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Effect Of Plant and Seasonal Diversity on the Chemical Composition of Pollen Grains in Honeybee Colonies

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ABSTRACT: Laboratory experiments were conducted at the College of Agriculture / University of Basrah to study the effect of vegetation and seasonal diversity on honeybee activity and the chemical composition of pollen grains during the period of November 2023 to the May 2024.

When analyzing the chemical composition of pollen grains, the average protein content was 20.9%, fat 5.5%, total carbohydrates 54.4%, ash 1.8%, and moisture 17.4%, with significant differences observed between the components of the different readings. Fourier Transform Infrared Spectroscopy (FTIR) technique was used to detect the active groups in pollen grains mixed with honey. Many active compounds appeared during the months of the study: November, December, January, February, March, April, and May, belonging to various chemical groups including (O-H), (C-H), (CH2), (C=O), (CH3), and (OH) and other groups in varying proportions for the months mentioned.

INTRODUCTION

Pollen is an important food source as it contains proteins, amino acids and micronutrients necessary to feed bee colonies. Worker bees collect it on their hind legs in a structure called a pollen basket and transport it to the hive, where it is stored and processed to make bee bread, which is used to feed the larvae. This integrated behavior reflects a mutually beneficial relationship, as the plant receives pollination, while the bees receive essential food resources (Roulston and Goodell, 2011).

Pollen and nectar play a crucial role in attracting pollinating insects, including honeybees, to flowers. Both pollen and nectar are key attractants for pollinators, promoting pollination and ensuring the survival of plant species. This relationship between plants and pollinators is part of a mutually beneficial evolutionary relationship between organisms, where each party benefits from the process (Morales and Calvino-Cancela, 2022).

Pollen and nectar, produced by flowering plants to attract pollinating insects, are vital elements and not merely byproducts of the plant. They constitute strategic tools that play a crucial biological role in enhancing the interaction between the plant and pollinators, as pollen provides the protein food necessary for the growth and reproduction of insects, especially honeybees (Nicolson and Human, 2013).

Pollen contains a wide range of active chemical compounds that directly affect honey quality. It is rich in sugars, proteins, amino acids, essential vitamins and minerals, as well as antioxidants such as phenolic compounds. These compounds play a significant role in enhancing the nutritional value of honey and improving its medicinal properties. Furthermore, variations in pollen sources lead to significant differences in the chemical composition of the honey produced (Topitzhofer *et al.*, 2019).

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The diversity of food sources leads to increased activity of bees outside the hive, as they make more daily visits to flowers to collect nectar and pollen, and work to store food in preparation for the winter period. Internal activity within the hive also benefits from this diversity by improving the nutrition of larvae and the queen, increasing wax production, and efficiently distributing food among worker bees (Brown *et al*., 2023).

Diverse vegetation provides a complete food supply of pollen and nectar, which contain proteins, amino acids, vitamins, and minerals essential for the proper nutrition of honeybee larvae. It also enhances the larvae's resistance to diseases and parasites. Furthermore, the balanced nutrition resulting from diverse plants contributes to increased queen activity, leading to the continuous production of healthy eggs and thus maintaining a stable colony population. All these factors make plant diversity a key element in maintaining brood health and ensuring the long-term productivity of bees (Kaluza *et al*., 2017).

The quantity and quality of pollen collected within the hive are important indicators of the bees' foraging efficiency and the diversity of their plant sources. Bees collect multiple types of pollen, which contributes to improved internal nutrition within the hive and increases the protein and mineral content of stored food. Furthermore, dietary diversity provides better support for larvae and the queen, and enhances the bees' resilience to disease and environmental stress. (Johnson and Lee, 2023).

Therefore, this study was conducted with the aim of evaluating the effect of plant diversity on honeybee activity during flowering seasons in Basrah Governorate / southern Iraq, and the effect of this seasonal variation on pollen collection and the diversity of its active compounds.

MATERIAL AND METHODS

The study samples (pollen and honey) were collected from local bee colonies in the College of Agriculture apiary - University of Basrah, Karma Ali site, which is surrounded by perennial trees, ornamental plants, field crops, vegetables, and others during the 2023-2024 season.

Chemical Composition of Pollen

Pollen samples were collected directly from beehives during the study period, taking into account different growing seasons to ensure representation of the seasonal diversity of plants cultivated in the region. Pollen was collected using special tweezers and then placed directly into sterile tubes. The samples were stored at -18°C until testing. Collection times were carefully chosen to coincide with peak bee activity. Chemical analyses were performed on the pollen grains to identify the proportions of their components, with three replicates per sample.

Moisture content estimation:

A suitable quantity of pollen mixture was taken, 2 grams were isolated, and placed in an electric oven at 100°C for 90 minutes. The moisture content was calculated according to the formula in A.O.A.C. (2020) and as in the equation below:

$$\text{Moisture \%} = \frac{\text{Weight of the sample before drying } \times \text{ Weight of the sample after drying}}{\text{Weight of the sample before drying}} \times 100$$

Protein percentage estimation:

The protein percentage was estimated based on the micro-Kjeldahl method using the Buchi 343 device and at a conversion factor of 6.25 (A.O.A.C. 2020).

Fat content determination:

The fat content was determined using a Soxhlet assay with petroleum ether as the extraction solvent (A.O.A.C. 2020).

Ash content (minerals):

The ash content was determined by burning 5 g of a pollen sample in an electric oven at 550°C for 10 hours until the sample turned white (A.O.A.C. 2020).

Total carbohydrate percentage:

The carbohydrate percentage was estimated using the following equation, as reported in Kalev and Shafir (2002).

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$$\text{Carbohydrates}\ \% = 100 - (Protein\% + fat\% + moisture\% + ash\%)$$

Active compounds in pollen identified by Fourier Transform Infrared Spectroscopy (FTIR):

Pollen samples from honey were taken and mixed separately with KBr, formed into dry discs, and their infrared spectrum was recorded using a Japanese Shimdzu FT-IR device belonging to the Chemistry Department/College of Education for Pure Sciences/University of Basrah.

Statistical Analysis:

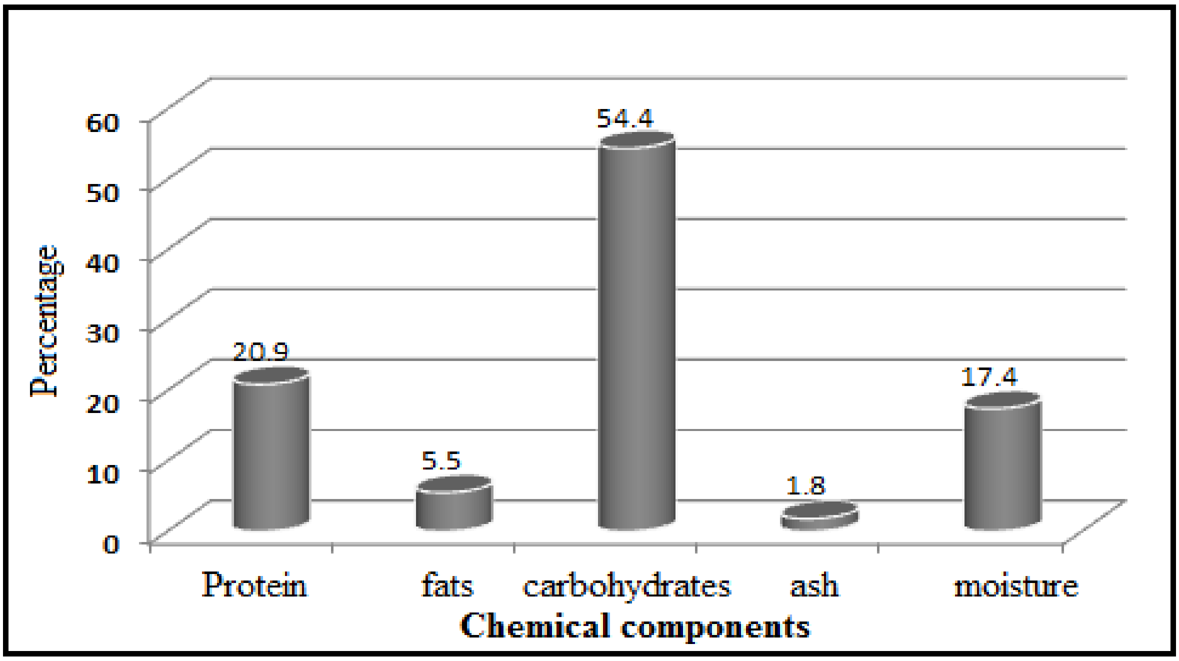
The experiments were carried out using a completely randomized design (CRD) and the Least Significant Difference (LSD) test at the 0.01 level, and were analyzed using the SPSS statistical software.

RESULTS AND DISSCUSION

Chemical content of pollen grains

Figure (1) shows the total content of chemical components in the pollen grain collections taken from beehives by direct collection method. The average protein content of the pollen grains was 20.9%, the percentage of fats was 5.5%, the percentage of total carbohydrates was 54.4%, while the percentage of ash was 1.8%, and the percentage of moisture was 17.4%.

It was found that the protein content in bee pollen ranges between 2.90-33.51% and depends on a range of factors, including the plant source (Somerville and Nicol, 2006). Kędzia and Hołderna-Kędzia (2005) also found that pollen contains protein at a rate of 22.7% of its natural components and has important biological properties that are necessary for bee nutrition.



L.S.D (P ≤0.01) = 3.77

Figure (1) Chemical content of pollen grains.

After the pollen collected by bees, it undergoes a series of processes that lead to the production of bee bread. Genetic factors also affect the pollen properties according to the plant source, and this effect leads to a difference in its nutritional content, which may affect the growth and activity of the colony members (Khachatryan *et al.,* 2008). The components of bee pollen depend directly on the plant source and its geographical origin, in addition to the type of soil, climatic factors, genetic factors, and the different methods of raising and the activity of the bees (Nogueira *et al.,* 2012; Silva *et al.,* 2014).

Active compounds in pollen grains identified by Fourier Transform Infrared Spectroscopy (FTIR) technology

Figures (2-8) and the accompanying Tables (1) and (2) show the most important locations of the infrared spectral bands representing the active groups in the pollen-honey mixture for the months of November, December, January, February, March, April, and May of the study period. Band groups appeared at wavenumbers 3390.86 - 3840.27 cm^{-1} during November, and similar bands appeared in December at wavenumber 3390.86 cm^{-1}, in January at wavenumber 3410.15 - 3849.92 cm^{-1}, and in February at wavenumber 3387.00 cm^{-1}. These bands also appeared in March, April, and May at wavenumbers 3381.21 cm^{-1}, 3379.29 cm^{-1}, and 3377.36 cm^{-1}, respectively. These are all types of The stretching vibration that belongs to the (O-H) group (Castiglioni *et al.,* 2019).

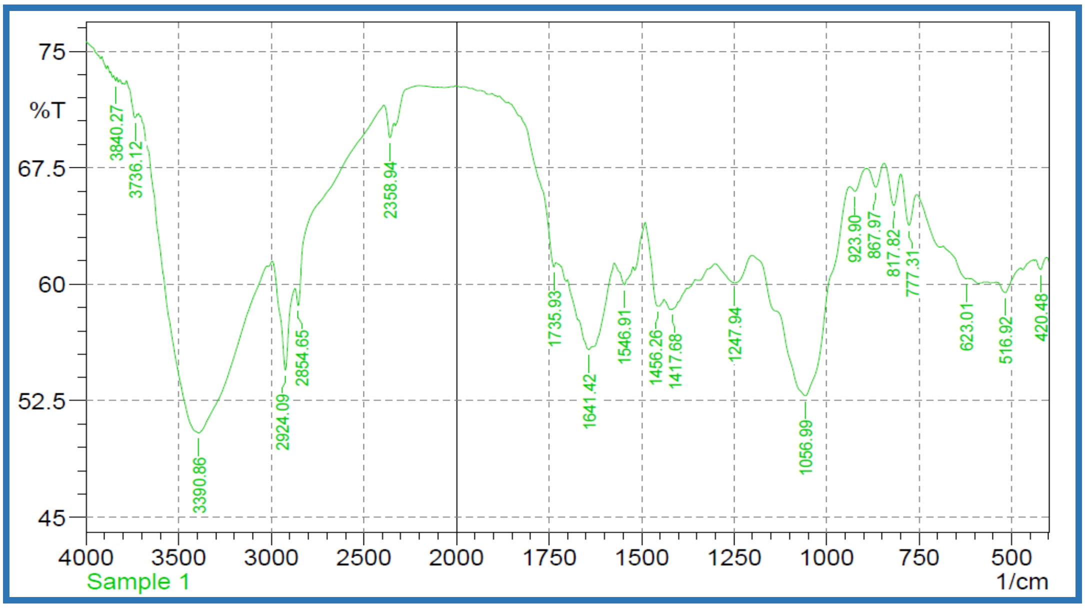


Figure (2): The active compounds of the pollen mixture identified using FTIR technology for the month of November.

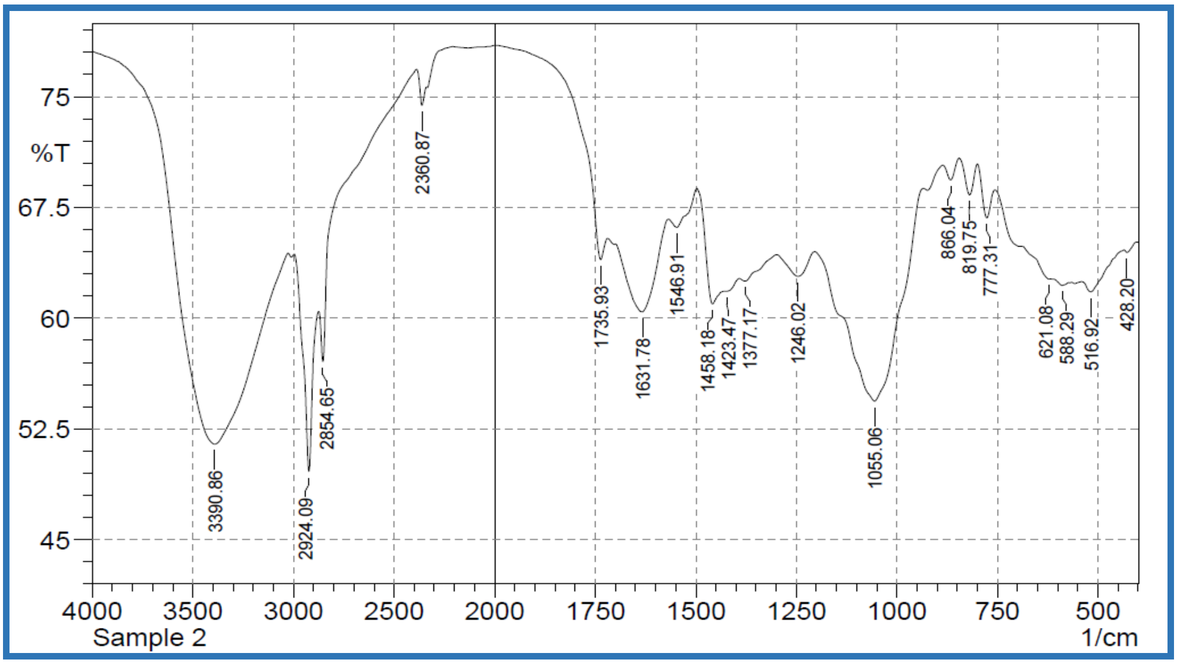


Figure (3): The active compounds of the pollen mixture identified using FTIR technology for the month of December.

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For the months of November, December, February, March, and April, the wavenumber 2924.09 cm^{-1} was the same, as were January and May, which had wavenumbers of 2920.23 and 2926.01 cm^{-1}, respectively. All of these belong to the (C-H) group (Svečnjak *et al.,* 2017).

Furthermore, spectral bands were formed, ranging from wavenumber 2358.94 to 2854.65 cm^{-1} in November to wavenumber 2291.43 cm^{-1} in May. These belong to the (CH_2) group, associated with hydrocarbons (Wu *et al.,* 2008).

A band belonging to the (C=O) group appeared in the pollen mixture with honey, which belongs to the carbon group of amino acids as well as flavonoid compounds, starting from the range of wave numbers 1641.42-1735.93 cm^{-1} for November and 1631.78-1735.93 cm^{-1} for December and the range 1639.49-1730.15 cm^{-1} for January, and the wave number 1647.21 cm^{-1} representing February, and the range 1649.14 -1735.93 cm^{-1} for March, reaching the wave number 1649.14 cm^{-1} representing April and May respectively.

The spectrum of the bands also appeared at the wavenumber range of 1417.68 -15546.91 cm^{-1} up to the range of 1413.82 - 1544.98 cm^{-1} for all months of the study respectively for the groups (C-H), (OH), (CH_3) found in both cellulose and lipids and the ketone groups of fructose and the aldehyde groups of glucose (Castiglioni *et al.,* 2019). A band also appeared at wavenumbers of 1377.17 cm^{-1} for January, 1348.24 cm^{-1} for February, 1348.24 cm^{-1} for April, and 1344.33 cm^{-1} for May respectively, which belong to the groups (-OH) and (C-OH) (Castiglioni *et al.,* 2019).

It was found that the bands ranging within the wavenumber intervals 923.90-1056.99 cm^{-1} reaching up to the range 921.97-1058.92 cm^{-1}, fall within the groups assigned to stretching vibrations of (C–C), (C–N), and (C–O) belonging to proteins and carbohydrates (Anjos *et al.,* 2017), in addition to the appearance of various bands belonging to other chemical groups. These results are consistent with those obtained by Meryem *et al.* (2022) through their identification of the active groups in an infrared spectroscopy of pollen-honey mixture taken from Moroccan bee colonies.

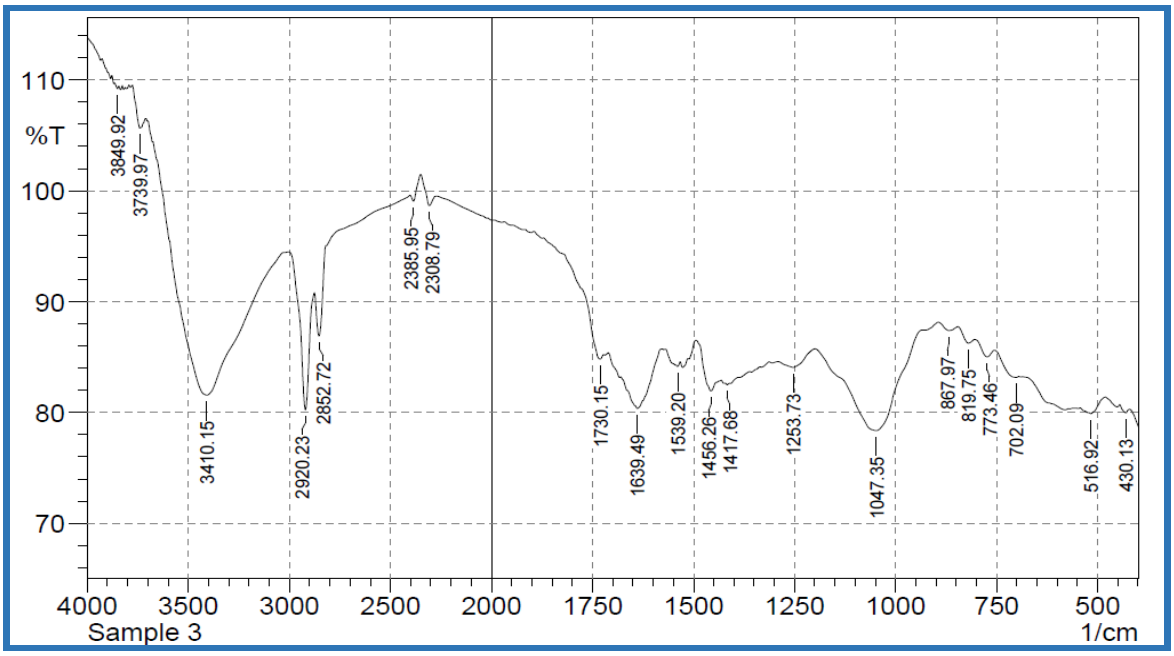

Figure (4): The active compounds of the pollen mixture identified using FTIR technology for the month of January.

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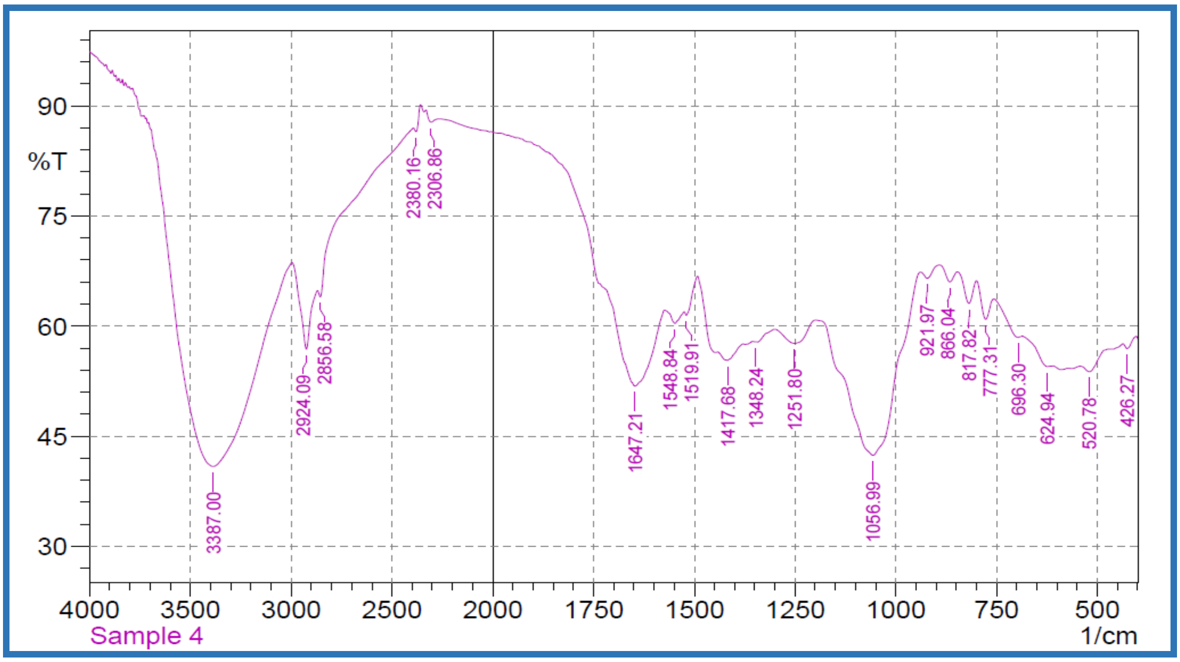

Figure (5): The active compounds of the pollen mixture identified by FTIR technology for the month of February.

Table (1) Active compounds of pollen mixture identified by FTIR for the months of November, December, January and February.

Active groups	November	December	January	February
ν (O-H) in H2O	3840.27-3390.86	3390.86	3849.92-3410.15	3387.00
ν (C-H)	2924.09	2924.09	2920.23	2924.09
(NH3)	2854.65-2358.94	2854.65-2360.87	2852.72-2308.79	2856.58-2306.86
δ (O-H)	1735.93-1641.42	1735.93-1631.78	1730.15-1639.49	1647.21
δ (O-CH) and δ (C-C-H)	15546.91-1417.68	1546.91-1423.47	1539.20-1417.68	1548.84-1417.68
δ (O-H)	---	1377.17	---	1348.24
δ (-OH) in C-OH group	1247.94-	1246.02	1253.73	1251.80
ν (C-O) in C-OH group or ν (C-C)	1056.99-923.90	1055.06	1047.35	1056.99-921.97
Other groups	876.97-420.48	866.04-428.20	867.97-430.13	866040-426.27

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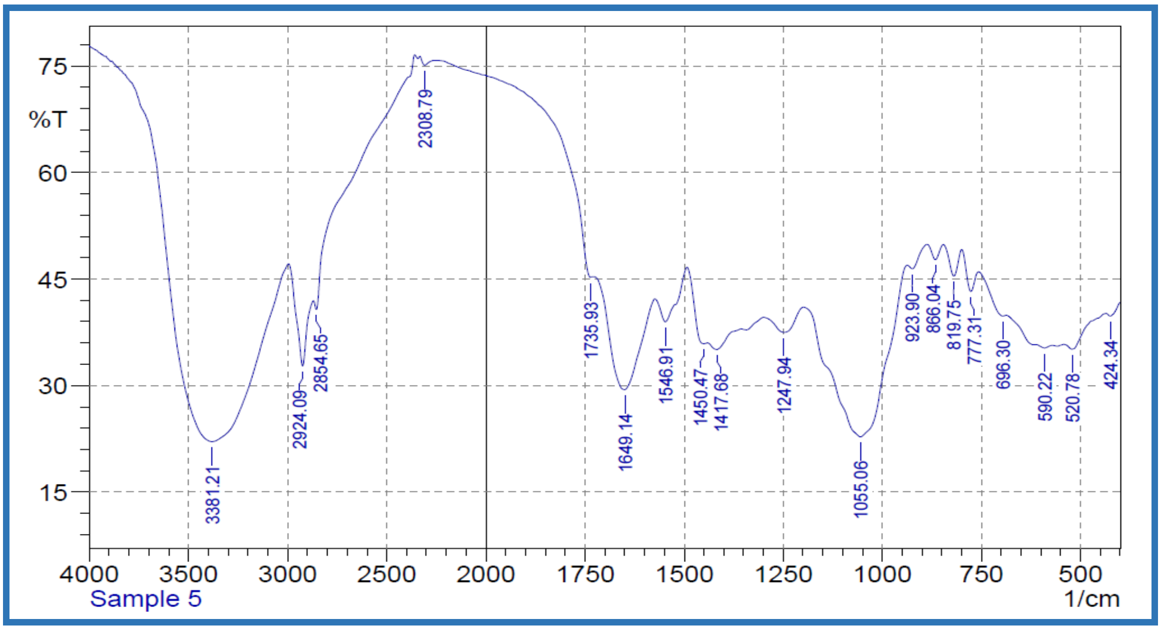

Figure (6): The active compounds of the pollen mixture identified by FTIR technology for the month of March.

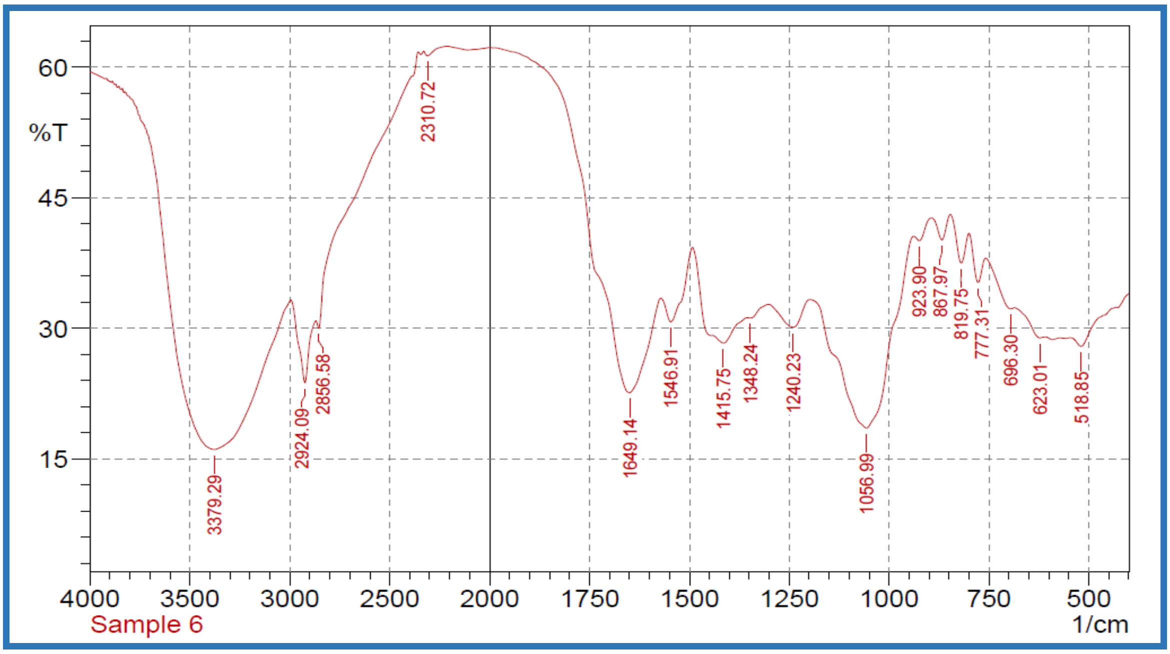

Figure (7): The active compounds of the pollen mixture identified by FTIR technology for the month of April.

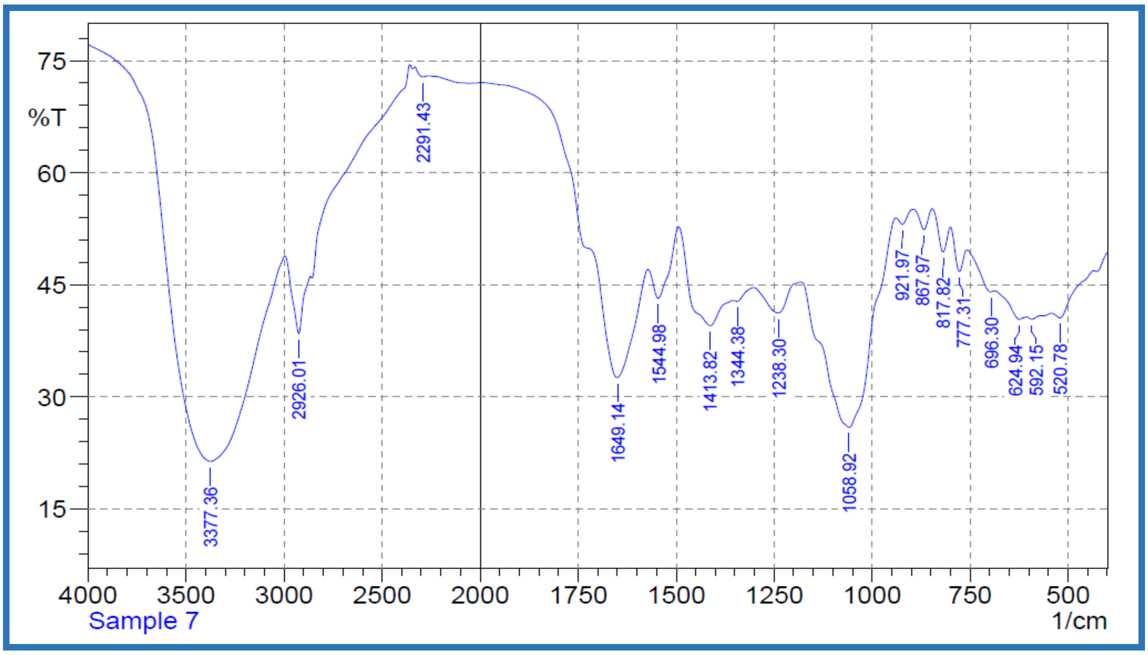

Figure (8): The active compounds of the pollen mixture identified by FTIR technology for the month of May.

Table (2) Active compounds of pollen mixture identified by FTIR for the months of March, April and May.

Active groups	March	April	May
ν (O-H) in H2O	3381.21	3379.29	3377.36
ν (C-H)	2924.90	2924.09	2926.01
(NH3)	2854.65-2308.79	2856.58-2310.72	2291.43
δ (O-H)	1735.93-1649.14	1649.14	1649.14
δ (O-CH) and δ (C-C-H)	1546.91-1417.68	1546.91-1415.75	1544.98-1413.82
δ (O-H)	---	1348.24	1344.33
δ (-OH) in C-OH group	1247.94	1240.23	1238.30
ν (C-O) in C-OH group or ν (C-C)	1055.06-923.90	1056.99-923.90	1058.92-921.97
Other groups	866.04-424.34	867.97-518.85	867.97-520.78

Honeybees are known to collect pollen from a single plant species or a group of flowering plant species (Cane and Sipes, 2006). The feeding of honeybees on flowers plays a fundamental role in the activity and vitality of colonies by providing the energy and biochemical components necessary for brood rearing, strengthening the body's immune system, and regulating the colony's temperature. Bees are fed from two main sources: nectar and pollen. Pollen provides bees with proteins, fats, vitamins, and minerals (Brodschneider and Crailsheim, 2010). Flowers have evolved to produce a harmony between pollen and nectar within their physiological structure to attract bees throughout the year and its seasons, such as bright colors, distinctive fragrance, and the availability of nectar rich in sugars (Potts *et al.,* 2010; Cresswell *et al.,* 2022).

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