CHARACTERIZATION OF HONEYS PRODUCED IN THE CENTER-WEST OF MOROCCO (AIT BAÂMRANE OF SIDI IFINI) USING MELISSOPALYNOLOGY

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Abstract. Honey is one of the most important products given by nature produced by bees from nectar and honeydew of various plants used by humans due to the health benefits it offers. For the characterization of honey, it is important to know the plants that participate in its production. In this study, the pollen analysis of 9 honey samples from central-western Morocco (Ait Baâmrane region) collected in three different zones, A (coast zone), B (plateaus and hills) and C (continental mountainous area) was carried out to determine the botanical sources involved in honey production in this region. To improve the pollen analysis, a factorial correspondence analysis (AFC) was used to determine the characteristic species of these three zones. Overall, 35 taxa belonging to 33 genera and 22 families were found in the samples. 23 taxa were identified in zone A samples, 20 in C and 16 in B. Of the nine samples studied, the summer samples were monofloral with *Euphorbia officinarum* subsp. *echinus* as the predominant pollen (between 25.71% 71.42%) while the spring samples were generally multifloral honeys (without dominance). They contained *Euphorbia regis-jubae*, *Papaver rhoeas*, *Echium petiolatum*, *Diplotaxis harra*, *Glebionis coronaria*, and *Calendula sp*. as secondary pollen with percentages ranging from 18.57% to 26.64%.
Keywords: *Euphorbia honeys, pollen spectrum, unifloral honey, melliferous plants*

Introduction

Honey is one of the most important products given by nature. It has been used by mankind since the beginning of time, due to the health benefits it offers, either as a nutrient or through its use in traditional medicine. It has anti-microbial, anti-inflammatory, anti-carcinogenic, antiatherogenic, anti-thrombotic and antioxidant effects as well as analgesic activities in the human organism (Siddiqui et al., 2017). It is a natural food consumed without any added ingredients, and is characterized by its complex composition, which varies according to the bee species, geographical region, and available floral source (Karabagias et al., 2014; Zhou et al., 2014).

Honey is produced by honeybees from nectar of plants, as well as from honey dew. Some of the components (carbohydrates, water, and traces of organic acids, enzymes, amino acids, pigments, pollen, and wax) are due to maturation of the honey, some are added by the bees and some of them are derived from the plants. Honey of the same floral

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source can vary due to seasonal climatic variations or to a different geographical origin (Anklam, 1998).

In addition to the nectar and honeydew that plants provide for bees as a source of honey production, they also produce pollen which is a very important element for bees. Honeybees collect pollen from a wide range of flowering plants, which usually fulfills their dietary requirements for proteins, lipids, minerals, and vitamins (Avni et al., 2014). Thus, in addition to its interest for the bees, the existence of pollen in the honey samples allows the identification of different melliferous species visited by the bees during foraging. Honey is studied by characterizing the pollen of the flowers it contains from a mellissopalynological analysis, with the aid of a microscope, characterizing pollen according to its size, shape, and surface patterns, as pores and thorns (Crane, 1980; Barth, 2004), which frequently correlates to the plant source of nectar. Therefore, the botanical and geographical origin of honey is determined by using the pollen analysis (Oddo et al., 2004; Juan-Borrás et al., 2014). Botanical origin is determined based on the relative frequencies of the pollen types of nectariferous species, using harmonized methods of melissopalynology (Ohe et al., 2004).

The region of Ait Baâmrane is characterized by an important vegetal diversity, which makes it among the preferable zones by the beekeepers given the beekeeping calendar that undergoes. The honey production depends on the annual climatic conditions, when they are favorable the type of honeys produced can be varied, as the Euphorbia honey (*E. officinarum* subsp. *echinus*), multifloral honey with the King Juba's Euphorbia species, honey of Eryngium, and honey of Thistle. In the last years with the climatic changes that the world undergoes, the region honey production is limited, generally in two types, multifloral honey during the spring and honey of Euphorbia species (*E. officinarum* subsp. *echinus*) during the summer. Euphorbia honey is known nationally by those advantages that allows, as its economic and food importance.

The current marketing of agri-food products is usually based on more in-depth studies, with specific characteristics such as: botanical and geographical origin, quality and safety, specific organoleptic or nutritional characteristics, among others (Juan-Borrás et al., 2014). Hence, the importance of making studies for honey at the international and national level. In Morocco, there are efforts to advance this sector, through studies that have been done on honey characterization (Terrab et al., 2014; Bettar et al., 2015; Chakir et al., 2016; Moujanni et al., 2017, 2018; Ait Abderrahim et al., 2019; Benjamaa et al., 2020). In general, the majority of this work is focused on antimicrobial activity and physicochemical characteristics. However, there is still a lack for studies on pollen analysis of honey.

Therefore, the objective of this study was to identify and enumerate all types of pollens present in honey samples collected in areas with different environmental conditions (coastal area, plateaus and hills, and the continental mountainous area) in the region of Ait Baâmrane and supply more information on their geographical and botanical origin.

Materials and methods

Study area

The study area was carried in the province of Sidi Ifni (Morocco) 29.383541° North, -10.168985° West and particularly in the confederation of Ait Baâmrane (*Fig. 1*). The population is about 127,781 inhabitants with a density of 49 ha/Km² and the area mainly practices agriculture, beekeeping, sea fishing, tourism, poultry and small ruminant

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breeding (Monographie Sidi Ifni, 2015). The climate in general is Mediterranean, with strong Saharan and Atlantic effects, with dry hot summers and wet cool winters. The region receives an average of 203 mm of rain per year. Average annual temperature is about 18.1 °C with a minimum of 14.4 °C in January and a maximum of 22.8 °C in August (Yous et al., 2022). Thus, the region is characterized by high levels of humidity throughout the year, with maximum values in summer (76%) and autumn (77%). The limitation of the area by the Mediterranean domain on one side and the Saharan domain on the other, allows it a varied climate and vegetation. It is characterized by the presence of the endemic tropical African species [*Argania spinosa* (L.) Skeels)], and by some thermophilous elements, compound by crassulescents often leafless [*Euphorbia officinarum* L. subsp. *echinus* (Hooker fil. & Cosson) Vindt, *Euphorbia regis-jubae* Webb & Berth., *Kleinia anteuphorbium* L. Haww., *Caralluma burchardii* N. E. BR. subsp. *maura* (Maire) Meve & F. Albers], which represents a specific entity from the physiognomic viewpoint, reminiscent of the infra-Canarian zone of vegetation which authors following Braun-Blanquet and Maire (1924) have become accustomed to calling 'the Argan sector' (Terrab et al., 2014).

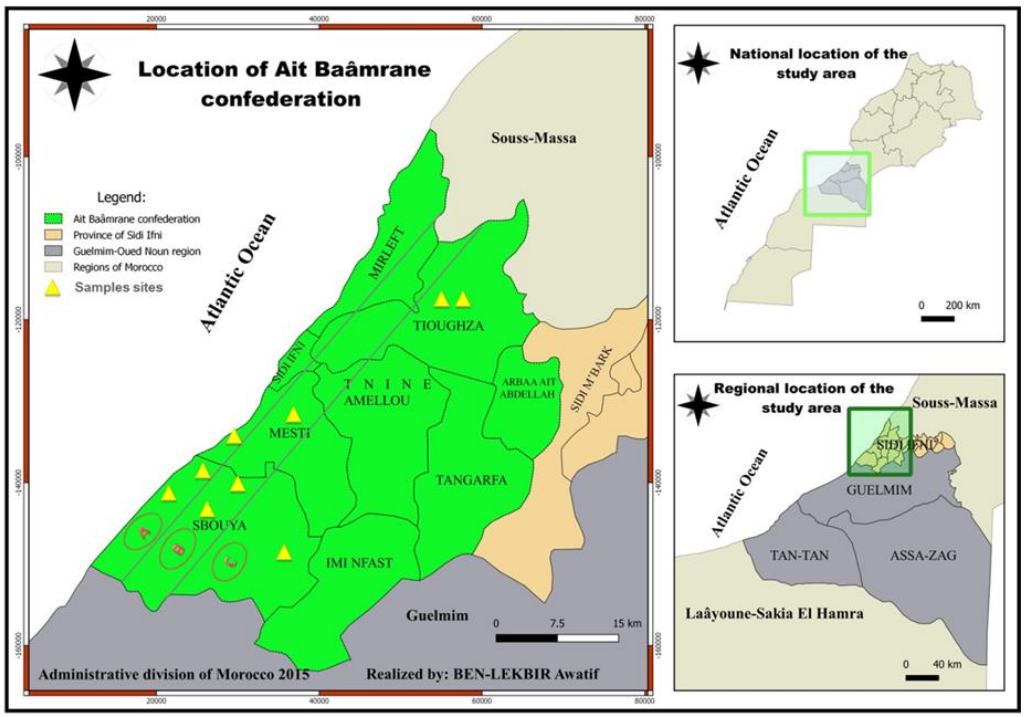

Figure 1. *Location map of the study area (Ait Baâmrane) and samples units (A, B and C)*

Among the Ait Baâmrane, traditional beekeeping is characterized by hives made of reeds (silts). They are generally sedentary and adapt to a local melliferous flora (*Fig. 2*).

Samples collection

The field work was carried out during the years 2016 to 2018. The sampling was done in three morphoclimatic units, zone A (coastal area), B (plateaus and hills) and C (continental mountainous area) having a strong impact on the vegetation and its physiognomy (*Fig. 3*).

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Figure 2. *Example of traditional fixed hives in the study area*

Figure 3. *The three morphoclimatic sampling units (A, B and C)*

Plants and flowers sampling

The floristic analysis was done according to the method adopted by Yous et al. (2022). A total of 90 floristic surveys were carried out in the sampling zones (*Fig. 3*); the releve was taken when the structure and physiognomy of vegetation or its habitat changed, over an area equivalent to the minimum area in the sense of Guinochet (1973), ranging from 100 to 400 m² depending on the structure of the vegetation and its environment. 30 surveys were conducted for each zone, spread over three representative sites in each. Thus, the surveys were conducted over the three years (2016, 2017, and 2018), with 10 surveys per area per year (*Table 1*). Surveys were conducted in all three years (2016, 2017, and 2018), with 10 surveys per zone per year. The collected plant samples were identified and preserved in the herbarium 'MARK' of Cadi Ayyad University.

The examination and identification of the geographical origin of a honey, requires the availability of a data bank on the melliferous species of the region as well as on their pollen grains. This requires the preparation of reference slides of pollen grains for the different honey plants in the study area. The collection of flowers was made in different landscape habitats (valleys, plateaus, mountains, plains, crop fields, fallow land, and allotment gardens with beehives), after numerous field trips. For the making of reference slides, the flowers were picked just before the opening of the flower bud. The hatching was done in the laboratory, which avoids the contamination of the collected pollen with that present in the environment. The species were identified and labeled by adding: name of the family, genus, species, in addition to the date and place of collection.

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Table 1. *Coordinates of the sampling sites for the floristic survey*

Floristic survey/ coordinates	Latitude	Longitude
SA1	29°27'49.99"N	-10°23'75.71"O
SA2	29°24'64.56"N	-10°27'62.19"O
SA3	29°23'12.51"N	-10°29'06.35"O
SB1	29°22'69.42"N	-10°26'95.18"O
SB2	29°22'69.93"N	-10°24'05.85"O
SB3	29°18'22.22"N	-10°24'05.85"O
SC1	29°30'24.72"N	-10°03'45.64"O
SC2	29°20'84.82"N	-10°14'29.65"O
SC3	29°18'14.25"N	-10°12'32.72"O

SA1: floristic survey on site 1 of zone A, SA2: floristic survey on site 2 of zone A, SA3: floristic survey on site 3 of zone A, SB1: floristic survey on site 1 of zone B, SB2: floristic survey on site 2 of zone B, SB3: floristic survey on site 3 of zone B, SC1: floristic survey on site 1 of zone C, SC2: floristic survey on site 2 of zone C, SC3: floristic survey on site 3 of zone C

Honey samples

Nine honey samples were collected between 2017 and 2018 during two different honeyflow periods. The first period is the spring honeyflow; it is characterized by the production of multifloral honey with the *Euphorbia regis-jubae* Webb and Berth, and the summer honeyflow, known by the production of honey from the *Euphorbia officinarum* subsp. *echinus* Hooker fil. & Cosson) Vindt. The collection was made in various areas according to the climate and reliefs (Zone A, Zone B and Zone C). Three samples were collected in each of these zones, two for the spring honey and one for the summer honey (*Table 2*). In order to be more representative of the area, the collection of honey samples was made from beekeepers with large hives, represented by a significant number of hives ranging from 80 to 175.

Table 2. *The origin, GPS coordinates, year of sampling and harvesting of honey samples*

Zones	Samples	Honey type	Latitude	Longitude	Collection date	Harvest date
Zone A						
	A1	Summer honey	29°27'32.14"N	-10°24'46.70 "O	2018	2018
	A2	Spring honey	29°24'69.53"N	-10°27'91.12"O	2017	2016
	A3	Spring honey	29°23'25.26"N	-10°29'16.10 "O	2018	2018
Zone B						
	B1	Spring honey	29°23'17.58"N	-10°26'63.27"O	2017	2016
	B2	Spring honey	29°22'66.91"N	-10°23'95.57 "O	2017	2016
	B3	Summer honey	29°18'01.45"N	-10°24'62.86 "O	2017	2016
Zone C						
	C1	Summer honey	29°29'47.63"N	-10°03'08.27 "O	2017	2016
	C2	Spring honey	29°20'85.84"N	-10°13'74.33 "O	2017	2017
	C3	Spring honey	29°18'01.87"N	-10°13'74.33 "O	2017	2017

A1: honey sample no. 1 from zone A, A2: honey sample no. 2 from zone A, A3: honey sample no. 3 from zone A, B1: honey sample no. 1 from zone B, B2: honey sample no. 2 from zone B, B3: honey sample no. 3 from zone B, C1: honey sample no. 1 from zone C, C2: honey sample no. 2 from zone C, C3: honey sample no. 3 from zone C

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Analysis of samples

Preparation of the reference slides

In the laboratory, flower anthers were placed in Petri dishes with distilled water and 95 °C alcohol to remove the fat and resin surrounding the pollen and to facilitate the opening of the anthers. Using forceps, anthers were crushed for pollen release. The remaining fragments of the anthers were removed, and the solution was transferred to centrifuge tubes. After that, the tubes were centrifuged at 2500 rpm for 10 min and the resulting pellet was subjected to acetolysis according to Erdtman (1952, 1960). The pellet was then put into slides, on which we added small squares of previously prepared glycerine gelatine. The slides were put on a warming plate (40°C), then they were left to dry for a few minutes. Next, they were covered with glass cover slips type 22 mm X 22 mm, and these were sealed with transparent nail enamel. In addition, each slide was labeled, the latter containing as information, the botanical family, name of species, the date, and the place of collection. Finally, the characterization and description of the pollen was done using a microscope with camera at 1000 × magnification. The different characteristics of pollen grains have been determined, according to Erdtman (1943, 1952); Hesse et al. (2008); and Punt et al. (1994).

Honey analysis

The pollen analysis of the honey samples collected was carried out using the method given by the International Commission of Bee Botany (Louveaux et al., 1978).

The identification of pollen grains was done by comparing them with our reference collection, in addition to the analysis of available databases and the following literature (Moore et al., 1991; Reille, 1992). Thus, the frequency of the different pollen types was determined by following the approach proposed by the International Commission for Bee Botany (ICBB) (Louveaux et al., 1978). For each sample, a minimum of 500 pollen grains was counted. The estimation of the frequencies of the different pollens has been done according to the following classes: predominant pollen (>45% of the total pollen detected in honey); secondary pollen (16–45%); minor important pollen (3–15%); minor pollen (<3%) (Louveaux et al., 1978).

Statistical analysis

Data analysis was done using R software version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). A factorial correspondence analysis (AFC) was done on a matrix (species/surveys) performed according to the frequency index determined by measuring the number of plant records containing the species in relation to the total number of records. Then, additional parameters represented by the ecological factors of each area (altitude, longitude, latitude, soil substrate and distance from the sea) were used as explanations of the plant distribution. Furthermore, Heat map combined to a hierarchical clustering were used to determine the relation honeys samples and plant species.

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Results

Floristic analysis

The floristic analysis of the area, allow the identification of 281 vascular species and subspecies, grouped into 187 genera belonging to 48 botanical families. The zone C had 47 families, followed by the zone B with 46 families, while zone A has only 43 families. Herbaceous and shrub species are the most represented, while trees are the weakest in the region. The vegetation cover is dominated by *Opuntia ficus-indica* in the three zones (A, B and C), and by *Argania spinosa* (a shrubby forest of climax argan trees) in zone C. In addition to these species, the characteristic species of these three areas were determined using a factorial correspondence analysis (*Fig. 4*).

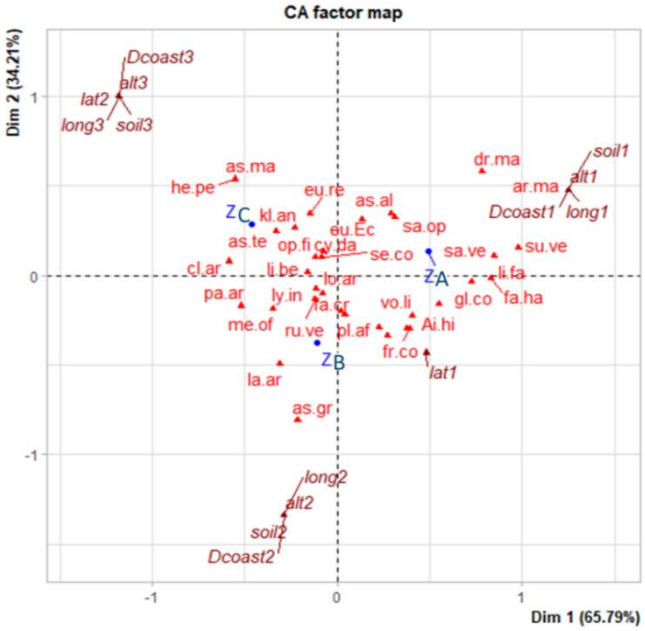

Figure 4. *Factorial correspondence analysis of species distribution frequencies based on floristic surveys, plan of axis 1 and 2; as.am: Asphodelus macrocarpus, cy.da: Cynodon dactylon, fa.ha: Fagonia harpago, co.al: Convolvulus althaeoides, kl.an: Kleinia anteuphorbium, sa.op: Salsola oppositifolia, vo.li: Volutaria lippii, ru.ve: Rumex vesicarius, op.fi: Asphodelus tenuifolius, dr.ma: Drimia maritima, lo.ar: Lotus arenarius, li.be: Limonium sinuatum subsp. beaumierianum, er.il: Eryngium ilicifolium, as.al: Asparagus albus, fr.co: Frankenia corymbosa, cl.ar: Cladanthus arabicus, he.pe: Helianthemum pergamaceum, eu.ec: Euphorbia officinarum subsp. echinus, gl.co: Glebionis coronaria, di.ha: Diplotaxis harra, eu.re: Euphorbia regis-jubae, se.co: Senecio glaucus subsp.coronopifolius, pl.af: Plantago afra, la.ar: Launaea arborescens, co.tr: Convolvulus trabutianus, ar.ma: Arthrocnemum macrostachyum, fa.cr: Fagonia cretica, li.fa: Limonium fallax, ai.hi: Aizoanthemopsis hispanica, me.of: Melilotus officinalis, as.te: Asphodelus tenuifolius, sa.ve: Salsola vermiculata*

The analysis carried out indicates that the two axes explain 100% of the total variance (65.79% for axis 1 and 34.21% for axis 2). Axis 1 would reflect a gradient of continentality and distance from the sea, with littoral zone A on the positive side of the graph and continental zones (B and C) on the negative side. The second axis seems to distribute the plant species according to their distribution frequencies in the total of the surveys. Thus, it highlights three groups:

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Zone A: Located along the Atlantic coast over a width of between 0 and 3 km from the sea. The altitude is between 0 and 100 m. It is a small plain, with an essentially clayey-sandy substrate, with a high salinity rate. The climate is of the subtropical Mediterranean type, and the atmospheric humidity is very high. The vegetation is a steppe dominated by species of the family Amaranthaceae: *Arthrocnemum macrostachyum*, *Salsola oppositifolia*, *Salsola vermiculata*, *Suaeda vera*, very adapted to salty coastal soils. In addition to, *Asparagus albus*, *Drimia maritima*, *Fagonia harpago*, *Limonium fallax*.

Zone B: These are plateaus and hills, on essentially granite substrate, giving skeletal and sandy soils. The altitude is between 100 and 400 m. The atmospheric humidity is still quite high, with a strong maritime influence. For the dominant species in this area, we find: *Asteriscus graveolens*, *Fagonia cretica*, *Launaea arborescens*, *Plantago afra*, and *Rumex vesicarius*.

Zone C: These are more continental reliefs, with substrates formed of sandstone and primary schites. The soils are clayey and with variable depths. The altitude is between 400 and 900 m. The climate is much more continental, of the Mediterranean type, with a more attenuated maritime influence. The characteristic species of zone C: *Argania spinosa*, *Asphodelus macrocarpus*, *Asphodelus tenuifolius*, *Helianthemum pergamaceum* subsp. *pergamaceum*, *Eryngium ilicifolium*, and *Kleinia anteuphorbium*.

Thus, the following floristic species were common to all three areas: *Opuntia ficus-indica*, *Cynodon dactylon*, *Euphorbia officinarum* subsp. *echinus*, *Euphorbia regis-jubae*, *Limonium sinuatum* subsp. *beaumierianum*, *Lotus arenarius*, and *Senecio glaucus* subsp.*coronopifolius*. And the common species between B and C are: *Cladanthus arabicus*, *Convolvulus althaeoides*, *Convolvulus trabutianus*, *Diplotaxis harra* subsp. *crassifolia*, *Lycium intricatum*, *Melilotus officinalis*, and *Paronychia argentea*. For the common species between A and B there are: *Aizoon hispanicum*, *Frankenia corymbosa*, *Glebionis coronaria*, *Volutaria lippii.*

According to the flowering calendar (*Table 3*), nectar and pollen supply occurs almost throughout the year for all three zones. It peaks in spring and summer, while it is less available during fall and winter.

Spring is characterized by the flowering of a large number of plants, the majority of which are annuals. Among the main plants that bloom in spring, the Euphorbiaceae *(Euphorbia regis-jubae)*, Aizoaceae *(Aizoon canariense*, *Aizoanthemopsis hispanica)*, Asteraceae *(Asteriscus graveolens*, *Calendula arvensis*, *Calendula suffruticosa*, *Cladanthus arabicus*, *Glebionis coronaria*, *Launaea arborescens)*, Cactaceae*(Opuntia ficus-indica)*, Caryophyllaceae *(Paronychia argentea)*, Boraginaceae *(Echium petiolatum)*, Cruciferae *(Diplotaxis harra* subsp. *Crassifolia)*, Labiatae *(Thymus maroccanus*, *Lavandula multifida)*, Leguminosae *(Genista tricuspidata*, *Lotus arenarius*, *Melilotus officinalis)*, Papaveraceae *(Papaver rhoeas)*, Sapotaceae *(Argania spinosa)* and Plumbaginaceae *(Limonium sinuatum)*.

During the summer, the supply of nectar and pollen is less diversified. However, it is a profitable period for the beekeepers, by the production of honey of Euphorbia. The latter is well known nationally for its qualities and benefits and sought after even beyond the borders of Morocco. The Euphorbia is very visited by the bees because of its important supply of nectar and pollen. Thus, during the summer, there are other plants in bloom like *Kleinia anteuphorbium*, *Salsola oppositifolia*, *Salsola vermiculata*, *Suaeda vera*, *Asparagus albus*, *Lycium intricatum*, *Ziziphus lotus*, *Convolvulus trabutianus* and *Eryngium tricuspidatum*.

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Table 3. *Flowering calendar of the Ait Baâmrane region*

Taxa / flowering period	January	February	March	April	May	June	July	August	September	October	November	December
Aizoaceae												
Aizoon canariense	■	■	■	■	■	■	■	■				■
Aizoanthemopsis hispanica	■	■	■	■	■	■	■	■	■	■	■	■
Amaranthaceae												
Arthrocnemum macrostachyum			■	■	■	■	■	■	■	■	■	
Chenopodium ambrosioides			■	■	■	■	■	■	■	■	■	
Salsola oppositifolia			■	■	■	■	■	■	■	■	■	
Salsola vermiculata			■	■	■	■	■	■	■	■	■	
Suaeda vera			■	■	■	■	■	■	■	■	■	
Anacardiaceae												
Searsia pentaphylla			■	■	■							
Searsia tripartita			■	■	■							
Asclepiadaceae												
Periploca angustifolia	■	■	■	■	■	■	■	■	■	■	■	■
Asparagaceae												
Asparagus albus						■	■	■	■	■	■	
Asphodelaceae												
Asphodelus macrocarpus			■	■	■	■	■	■				
Asphodelus tenuifolius			■	■	■	■	■	■				
Asteraceae												
Asteriscus graveolens	■	■	■	■	■							■
Calendula arvensis	■	■	■	■	■	■	■	■				■
Calendula suffruticosa	■	■	■	■	■	■	■	■				■
Cladanthus arabicus			■	■	■	■	■	■				
Glebionis coronaria	■	■	■	■	■							■
Glebionis segetum	■	■	■	■	■							■
Kleinia anteuphorbium			■	■	■	■	■	■	■	■	■	

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Taxa / flowering period	January	February	March	April	May	June	July	August	September	October	November	December
Launaea arborescens			■	■	■	■	■	■	■	■	■	
Senecio glaucus subsp. *coronopifolius*	■	■	■	■	■	■	■	■				■
Boraginaceae												
Echium petiolatum			■	■	■							
Cactaceae												
Opuntia ficus-indica			■	■	■	■	■	■				
Caryophyllaceae												
Paronychia argentea	■	■	■	■	■	■	■	■				■
Silene virescens	■	■	■	■	■	■	■	■				■
Silene vulgaris	■	■	■	■	■	■	■	■				■
Cistaceae												
Cistus salviifolius			■	■	■							
Helianthemum pergamaceum	■	■	■	■	■							■
Convolvulaceae												
Convolvulus althaeoides			■	■	■	■	■	■				
Convolvulus trabutianus	■	■	■	■	■	■	■	■	■	■	■	■
Cruciferae												
Diplotaxis harra subsp. *crassifolia*	■	■	■	■	■	■	■	■				■
Euphorbiaceae												
Euphorbia officinarum subsp. *echinus*						■	■	■	■	■		
Euphorbia regis-jubae			■	■	■							
Frankeniaceae												
Frankenia corymbosa			■	■	■							
Frankenia pulverulenta			■	■	■	■	■	■	■	■	■	
Labiatae												
Lavandula multifida			■	■	■				■	■	■	
Thymus maroccanus			■	■	■							
Leguminosae												
Acacia gummifera			■	■	■	■	■	■	■	■	■	

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Taxa / flowering period	January	February	March	April	May	June	July	August	September	October	November	December
Ceratonia siliqua	■	■							■	■	■	■
Genista tricuspidata			■	■	■							
Lotus arenarius	■	■	■	■	■	■	■	■				■
Melilotus officinalis			■	■	■							
Papaveraceae												
Papaver rhoeas			■	■	■	■	■	■				
Plantaginaceae												
Plantago afra			■	■	■	■	■	■				
Plumbaginaceae												
Limonium fallax			■	■	■							
Limonium sinuatum subsp. *beaumierianum*	■	■	■	■	■							■
Polygonaceae												
Rumex vesicarius			■	■	■							
Rhamnaceae												
Ziziphus lotus			■	■	■	■	■	■				
Sapotaceae												
Argania spinosa			■	■	■	■	■	■	■			
Solanaceae												
Lycium intricatum			■	■	■	■	■	■	■	■	■	
Umbelliferea												
Eryngium ilicifolium			■	■	■	■	■	■				
Eryngium tricuspidatum						■	■	■				
Foeniculum vulgare			■	■	■	■	■	■				
Zygophyllaceae												
Fagonia cretica			■	■	■							
Zygophyllum gaetulum						■	■	■	■	■	■	

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Pollen analysis

The pollen analysis of nine honey samples allowed to identify 35 taxa distributed in the nine honey samples analyzed with different frequencies ranging from 0.25% to 92% (*Table 4*).

Table 4. *Frequency of taxa in the 9 honeys samples analyzed*

Taxa code	Plant taxa	A1	A2	A3	B1	B2	B3	C1	C2	C3
Ai.sp	*Aizoon sp*	3,7		4,09		4,55			1,54	
As.ma	*Asphodelus macrocarpus*					3,64				
As.ca	*Calendula sp.*	9,88	0,38	7,73	2		4,29	8,33	26,6	
Cer.si	*Ceratonia siliqua*						5,71			
Ci.sa	*Cistus salviifolius*		0,5	6,82						
Ci.sp	*Citrus sp.*		3,75							
Co.al	*Convolvulus althaeoides*			1,36						1,02
Con.tr	*Convolvulus trabutianus*							3,57		
Cor.sp	*Coronilla viminalis*			8,18						
As.cr	*Crepis sp.*	1,23	0,63	10,5					2,7	
Dip.ha	*Diplotaxis harra*	11,1		10		63,6	18,6		8,49	
Ech.sp	*Echium petiolatum*	1,85			92		14,3		3,86	
Ery.sp	*Eryngium sp.*				4	6,36	5,71	10,7	0,77	
Euc.sp	*Eucalyptus sp*						2,86			
Eu.ec	*Euphorbia officinarum* subsp. *echinus*	39,5					25,7	71,4		
Eup.re	*Euphorbia regis-jubae*			5,91					19,7	3,44
Fo.vu	*Foeniculum vulgare*					7,27				
Gen.sp	*Genista sp.*			12,7				3,57		1,34
Gl.co	*Glebionis coronaria*		0,75	9,09		6,36	2,86		19,3	2,04
Hel.sp	*Helianthemum sp.*									0,38
Kle.an	*Kleinia anteuphorbium*			3,18						
Lam.la	*Lavandula sp.*				2					
Lim.sp	*Limonium sp.*		0,25	2,27						
Lin.sp	*Linum sp.*		1,25						0,77	
Lot.sp	*Lotus sp.*							2,38	0,77	
Lam.me	*Mentha sp*								11,2	
Me.of	*Melilotus officinalis*					7,27				
Pa.rh	*Papaver rhoeas*		1,5	14,1						90
Par.ar	*Paronychia argentea*	6,79							3,09	
Pla.sp	*Plantago sp.*						10			
Po.sp	*Polygonum sp.*		0,38	2,27					1,16	
Sa.ve	*Salsola vermiculata*	15,4					10			1,78
Si.sp	*Silene sp.*		90							
Th.ga	*Thapsia garganica*		0,63	1,82						
Thy.sp	*Thymus sp.*	10,5				0,91				

The 35 taxa identified belong to 33 genera and 22 families. Leguminosae is the most represented family with 5 taxa, followed by the Asteraceae with 4 taxa. In third place come the Labiatae and Umbelliferae with 3 taxa each, then the Euphorbiaceae, Convolvulaceae, Caryophyllaceae, and Cistaceae with 2 taxa. The rest of the families are represented by 1 taxon (*Fig. 5*). The pollens of herbaceous species are the most represented, followed by shrubs and lastly the trees.

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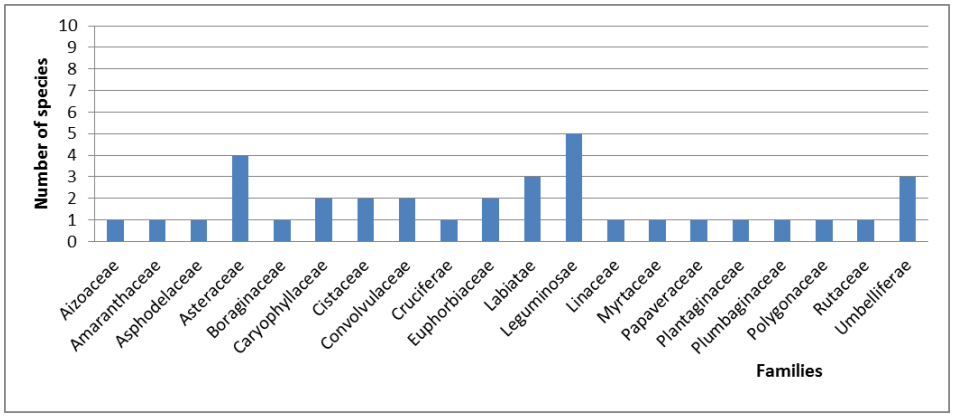

Figure 5. *Specific diversity of families according to taxa found in all analyzed honey samples*

The number of taxa per sample varied from 4–15. The most important are: *Calendula sp*. in 7 samples (77, 78%), *Glebionis coronaria* in 6(66, 67%), *Diplotaxis harra* and *Eryngium sp*. in 5 samples (55, 56% each). Then come *Crepis sp*., *Echium petiolatum*, and *Aizoon sp*. in 4 (44, 44% each). For *Euphorbia officinarum* subsp. *echinus*, *Euphorbia regis-jubae*, *Genista sp*., *Papaver rhoeas*, *Polygonum sp*. and *Salsola vermiculata* were found in 3 (33, 33% each). The rest of the taxa were found in two or one sample (*Fig. 6*).

The honey samples were collected in two honeydew periods, the first one was during the spring when the flowering took place for all flower, and the second one during the summer when the flowering was dominated by *Euphorbia officinarum* subsp. *echinus*.

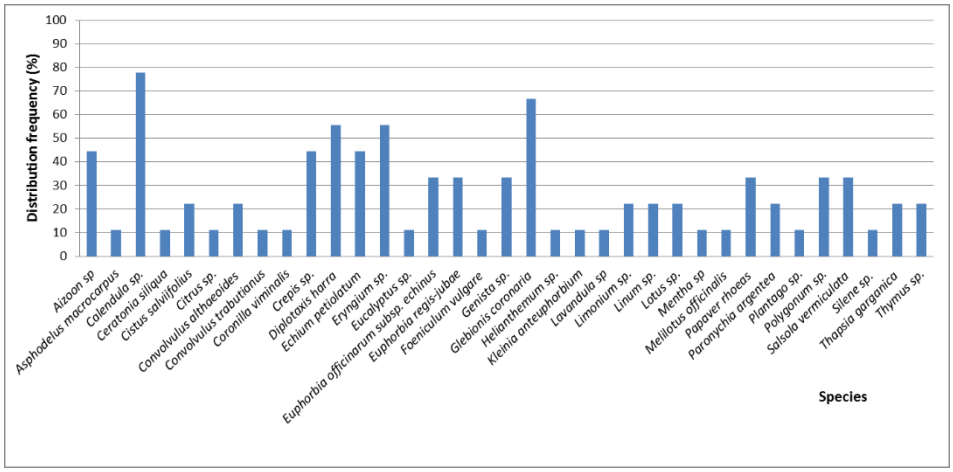

Figure 6. *Distribution frequency of species in the nine honey samples*

Pollen spectra

The analysis of the pollen spectrum shows that for the summer honeys, all samples (A1, C1, and B3) are monofloral honeys of *Euphorbia officinarum* subsp. *echinus* with percentages of 39.51%, 71.43% and 25.71%, respectively. According to Terrab et al. (2014) a minimum of 25% pollen from *Euphorbia sp*. (after eliminating the pollen grains from anemophilous and nectarless plants) was required to designate these honeys as unifloral from a melissopalynological point of view.

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For the spring honeys (B1, B2, A2, C2, C3 and A3), 3 samples (C2, C3, and A3) are multifloral without pollen dominance and are characterized by the presence of secondary pollens (*Euphorbia regis-jubae*, *Papaver rhoeas*, *Echium petiolatum*, *Diplotaxis harra*, *Glebionis coronaria*, and *Calendula sp*.) with pollen frequencies between 16 and 45%, important (tertiary 3-15%) and rare (rare to -3%) single pollens. The 3 remaining samples are dominated by *Echium petiolatum* (B1) with 92 %, *Diplotaxis harra* (B2) with 63.64% and *Silene sp*. (A2) with 90%. *Papaver rhoeas* is not nectariferous but represents a very good pollen source for bees (*Fig. 7*). *Figure 8* represents the pollen grain of the main taxa identified in the honeys analyzed.

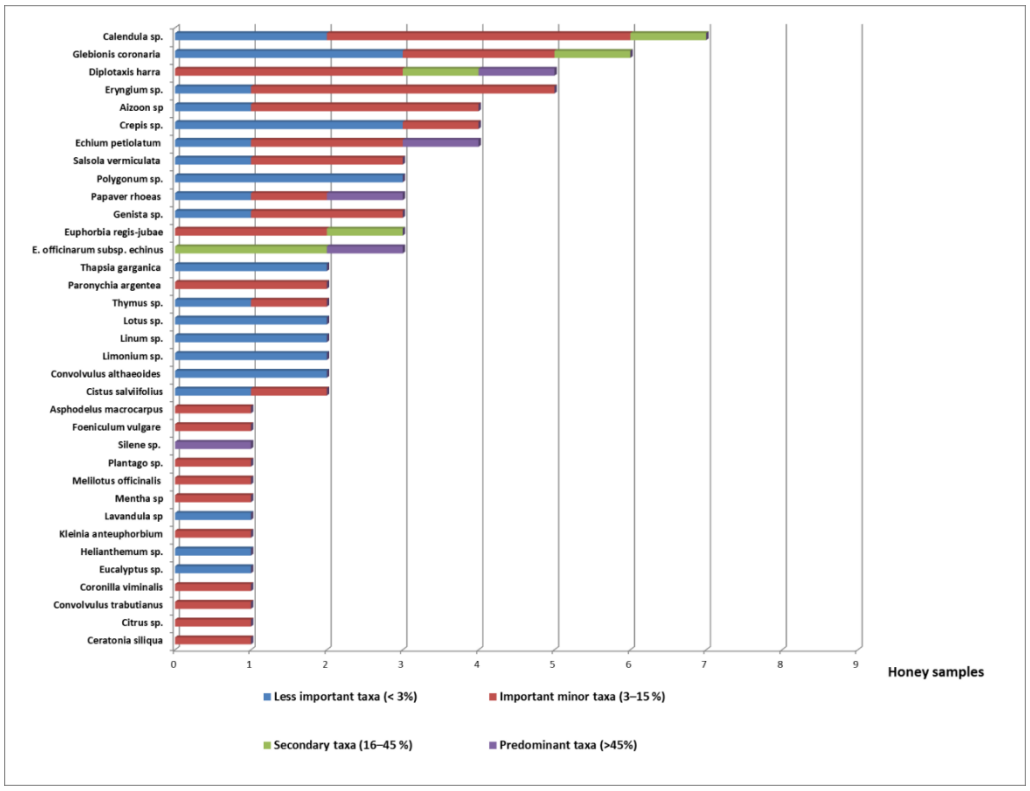

Figure 7. *presence of 35 pollen taxa identified in the honey samples represented in four classes of pollen frequency*

In order to visualize the most diverse honey samples analyzed in terms of pollen taxa, and also to determine the similarity between the samples, a heat map representation (*Fig. 9*) was made.

According to the characterization of the similarity of the samples in taxa found by heat map analysis, two groups of honey samples were observed:

Group 1: represented by the samples (A1, C1, and B3), which are unifloral honeys of the summer; they are characterized by the dominance of the pollen of *Euphorbia officinarum subsp. echinus* accompanied by other species essentially, *Eryngium sp*., *Convolvulus trabutianus*, *Salsola vermiculata*. The sample B3 is the most diverse with 10 taxa, followed by A1 with 9 taxa, and at the end C1 with 6 taxa.

Group 2: presented by the honeys (B1, B2, A2, C2, C3, and A3), which are spring honeys. A3, C2 and B2 honeys are the richest and most diversified in pollen taxa, with 15, 13, and 8 taxa successively, while B1, A2 and C3 honeys appear less rich.

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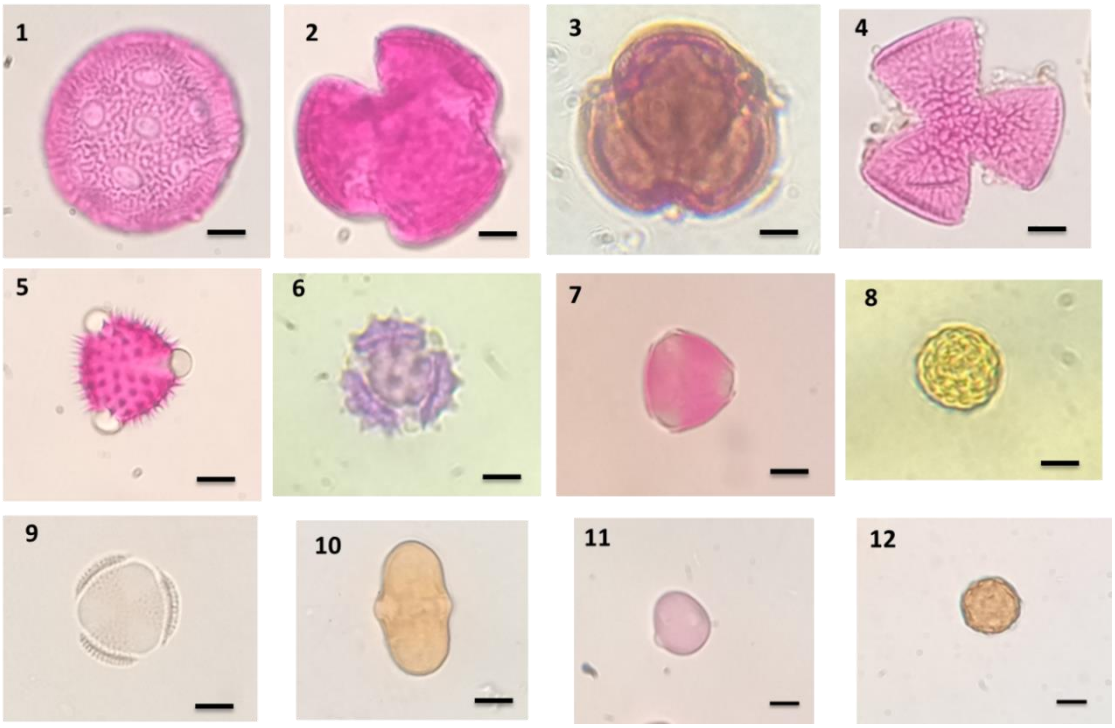

Figure 8. *Pollen grain of the main taxa identified in the honeys analyzed: (1) Silene vulgaris, (2) Euphorbia regis-jubae, (3) Euphorbia officinarum subsp. Echinus, (4) Limonium fallax, (5) Calendula arvensis, (6) Glebionis coronaria, (7) Papaver rhoeas, (8) Salsola vermiculata, (9) Diplotaxis harra subsp. crassifolia, (10) Eryngium ilycifolium, (11) Echium petiolatum, (12) Paronychia argentea ; scale bar = 10 µm. Pollen in equatorial view: 10 and 11; Pollen in polar view: 2, 3, 4, 5, 6, 7 and 9; Circular shape: 1, 8 and 12*

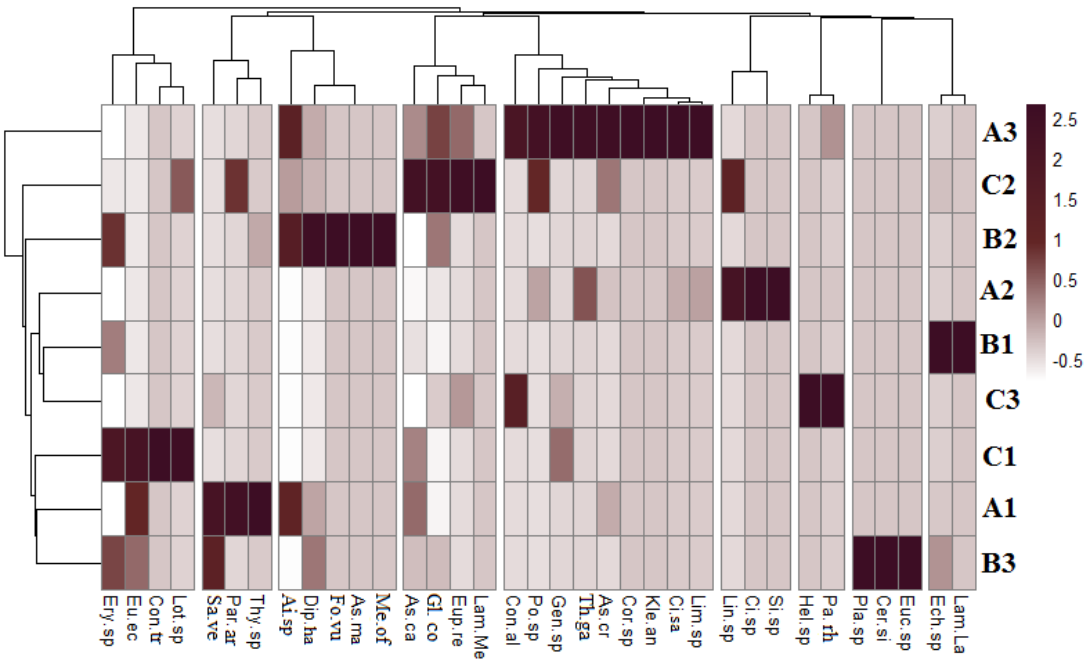

Figure 9. *Heat map of pollen grain richness in the 9 honey samples. The intensity value describes the percentage of taxa in each sample*

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Tables 5, *6*, and *7* represent the taxa found in the honeys analyzed and the characteristic species of zones A, B, and C as well as their beekeeping interest as nectariferous (N), polliniferous (P) or both (NP).

Table 5. *Taxa found in honeys and characteristic species of zone A and their beekeeping interest*

Species in honey	Beekeeping interest	Characteristic species	Beekeeping interest
Aizoon sp	NP	*Arthrocnemum macrostachyum*	P
Calendula sp.	NP	*Asparagus albus*	NP
Cistus salviifolius	P	*Chenopodium ambrosioides*	P
Citrus sp.	NP	*Cynodon dactylon*	P
Convolvulus althaeoides	NP	*Drimia maritima*	NP
Coronilla viminalis	NP	*Euphorbia officinarum* subsp. *Echinus*	NP
Crepis sp.	NP	*Euphorbia regis-jubae*	NP
Diplotaxis harra	NP	*Fagonia harpago*	NP
Echium petiolatum	NP	*Frankenia corymbosa*	NP
Euphorbia officinarum subsp. *echinus*	NP	*Glebionis coronaria*	NP
Euphorbia regis-jubae	NP	*Limonium fallax*	NP
Genista sp.	P	*Opuntia ficus-indica*	NP
Glebionis coronaria	NP	*Salsola oppositifolia*	NP
Kleinia anteuphorbium	NP	*Salsola vermiculata*	NP
Limonium sp.	NP	*Senecio glaucus* subsp.*coronopifolius*	NP
Linum sp.	NP	*Suaeda vera*	P
Papaver rhoeas	p		
Paronychia argentea	NP		
Polygonum sp.	NP		
Salsola vermiculata	NP		
Silene sp.	NP		
Thapsia garganica	NP		
Thymus sp.	NP		

P: polliniferous, N: nectariferous, NP: polliniferous and nectariferous

For the species found in the analyzed honey samples, 23 taxa were identified in zone A for three honey samples (A1, A2, and A3), 16 in zone B for samples B1, B2, and B3, while in zone C, 20 taxa were found in samples C1, C2, and C3.

The 35 taxa identified in the nine samples of honey analyzed are distributed differently in the three zones. Among these taxa, there are those that are found only in one area, and others that are common between the three areas.

For species found only in zone A, there are *Cistus salviifolius*, *Citrus sp*., *Coronilla sp*., *Limonium sp*., *Silene sp*., and *Thapsia garganica*. For those found in zone B only, *Asphodelus macrocarpus*, *Ceratonia siliqua*, *Eucalyptus sp*., *Lavandula sp*., *Melilotus officinalis*, and *Plantago sp*, regarding the zone C, there are *Convolvulus trabutianus*, *Helianthemum sp*., *Lotus sp*., and *Mentha sp*.

The following species are those common to all three areas: *Aizoon sp*, *Calendula sp*. *Diplotaxis harra*, *Echium petiolatum*, *Euphorbia officinarum* subsp. *echinus*, *Glebionis coronaria*, *Salsola vermiculata*.

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Table 6. *Taxa found in honeys and characteristic species of zone B and their beekeeping interest*

Species in honey	Beekeeping interest	Characteristic species	Beekeeping interest
Aizoon sp	NP	*Asteriscus graveolens*	NP
Asphodelus macrocarpus	NP	*Cladanthus arabicus*	NP
Calendula sp.	NP	*Convolvulus althaeoides*	NP
Ceratonia siliqua	N	*Convolvulus trabutianus*	NP
Diplotaxis harra	NP	*Cynodon dactylon*	P
Echium petiolatum	NP	*Diplotaxis harra* subsp. *crassifolia*	NP
Eryngium sp.	NP	*Euphorbia officinarum* subsp. *echinus*	NP
Eucalyptus sp.	NP	*Euphorbia regis-jubae*	NP
Euphorbia officinarum subsp. *echinus*	NP	*Fagonia cretica*	NP
Foeniculum vulgare	NP	*Frankenia corymbosa*	NP
Glebionis coronaria	NP	*Kleinia anteuphorbium*	NP
Lavandula sp	NP	*Launaea arborescens*	NP
Melilotus officinalis	NP	*Melilotus officinalis*	NP
Plantago sp.	P	*Opuntia ficus-indica*	NP
Salsola vermiculata	NP	*Paronychia argentea*	NP
Thymus sp.	NP	*Plantago afra*	P
		Rumex vesicarius	P
		Senecio glaucus subsp.*coronopifolius*	NP

P: polliniferous, N: nectariferous, NP: polliniferous and nectariferous

Table 7. *Taxa found in honeys and characteristic species of zone C and their beekeeping interest*

Species in honey	Beekeeping interest	Characteristic species	Beekeeping interest
Aizoon sp	NP	*Asphodelus macrocarpus*	NP
Calendula sp.	NP	*Asphodelus tenuifolius*	NP
Convolvulus althaeoides	NP	*Cladanthus arabicus*	NP
Convolvulus trabutianus	NP	*Convolvulus althaeoides*	NP
Crepis sp.	NP	*Convolvulus trabutianus*	NP
Diplotaxis harra	NP	*Cynodon dactylon*	P
Echium petiolatum	NP	*Diplotaxis harra* subsp. *crassifolia*	NP
Euphorbia officinarum subsp. *echinus*	NP	*Eryngium ilicifolium*	NP
Euphorbia regis-jubae	NP	*Euphorbia officinarum* subsp. *Echinus*	NP
Genista sp.	P	*Euphorbia regis-jubae*	NP
Glebionis coronaria	NP	*Fagonia cretica*	NP
Helianthemum sp.	NP	*Helianthemum pergamaceum* subsp. *pergamaceum*	NP
Linum sp.	NP	*Kleinia anteuphorbium*	NP
Lotus sp.	NP	*Limonium sinuatum* subsp. *beaumierianum*	NP
Mentha sp	NP	*Melilotus officinalis*	NP
Papaver rhoeas	P	*Opuntia ficus-indica*	NP
Paronychia argentea	NP	*Paronychia argentea*	NP
Polygonum sp.	NP	*Senecio glaucus* subsp.*coronopifolius*	NP
Salsola vermiculata	NP		
Eryngium sp.	NP		

P: polliniferous, N: nectariferous, NP: polliniferous and nectariferous

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In addition, several taxa are found in zone A and C at the same time, such as *Convolvulus althaeoides, Crepis sp., Euphorbia regis-jubae, Genista sp., Linum sp. Papaver rhoeas, Paronychia argentea, Polygonum sp.*

The choice of the location of the hives is made according to their needs in nectar and pollen, hence the importance of knowing the melliferous plants of a given area by the beekeeper. According to the floristic analysis and the pollen spectrum of the analyzed honeys, the main melliferous plants in the region of Ait Baâmrane on the three areas studied are presented in *Table 8*. This can help the beekeeper to choose the places of production and the location of his hives.

Table 8. *Main melliferous plants in the region of Ait Baâmrane*

Location	Season	Pollen types
Zone A	Summer	*Euphorbia officinarum* subsp. *echinus* *Salsola vermiculata* *Diplotaxis harra* *Thymus sp.* *Calendula sp.* *Paronychia argentea*
	Spring	*Silene sp.* *Papaver rhoeas* *Genista sp.* *Crepis sp.* *Diplotaxis harra* *Glebionis coronaria* *Coronilla viminalis* *Calendula sp.* *Cistus salviifolius* *Euphorbia regis-jubae* *Kleinia anteuphorbium*
Zone B	Summer	*Euphorbia officinarum* subsp. *echinus* *Diplotaxis harra* *Echium petiolatum* *Plantago sp.* *Salsola vermiculata* *Eryngium sp.*
	Spring	*Echium petiolatum* *Diplotaxis harra* *Foeniculum vulgare* *Melilotus officinalis* *Eryngium sp.* *Glebionis coronaria* *Aizoon sp*
Zone C	Summer	*Euphorbia officinarum* subsp. *echinus* *Eryngium sp.* *Calendula sp.* *Convolvulus trabutianus*
	Spring	*Papaver rhoeas* *Calendula sp.* *Euphorbia regis-jubae* *Glebionis coronaria* *Mentha sp* *Diplotaxis harra* *Echium petiolatum* *Paronychia argentea*

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Discussion

According to *Figure 3* (factorial analysis of correspondences), there is a floristic diversity between the three zones A, B and C, this is due to the environmental conditions that differ by the reliefs, substrates, as well as the distance to the sea. This is also visible in the pollen spectrum of the honey samples analyzed in the three areas (A, B and C), where there is a difference in the pollens of the taxa found in these three areas.

Melliferous plants vary from one area to another depending on the floristic composition and environmental conditions (biotic, climatic and ecological factors) (Smart et al., 2017; Milla et al., 2022).

Concerning the honey samples, they were collected from beekeepers with traditional sedentary hives that adapt to local vegetation. This makes it possible to limit the effects of mobility of the hives, and thus the effects of the anthropic factors which can influence the results. In addition, each sample represents the honeys of several hives (between 80 and 175 hives per site) and knowing that the hives of the same site forage for different taxa during the day, and that we have identified all the taxa present in the honey (polliniferous and nectariferous), a honey sample thus contains several pollen spectra that reflect the vegetal diversity of the studied sites. Thus, for each site, there are 2 spring samples and 1 summer sample. The spring samples were taken in neighboring apiaries, and we preferred to separate them to have an idea of the pollen spectra reflecting the very remarkable floral diversity. During the summer, the scarcity of flowering plants forces bees to diversify their supply of pollen and nectar, and to have almost identical pollen spectra in hives at the same site. For this reason, a single mixed sample of several honeys from different hives was chosen.

Regarding the diversity of the honey samples in pollen taxa in the three zones, zone A is the most diversified with 23 taxa, followed by zone C with 20 taxa and zone B with 16 taxa. This can be explained by the fact that the samples of honey are collected in traditional hives that are characterized in the Ait Baâmrane of Sidi Ifni by hives made of reed (the silts) generally sedentary adapt to a local floristic richness limited in space.

In addition, according to tables 3, 4 and 5, this difference can be justified by considering that zone A is characterized by the abundance of polliniferous species of the family Chenopodiaceae and Amarantaceae (*Chenopodium ambrosioides*, *Suaeda vera*, *Arthrocnemum macrostachyum*) and poor in plants with high value as nectariferous resources which obliges the bees to look for several species for their needs in nectar. Contrary to zone B and C where nectar resources are important and varied, which favors a selection of plants foraged by bees, given their abundance, long flowering period or plants with high melliferous value.

Comparing the results of the floristic and pollen analysis, there is a significant number of plants that are in bloom, while their pollen is not present in the honeys analyzed. This can be explained by the selection made by foraging bees according to the quantity, quality and composition of nectar and pollen.

According to Mc Tellaria (1993); Lobreau-Callen (1994) and Coulibaly et al. (2019), the floristic diversity in honey plants between different areas is due to the selection of species to be foraged by bees. This selection is influenced by the floral morphology, phenology, and floristic composition of the environment (Steffan-Dewenter and Kuhn, 2003; Requier et al., 2015; Nuernberger et al., 2019; Cengiz et al., 2021).

The region of Ait Baâmrane is generally known by production of two types of honey, multifloral honey in spring and monofloral honey of *Euphorbia officinarum* subsp. e*chinus* in summer, which is characteristic of the area. This was confirmed by the pollen

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analysis made on the samples of honeys collected in the area, whose summer samples are honeys with a dominance of Euphorbia, and spring honeys whose samples do not have dominance (multifloral honey). Moreover, this is due to the fact that in spring there is the flowering of a large number of plants and the majority of which are annual plants. On the other hand, in summer where a lack of flowering plants was observed and of which the principal flowering plant is the *Euphorbia officinarum* subsp. *echinus.*

In the summer honeys *E. officinarum* subsp. *echinus* is the most representative species as it is dominant in all the honeys analyzed, its relative pollen frequency reaches 71.42% for the sample (2), and it is an important source of nectar in this region during the summer. Follow-up of taxa with tertiary pollen frequencies (between 3 and 15 %), the most frequent being *Eryngium* sp., *Echium petiolatum*, *Diplotaxis harra*, *Salsola vermiculata*, *Calendula* sp., and *Convolvulus trabutianus*.

Spring honeys (multifloral honeys) generally honeys without pollen dominance except for the samples (B1, B2, and A2) which are dominated by *Echium petiolatum*, *Diplotaxis harra*, and *Silene sp*. They are characterized by the presence of secondary pollens mainly, *Euphorbia regis-jubae*, *Diplotaxis harra*, and Asteraceae (*Calendula sp*., *Glebionis coronaria*) with percentages ranging from 18.57% to 26.64%. Thus, tertiary pollens of which the most frequent are *Aizoon sp*., *Paronychia argentea*, and rare pollens essentially, *Convolvulus althaeoides*, *Limonium sp*, *Thapsia garganica*, *Crepis sp*., and *Polygonum sp*. The species, *Papaver rhoeas*, *Cistus salviifolius*, and *Plantago afra*, are not nectariferous, but are very good sources of pollen for bees.

The dominance of the samples (B1, B2, and A2) by *Echium petiolatum*, *Diplotaxis harra*, and *Silene sp* can be explained by the supply of nectar and pollen to the bees by these species during the foraging period, as traditional hives adapt to local and limited floristic resources in the surroundings of these hives.

Thus, bees are very attracted to Echium for its nectar produced in large quantities. For *Diplotaxis harra* and *Silene sp*, according to the flora of Morocco they bloom during winter, spring and summer, and thus provide pollen and nectar for a long time for the bees.

In terms of comparison with other studies in the same or neighboring regions. The pollen spectra recovered in our study are close to those obtained by Terrab et al. (2014) in the same region. They found that, for *Euphorbia officinarum* honey, the most characteristic accompanying species are *Eryngium ilycifolium*, present in > 90% of the samples, followed by *Bellis* sp., *Capsella* f. and *Reseda* sp. (85%). However, for the *E. regis-jubae* honeys, the most characteristic accompanying species are *Crepis* f., present in 100% of the samples, followed by *Anthemis* f., *Bellis* sp., *Capsella* f., *Coronilla viminalis*, *Lotus* f. and *Limonium* f. pollen type (95%), and by *Kleinia anteuphorbium* and *Paronychia argentea* (90%). Thus, they differ from those found by Boutoub et al. (2021) in a sample (M1) collected in the region (Guelmim-Oued Noun region and closer to Ait Baâmrane), and whose most predominant pollens are: *E. officinarum* (55.67% ± 1.78), *Quercus rotundifolia* (10.31% ±0.35), *Genista hirsuta* (6.88% ± 0.79), *Thymus lotocephalus* (4.95%± 0.2), *Cistus albidus* (4.08% ± 0.47), *Ilex aquifolium* (2.97% ± 0.10), *Malus sylvestris*(2.9% ± 0.15), *Malus domestica* (2.83% ± 0.75), *Eucalyptus cinerea* (2.32% ± 0.34), *Cistus crepis* (2.1% ± 0.35), *Campanula primulifolia* (1.64% ± 0.11), *Lavandula viridis* (1.46% ±0.13).

In addition, the results obtained in this study are far from those obtained by Ihitassen et al. (2019) in the region of Azilal (Morocco), whose the most common pollens are those of *Quercus ilex* (36 samples), *Papaver* sp. (30 samples), *Euphorbia resinifera* (25

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samples) with a percentage ranging from less than 1 to 37%. *Olea europaea* is present in 23 samples, *Chamaerops humilis* in 17 and *Echium sp*, *Hirschfeldia incana* and *Reseda sp.* in 16 samples.

Conclusions

This study describes the pollen characteristics of 9 honey samples from 3 areas. (Coast zone, plateaus and hills, continental mountainous area) located in central-west Morocco (Ait Baâmrane region). The pollen analysis of the nine samples revealed that three samples of summer honeys are monofloral (*Euphorbia officinarum* subsp. *echinus*) they confirm the importance of this taxon as the main melliferous source in this region, while for the six spring samples three are multifloral and three are dominated by *Diplotaxis harra, Echium petiolatum* and *Silene sp*. This analysis identified 35 plant taxa belonging to 33 genera and 22 families foraged by bees. The melissopalynological analysis showed that the most dominating pollens in honey samples were Leguminosae, Asteraceae, Labiatae, Umbelliferae and Euphorbiaceae.

The predominant species in these honeys are: E*uphorbia officinarum* subsp. *echinus*, *Euphorbia regis-jubae*, *Papaver rhoeas*, *Echium petiolatum*, *Eryngium sp*., *Diplotaxis harr*a, *Glebionis coronaria*, and *Calendula sp*. The species richness varies from 4 to 15 taxa per honey sample. Zone A honeys are the richest in plant taxa with 23 taxa followed by zone C with 20 and zone B with 16 taxa.

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