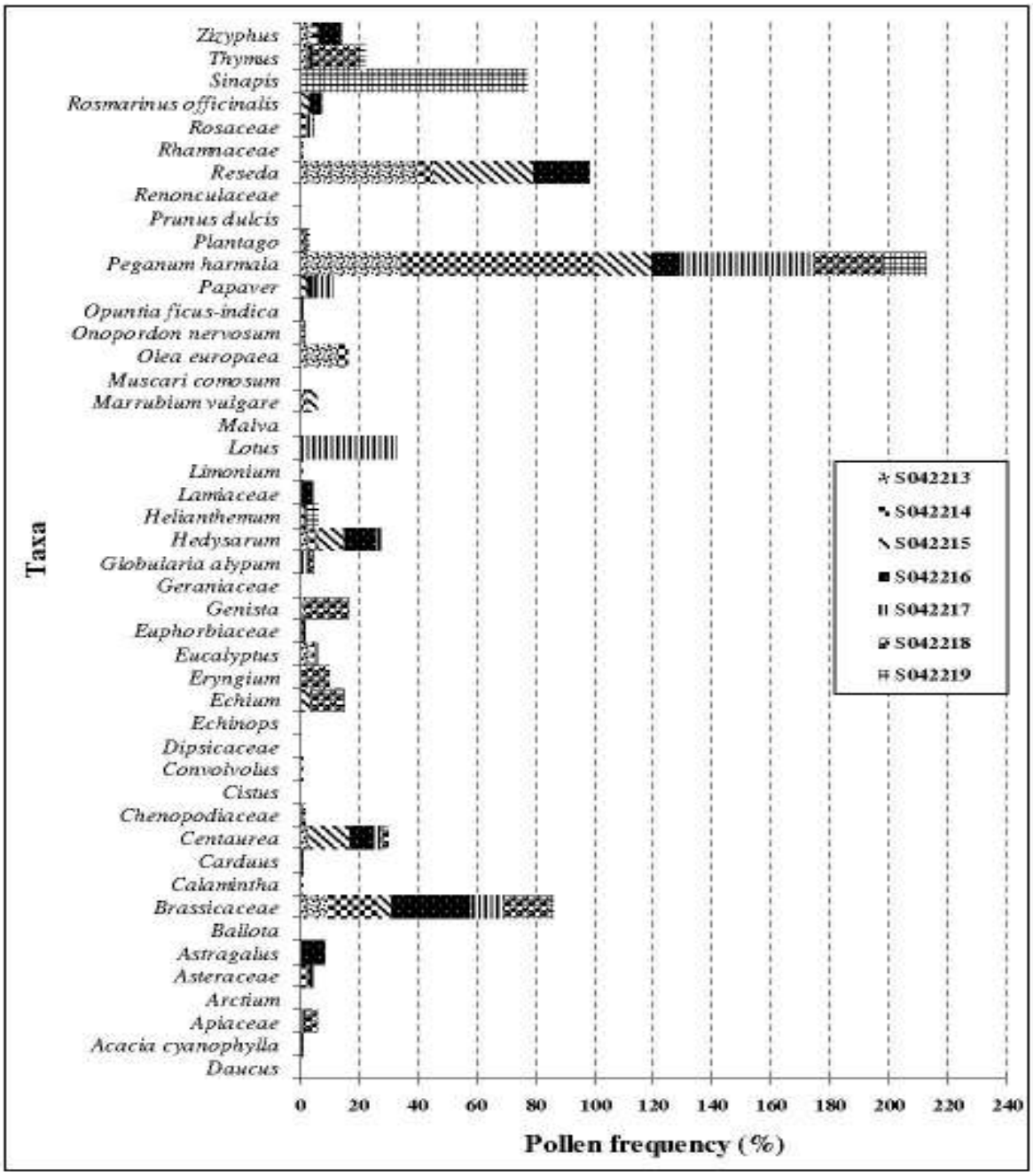

Figure 2: Pollen spectrum of honeys produced in Southwest of Kef.

Figure 3 represents the flowering calendar of the taxa determined in the pollen spectrum of the studied honeys. The spring and the beginning of summer constitute the main beekeeping season.

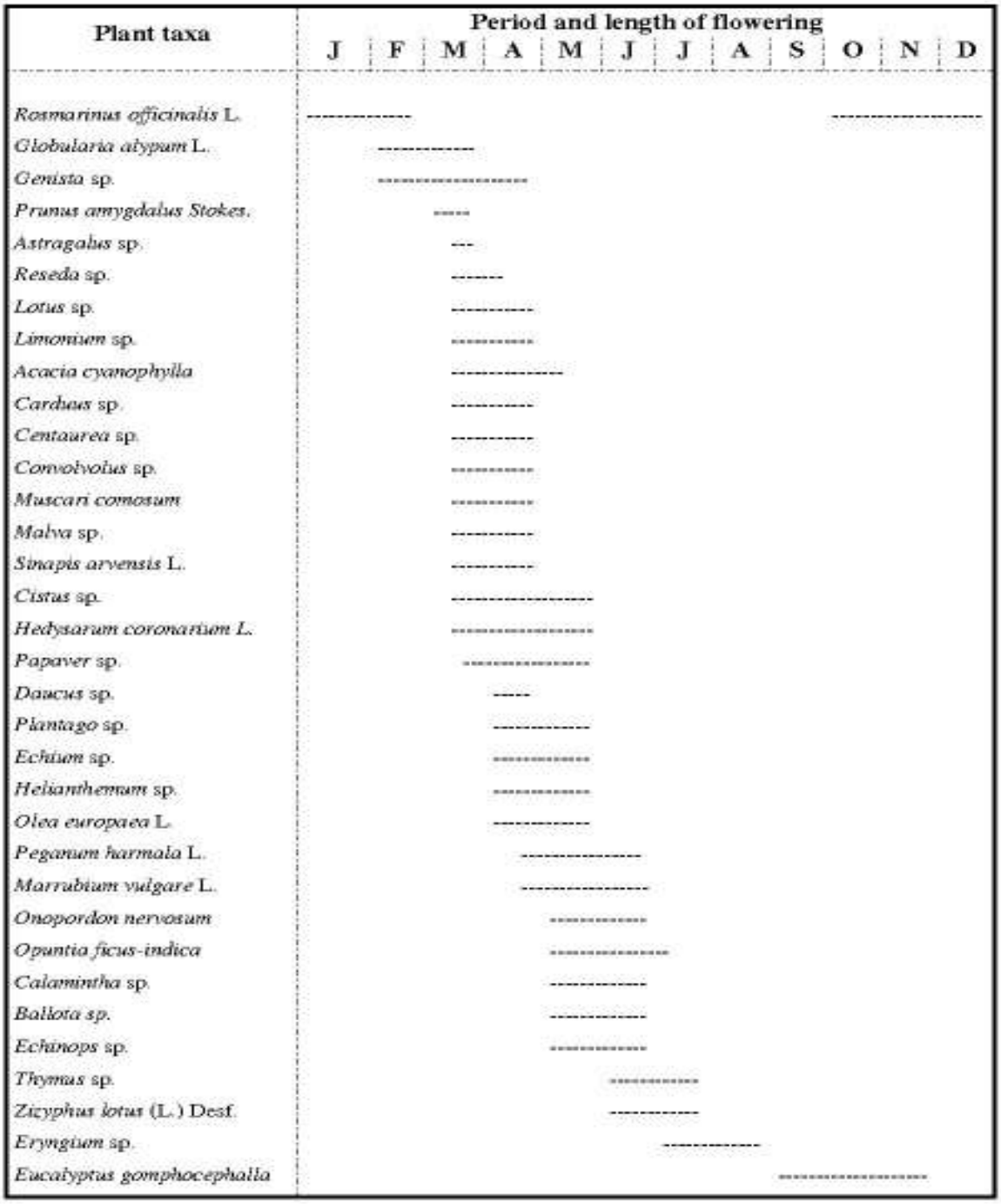

Figure 3: Flowering calendar of melliferous plants in the Southwest of Kef.

Discussion:

Physicochemical properties of honeys:

For five samples (S042213, S042214, S042215, S042216 and S042219), the entire parameters checked meet perfectly the norms required by the *Codex Alimentarius* for the appellation “honey”. They are also keeping with the norms of the European Honey Directive of 2001 (Official Journal of the EC, December 20, 2001) and to the decree of June 30, 2003 of the French Official Journal. This latter is more restrictive than the *Codex Alimentarius* for the HMF content, which must be less than 40 mg/kg, except for honeys from tropical regions where the HMF value can reach 80 mg/kg [4]. The water content (≤18%), the concentration of HMF (≤ 40 mg/kg) and the diastase activity values (≥ 8 in units of Schade) recorded are those of honeys of high quality. Moreover, the values of electrical activity (314 – 477 – 319 - 586 < 800 µS/cm) and free acidity (14.2 – 16.2 –12.5 – 9.9 < 50 mEq/kg) as well as the sugar spectrum of the samples S042213, S042214, S042216 and S042219, are typical of blossom honeys. The sugars detected in these samples constitute the most important part with the respective concentrations 78.1% - 74.6% - 79.7% and 78.4. The proportions sum of reducing majority sugars, fructose and glucose, is above 60% (72.5% - 67.8% - 69.4% and 60.3%) indicating the floral origin of the honeys [4]. The proline contents (740.6 – 597.3 – 465.3 and 529.7 mg/kg > 200 mg/kg) of these four honey samples are excellent, asserting their ripeness and the absence of adulteration [4-22]. As for the case of sample S042215, the electrical activity and acidity values are those of blossom honey. Nevertheless, the sugar spectrum shows exceptionally high sucrose content (14.4%). Indeed, the legislation provides an apparent content value of this disaccharide not exceeding 5% [2-4-23]. On the other hand, some diversions can occur in unifloral honeys such as those of *Robinia*, *Lavandula*, *Salvia*, *Rosmarinus*, *Medicago*, *Hedysarum*, *Onobrychis*, *Calluna* and *Citrus,* which have high sucrose content (10%). Some honeydew or blends of honeydew and blossom can have also extremely high sucrose content reaching even 15% [4-24]. Furthermore, the addition of sucrose syrup, having a similar composition to honey, can raise artificially this ratio [25-26]. However, the honey sample S042215 has a relatively high diastasic rating equal to 16.4 in units of Schade (table II). In this connection, it has been shown that sometimes, and during a strong nectar production, the raw material influence is so intense that it persists in the sugar spectrum of the honey in spite of leveling action of bee gland secretions [24]. In that case, the sugar spectrum can differ from the basic type one by higher sucrose content with corollary an abnormally low proportion of glucose plus fructose (in our case 59%). Additionally, the content of proline is excellent (483 mg/kg) and that of turanose, scarce sugar but constant in honeys, is normal and not affected (1.4%). All these elements correlated with the quite low moisture rate (16%) lead to suppose that the sample S042215 is correctly unprocessed (natural) honey with exceptionally high sucrose content. This statement of fact is supported by the microscopic examination where non-sign of adulteration has been detected. Similarly, the sugar spectrum of honey S042219 shows relatively high content of sucrose (4.5%), although it complies with legislation, as well as of maltose (6.8%). For this latter, there is non-legal norm. Indeed, contrary to sucrose, all the honeys contain maltose at variable concentrations reaching 7.5% in some cases

[5-23]. These values correlated with the low moisture rate (16.4%) and relatively low, but correct, content of amylase (8.5 in units of Schade), indicating that during the ripening process of this honey, the raw material (nectar) has not been well kneaded by bees [24]. The moisture rate recorded in honey S042217 is very high (21.8%) and does not comply with the *Codex Alimentarius* neither the European Honey Directive [4-5-22]. This fact can be essentially attributed to very early harvest and humid climate. The major risk of such honey is yeast fermentation causing its profound and irreversible spoiling. Indeed, under polarizing microscope, a marked presence of yeast has been observed. The two samples S042217 and S042218 have also HMF values over 40 mg/kg (45.3 mg/kg) and low diastasic ratings, respectively 3 and 7.5 in units of Schade. These values seem to be consequences of possible deterioration of honeys that happened during a heating or a long conservation in a hot place [5-22-27-28-29-30]. The other controlled parameters such as electrical conductivity, acidity and proline contents which are excellent (827.6 et 536.2 mg/kg), meet perfectly the norms of the *Codex Alimentarius* and the European Honey Directive for the appellation of “honey” indicating that they are typical blossom honeys.

Melissopalynological analysis :

The pollen analysis shows that *Peganum harmala* L. and Brassicaceae are the most frequent and predominant forms, found in all samples. To a lesser extent, *Reseda* L., *Thymus* L., *Hedysarum coronarium* L., *Eucalyptus* and *Centaurea* L. are met in five honeys. Moreover, we have identified a great number of taxa having important minor or minor pollen such us *Marrubium vulgare* L., *Rosmarinus officinalis* L., *Zizyphus* L., *Carduus* L., *Onopordon* L., *Daucus* L., *Eryngium* L., *Echium* Tourn., *Malva* L., *Astragalus* L., *Genista* L., Renonculaceae, Rosaceae, Euphorbiaceae, etc. Secondary pollen forms found are those of *Reseda* L. (40%, 34%, 19%), *Peganum harmala* L. (34%, 24%, 19%), *Lotus* L. (33%), Brassicaceae (27%, 16%) and *Thymus* L. (16%).
Nevertheless, floral species having predominant pollen are very few in number. *Sinapis arvensis* L., which has been found in sample S042219 at rate of 77%, and *Peganum harmala* L. appeared in both honeys S042214 and S042217 at respective rates of 66% and 45. Theses observations lead to precise that *Peganum harmala* L. or “Harmel” is a plant species that is well spread over the whole region of Southwest of Kef. If its pollen is not predominant, it appears as secondary (34%, 24%, 19%) or important minor (14%, 10%) pollen but on no account as minor rare pollen (< 3%). It is the most frequently occurring pollen form in all honey samples studied and the taxon seems to be a good quality nectar source which is well exploited by honeybees. *Sinapis arvensis* L. is also a common plant species in the region and tends to grow thoroughly in the fields of cereals and the fallow lands forming vast and very concentrated populations. When it is fully blooming, this plant species, which the melliferous value is well known [31], gives a bright yellow shade and seems to have a very particular attractiveness on honeybees.
To have a botanical appellation, these three unifloral honeys derived from *Peganum harmala* L. and *Sinapis arvensis* L. might have constant and characterized physicochemical and organoleptic properties. On the other hand, the pollen spectrum shows that the flora found within the composition of studied honeys is mainly spontaneous. Very few pollen forms of cultivated plants are found, it is notably the case

of Rosaceae (fruit trees), not determined accurately, or *Prunus amygdalus* Stokes, not exceeding the rate of isolated rare pollen. In addition to nectariferous plants, some wind-pollinated or nectarless plants as *Olea europaea* L., *Acacia cyanophylla*, *Papaver* L., *Helianthemum* Mill., *Cistus* L., *Plantago L.* and Chenopodiaceae type, have been also taken down. They represent 15% of all detected forms and have rare (4%, 7%, 13%) or sporadic (< 3%) pollen grains. These taxa are nectarless but their flowers seem to attract honeybees that visit them to gather pollen even they do not fit to entomophily [32-33-34].

Flowering calendar of melliferous plants in the Southwest of Kef:

According to the flowering calendar (figure 3), the Southwest of Kef is characterized by a late spring season. Indeed, the cold and frost, which is quiet frequent and late, could appear until April in the plains causing a spring growth delay of plants and consequently a shortening of the flowering length. The spring and the beginning of summer constitute the main beekeeping season. The nectariferous resources offered during the period of mi-March to the end of June are predominant and match with the great period of nectar productions. They are spring species blooming during the months preceding the honey harvest (from mid to end of July). However, species flowering in autumn are very scarce yet they are essential for internal needs of the colony and the setting up of winter stock. This fact can be due to the floristic composition of the region that is impoverished in autumn because of the harsh and continental climate. Furthermore, the autumn flowering concerns generally trees and shrubs that are, in the studied area, spread only in forest phytosociological communities. The distance between these trees and the hives can also explain their lesser attraction by honeybees. The flowering calendar reveals two alternatives: Eucalyptus and mainly Rosemary. This shrub can take advantage of stormy rains of summer or early rains of autumn to thrive and flower during several months allowing to honeybees a good wintering. Finally, it is advisable to emphasize that to pass December-January mark, the beekeepers using traditional methods, are found to be compelled to leave one part of honey in the beehives to meet the dietary needs of bees during winter.

Conclusion:

The flora found within the composition of honeys of Southwest of Kef is wealthy and diversified. The representation and the abundance of *Peganum harmala* L. are striking features of the different studied honeys that arouse a great interest. Indeed, the honey of “Harmel” which has been also named among the honeys of the “Algerian Canstantinois” [33] or those of Turkey [35] is a scarce product in Tunisia. Moreover, this honey is appreciated too much owing to its numerous ancestral medicinal virtues in view of the fact that the plant species, from which is originating is considered as an authentic panacea by North African people. Admittedly, this gap is proving to be very promising especially in the perspective of the creation of controlled origin appellations. The granting of such appellations would promote the honeys, natural local products, and would furthermore allow a distinction of national products from those imported.

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