الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research
جامعة زيان عاشور- الجلفة
University Ziane Achour – Djelfa
كلية علوم الطبيعة والحياة
Faculty of Natural and Life Sciences
قسم العلوم البيولوجية
Department of Biological Sciences

Graduation Project

With a view to obtaining the Master's degree

Sector: Biological sciences

Specialty: Applied Microbiology

Theme

Microbiological quality, physicochemical properties, and pollen profile of Jujube honey

Presented by: REBHI Imane Hadjer

Before the jury composed of:

President:	**M. NAAS O.**	**Univ. Djelfa**
Supervisor:	**M. MEKIOUS S.**	**Univ. Djelfa**
Co-supervisor:	**Mr. LAHRECH T.**	**Univ. Djelfa**
Examinator:	**Mr. KACIMI M.**	**Univ. Djelfa**

Academic year: 2023/2024

{And your Lord inspired the bees: “Make ˹your˺ homes in the mountains, the trees, and in what people construct, and feed from ˹the flower of˺ any fruit ˹you please˺ and follow the ways your Lord has made easy for you.” From their bellies comes forth liquid of varying colours, in which there is healing for people. Surely in this is a sign for those who reflect.}- The **Qur'an 16:68-69** (Translated by Dr. Mustafa Khattab, The Clear Quran)

Acknowledgements

I would like to express my sincere gratitude to

Allah, I am thankful for the guidance You have sent through Your prophets and the wisdom contained in the Quran. Your words have illuminated my path, strengthened my faith, and instilled in me a profound sense of purpose.

My Supervisor, **Mekious Scherazad**, for her guidance, expertise, and mentorship throughout this research project. Her insightful feedback, continuous encouragement, and unwavering support have been instrumental in shaping the success of this work.

My co-Supervisor, **Lahrech Talal**, for his invaluable input, constructive criticism, and collaborative efforts. His unique perspectives and technical expertise have been instrumental in enhancing the quality and depth of this research.

all **the professors of the faculty**, for their dedication to their work, and their immense contribution to my knowledge and experience throughout the years. Their great example serves as an encouragement in my academic journey.

Professor **Kacimi Mohamed**, for organizing and dedicating his time for the training sessions on the realization of scientific research. His valuable knowledge and expertise were great contributions to the realization of this project.

Professor **Belli Zoubida**, for the profound impact she has had on my academic journey. Her inspiring lectures, thoughtful mentorship, and unwavering commitment to student success have left a great mark in my life.

The ACQCP Laboratory Directrice, technicians and staff, whose willingness to share their facilities, knowledge and expertise has been instrumental in the successful completion of the experimental work, especially **Fatma**, her mentorship and guidance during the experiments contributed to the experiences I've gained during this project.

My dear friends, **Nesrine, Amira, and Chahinaze**, for their continuous support and assistance throughout the period of this project.

The administrative body, laboratory staff, and all the employees of the faculty, who dedicate their time and effort to provide a suitable environment for education and scientific research.

Dedication

In the name of **Allah**, the Most Gracious, the Most Merciful

I humbly offer my deepest gratitude and heartfelt thanks to **Allah**, the Most Compassionate and Merciful, for guiding me, granting me strength, and blessing me with opportunities to grow and succeed.

To my **beloved parents**, your unwavering love, endless support, and invaluable guidance have been the foundation of my journey. Your sacrifices, wisdom, and encouragement have shaped me into the person I am today. I am forever grateful for your boundless love and belief in me.

To my **dear siblings**, your companionship, understanding, and shared experiences have enriched my life in countless ways. Your support, laughter, and camaraderie have been a source of strength and joy throughout my endeavors. I cherish the bond we share and the memories we have created together.

To my **family**, your love, encouragement, and presence have been a source of comfort and inspiration. Your support has fueled my determination and resilience. I am grateful for the unity, warmth, and sense of belonging that you have provided me with.

To my **dear friends**, your friendship, loyalty, and encouragement have been a source of light and positivity in my life. The memories we shared throughout the years are a treasure that I will cherish for the rest of my life, your support has lifted me up during both challenges and triumphs. I am thankful for the bond we share and the strength we draw from each other.

In this journey of life, I am blessed to have been surrounded by such incredible individuals who have shaped my path, supported my dreams, and enriched my experiences. Eternally grateful to **Allah** for blessing me with these bonds.

Table of Contents

Appendix

ملخص

Abstract

Résumé

List of Abbreviations

DLA	**:**	Desoxycholate Lactose Agar.
EC	**:**	Electrical Conductivity.
ECD	**:**	European Council Directive.
FA	**:**	Free Acidity.
LM	**:**	Liver Meat.
MC	**:**	Moisture Content.
OGA	**:**	Oxytetracycline Glucose Agar.
PCA	**:**	Plate Count Agar.
SAS	**:**	Sulfite-reducing Anaerobic Spores.
TAMF	**:**	Total Aerobic Mesophilic Flora.
TC	**:**	Total Coliforms.
TTC	**:**	Thermotolerant Coliforms.
YM	**:**	Yeast and Mold.

List of Figures

List of Tables

Introduction

Honey, as defined by the Codex Alimentarius, “is a natural sweet substance produced by honeybees from the nectar of flowers or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature.” Its sweet taste, energy content, and various health benefits have made it a popular food and natural remedy for thousands of years, and ongoing scientific research continues to uncover its potential health and therapeutic properties **(Codex Alimentarius, 2001)**.

Among Muslims, honey is considered a cure for mankind according to the Holy Quran **(Qur’an 16:69)** and the hadith (sayings) of the prophet Muhammad peace be upon him **(Sahih al-Bukhari 5680)**. This association of honey with spiritual nourishment, health benefits, and its mention as a food of Paradise highlights its significance in Islamic culture and tradition. The sidr, also known as the lote tree, is mentioned in several verses in the Quran describing it as a tree of paradise, near the "Garden of Refuge" and the "Utmost Boundary", several authentic hadiths also mention the use of sidr for purification and cleansing.

Algeria's honey quality is intricately linked to its diverse climate and geography which significantly affects honey's properties as highlighted by **Homrani et al. (2020)**. The country's varied terrain, from the Sahara Desert in the south to the Atlas Mountains in the north, and the Mediterranean coastline in between, provides a rich tapestry of botanical sources for honey production. This diversity contributes to the distinct taste, color, and aroma of Algerian honey **(Ingram et al., 2020)**.

Among the various honey types produced in Algeria, Jujube honey, also known as sidr honey, is one of the most popular natural products in the country due to its unique properties and nutritional value, it is produced from the nectar of *Ziziphus lotus*, and it possesses various health benefits, including antimicrobial, antioxidant, and anti-inflammatory properties, and it is used in traditional medicine to treat wounds, burns, digestive issues, respiratory infections and various illnesses **(Yupanqui Mieles et al., 2022)**.

Honey production in Algeria is a growing sector with a rich history, but it faces several challenges related to quality, disease, genetic quality of bees, competition with imported honey, and the absence of a legal framework recognizing quality. The variability of its quality can be attributed to the methods used, with some producers adhering to traditional methods and others using modern techniques **(Pelletan, 2017)**.

Honey quality regulations are established by the European Honey Directive **(Council Directive, 2002)** and the Codex Alimentarius Standard for Honey **(Codex Alimentarius, 2001)**, The International Honey Commission (IHC) was formed in 1990 to revise the methods and standards for honey quality and has compiled the methods of analysis currently used in routine honey control and carried out ring trials to establish precision parameters **(Bogdanov, 2001)**, many countries have also established their own national regulations for honey, including limits on the characteristics of specific monofloral honey types. However, this has led to differences in legislation and standards between countries, causing issues with fair competition, consumer confusion, and trade barriers **(Thrasyvoulou et al., 2018)**.

Various physicochemical, microbiological, and microscopic analyses were used in this study to investigate honey's botanical origin and quality. Moisture content, electrical conductivity, pH and free acidity are the physicochemical parameters selected for the analyses, the microbiological quality analyses examined the total aerobic mesophilic flora, total and thermotolerant coliforms, sulfite-reducing anaerobic spores, yeast and mold, while the pollen composition was determined through qualitative pollen analysis.

This thesis aims to provide a comprehensive analysis of the physicochemical properties, microbiological quality, and pollen profile of Jujube honey sampled from different regions of Algeria, to assess its quality, authenticity, and safety for consumption while highlighting the importance of quality control regulations, and help the valorization and promotion of local Jujube honey production and consumption.

1. Literature review

Several studies, in Algeria and internationally, have been conducted to assess the quality and characteristics of Jujube honey. Some of these studies are mentioned below for a comprehensive understanding of the characteristics of Jujube honey:

→ Pollen profile:

A study done by **Kaid (2021)** analyzed the pollen composition of three Jujube honey samples from Algeria, the results showed that *Ziziphus sp.* was an accompanying/secondary pollen with percentages ranging from 38.4% to 40.5%, other accompanying pollens included *Cytisus striatus* type and *Eucalyptus sp.* . On the other hand, **Mekious et al. (2022)** found that *Ziziphus lotus* was present and predominant in all of the samples analyzed with a maximum recorded percentage reaching 97.1%, secondary pollens present were *Thapsia garganica, Euphorbia bupleuroides, Retama raetam,* and *Peganum harmala* . **Zerrouk et al. (2017)** also found that *Ziziphus lotus* was present and predominant in all the samples, and secondary pollens observed were *Peganum harmala,* and *Olea europaea*.

→ Physicochemical analyses:

Results observed in different studies showed that Jujube honey is characterized by a low moisture content with mean values ranging from 13,93%-15.1%, and pH mean values from 5.17-5.5 **(Zerrouk et al., 2017) (Mekious et al., 2015)**, different values were observed by **Adjlane et al. (2014)**, with an average pH of 3.87, and a moisture content mean value of 17.43%. **Zerrouk et al. (2017)** also analyzed electrical conductivity and free acidity values of Jujube honey and found mean values of 0,47 mS/cm and 5,18 meq/kg ,respectively, while **Mekious et al. (2015)** observed higher values of 0.654 mS/cm and 12.5 meq/kg, respectively. All the results mentioned above conform to the **(Codex Alimentarius, 2001)**'s and **(Council Directive, 2002)**'s standards.

→ Microbiological quality analyses:

A study done by **Adjlane et al. (2014)** analyzed 3 samples of Jujube honey collected from Djelfa in 2010, the **total aerobic mesophilic flora** count results showed that one sample had a concentration greater than 1,000 $CFU.g^{-1}$ and was considered of poor quality, while two Jujube samples had a concentration below 10 $CFU.g^{-1}$, **Coliforms and Sulfite-reducing Clostridia** were absent in all samples, the count of **osmophilic yeast** showed

that the Jujube honey samples had a concentration of 9.10^2 yeast.g^{-1} which is a high concentration that can alter the quality of the honey**. El Menyiy et al. (2020)** analyzed one Jujube honey sample and observed the absence of all germs analyzed (TAMF, TC, TTC, SAS, and YM).

2. Materials and methods

2.1. Samples and sampling regions

Six samples of local Jujube honey were collected from different regions in Algeria as shown in **Table 1**. The honey samples were kept in the dark at room temperature until the analysis. The number of samples was limited due to the time constraints, and the low production yield from last year because of the harsh climatic conditions, land degradation and desertification which affected the flora.

Table 1. Samples' regions and characteristics.

N° sample	Region	Condition	Harvest year
1	Laghouat	Liquid	2023
2	Biskra	Liquid	2023
3	Djelfa	Liquid	2023
4	Naama	Liquid	2023
5	Bordj Badji Mokhtar	Liquid	2023
6	Messaad	Liquid	2022

The sampling regions are: Laghouat, Biskra, Djelfa, Naama, Bordj Badji Mokhtar and Messaad. Each region is highlighted in the map below **(Figure 1)**.

A literature review was conducted on the flora of the regions to identify potential geographical markers.

The region of Biskra is situated in Northeastern Algeria, serving as a transition zone between the Northern mountainous area and the Southern Saharan highlands. It covers an area of 21,671.2 km² with an altitude of 124 m and is predominantly utilized for agriculture, particularly date palm cultivation within oasis ecosystems. Climatically, Biskra experiences a hot and extremely dry summer, contrasted by cold winters with subfreezing temperatures. A study identified 45 flora species belonging to 41 genera and 26 families in Biskra, with *Chenopodiaceae, Fabaceae, Brassicaceae, Asteraceae, Lamiaceae,* and *Zygophyllaceae* being the most represented in the region **(Nacima, 2016)**.

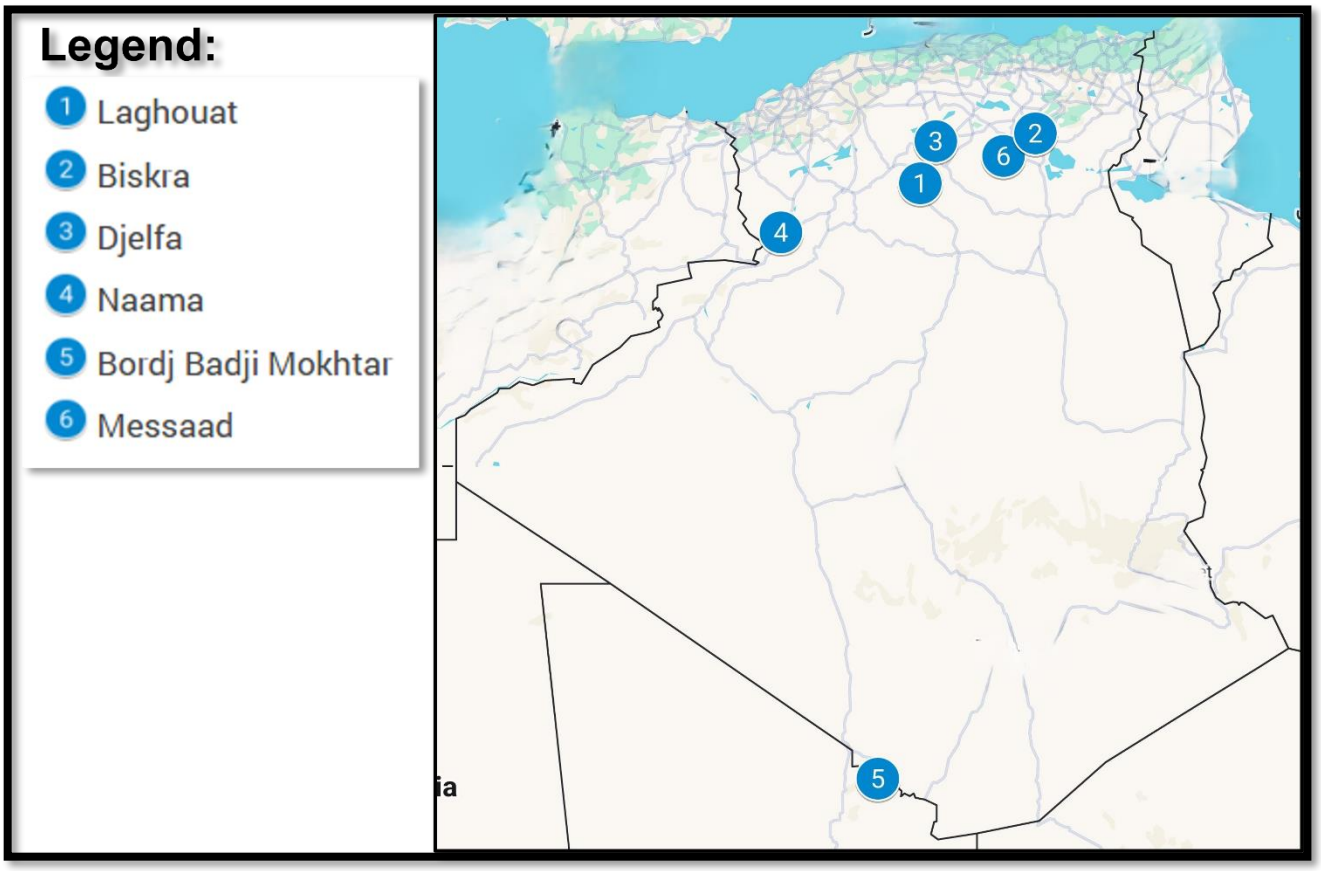

Figure 1. Map of sampling regions (Google maps, 2024).

Djelfa, a semi-arid region located in north-central Algeria, is characterized by a unique bioclimatic and geographic nature. The region falls within the steppe ecosystem, with a significant portion of its territory comprising of steppe vegetation. The area is known for its diverse flora, with a study identifying 127 plant taxa belonging to 33 families, dominated by *Asteraceae* and *Poaceae* **(Habib et al., 2020)**.

Naama is situated in the southwest of Algeria, between the Saharan Atlas and the Tal Atlas, it has a cold semi-arid climate, bordering on a hot semi-arid climate. 135 taxa, divided into 36 families and 105 genera were identified in the regions with *Asteraceae*, *Poaceae, Fabaceae* and *Brassicaceae* being the most represented **(Habib et al., 2020)**.

Bordj Badji Mokhtar is a province located in the Algerian Sahara, it has a Saharan climate, characterized by very high temperatures and low rainfall. The common vegetation includes desert-adapted plants such as *acacia* trees, tamarisks (specifically *Tamarix* species), date palms, and various types of desert shrubs.

Messaad is located in the Saharan Atlas Mountains of Algeria, which is characterized by a semi-arid to arid climate. The region experiences high temperatures and low precipitation, with an average annual rainfall of around 200 mm **(Mallem, 2018)**. The vegetation in Messaad is adapted to the harsh bioclimatic conditions. The Djebel Messaad area is known for its rich flora, with about 97 species identified across 27 botanical families. The *Asteraceae* family, to which *Echinops* and *Centaurea* belong, is notably represented in the region. Other species such as those from the *Brassicaceae* family and *Thapicia garganica* also exist in this diverse ecological area **(Laid et al., 2014) (Abdelghani et al., 2016)**.

2.2. Pollen profile analysis

Pollen analysis is a vital tool for the honey industry, providing insights into the botanical and geographical source of honey to ensure its authenticity.

The qualitative pollen analysis was conducted in the university of Djelfa, faculty of Natural and life sciences' laboratory. It was realized according to the methods established by the International Commission of Apicultural Botany, described by **Louveaux et al. (1978)**, 10g of honey was dissolved in 20ml of distilled water on a hot plate (temperature below 40°C)**,** the solution was then centrifuged for 10 minutes at 3000 rpm, the supernatant is removed and 10mL of distilled water is added, the solution was centrifuged again for 10 minutes (3000 rpm)**.** The process was repeated several times to remove the sugar from the sample **(Figure 2)**.

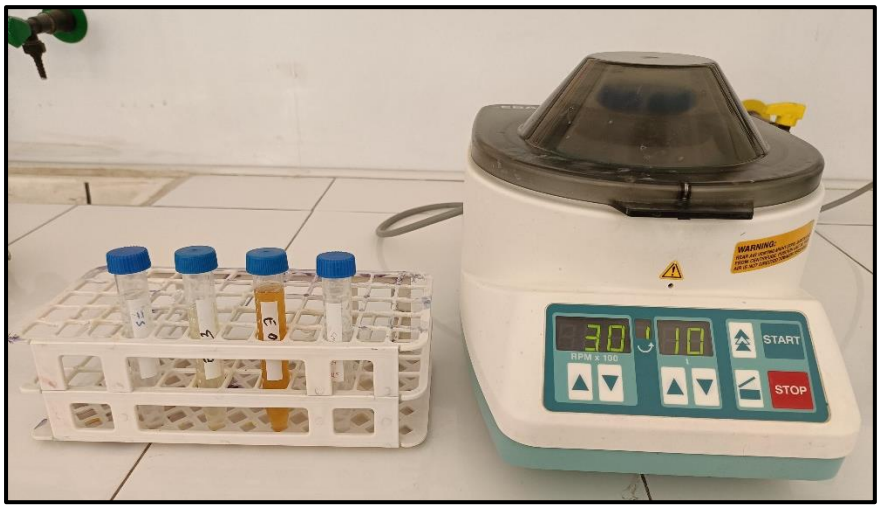

Figure 2. Centrifugation of the samples (original image 2024).

The entire sediment is then placed in a slide using a pipette, and the slide is heated until complete dryness, a drop of glycerol gelatin is added, and the cover glass is placed on the slide **(Figure 3)**. The prepared slides are then examined using a microscope to identify the different types of pollen grains present, this involves counting and identifying the pollen grains in 10 field views. This makes it possible to differentiate the varieties of pollen grains present by determining their frequency expressed as a relative percentage compared to the total number of pollen grains counted in a sample and the distribution frequency **(Figure 4)**.

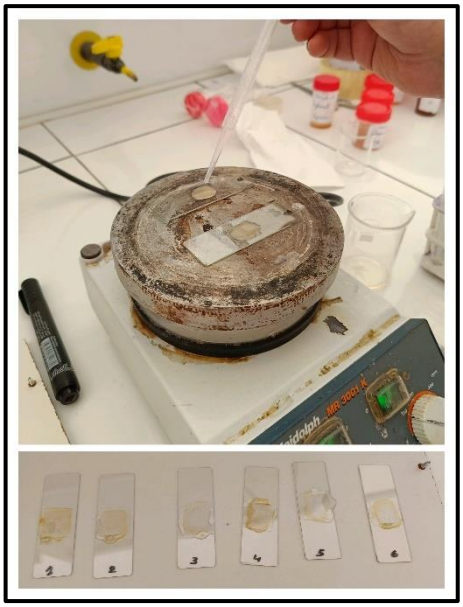

Figure 3. Slide preparation (original image 2024).

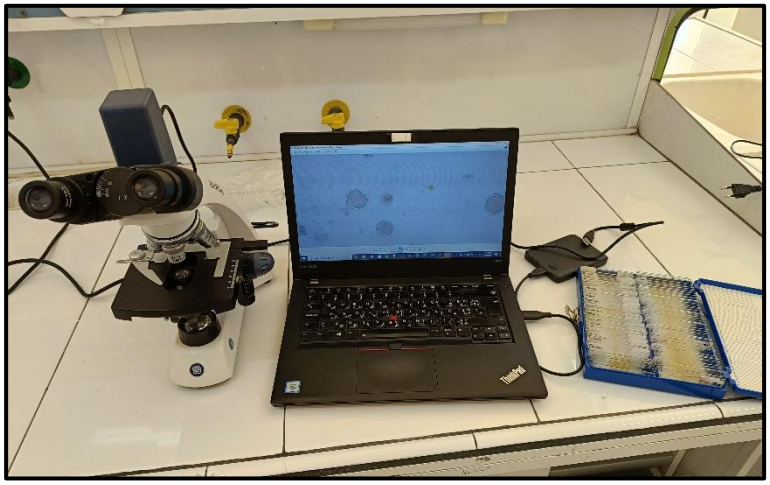

Figure 4. Microscopic analysis (original image 2024).

In our case, the determination of pollen frequency classes was done by counting approximately 500 pollens in each sample. The relative frequency classes were determined according to the international melissopalynological nomenclature.

The pollen frequency classes are as follows:

- **Predominant Pollen:** Pollen type representing more than 45% of the total pollen grains counted is considered the predominant pollen in honey.
- **Secondary Pollen:** Pollen type representing 16-45% of the total pollen grains counted is classified as secondary pollen.
- **Important Minor Pollen:** Pollen type representing 3-15% of the total pollen grains counted is categorized as important minor pollen.
- **Minor Pollen:** Pollen type representing less than 3% of the total pollen grains counted is classified as minor pollen.

The distribution frequency of the pollen types (taxa) in the samples was calculated based on **Feller-Demalsy et al. (1987)**:

- **Very frequent taxa:** present in more than 50% of samples
- **Frequent taxa:** present in 20 to 50% of samples
- **Infrequent taxa:** present in 10 to 20% of samples
- **Rare taxa:** present in less than 10% of samples

The identification of pollen types is carried out by comparing the morphology and dimensions of the pollen grains observed by a light microscope in our samples with those of the reference pollen microphotographs established by **Ricciardelli (1998)**.

2.3. Physicochemical analysis

Physicochemical properties influence the taste, texture, and shelf-life of honey, making it essential to ensure they comply with quality standards to maintain the integrity of the product and ensure consumer safety **(Robin Lim et al., 2022)**. Understanding these properties also aids in detecting adulteration and determining the botanical and geographical origins of honey, which can impact honey's flavor profile and medicinal properties **(Raweh et al., 2023)**.

The physicochemical properties assessed in this study were the moisture content, electrical conductivity, pH and free acidity. These properties provide insights into the purity, freshness, and nutritional value of honey **(Getachew et al., 2014)** and they were selected based on the available resources. The physicochemical analyses were conducted in the Algerian Center for Quality Control and Packaging in Djelfa. They were carried out according to the International Honey Commission **(IHC)** recommendations **(Bogdanov, 2001)**.

2.3.1 Determination of moisture content (refractometric method)

The refractive index was determined using the Abbe refractometer (ATAGO) **(Figure 5)**, after homogenization of the honey sample, a few drops are applied to the prism and the reading is taken after a few minutes. The RI is converted to moisture content percentage using the Chataway table **(Appendix A)**.

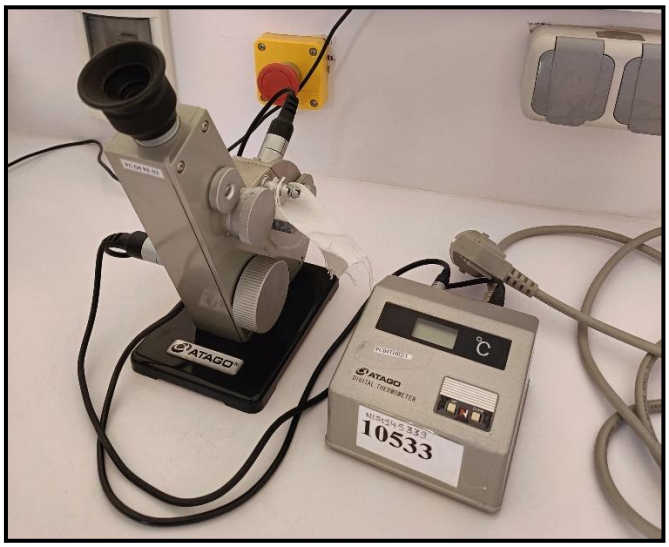

Figure 5. ATAGO Abbe refractometer (original image 2024).

The MC of honey is a critical parameter that influences its stability, resistance to fermentation, and overall quality. Maintaining MC within recommended standards is vital to

prevent fermentation and ensure honey quality. Different types of honey have specific MC ranges that need to be adhered to for optimal quality.

2.3.2 Determination of pH

The pH of honey is essential as it influences its taste, inhibits microbial growth, and affects its stability and shelf life. The acidity of honey plays a crucial role in inhibiting the growth of bacteria and fungi, making it a natural preservative. Additionally, the low pH of honey is essential for its unique flavor, texture, and overall quality. Research has shown that the pH of honey can vary depending on factors such as botanical origin, geographical location, and processing methods.

To determine the pH, the samples are homogenized, and 10g of each sample is dissolved in 75ml of distilled water, a sensION pH meter (calibrated) is used to determine the pH **(Figure 6)**.

Figure 6. sensION pH meter (original image 2024).

2.3.3 Determination of free acidity by titration to pH 8.3

Free acidity in honey is a key parameter that indicates the condition and quality of honey. It is linked to the natural presence of organic acids in honey, which can affect its taste, stability, and safety. High levels of FA may suggest that honey has started to ferment, leading to the production of organic acids.

The determination of FA is done as follows: The initial pH is recorded (the same process as the pH determination), and the solution is titrated with 0.1M NaOH to a pH of 8.3, the volume of the titrant used during the process is recorded **(Figure 7)**.

Figure 7. Titration of the sample using 0.1M NaOH (original image 2024).

The FA is calculated using the following equation:

$$\textbf{Free Acidity}\ (\text{meq/kg}) = \mathbf{V_{NaOH}} \times 10$$

-V_{NaOH} : number of mL of 0.1M NaOH used during the titration of 10g of honey.

-10 is the conversion factor to express acidity levels as a concentration relative to the weight of the sample.

2.3.4 Determination of electrical conductivity

The electrical conductivity of honey is a critical parameter in honey quality control and authentication, providing valuable information about the botanical origin, nutritional value, and overall quality of the honey.

The EC of honey is measured with a 20% (w/v) honey solution at 20°C ± 0.5°C, 10g of the sample was dissolved in 50mL of distilled water, the EC of the solution was determined

using a sensION conductivity meter **(Figure 8)**. The results are expressed in milliSiemens per centimetre (mS/cm).

Figure 8. sensION conductivity meter (original image 2024).

2.4. Microbiological analyses

To evaluate the microbiological quality of our honey samples several parameters were selected based on the information available about the different sources of contamination in honey, the germs concerned by this study are:

Total aerobic mesophilic germs, which is an indicator of the general microbiological quality of our samples. Total and Thermotolerant Coliforms, which are indicators of the sanitary quality and conditions during the processing of the product, and could suggest the presence of pathogenic bacteria **(Snowdon and Cliver, 1996)**. Sulfite-reducing spores, which are indicators of contamination and could possibly lead to health hazards **(Velásquez Giraldo et al., 2013)**. Yeast and mold, which are indicators of potential fermentation, deterioration, and toxicity of products **(Ananias et al., 2014)**.

The microbiological analyses were conducted in the university of Djelfa, faculty of natural and life sciences' laboratory **(Figure 9)**. Sample preparation, and the different enumeration and detection tests were carried out according to their respective ISO recommendations. The manipulations were carried out under aseptic conditions (within the sterile zone of a Bunsen burner).

2.4.1 Sample preparation

The preparation was carried out according to **(ISO, 2017)** standard. By transferring 10g of honey into a sterile bottle containing 90 ml of Peptone water (Tryptone Water) the mother suspension was obtained (10^{-1}). The mother suspension was homogenized, and 1 ml was transferred into the 1st tube to obtain the 10^{-2} dilution. The 1st tube (10^{-1} dilution) was homogenized using a vortex, and 1 ml was transferred into the second tube to obtain the 10^{-3} dilution.

2.4.2 Enumeration of Total Aerobic Mesophilic Flora

The enumeration was carried out according to **(ISO, 2013)** standard:

From each decimal dilution, 1 ml was transferred into a Petri plate. Approximately 15 ml of PCA, supercooled to 45±1°C, was added to each plate. Circular movements back and forth in the shape of eight were performed with each plate to allow the inoculum to mix with the agar.

The plates were left until solidification, and then incubated in an inverted position at 30°C. The reading was performed after 72 hours, TAMF colonies appear in a mass lenticular form, plates containing between 30 and 300 colonies were enumerated.

2.4.3 Enumeration of Sulfite-reducing Anaerobic Spores

The enumeration was carried out according to **(*ISO*, 2023)** standard:

A bottle of LM agar was melted and then supercooled to 45°C, 2.5ml of iron alum and 5ml of sodium sulphite was added to the media (for 250ml of media) and mixed carefully and aseptically. The medium was maintained in an oven or in a water bath at 45°C until the time of use. 1 ml of each dilution was transferred into a sterile screw tube. The tubes were subjected to heating at 80°C for 8-10 minutes, then immediately cooled under tap water, to eliminate the vegetative forms and keep only the sporulated forms. The contents of each tube are poured in petri plates, and approximately 15 ml LM agar is added to each plate. Circular movements back and forth in the shape of eight were performed to allow the inoculum to mix with the agar, the plates are then left to solidify.

The plates are put inside a sterile glass jar, and a candle is lit up inside it to produce an anaerobic environment (due to the lack of an anaerobic incubator). The jars were Incubated at 37°C for 16 hours (obligatory first reading), then 24 or 48 hours at most. The first reading must be done after 16 hours because the colonies of Sulphite-reducing Clostridium are invasive and could result in a completely black plate making interpretation difficult or even impossible. If there is no characteristic colony, re-incubate the plates and take a second reading after 24 hours or 48 hours at most. The spores appear as colonies surrounded by a black halo.

2.4.4 Enumeration of Total Coliforms and Thermotolerant Coliforms

The enumeration was carried out according to **(ISO, 2006)** standard: From each decimal dilution (10-3 to 10-1), 1 ml was transferred into two petri plates (1 plate for TC test and 1 plate for TTC test), approximately 15 ml of DLA, supercooled to 45±1°C, was added to each plate. Circular movements back and forth in the shape of eight were performed to allow the inoculum to mix with the agar, the plates were then left to solidify.

The plates were incubated in an inverted position at 37°C for TC test and at 44°C for TTC, the reading was done after 24h for TTC, 24 to 48 hours for TC. Plates containing between 15 and 150 colonies are enumerated.

2.4.5 Enumeration of yeasts and molds

The enumeration was carried out according to (ISO, 2008) standard: OGA was melted and supercooled to 45°C. Approximately 15 ml of the medium was poured into petri plates and then left to solidify. 0.1ml of each dilution was transferred to the surface of the petri plates containing the OGA agar, and a sterile rake was used to distribute the inoculum over the entire surface.

The plates are incubated in an inverted position for 5 days at 25°C. Plates containing between 15 and 150 colonies were enumerated.

2.4.6 Expression of results

All results were calculated using the following equation **(*ISO*, 2007)**:

$$N = \frac{\sum C}{V(n1 + n2 \cdot 0.1)d}$$

N: Number of coliforms per gram of honey

C: Number of colonies on each plate.

V: Volume inoculated.

$n1$: number of essays from the first dilution.

$n2$: number of essays from the second dilution.

d: the first dilution retained.

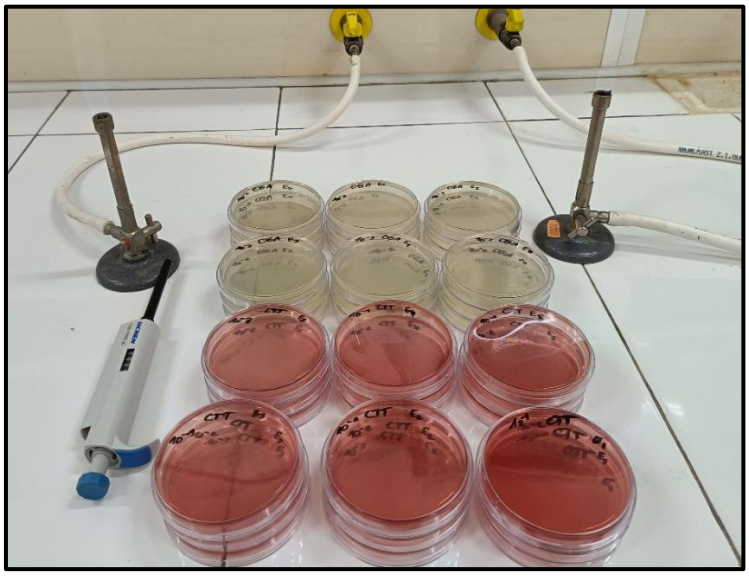

Figure 9. Plates preparation for Microbiological analyses (original image 2024).

3. Results and interpretations

3.1. Pollen profile

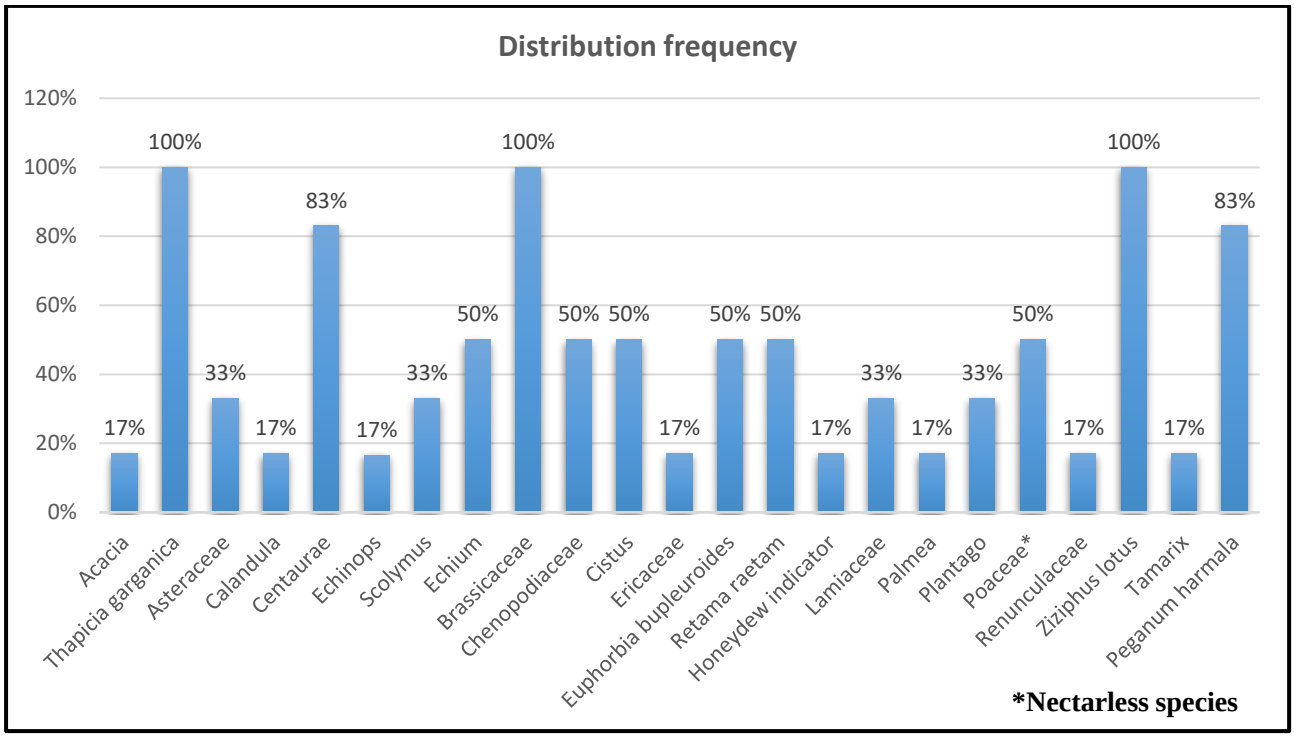

Figure 10. Distribution frequency of pollen taxa in the samples.

Our samples presented a diverse pollen composition with 33 taxa identified. The frequency of pollen taxa distribution is presented in **Figure 10**, *Ziziphus lotus*, *Thapicia garganica and Brassicaceae* have a distribution frequency of 100% indicating they are present in all 6 samples, they are followed by *Poaceae*, *Peganum harmala*, *Chenopodiaceae*, *Cistus*, *Retama raetam*, *Euphorbia bupleuroides*, *Echium, and Centaurae*, all of which have a distribution frequency above 50% making them very frequent taxa, these taxa are common in the arid and semi-arid regions and well-documented in our sampling regions. For Frequent taxa, *Lamiaceae*, *Plantago*, *Scolymus*, and *Asteraceae* are present in 33% of our samples. The rest of the taxa are infrequent (<20%).

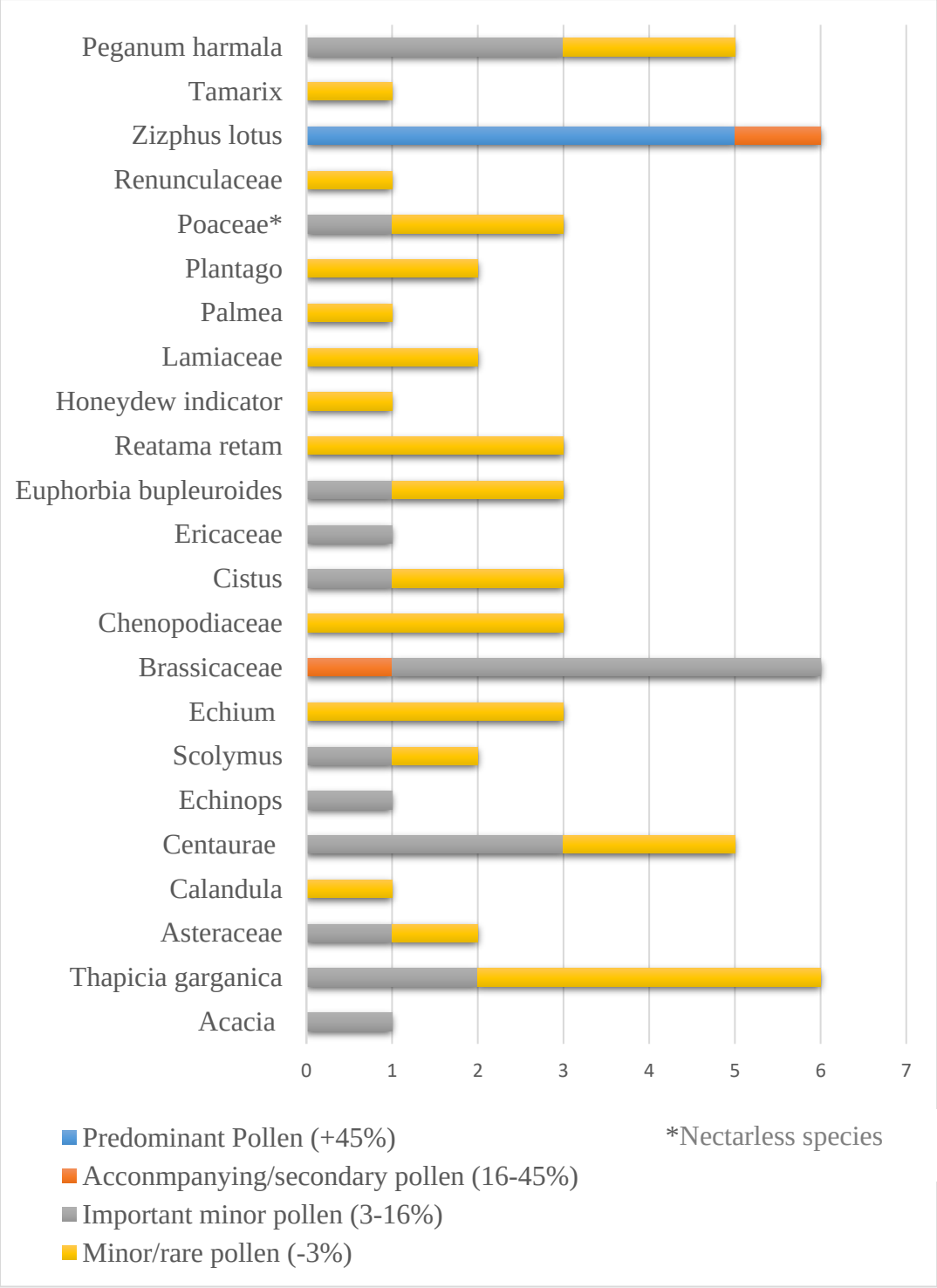

Figure 11. Identified pollen taxa as frequency classes.

The frequency classes of the different taxa identified is presented in **Figure 11**. The results indicate the presence of *Ziziphus lotus* (Jujube pollen grains) as a predominante pollen in 5 of the samples, this result is as expected and it validates the Jujube honey label declared by the sellers. On the other hand, *Ziziphus lotus* was observed as a secondary pollen in one

sample with no other dominant pollen observed, this indicates that one of our samples is a different honey type and not Jujube honey.

Brassicaceae and *Thapicia garganica* were detected in all the samples, being mostly represented as important minor pollen and minor rare pollen, respectively. *Brassicaceae* is adapted to arid and Saharian climates, and *Thapicia garganica* species are well-documented in the arid and semi-arid regions, which explains their presence in the samples. *Centaurae* was present in 5 of the samples and was an important minor pollen in 3 of them, this is explained by the fact that this species is common in arid and semi-arid regions and is well adapted to dry environments.

Several images were taken during the microscopic observation of the slides using the Euromax microscope Bioblue series, which are showcased below along with each sample's pollen composition, the enumeration was done at 40X magnification.

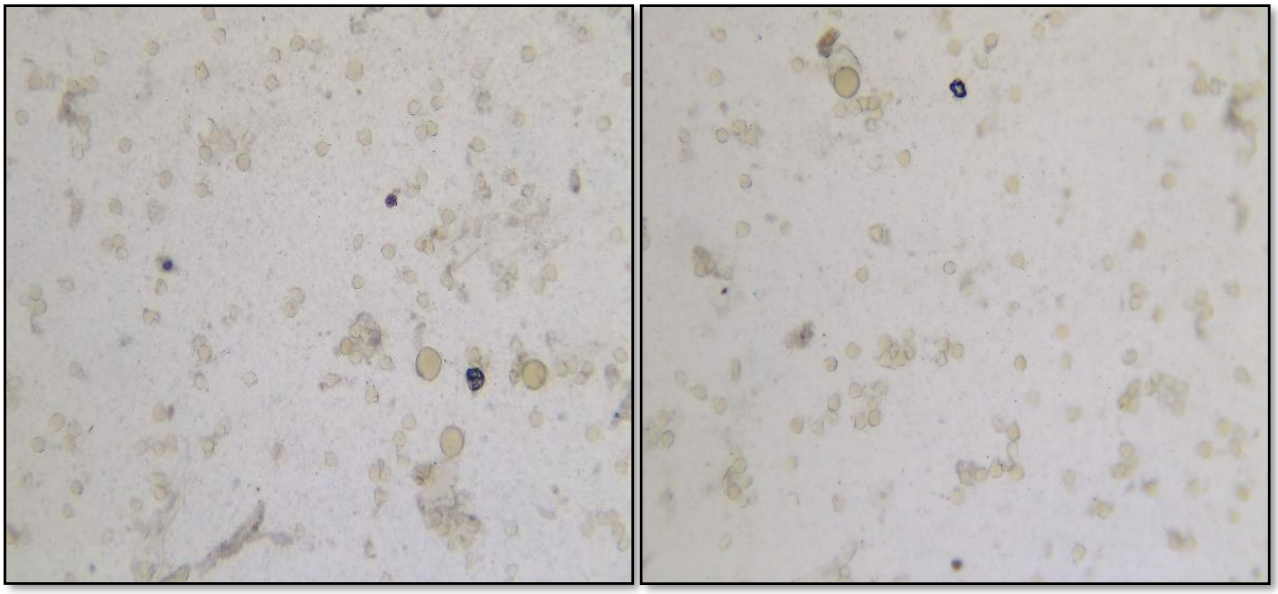

Figure 12. Microscopic observation of sample 01 (original image 2024).

Sample 01 was relatively clean and didn't contain a lot of debris and sand as shown in **Figure 12**, this can indicate that good handling practices are implemented, it can also indicate that the sample was filtered to remove sand and big objects.

The sample had a variety of pollen grains **(Figure 13)** with *Ziziphus lotus* (Jujube pollen grains) being the dominant pollen in terms of frequency confirming its botanical origin as Jujube honey. *Brassicaceae*, *Poaceae*, and *Centaurae* are important minor pollens, these can be considered as characteristic species of the region since they are all xerophytic or show xerophytic characteristics.

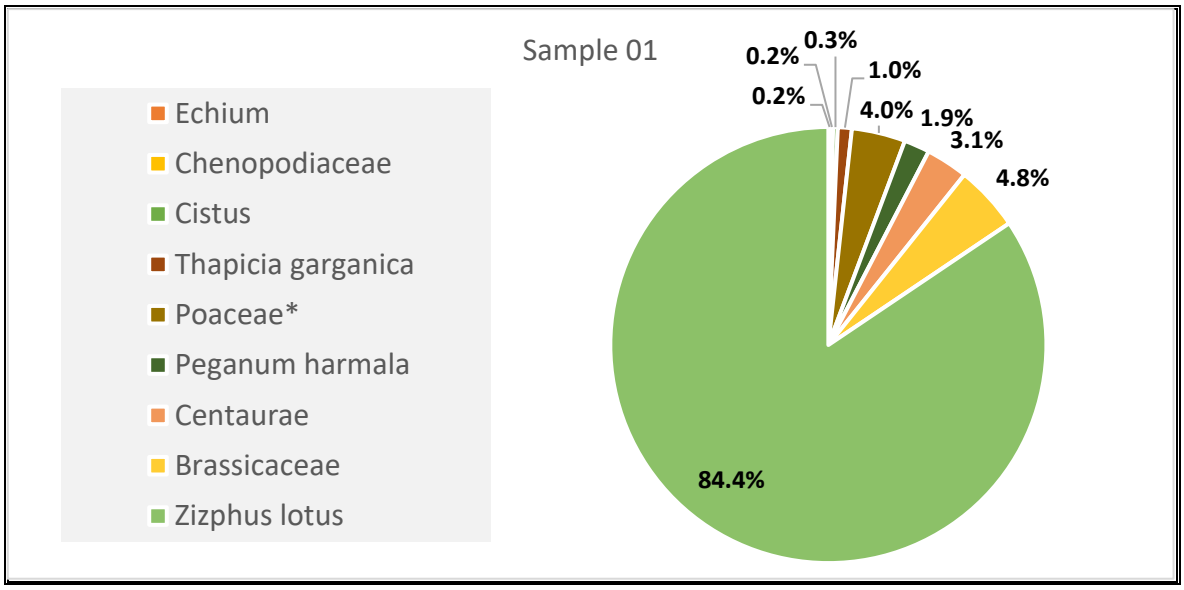

Figure 13. Pollen Taxa frequencies of sample 01.

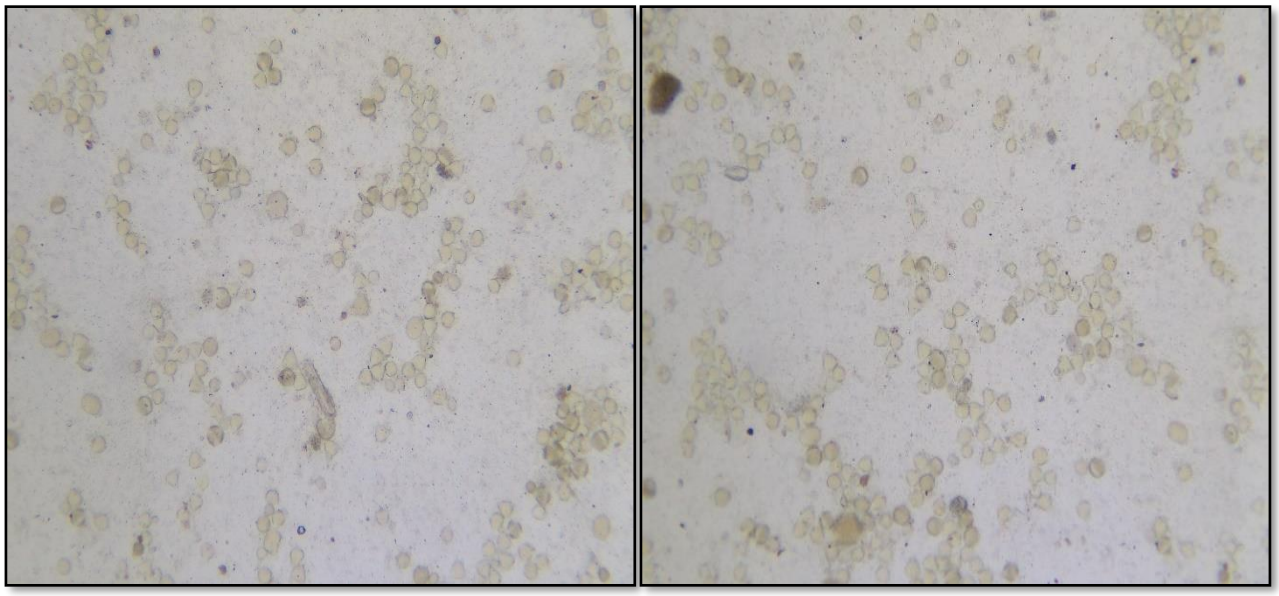

Figure 14. Microscopic observation of sample 02 (original image 2024).

Sample 02 contained some debris, sand and other impurities as shown in **Figure 14**, these impurities can originate from natural sources and occurrences like the sand carried by honeybees and the wind, especially due to the environment of the production regions (arid, semi-arid, and Saharian where sand is ubiquitous), or it can originate from improper handling and storage of honey. This can also indicate that the sample was not filtered.

Samples 02 also showed a great frequency of *Ziziphus lotus* **(Figure 15)**, this confirms its botanical origin, it also contained some important minor pollens *Centaurae*, *Brassicaceae*,

and *Peganum harmala*, which are characteristic species of the region of Biskra, this confirms the geographical origin of the sample.

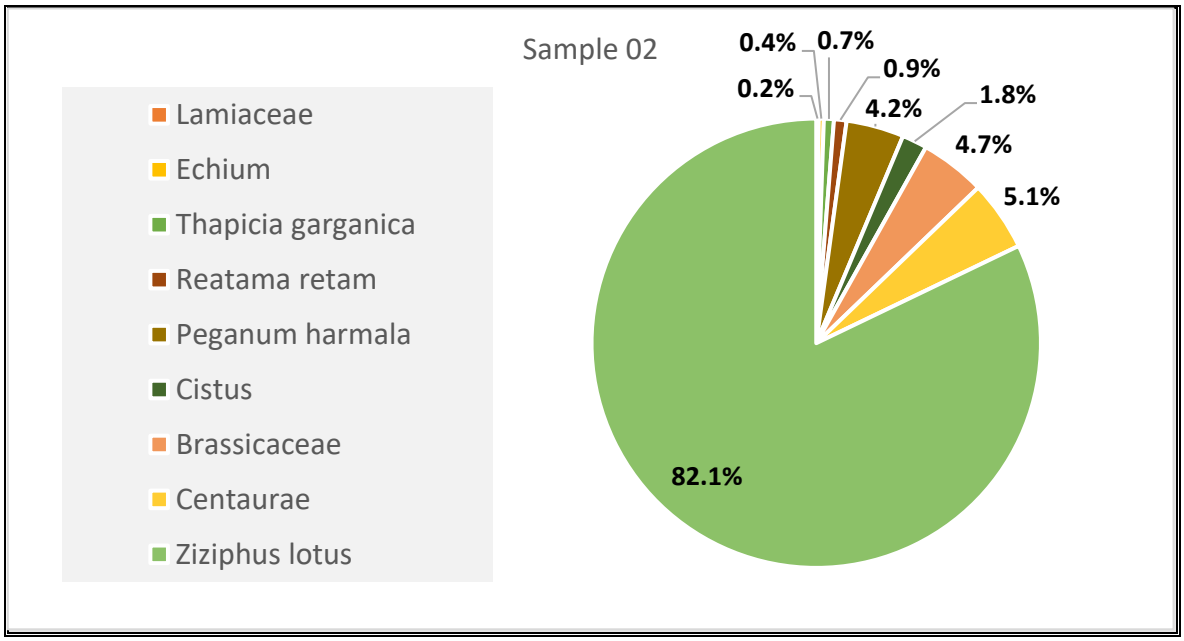

Figure 15. Pollen Taxa frequencies of sample 02.

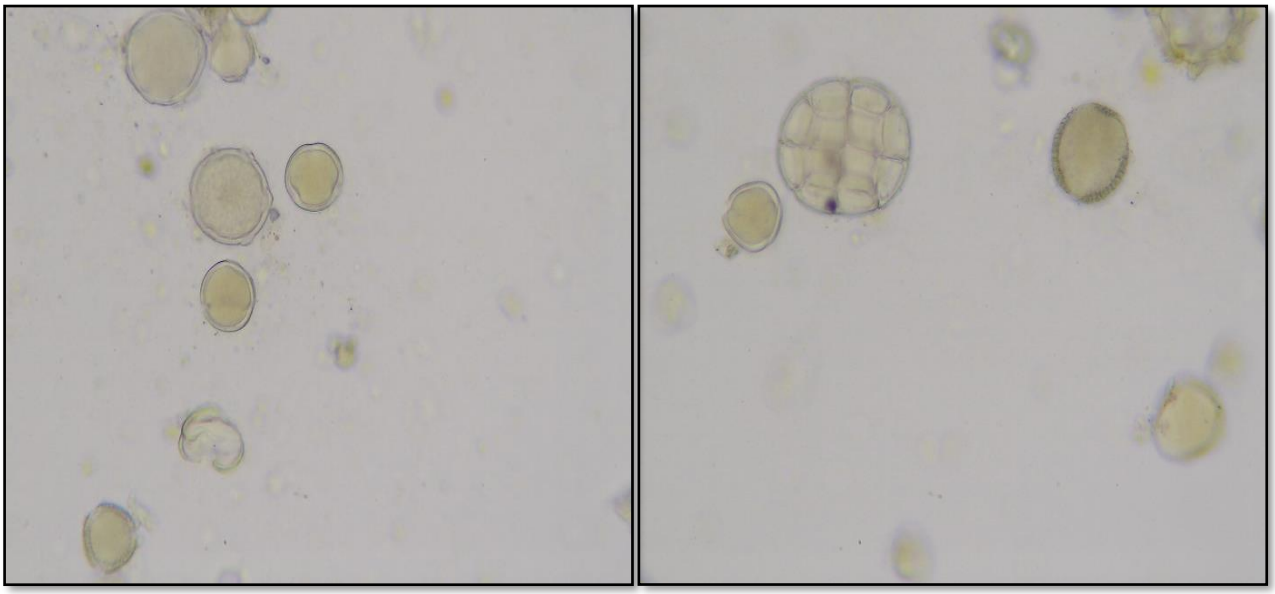

Figure 16. Microscopic observation of sample 03 (original image 2024).

Sample 03 was also clean, no debris or sand were observed as shown in **Figure 16,** this can indicate that the sample has undergone filtration to remove sand and other impurities, and it can also indicate the use of proper handling practices.

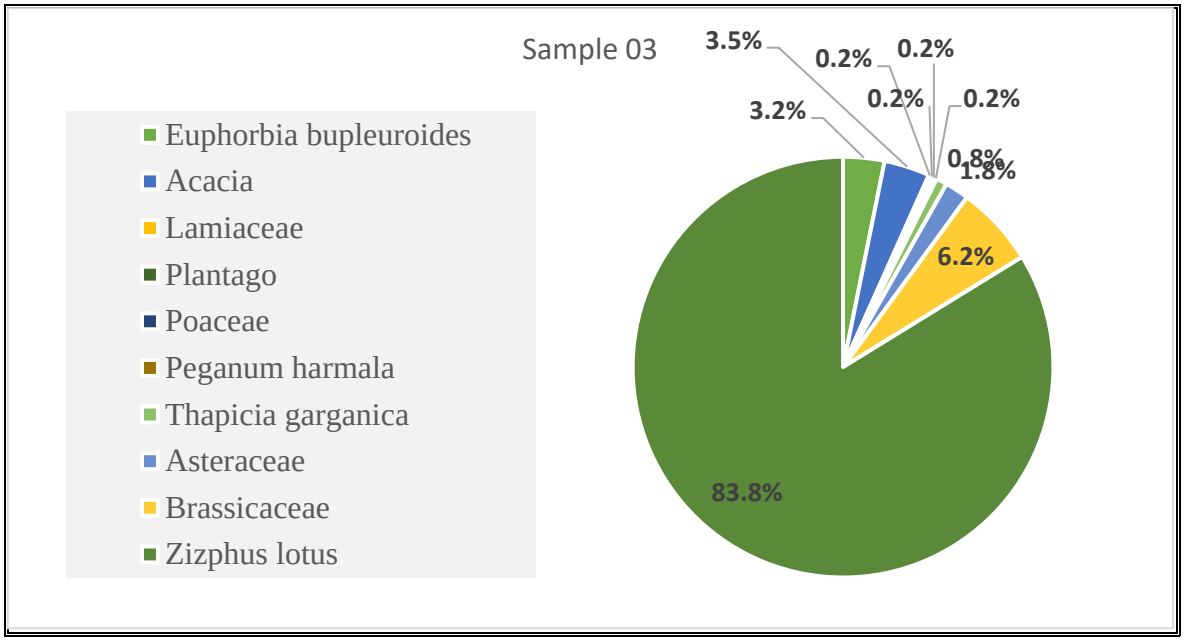

Figure 17. Pollen Taxa frequencies of sample 03.

Sample 03 shows a dominance of *Ziziphus lotus* validating its botanical origin **(Figure 17)**, it also contained *Brassicaceae* as an important minor pollen similar to the previous samples, additionally *Acacia* and *Euphorbia bupleuriodes* were also observed as important minor pollens, these are not the most common species in Djelfa, and this is confirmed by the results (low frequency).

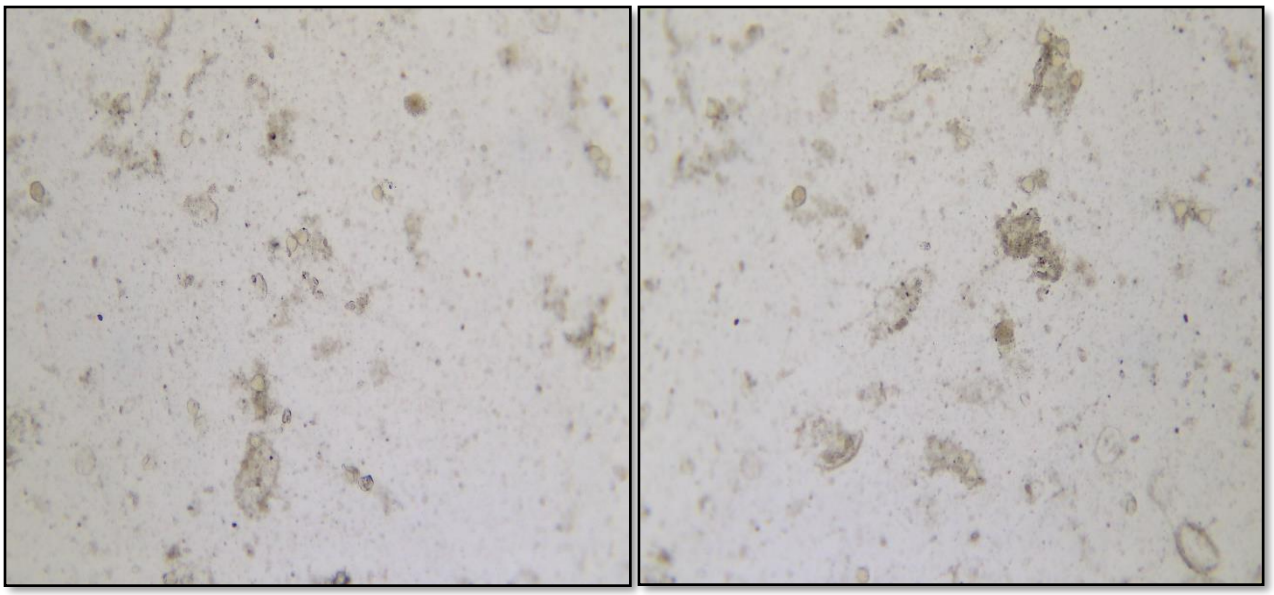

Figure 18. Microscopic observation of sample 04 (original image 2024).

Sample 04 was concentrated with debris, sand and other impurities as observed in **Figure 18,** not many pollen grains were observed either which could indicate adulteration.

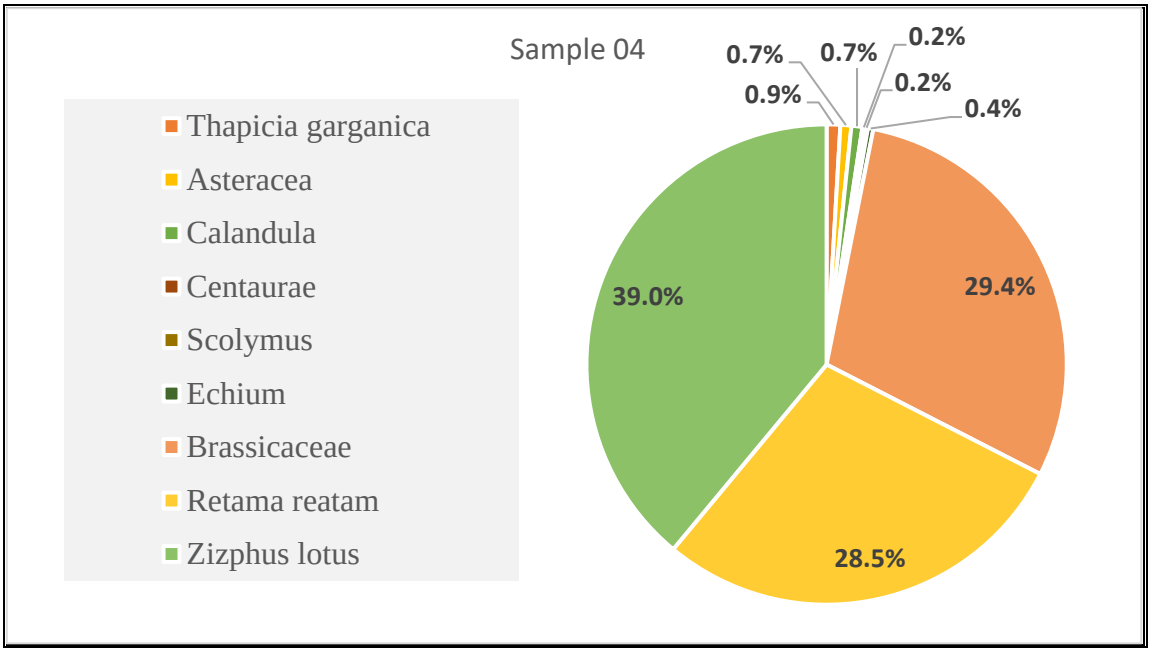

Figure 19. Pollen Taxa frequencies of sample 04.

Sample 04 contained a comparatively low abundance of *Ziziphus lotus* (secondary pollen) **(Figure 19)**, it also contained *Brassicaceae* and *Retama reatam* as secondary pollens with no predominant pollen observed, this indicates that it is a multifloral honey and not a Jujube honey. *Brassicaceae* and *Retama reatam* are largely represented species of the region of Naama, our results affirm this information and confirm the geographical origin of the sample.

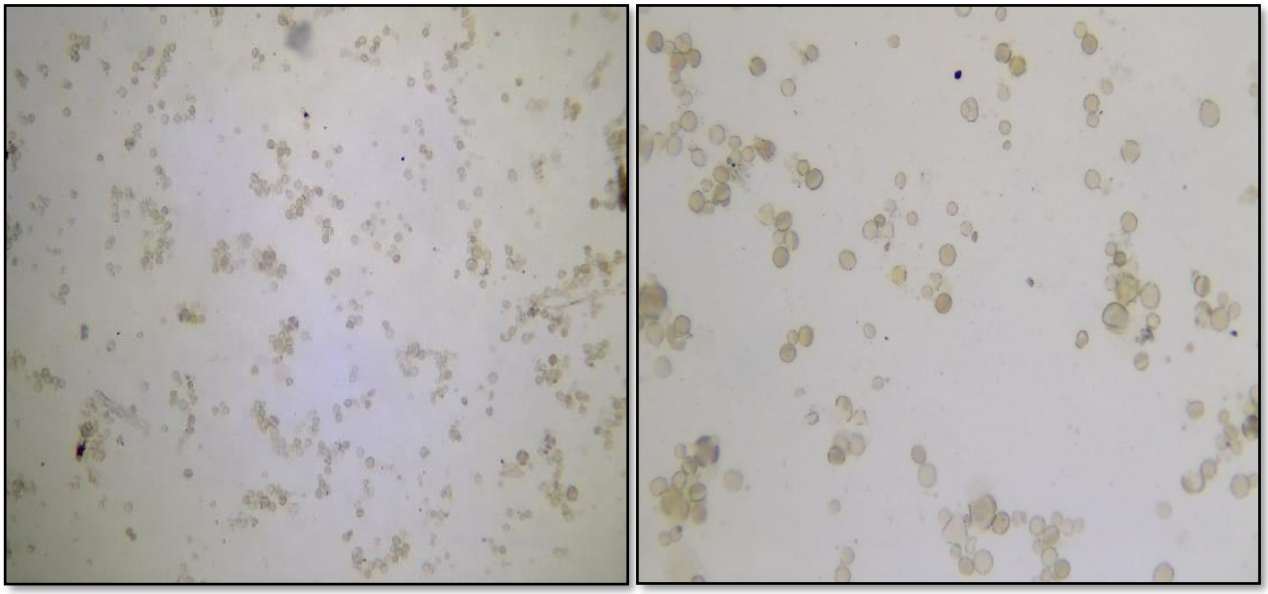

Figure 20. Microscopic observation of sample 05 (original image 2024).

Sample 05 was clean with no debris or sand observed as shown in **Figure 20,** which can indicate that the sample has been filtered to remove the impurities, and/or proper handling practices were implemented.

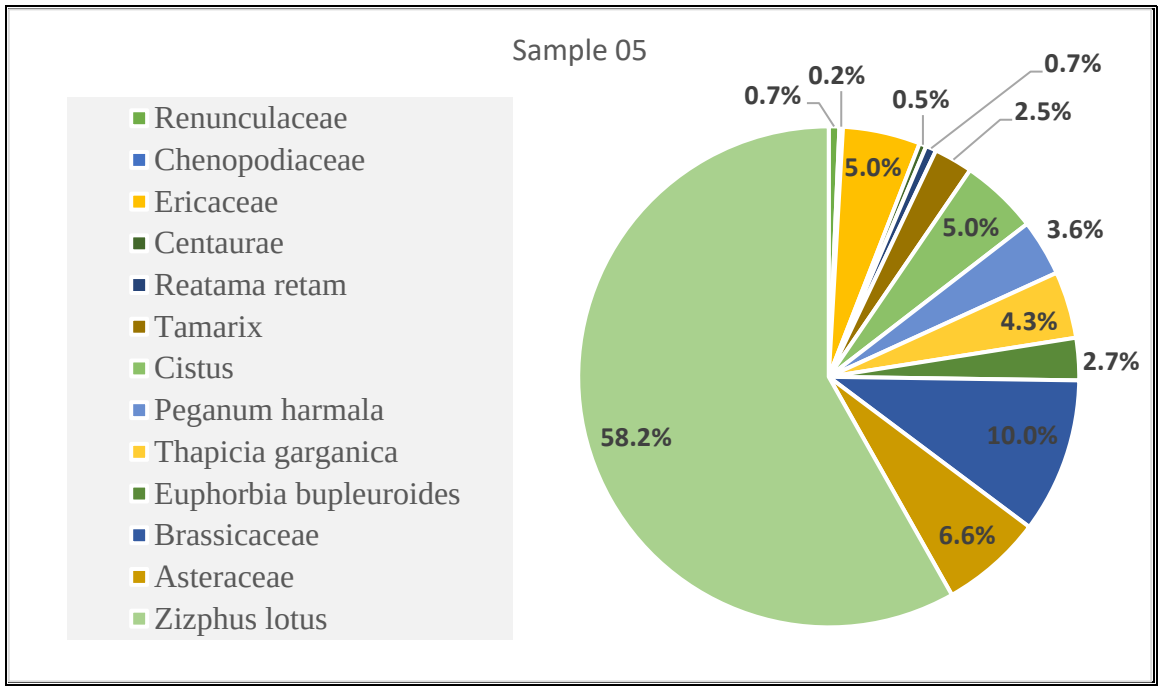

Figure 21. Pollen Taxa frequencies of sample 05.

Sample 05 shows a more divers pollen composition **(Figure 21)** with 13 different taxa identified in total, *Ziziphus lotus* was observed as the predominant pollen confirming the botanical origin of the sample, it also contained 6 important minor pollens, *Brassicaceae, Asteraceae, and Peganum harmala* are present in the Saharian zones but species like *Ericaceae* (typically found in more temperate climates), *Cistus* (Mediterranean plants), and *Thapicia garganica* are not well adapted to the climate and the region of Bordj Badji Mokhtar, and do not have a well-documented presence in the Sahara, their presence in the sample could indicate adulteration. The lack of recent studies of the flora in the region renders it impossible to make any objective explanations.

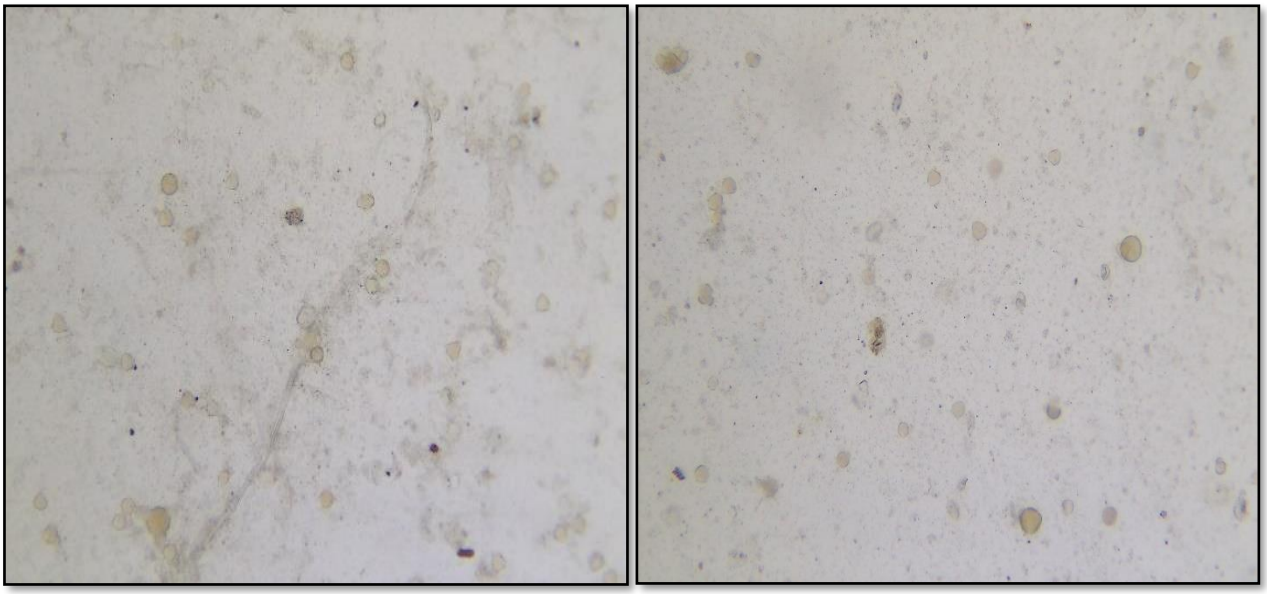

Figure 22. Microscopic observation of sample 06 (original image 2024).

Sample 06 contained a high concentration of sand and other pollutants **(Figure 22)**, which could indicate the improper handling of the product during the harvest and manipulation, we can also conclude that no filtration process was performed. A few honeydew indicators were also observed in this sample.

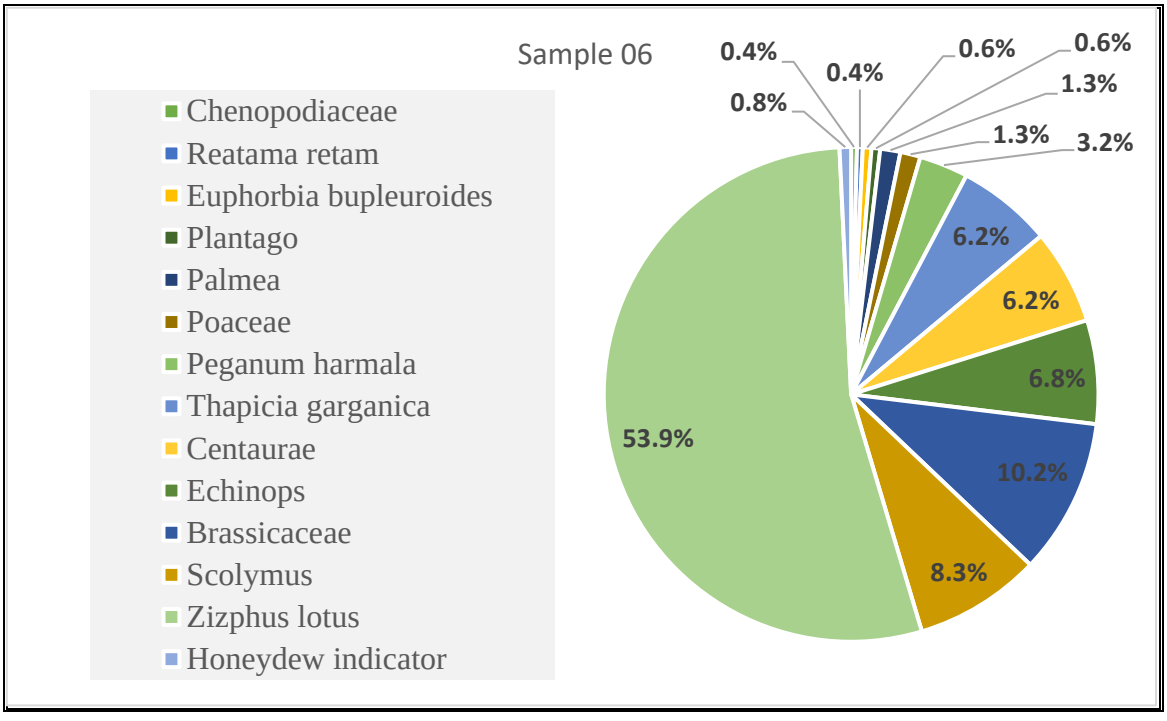

Figure 23. Pollen Taxa frequencies of sample 06.

Sample 06 also presented 13 different pollen taxa with *Ziziphus lotus* dominating the chart and validating its botanical origin **(Figure 23)**, this sample also contained 6 important minor pollens, *Brassicaceae, Scolymus, Echinops, Centaurae,* and *Thapicia garganica*, all of which are native to the region. The sample also contained a few honeydew indicators but not in sufficient frequencies to invalidate its nectar origin.

Arid, semi-arid and Saharian regions naturally have lower plant diversity compared to more temperate or tropical regions **(Ochungo et al., 2021)**. With fewer plant species available, honeybees have access to a smaller pool of pollen sources especially during the dry season when jujube honey is produced and harvested. This explains why our samples contained a narrow range of pollen taxa compared to the results obtained by **Laouar (2016)** through the analysis of honey originating from the northeastern regions of Algeria .

3.2. Physicochemical characteristics

The physicochemical analysis results are presented and explained below.

3.2.1 Determination of moisture content (refractometric method)

The **moisture content** of our samples ranged from 11.9% to 14.6% with an average of 13.25% as presented in **Figure 24**, this conforms to the **Codex Alimentarius' (2001)** and **European Council Directive's (2002)** standards (**Appendix B**), which imposes that MC in blossom/nectar honey should not exceed 20%.

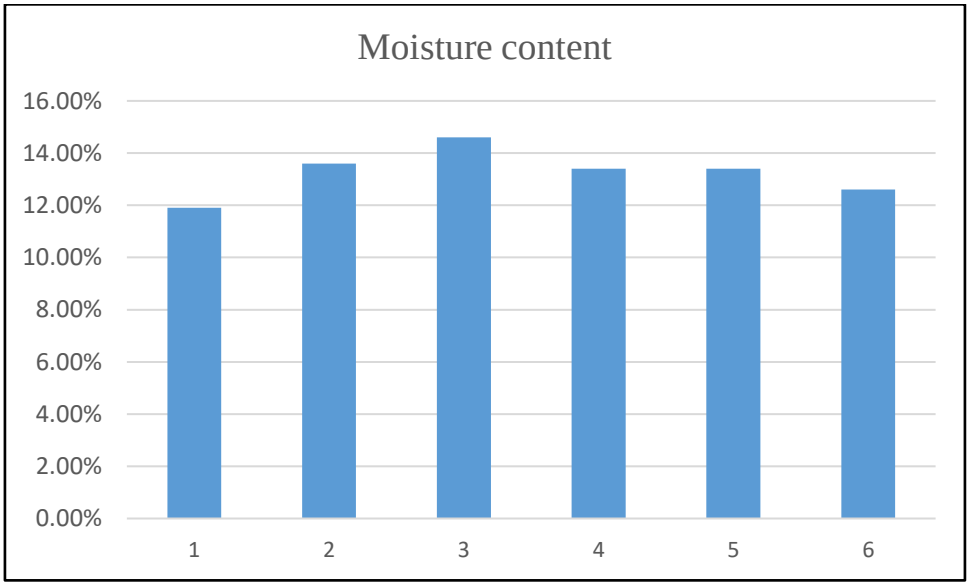

Figure 24. Moisture content of the honey samples.

Our results are similar to the work **of Mekious et al.(2015)**, while the results obtained by **Zerrouk et al.(2017)** and **Adjlane et al.(2014)** were higher. The low MC of honey is a result of the process of nectar collection and evaporation by bees, and it creates an environment that is not conducive to the growth of most microorganisms which makes it less susceptible to fermentation and spoilage by yeast and bacteria.

3.2.2 Determination of pH

The **pH** ranged from 3.75 to 6.12 with a mean of value of 4.9 as presented in **Figure 25**, samples 1,2,and 4 showed a higher pH compared to the other samples in this study and the study done by **Adjlane et al.(2014)** where all his samples had a pH below 5, while **Zerrouk et al.(2017)** observed relatively high values of pH . The ECD and Codex do not

impose a maximum allowed value for honey pH, but a low pH is beneficial in preventing the growth of microorganisms **(Ratiu et al., 2019)**.

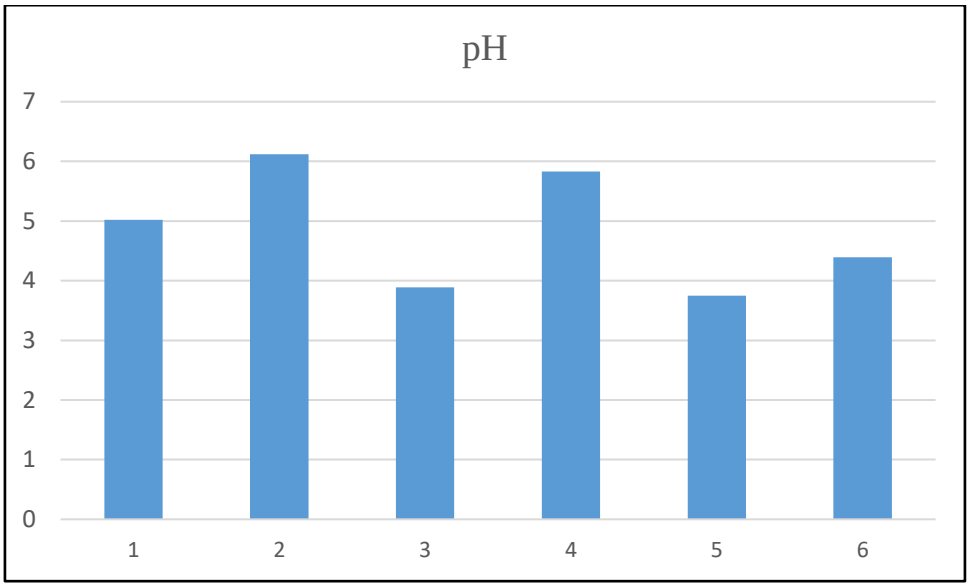

Figure 25. pH of the honey samples.

3.2.3 Determination of free acidity by titration to pH 8.3

F**ree acidity** ranged from 7 to 17.5 meq/kg, with a mean of 11.8 meq/kg as shown in **Figure 26**, which was within the internationally accepted limit. In our case, FA values are low which indicates that the honey has not undergone significant fermentation or spoilage processes, maintaining its natural composition and flavor profile.

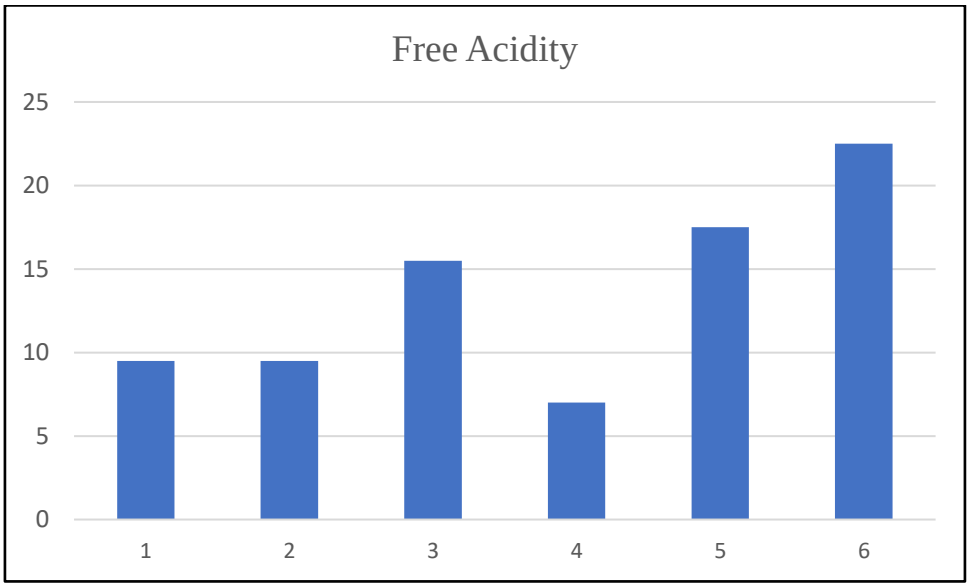

Figure 26. Free acidity of the honey samples.

3.2.4 Determination of electrical conductivity

Lastly, the **electrical conductivity** ranged from 0.257 to 0.566 mS/cm with a mean value of 0.456 mS/cm **(Figure 27)**, and was in conformity with the Codex and ECD's standards. **Mekious et al. (2015)** had a similar result with EC values ranging from 0,27-0,58 mS/cm for Jujube honey samples while **Zerrouk et al. (2017)** had a higher mean value of 0.654 mS/cm.

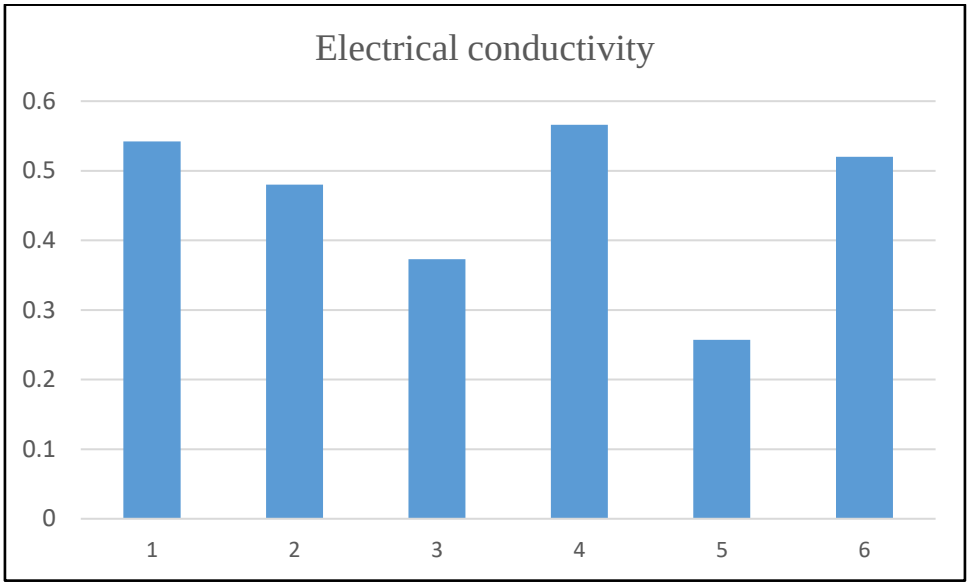

Figure 27. Electrical conductivity of the honey samples.

EC serves as a criterion for determining the botanical origin of honey and is commonly used in routine honey quality control and purity assessments to distinguish between nectar honey and honeydew, nectar honeys have values lower than 0.6 mS/cm **(Mekious et al., 2015)**.

3.3. Microbiological quality

The microbiological quality analysis results are presented in Table 2.

Table 2. Microbiological analyses results

N° sample	**TMAF** (CFU/g)	**Total Coliforms** (TC/g)	**Thermotolerant Coliforms** (TTC/g)	**Sulfite-reducing Anaerobic spores** (SAS/g)	**Yeast and mold** (YM/g)
01	$2.7\ 10^2$	100	60	0	$4.1\ 10^2$
02	$9.2\ 10^2$	40	0	0	$1.2\ 10^3$
03	$1.8\ 10^2$	$2\ 10^2$	0	0	40
04	$5\ 10^2$	$4\ 10^2$	10	0	$1.5\ 10^3$
05	$1.6\ 10^3$	20	0	0	10^3
06	$1.7\ 10^3$	10	10	0	10

The absence of standard limits renders the evaluation and interpretation of these results difficult; several different recommendations were considered (national and international) (**Appendix C**).

The Total Aerobic Mesophilic Flora count for samples 1,2,3, and 4 was below 10^3 CFU/g, which is the microbiological limit value according to French standard **(El Menyiy et al., 2020)**, while samples 5 and 6 had a higher count indicating a poor microbiological quality. However, sample 5 can be considered of good microbiological quality due to the absence of fecal contamination according to the standard recommended by **Snowdon and Cliver (1996)**.

A TAMF above the recommended limits indicates non hygienic practices during the production and storage or contamination from natural sources such as pollen, bees' digestive tracts, dust, air…

The variation in the TAMF concentration between the samples can be attributed to the difference in the bioclimatic and biogeographic zones of sampling regions, a study have shown that honeys from tropical climate zones had different microbiological quality compared to honeys from temperate climate zones, with 70% of tropical honeys having higher total viable bacterial counts **(Rosiak et al., 2021)**. These findings highlight the importance of considering the biogeographical origins and bioclimatic conditions when assessing the

microbiological safety and quality of honey. Differences in water content between climatic zones appear to be the main factor driving variations in microbial counts in honey.

A similar result was recorded by **Adjlane et al. (2014)** who reported that two Jujube honey samples from the region of Djelfa showed a good microbiological quality with a TAMF concentration below 10 CFU/g while only one sample had a higher count indicating its poor quality. While, **El Menyiy et al. (2020)** reported that a sample of Jujube honey showed a complete absence of TAMF. As for **Laredj and Waffa (2017)** only two of his samples showed a count above the limit.

The Total Coliform count in samples 2, 5, and 6 was below 100 coliforms/g, while samples 1,3,4 had 100 or more coliforms/g. Thermotolerant Coliforms were not detected in sample 2,3, and 5, while samples 1, 4, and 6 contained a varying number of TTC ranging from 10 to 60. The presence of coliforms in honey can indicate fecal contamination and poor hygienic conditions during processing, while their absence is an indication of the sanitary quality of the analyzed honey samples. The absence of TTC in sample 2,3, and 5 indicates they could be considered of good microbiological quality.

The microbiological limit for coliforms in honey varies depending on the region and regulations. In general, the World Health Organization (WHO) recommends that honey should not contain any detectable coliforms, this is because coliforms are commonly associated with fecal contamination and can indicate poor handling practices or contamination during processing. In the European Union, the maximum permitted level of coliforms in honey is 0 Coliforms/g, which is in line with WHO guidelines **(Snowdon and Cliver, 1996)**.

The absence of Coliforms was reported by **El Menyiy et al. (2020)** and Pereira et al. (2021) for all honey samples including Jujube honey, while the presence of coliforms was reported by **Omafuvbe and Akanbi (2009)** in Nigeria with a value of 30 cfu/g ,the botanical origin of their honey samples was not mentioned.

Sulfite-reducing anaerobic spores were not detected in any of the tested samples, this was also observed in other studies such as **Adjlane et al. (2014)** in Algeria (which included Jujube honey and other types of honey), and **Landeka et al. (2022)** in Bosnia. In the context of honey quality, their absence is often linked to the application of good manufacturing and hygienic practices during honey production and handling, proper storage conditions, and good

practices. The presence of these microorganisms can indicate contamination or pollution, which is a significant concern in honey production.

Health Protection Agency (2009) recommends a count below 10 CFU/g for Sulphite-reducing clostridia, no other recommendations were set in place on the national and international level.

Yeast and mold (YM) results showed that samples 3, and 6 presented a count of 40 and 10 YM/g, respectively, which conforms to the Microbiological limit set by the Algerian Official Journal (2017) which states that honey shouldn't contain more than 100 YM/g, on the other hand, samples 1,2,4,and 5 all surpassed the microbiological limit indicating they are more susceptible to fermentation risks. However, despite the high count of YM, our samples have a low moisture content ranging from 11.9% to 14.6%, generally, honeys with a moisture content below 17% are less susceptible to fermentation due to the inhibitory effect of low water content on yeast growth, making it more resistant to spoilage and maintaining its quality over time.

Several studies have investigated the YM count in different honey types, a study done by **Laredj and Waffa (2017)** in Algeria, detected low counts below the limit (approximately 100 cfu/g) in seven samples indicating low potential for deterioration while the rest of the samples had really high counts ranging between 620 to 960. **Ananias et al. (2014)** found that 20% of samples showed a YM count above the microbiological established limit. A study done by **Estevinho et al. (2012)** in Portugal had very low counts of YM with mean values of 5.5 YM/g. Similarly, **Tornuk et al. (2013)'s** samples showed very low counts. A study done by (Kiš et al., 2018) in Croatia showed that 7 samples of honey had a YM count below 100 YM/g while 13 samples had counts above the limit, the highest count being 1300 (yeast/g).

3.4. Important remarks

Overall, the results for the analyses were in the norm and showed minor variabilities in most parameters, the variabilities could have been further analyzed and scrutinized if more units and samples were collected in similar regions but ,as mentioned in the samples and sampling regions section, this was not possible due to the time constraints of the project, which prevented us from obtaining fresh samples during the harvest period, and the low production yield of Jujube honey from last year making it scarce in the market. The low number of samples also renders statistical analysis insignificant by making it harder to detect meaningful differences even if they exist, as it increases the risk of false positive and false negative results , and limiting the ability to generalize findings to the broader population.

The pollen analysis results mostly conformed to the declared botanical and geographical origins of our samples, as for sample 5, the presence of certain species had to be addressed, while these plants are not typical of the central Sahara, their presence in peripheral or unique microclimates within the Sahara's larger ecosystem is possible. They might be found in regions where specific conditions, such as elevation, moisture, and soil type provide a more hospitable environment compared to the harsh, central desert conditions. The lack of recent studies on the region’s floral diversity makes interpretation difficult. Adulteration is also a possible explanation, **Copeland (2020)** revealed that honey is one of the most adulterated foods in the world, since adulteration and misrepresentation of honey is not easy to detect the fraudulent practice has become more widespread, this doesn’t only affect the apiculture business by driving honey prices down, but it also reduces the quality of the product and could put the consumer’s health at risk.

The physicochemical properties conformed to Codex’s and ECD’s standards, the analyses indicated great potential for the commercialization of Algerian Jujube honey, nationally and internationally, should the microbiological quality improve.

The microbiological quality results in particular showed great contrast among the samples, the lack of official standards for most of the tests rendered the interpretation and evaluation difficult, germs concerned in this study are all essential for the evaluation and quality control of honey to ensure its safety for consumption, despite that, the official journal only imposes a limit for YM, on the international level the limits were scattered and hard to gather with each country imposing limits on one or two parameters only, in particular, the only limit set for SAS was by **Health Protection Agency (2009)** a governmental organization

which has been dissolved in 2013. The health risks associated with SAS in honey are primarily related to the potential presence of *Clostridium botulinum* and *Clostridium perfringens* spores, these bacteria can produce toxins that can cause botulism, a potentially fatal illness. This lack of regulations along with the potential risks highlights the importance of establishing microbiological criteria and analysis methodologies for honey, at the national and international level, to ensure public health and consumer safety.

For an objective and just evaluation, it is important to consider different parameters and their influence on the product and on each other, during the interpretation of the results it was necessary to consider the effect of each property and parameter on the result as well as the bioclimatic and geographic origin of the sample. This allowed for a more comprehensive understanding of the results.

Conclusion

This study examined the Microbiological quality and physicochemical properties of Jujube honey to assess its quality, while the pollen profile was investigated to confirm the botanical and geographical origin of each sample. The honey samples were collected from different regions in the country in order to provide a representative and comprehensive overview of the quality and authenticity of Jujube honey in Algeria, and to highlight the effect of the regions' bioclimatic conditions and biogeographic origins on the samples' properties and quality.

The pollen profile of our samples showed a rich composition of different pollen families, *ziziphus lotus* pollen frequency dominated in five of the samples confirming their botanical origin, and one of the samples was identified as multifloral. The analysis of pollen frequencies also allowed the confirmation of the geographical origin of the samples by referencing characteristic pollen species of each region.

The physicochemical parameters investigated were moisture content, pH, free acidity, and electrical conductivity. The results showed that all our samples conform to the Codex's and ECD's standards. Our samples had a low MC well below 15%, this indicates that they are less susceptible to fermentation and deterioration, allowing them to be stored for longer periods of time. The pH values varied between 3.75 to 6.12, despite the lack of a standard for the pH of honey, a low pH is a key factor contributing to its antibacterial properties, taste, shelf life and overall quality. FA values were all below 50 meq/Kg which is the recommended standard limit by Codex and ECD, this indicates that the honey is fresh and has not undergone significant fermentation or aging, sample 6, which has been harvested in 2022 unlike the rest of the samples (2023) shows the highest free acidity value (22,5 meq/kg). EC results conformed to the standards with values well below 0.8mS/cm, which confirms the botanical origin of our samples as nectar honeys according to the Codex Alimentarius.

The microbiological quality of our samples was evaluated based on the detection and enumeration of the Total aerobic mesophilic flora, total and thermotolerant coliforms, sulfite-reducing anaerobic spores, and yeast and mold. The microbiological quality of the samples varied, with samples 2,3, and 5 having good results in most of the tests, while samples 1,4, and 6 showed a presence of TTC. TAMF count results showed that samples 1,2,3, and 4 conformed to the French standard (<1000 cfu/g), while sample 5 was above the standard but conforms to the recommendations of **Snowdon and Cliver (1996)**. TC were present in all the samples in varying numbers with samples 3, and 4 having counts above 100 TC/g, TTC was present in samples 1,4, and 6, but absent in the rest of the samples. There is no standard limit for TC in honey, but several international entities have imposed

strict limits for TTC, indicating their obligatory absence, this shows that samples 1,4, and 6 suffered from poor sanitation practices during honey production, processing, or handling. The samples presented a complete absence of SAS which conforms to the standard set by Health Protection Agency.

These results allowed us to confirm the botanical and geographical origin of our samples indicating good ethics of beekeepers and sellers who declared the correct honey type, the results also showed that most of our samples are of good quality, some samples showed high concentrations of specific germs which indicated the absence of sanitary and hygienic practices.

This study is a contribution to the knowledge and understanding of the characteristics of Algerian Jujube honey and its botanical origin, by highlighting its unique qualities we hope to aid in the valorization and promotion of local production and consumption of this natural product. The pollen profile findings are also a contribution to the knowledge of the flora in the regions concerned in this study and could aid in the selection of botanical taxa usable as geographic markers. By dedicating this work to the consumers and producers of honey, we hope to promote informed purchasing decisions, and better hygienic and sanitary practices.

By addressing concerns about the lack of honey quality regulations and advocating for safer honey products, we seek to open new avenues for quality evaluation studies and raise interest in the research and development of quality standards in the national and international level.

References

- Abdelghani, Z., Fateh, M., Benkherif, M., Djamel, S., & Fenni, M. (2016). Diversity and plant distribution according to the topographical factors in Djebel Messaad forest (M'Sila—Algeria). *Advances in Environmental Biology (AEB)*, *10*, 27–38.
- Adjlane, N., Haddad, N., Laid Ameur, K., Kesraoui, S., & Moussaoui, D. (2014). Physicochemical and Microbiological Characteristics of Some Samples of Honey Produced by Beekeepers In Algeria. *Acta Technologica Agriculturae*, *17*(1), 1–5. https://doi.org/10.2478/ata-2014-0001
- Ananias, K. R., de Melo, A. A. M., & de Moura, C. J. (2014). Analysis of moisture content, acidity and contamination by yeast and molds in Apis mellifera L. honey from central Brazil. *Brazilian Journal of Microbiology*, *44*(3), 679–683.
- Bogdanov, S. (2001). Harmonised methods of the International Honey Commission. *International Honey Commission (IHC)*, 1–62.
- Codex Alimentarius. (2001). Revised Codex Standard for Honey Codex Stan 12-1981, Rev. 1 (1987), Rev. 2 (2001). *Codex Standard*, *12*, 1–7.
- Copeland, C. (2020, September 26). *Honey is one of the most faked foods in the world, and the US government isn't doing much to fix it*. Business Insider. https://www.businessinsider.com/fake-honey-problems-how-it-works-2020-9
- Council Directive 2001/110/EC Relating to Honey., 47 (2002).
- El Menyiy, N., Akdad, M., Elamine, Y., & Lyoussi, B. (2020). Microbiological Quality, Physicochemical Properties, and Antioxidant Capacity of Honey Samples Commercialized in the Moroccan Errachidia Region. *Journal of Food Quality*, *2020*, e7383018. https://doi.org/10.1155/2020/7383018

- Estevinho, L. M., Feás, X., Seijas, J. A., & Pilar Vázquez-Tato, M. (2012). Organic honey from Trás-Os-Montes region (Portugal): Chemical, palynological, microbiological and bioactive compounds characterization. *Food and Chemical Toxicology*, *50*(2), 258–264. https://doi.org/10.1016/j.fct.2011.10.034
- Feller-Demalsy, M.-J., Parent, J., & Strachan, A. A. (1987). Microscopic Analysis of Honeys from Alberta, Canada. *Journal of Apicultural Research*, *26*(2), 123–132. https://doi.org/10.1080/00218839.1987.11100748
- Getachew, A., Assefa, D., & Tajebe, Z. (2014). PHYSICO-CHEMICAL PROPERTIES OF HONEY PRODUCED IN MASHA, GESHA, AND SHEKO DISTRICTS IN SOUTHWESTERN ETHIOPIA. *Current Research in Agricultural Sciences*.
- Habib, N., Regagba, Z., Miara, M. D., Hammou, M. A., & Snorek, J. (2020). Floristic diversity of steppe vegetation in the region of Djelfa, North-West Algeria. *Acta Botanica Malacitana*, *45*, 37–46. https://doi.org/10.24310/abm.v45i.7987
- Health Protection Agency. (2009). *Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market*.
- Homrani, M., Escuredo, O., Rodríguez-Flores, M. S., Fatiha, D., Mohammed, B., Homrani, A., & Seijo, M. C. (2020). Botanical Origin, Pollen Profile, and Physicochemical Properties of Algerian Honey from Different Bioclimatic Areas. *Foods*, *9*(7), Article 7. https://doi.org/10.3390/foods9070938
- Ingram, V., Hansen, M. E., & Bosselmann, A. S. (2020). To Label or Not? Governing the Costs and Benefits of Geographic Indication of an African Forest Honey Value Chain. *Frontiers in Forests and Global Change*, *3*. https://doi.org/10.3389/ffgc.2020.00102
- Kaid, S. (2021). *Quality evaluation of Algerian honeys: Eucalyptus, Jujube, Euphorbia and multiflora*.

- Kiš, M., Furmeg, S., Jaki Tkalec, V., Zadravec, M., Denžić Lugomer, M., Končurat, A., Benić, M., & Pavliček, D. (2018). Characterisation of Croatian honey by physicochemical and microbiological parameters with mold identification. *Journal of Food Safety*, *38*(5), e12492. https://doi.org/10.1111/jfs.12492
- Laid, B., Rebbas, K., Mouloud, G., Rabah, B., Brini, F., & Bouzerzour, H. (2014). Ethnobotanical study of medicinal plants in Jebel Messaad region (M'sila, Algeria). *Global Journal of Research on Medicinal Plants and Indigenous Medicine*, *3*, 445–459.
- Landeka, V., Cvrtila, Ž., Kozačinski, L., Drmać, M., Sesar, A., & Aljičević, M. (2022). Microbiological and physicochemical quality of honey in Bosnia and Herzegovina. *Veterinarska Stanica*, *53*(5), 561–571. https://doi.org/10.46419/vs.53.5.5
- Laouar, H. (2016). *Analyses polliniques et physico-chimiques des miels du Nord Est algérien*. Université Badji Mokhtar.
- Laredj, H., & Waffa, R. (2017). Microbiological and Physicochemical Characterization of Honeys from the Tiaret Region of Algeria. *Asian Journal of Pharmaceutical Research and Health Care*, *9*(3), 85. https://doi.org/10.18311/ajprhc/2017/15698
- Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of Melissopalynology. *Bee World*. https://www.tandfonline.com/doi/abs/10.1080/0005772X.1978.11097714
- Mallem, H. (2018). *ETUDE DU POTENTIEL DE LA VEGETATION STEPPIQUE DANS LA LUTTE CONTRE LA DEFLATION EOLIENNE*.
- Mekious, S., Houman, Z., Bruneau, É., Masseaux, C., Guillet, A., & Hance, T. (2015). Caractérisation des miels produits dans la région steppique de Djelfa en Algérie. *BASE*. https://popups.uliege.be/1780-4507/index.php?id=12213

- Mekious, S., MASSEAUX, C., LOUCIF-AYAD, W., & Vercelli, M. (2022). Pollen quality and sensory attributes of Algerian jujube (Ziziphus lotus (L.) Lam.) honeys. *Acta Agriculturae Slovenica*, *118*, 1. https://doi.org/10.14720/aas.2022.118.3.2690
- *Microbiology of food and animal feeding stuffs ,General requirements and guidance for microbiological examinations*. (2007).
- *Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coliforms ,Colony-count technique* (4832). (2006).
- *Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of yeasts and moulds, Part 2: Colony count technique in products with water activity less than or equal to 0,95* (21527–2). (2008).
- *Microbiology of the food chain: Horizontal method for the detection and enumeration of Clostridium spp. —Part 1: Enumeration of sulfite-reducing Clostridium spp. By colony-count technique* (15213–1). (2023).
- *Microbiology of the food chain: Horizontal method for the enumeration of microorganisms, Part 2: Colony count at 30 °C by the surface plating technique* (4833–2). (2013).
- *Microbiology of the food chain—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 1: General rules for the preparation of the initial suspension and decimal dilutions* (6887–1). (2017).
- Nacima, D. D. (2016). Importance of spontaneous plants of steppe arid regions ouled djellel biskra (Algeria). *International Journal of Botany*, *1*, 2455–2541.
- Ochungo, P., Veldtman, R., Kinyanjui, R., Abdel-Rahman, E. M., Muli, E., Muturi, M. N. K., Lattorff, H. M. G., & Landmann, T. (2021). Pollen diversity and protein content in differentially degraded semi-arid landscapes in Kenya. *Journal of Apicultural Research*, *60*(5), 828–841. https://doi.org/10.1080/00218839.2021.1899656

- Official Journal. (2017, July 2). *Official Journal of the democratic and popular Algerian republic*.
- Omafuvbe, B., & Akanbi, O. (2009). Microbiological and physico-chemical properties of some commercial Nigerian honey. *African Journal of Microbiology Research*, *3*.
- Pelletan, J. (2017, March 2). *Beekeeping—Objective professionalization [Algeria]*. Fert. https://www.fert.fr/apiculture-professionnalisation-algerie/
- Pereira, I. A., Moraes, L. A. D., Souza, M. D. A., Finger, R. M., Custódio, F. B., & Teixeira, F. M. (2021). Microbiological quality of honey from family agriculture in the district of cantagalo-rio das ostras-rj. *Journal of Agricultural Sciences Research (2764-0973)*, *1*(2), 2–18. https://doi.org/10.22533/at.ed.9732111115
- Ratiu, I. A., Al-Suod, H., Bukowska, M., Ligor, M., & Buszewski, B. (2019). Correlation Study of Honey Regarding their Physicochemical Properties and Sugars and Cyclitols Content. *Molecules*, *25*(1), 34. https://doi.org/10.3390/molecules25010034
- Raweh, H. S. A., Badjah-Hadj-Ahmed, A. Y., Iqbal, J., & Alqarni, A. S. (2023). Physicochemical Composition of Local and Imported Honeys Associated with Quality Standards. *Foods*, *12*(11), Article 11. https://doi.org/10.3390/foods12112181
- Ricciardelli, D. G. (1998). *Mediterranean Melissopalynology*. Università degli studi di Perugia, Facoltà di agraria, Istituto di entomologia agraria.
- Robin Lim, A. H., Sam, L. M., Gobilik, J., Ador, K., Choon, J. L. N., Majampan, J., & Benedick, S. (2022). Physicochemical Properties of Honey from Contract Beekeepers, Street Vendors and Branded Honey in Sabah, Malaysia. *Tropical Life Sciences Research*, *33*(3), 61–83. https://doi.org/10.21315/tlsr2022.33.3.5
- Rosiak, E., Madras-Majewska, B., Teper, D., Łepecka, A., & Zielińska, D. (2021). Cluster Analysis Classification of Honey from Two Different Climatic Zones Based on Selected

Physicochemical and of Microbiological Parameters. *Molecules*, *26*(8), Article 8. https://doi.org/10.3390/molecules26082361

- Snowdon, J. A., & Cliver, D. O. (1996). Microorganisms in honey. *International Journal of Food Microbiology*, *31*(1–3), 1–26. https://doi.org/10.1016/0168-1605(96)00970-1
- Thrasyvoulou, A., Tananaki, C., Goras, G., Karazafiris, E., Dimou, M., Liolios, V., Kanelis, D., & Gounari, S. (2018). Legislation of honey criteria and standards. *Journal of Apicultural Research*, *57*(1), 88–96. https://doi.org/10.1080/00218839.2017.1411181
- Tornuk, F., Karaman, S., Ozturk, I., Toker, O. S., Tastemur, B., Sagdic, O., Dogan, M., & Kayacier, A. (2013). Quality characterization of artisanal and retail Turkish blossom honeys: Determination of physicochemical, microbiological, bioactive properties and aroma profile. *Industrial Crops and Products*, *46*, 124–131. https://doi.org/10.1016/j.indcrop.2012.12.042
- Velásquez Giraldo, A. M., Vélez Acosta, L. M., & Zuluaga Gallego, R. (2013). Physicochemical and Microbiological Characterization of Apis mellifera sp. Honey from Southwest of Antioquia in Colombia. *Ingeniería y Ciencia*, *9*(18), 61–74.
- Yupanqui Mieles, J., Vyas, C., Aslan, E., Humphreys, G., Diver, C., & Bartolo, P. (2022). Honey: An Advanced Antimicrobial and Wound Healing Biomaterial for Tissue Engineering Applications. *Pharmaceutics*, *14*(8), 1663. https://doi.org/10.3390/pharmaceutics14081663
- Zerrouk, S., M. Carmen, S.-C., Escuredo, O., & Flores, R. (2017). Characterization of Ziziphus lotus (jujube) honey produced in Algeria. *Journal of Apicultural Research*, *57*, 1–9. https://doi.org/10.1080/00218839.2017.1399663

Appendix

Appendix A. Chataway conversion table :

Water Content,	Refractive Index	Water Content	Refractive Index
g/100 g	20°C	g/100 g	20°C
13.0	1.5044	19.0	1.4890
13.2	1.5038	19.2	1.4885
13.4	1.5033	19.4	1.4880
13.6	1.5028	19.6	1.4875
13.8	1.5023	19.8	1.4870
14.0	1.5018	20.0	1.4865
14.2	1.5012	20.2	1.4860
14.4	1.5007	20.4	1.4855
14.6	1.5002	20.6	1.4850
14.8	1.4997	20.8	1.4845
15.0	1.4992	21.0	1.4840
15.2	1.4987	21.2	1.4835
15.4	1.4982	21.4	1.4830
15.6	1.4976	21.6	1.4825
15.8	1.4971	21.8	1.4820
16.0	1.4966	22.0	1.4815
16.2	1.4961	22.2	1.4810
16.4	1.4956	22.4	1.4805
16.6	1.4951	22.6	1.4800
16.8	1.4946	22.8	1.4795
17.0	1.4940	23.0	1.4790
17.2	1.4935	23.2	1.4785
17.4	1.4930	23.4	1.4780
17.6	1.4925	23.6	1.4775
17.8	1.4920	23.8	1.4770
18.0	1.4915	24.0	1.4765
18.2	1.4910	24.2	1.4760
18.4	1.4905	24.4	1.4755
18.6	1.4900	24.6	1.4750
18.8	1.4895	24.8	1.4745
		25.0	1.4740

Appendix B. Codex and EU directive standards :

Parameter	Honey type	Codex	EU directive
Moisture content	Honeys not listed below	not more than 20%	not more than 20%
	Heather honey (Calluna)	not more than 23%	not more than 23%
	baker's honey from heather (Calluna)		not more than 25 %
Electrical conductivity	honey not listed below	not more than 0.8 mS/cm	not more than 0.8 mS/cm
	honeydew and chestnut honey and blends of these with exceptions*	not less than 0.8 mS/cm	not less than 0.8 mS/cm
Free Acidity	in general	not more than 50 meq/kg	not more than 50 meq/kg
	baker's honey		not more than 80 meq/kg

* **Exceptions :** Strawberry tree (Arbutus unedo), Bell Heather (Erica), Eucalyptus, Lime (Tilia spp), Ling Heather (Calluna vulgaris) Manuka or Jelly bush (Leptospermum), Tea tree (Melaleuca spp).

Appendix C. Microbiological limits:

Germs	Standard limit	Source
Total aerobic mesophilic flora	<1000 cfu/g <10000 cfu/g	French standard 2003 Snowdon and Cliver, 1996 Health Protection Agency
Thermotolerant coliforms	Abs	European Union WHO
Sulfite-reducing anaerobic spores	10 cfu/g	Health Protection Agency
Yeast and mold	<100 YM/g	Algerian standard Official Journal (2017)

ملخص

أجريت هذه الدراسة على 6 عينات من عسل السدر تم جمعها من مناطق مختلفة من الجزائر لفحص خصائصها الميكروبيولوجية والفيزيوكيميائية ومحتواها من حبوب اللقاح. تم إجراء التحليل النوعي لحبوب اللقاح بهدف تأكيد الأصل النباتي لعيناتنا. تم تحديد الجودة الميكروبيولوجية من خلال تعداد وكشف: اجمالي الميكروبات الهوائية اليفة الحرارة المعتدلة، القولونيات الكلية ومتحملة الحرارة، الابواغ اللاهوائية المختزلة للكبريتيت، الخميرة والعفن. العوامل الفيزيائية والكيميائية التي تمت دراستها هي محتوى الرطوبة، الناقلية الكهربائي، درجة الحموضة والحموضة الحرة. أظهرت النتائج أن العينات 1،2،3،5، و6 تحتوي على نسبة عالية من حبوب *Ziziphus lotus* (< 50٪) مما يؤكد أصلها النباتي كعسل سدر. ومع ذلك، كانت العينة 4 تحتوي على نسبة أقل من حبوب *Ziziphus lotus* (39٪) بالإضافة إلى أنواع حبوب لقاح أخرى بنسب مماثلة، مما يشير إلى أنه عسل متعدد الأزهار. أظهرت النتائج المتعلقة بالمعايير الفيزيوكيميائية أن عيناتنا تتوافق مع معايير الدستور الغذائي وتوجيهات المجلس الأوروبي. فيما يتعلق بالمعايير الميكروبيولوجية، أظهرت النتائج أن العينات 1، 2، 3، 4، و5 كان تركيزها من إجمالي الميكروبات الهوائية اليفة الحرارة المعتدلة أقل من الحد الميكروبيولوجي المفروض، فقط العينة 6 أظهرت تركيزا أعلى. كانت القولونيات الكلية موجودة في جميع العينات بتركيزات متفاوتة، في حين كانت القولونيات المقاومة للحرارة موجودة فقط في العينات 1، 4، و6 مما يشير إلى أنها ذات نوعية رديئة. ولم يتم الكشف عن الأبواغ اللاهوائية المختزلة للكبريتيت في أي من العينات، في حين ان عدد الخميرة والعفن تباين، حيث أظهرت العينات 1 و2 و4 و5 أعدادًا أعلى من الحد المفروض. يعد عدم وجود قوانين صارمة لمراقبة جودة العسل على المستوى الوطني والدولي مشكلة يجب معالجتها بشكل صحيح لتحسين جودة العسل وحماية المستهلك.

الكلمات المفتاحية: عسل السدر، *Ziziphus lotus*، تحليل حبوب اللقاح، تحاليل فيزيوكيميائية، تحاليل ميكروبيولوجية.

Abstract

This study was carried out on 6 samples of Jujube honey collected in different regions of Algeria to examine their microbiological, pollen and physicochemical properties. Pollen qualitative analysis was carried out with the aim of confirming the botanical origin of our samples. Microbiological quality was determined by the enumeration and detection of: total aerobic mesophilic flora, total and thermotolerant coliforms, anaerobic sulfite-reducing spores, yeasts and molds. The physicochemical parameters studied are moisture content, electrical conductivity, pH and free acidity. The results showed that samples 1, 2, 3, 5 and 6 all had a high frequency of Ziziphus lotus (>50%) confirming their botanical origin as Jujube honeys. However, sample 4 had a lower frequency of Ziziphus lotus (39%) as well as other pollen species at similar frequencies, indicating that it is a multifloral honey. The results relating to the physicochemical parameters showed that our samples comply with Codex standards and European Council directives. Concerning the microbiological parameters, the results showed that samples 1, 2, 3, 4 and 5 had a concentration of total mesophilic aerobic flora lower than the imposed microbiological limit, only sample 6 presented a higher concentration. Total coliforms were present in all samples at varying concentrations, while thermotolerant coliforms were only present in samples 1, 4 and 6, indicating that they are of poor quality. Anaerobic sulfite-reducing spores were not detected in any of the samples, while the yeast and mold count varied, with samples 1, 2, 4 and 5 showing counts above the imposed limit. The lack of strict regulations for honey quality control at the national and international levels is a problem that should be addressed properly to enhance honey's quality and protect the consumer.

Keywords: Jujube honey, *Ziziphus lotus*, pollen analysis, physicochemical analyses, microbiological analyses.

Résumé

Cette étude a été menée sur 6 échantillons de miel de Jujubier collectés dans différentes régions d'Algérie pour examiner leurs propriétés microbiologiques, polliniques et physicochimiques. L'analyse qualitative du pollen a été réalisée dans le but de confirmer l'origine botanique de nos échantillons. La qualité microbiologique a été déterminée par le dénombrement et la détection de : la flore mésophile aérobie totale, les coliformes totaux et thermotolérants, des spores anaérobies sulfito-réducteurs, les levures et les moisissures. Les paramètres physicochimiques étudiés sont la teneur en humidité, la conductivité électrique, le pH et l'acidité libre. Les résultats ont montré que les échantillons 1, 2, 3, 5 et 6 avaient tous une fréquence élevée de Ziziphus lotus (>50%) confirmant leur origine botanique en tant que miels de Jujubier. Cependant, l'échantillon 4 présentait une fréquence plus faible de Ziziphus lotus (39 %) ainsi que d'autres espèces de pollen à des fréquences similaires, ce qui indique qu'il s'agit d'un miel multifloral. Les résultats relatifs au paramètres physico-chimique ont montré que nos échantillons sont conformes aux normes du Codex et des directives du Conseil européen. Concernant les paramètres microbiologiques, Les résultats ont montré que les échantillons 1, 2, 3, 4 et 5 avaient une concentration de flore aérobie mésophile total inférieure à la limite microbiologique imposée, seul l'échantillon 6 présentait une concentration plus élevée. Les coliformes totaux étaient présents dans tous les échantillons à des concentrations variables, tandis que les coliformes thermotolérants n'étaient présents que dans les échantillons 1, 4 et 6, ce qui indique qu'ils sont de mauvaise qualité. Les spores anaérobies sulfito-réducteur n'ont été détectées dans aucun des échantillons, tandis que le nombre de levures et de moisissures variait, les échantillons 1, 2, 4 et 5 montrant des comptes supérieurs à la limite imposée. L'absence de réglementations strictes en matière de contrôle de la qualité du miel aux niveaux national et international est un problème qui devrait être traité correctement pour valoriser et protéger les miels et le consommateur.

Mots clés : Miel de Jujubier, *Ziziphus lotus*, analyse pollinique, analyses physicochimiques, analyses microbiologiques.