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# Pollen Analysis of Natural Honey Collected from District Dadu, Sindh Pakistan

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**Abstract:** The present study aims to investigate different samples of honey from District Dadu Sindh for its pollen analysis. For this investigation, eight different locations were selected for honey sampling. The evaluation of different pollen parameters of several honey samplesrevealed variation in terms of quantity, quality and pollen parameters, such as pollen count, pollen size, pollen shape number and position of the opening of the pollen. Based on their mellissopalynological research, 25 plant species belonging to 18 different families were identified. Of 18 families, *Mimosaceae* was observed as the most dominant family in the area sampled with its three representatives. Nevertheless*; Rhamnaceae*, *Fabaceae* and *Myrtaceae*, were registered as the second main families in this contest with two species of each. *Zizyphus jujuba* was noted as the most attractive taxa for bees in the sampled area because it was present in four samples out of a totaleight. *Dalbergia sissoo, Acacia niloticaand Lawsonia alba* were graded as the second leading taxon in the area in which they were present in three of eight samples.*Capparis decidua, Mangifera indica, Psidium guajava, Rosa sp., Azadirachta indica* and *Brassica campestris* were present in two samples and thus occupied third leading taxon in the sampling area. The study would be helpful for botanists in general and melissopalynologists particularly to promote honey industry in the area.

**Keywords:** *Mellissopalynology, DistrictDadu; Natural Honey, Pollen*

## 1. INTRODUCTION

Microscopic examination of honey pollen provides authentic information regarding the floral sources which bees are using and gives clear indication about botanical and geographical origin of honey. This information is often used to assess the quality of honey and the season of honey production, because it is correlated with flowering period. Variation in honey color, density, taste, and pollen contents depend upon the diversity in nectar source, seasonal change and distribution of plant tax a in a particular region (Babi *et al.,* 2008).

As nectar and pollen are the major sources of the food for honeybee. It is estimated that bee larva from egg to maturity requires about 142m-l of honey (Winston et al.,1989). Pollen is the major source of protein, minerals, fatty acidic, and vitamins. It is very important for the growth of larva and young adult bees. The collection of the pollen is done by worker bees of the colony when the workers are "loaded" with pollen they return to their hives. Once at hive, workers pack pollen into the comb, phytocidial acid is added to prevent bacterial growth and pollen germination, when pollen completely proceed for storage, the pollen comb is referred as "bee-bread"(Baum. 2011) Pollen analysis plays vital role in the assessment of honey, the presence of different types of pollen in honey is the reflection of floral nectar source utilized by bee to produce honey. Relative pollen frequency is often used to verify the major and minor nectar source of honey.

This information is important, especially for commercial purposes, because honey made from specific plant species, commands a premium price. Identifying and quantifying the pollen in honey samples is one of the best way to determine range of a nectar source used to produce honey. An additional reason that the pollen analysis of the honey is often required to identify geographical source of origin (Bryant .2001).

**MELLISSOPALYNOLOGY** is applied branch of palynology which deals with the study of pollen in honey samples and its applications in apiculture. Thus, mellissopalynology is concerned with the identification of pollen in honey (Bhargava. 2009). Mellissopalynology and mellittopalynology are synonyms because both; **Mellissa** or **melitta** means **"a bee".** Nowadays, study of pollen in honey is considered as an important area of research. Various pollen morphological features such as; symmetry, shape, size, aperture pattern and exine configuration are reliable source for the taxonomic assessment of the plants (Noor *et al.,* 2017). Each of plant taxa possess different pollenomorphic characteristics which may be used for authentic identification of plant species, not only this but even composition of pollen in honey is greatly valuable in identifying of a botanical and geographical origin of honeyvarious honey (Atrouse.2004).

## INTRODUCTIONTOSITEAREA

British administration constituted *DADU* as a district in

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1933 by merging two tehsils (KotriandKohistan) form Karachi district and five tehsils (Mehar, Khairpur Nathan Shah, Johi, Sehwan and Dadu) from Larkana district. District Dadu shares its boundaries with districts; NausheroFeroze in the East, ShahdadKot at Qambar in the North, Jamshoro in the South and in the West Kirthar range is located. The total area of this district is 19,070 square kilometers, which is divided into seven talukas i.e. Johi, Khaipur Nathan Shah, Mehar, Phulji Station, Beto Jatoi, TharriMohabat and Sargari. It remained the largest district until December, 2004 when it was again pronged, in order to create a new district with the name of Jamshoro, on December, 13, 2004. According to the census of 1998, the total population of this district is approximately 1,688,810. Population density of this district is 88.6 person / square kilometers. Form the total population 79% is living in the rural areas while 21% of the total population is the resident of urban areas. Cultivated crops of this district are; wheat, rice cotton and sugar-cane. Most important plants of this district are*Acacia nilotica*, *Azadirachtaindica*, *Psidium guajava*, *Prosopis glandulosa*, *Lawsonia alba*, *Zizyphus jujuba*, *Dalbergia sisso* and *Mangifera indica*. All these are high ranked commercially and medicinally valuable plants. Major cereal crops in the area are *oryza satavium*, *triticum indicum*,and *Zea-mays*. Among the pulses *Arachis hypogaea* and *Vigna radiata* are very common however, chief vegetables of this area are *Solanum tubrisum*, *Lycopersicum esculentum*, *Capsicumannuum*, *Solanum malongena*, *and Allium cepa*. As compared to all vegetables; chilies and tomatoes are cultivated at large scale in this area.

Literature stated that several mellissopalynological studies have been conducted worldwide, especially in; New Zealand, Brazil, UK, Australia, Turkey and in India. Presently, India is considered as the world's largest honey contributing state. Unfortunately, no remarkable work has been reported across Pakistan. Therefore, the present work is carried out to determine the critical analysis of various honey samples in order to identify pollen flora of district Dadu, Sindh, Pakistan.

## 2. MATERIAL AND METHODS

Eight honey samples were collected from different localities of district Dadu i-e; Johi, Khairpur Nathan Shah, Mehar, Seeta Road, Phulji Station, TharriMuhabbat, Khanpur and BetoJatoi. The collection was made during March-May and September-October, 2012-2013. The collection of honey sampling was started from Johi. All samples were processed from plant species. All honey samples were collected in the sterilized polyethylene bottles from the beehives. Thereafter, honey samples were filtered through single thickness fine cloth to remove all suspended particles such as; beeswax, dirt and other impurities. All samples were stored in air tight container at room temperature for further investigation. Then the samples were brought into the research laboratory of Institute of Biotechnology and Genetic Engineering, University of Sindh Jamshoro. Samples were acetalized one by one and slides were prepared from each sample following acetolysis method adopted from Erdtman with slight modification.The followed procedure is mentioned below.

About 5 ml. of honey was dissolved in 10 ml. of distiled water, centrifuged for 10 minutes at 2500 rpm and exrtra water above the sediment was poured.Samples were treated with 5 ml.of glacial acetic acid, cetrifuged at 2500 rpm for 10 minutes.The sediments were acetalized at a ratio of (9:1).One part of concentrated Suphuric acid was added to the 9 parts of acetic anhydride. To avoid anyexothermic reaction, sulphuric acid was added drop wise to the acetic anhydride and then after, the acetalized mixtures were warmed in water bath for 5to 6 minutes or until the water turned chestnut brown in appearance. After cooling,mixtures were cetrifuged again at 2500 rpm for 10 minutes. The supernatant material from the samples was decanted off in order to remove acid.

Sediments were further treated with (GAA) and centrifuged again at 2500 rpm for 10 minutes. Samples were washed again with distlled water three or four times. After each rinsing they were cntrifuged at 2500 rpm for 10 minutes.

Finally 50% glycerinwas added to the residue (prepared in distlled water) and centrifuged for 10 minutes at 3500 rpm.The supernatant liquid was decanted off. Later on few dropes of phenol were also added in order to protect the samples from microbial decomposition.

### PREPARATIONOFSLIDES

Microscopic slides were prepared from each of the treated pollinoferous material.Each slide was labelled with specific code of its locality specifically; at least ten slides were prepared from each sample, using wet mount technique (Atrouse, Oran, 2004). The pollen sediment was taken on a pallet of glycerin jelly and transferred to the centre of the slides of **75× 25** mm size and was **0.8** mm in thickness. After warming it gently, the melted jelly with pollinic material was covered by cover glass (22 mm). An extra care was taken while placing the cover glass on sediment just to avoid bubble formation.The cover glass was sealed later with paraffin wax. By adopting such procedure permanent slides were made andexamined microscopically.

## MICROSCOPYANDMICROPHOTOGRAPHY

Slides representing each sample were then examined using Olympus BX 50 microscope at manificationof 40x and 100x and microphotographs of pollensfrom each slide were taken with the help of an Olympus camera Model U-CMAD 2 and all pollen morphplogical characteristics were keenly examined. The pollen record of the each sample was maintained carefully.

## MEASUREMENT

Pollen, were measured with the help of an ocular Division (Erma) and unit of measuring was converted into µm ( milimicron), at least 15 pollen grains from each sample were measured. The terminology of pollen used in the present study is according to the researchers like Erdtman (1952) Kremp (1965) and Faegri (1964).

## POLLENCOUNT

For pollen count per 5 ml of honey, the method of J.Louveaux (1970) was followed. The quantitive analysis of the pollen was done, based on the method recommended by International Commission for Bee Botnay (ICBB). Counts were taken randomly. Covering the maximum mounted area to avoid repetition. Once the pollen were identified then were counted. Based on pollen frequency classes they were placed into following 5 categaries;

1) Predominant pollen (> 45%)
2) Secondary pollen type: (16-44%)
3) Important minor pollen types (3-15%)
4) Minor pollen types (< 3%) and
5) Pollen present (<1%)

## IDENTIFICATIONOFPOLLENFROMEACHHONEYSAMPLE

Pollen taxa were idetified from each of the traeted honey sample with the help of authentic available litrature, pollen atlas and published research floras, in which brief descriptions of observed honey pollen is given. For pollen identification mostly the help was taken fromthe work of Wodehouse (1935) and Martin (2017).Pollen morphological characters were also referred withH.P. Gupta (1986), with pollen atlas of P.A .Collinvaux (2003).and an atlas of air born pollen byH. Hyde (1958).For extensive description **(Table: 1).**

**Table 1: Collection Sites of Honey Samples from District Dadu**

| SAMPLE NO. | LOCALITY | LAT:26.02° N LNG:67.48° E | DATE OF COLLECTION | SOURCE OF HONEY |
|---|---|---|---|---|
| **D1** | Johi | 20 KM EAST | 6-10-2012 | *(Salvadorapersica)* |
| **D2** | K.N shah | 30 KM NORTH | 9-10-2012 | *(Mangiferaindica)* |
| **D3** | Mehar | 10 KM NORTH | 13-10-2012 | *(Dalbergiasissoo)* |
| **D4** | Seeta Road | 30 KM NORTH WEST | 14-10-2012 | *(Acacia nilotica)* |
| **D5** | Phulji Station | 10 KM EAST | 16-10-2012 | *(Zizypusjujuba)* |
| **D6** | Tharri Muhabbat | 50KM NORTH | 19-10-2012 | *(Prosopisglandulosa)* |
| **D7** | Khanpur | 35 KM NORTH | 21-10-2012 | *(Albizzia lebbeck)* |
| **D8** | Beto Jatoi | 46 KM NORTH | 24-10-2012 | *(Acacia nilotica)* |

**Note:** LAT= *Latitude*; LNG= *Longitudes*; D= *Dadu***Source:** *Developed by the researcher*

## 3. RESULTS

**Table: 2: The Results of Pollen Analysis of Natural Honey Collected from District Dadu, Sindh Pakistan**

| Sample No. | Total pollen count per 5 ml | Pollen characteristics | | | Species | Family | Honey Type |
|---|---|---|---|---|---|---|---|
| | | Pollen sizein (µ) | Pollen Shape | Pollen Aperture | | | |
| **01 Johi** | 30000 | 39 – 45 L<br>28 – 34 B | Prolate to per prolate | Tricolporatecolpi long | *Lathyrus sativus* | Fabaceae | Unifloral |
| | | 39 – 45 L<br>25– 31 B | Prolate | Tricolpate, colpi long | *Dalbergia sissoo* | Fabaceae | |
| | | 10 – 16 L<br>10 – 16 B | Spheroidal | Heteropolar, Diporate | *Ficus religiosa* | Moraceae | |
| | | 52 – 58 L<br>42– 48 B | Sub prolate | Inaperturate | *Acacia nilotica* | Mimosaceae | |
| | | 18 – 23 L<br>13– 17 B | Prolate | Tricolporate | *Capparis decidua* | Capparidaceae | |
| **02 K.N. Shah** | 34000 | 25 – 30 L<br>12 – 18 B | Oblate | Tricolporate, colpi long | *Psidium guajava* | Myrtaceae | Multi-floral |
| | | 35 – 41 L<br>15– 21 B | Prolate | Tricolporate | *Mangifera indica* | Anacardaceae | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 18 – 22 L 16–21 B | Prolate spheroidal | Tricolporate | *Lawsonia alba* | Lythraceae | |
| | | 32 – 38 L 22– 28 B | Sub oblate | Tricolporate | *Zizyphus jujuba* | Rhamnaceae | |
| | | 21 – 27 L 13– 19 B | Oblate | Tricolporate ,longicolpate | *Syzygiun cumini* | Myrtaceae | |
| **03 Mehar** | 28000 | 35 – 4 L 15 – 21 B | Prolate | Tricolporate | *Mangifera indica* | Anacardaceae | Multi-floral |
| | | 25 – 31 L 13– 19 B | Oblate | Tricolporate, colpi long | *Psidium guajava* | Myrtaceae | |
| | | 17 – 22 L 15– 21 B | Prolate, Spheroidal | Tricolporate | *Lawsonia alba* | Lythraceae | |
| | | 39 – 45 L 37– 42 B | Prolate Spheroidal | Tetracolporate | *Azadirachta indica* | Meliaceae | |
| | | 22 – 27 L 17– 22 B | Prolate spheroidal | Punctitegillate, furrows long | *Solanum melongena* | Solanaceae | |
| **04 Seeta Road** | 26000 | 19 – 25 L 13 - 19 B | Sub prolate | Tricolporate | *Prosopis glandulosa* | Mimosaceae | Multi-floral |
| | | 33 – 39 L 21 – 27 B | Sub oblate | Tricolporate | *Zizypus jujuba* | Rhamnaceae | |
| | | 47 – 53 L 37 – 43 B | Sub prolate | Inaperturate | *Acacia nilotica* | Mimosaceae | |
| | | 17 – 23 L 11 – 17 B | Oblate | Triporate | *Ficus bengalensis* | Moraceae | |
| | | 20 – 26 L 14 – 20 B | Prolate | Tricolporate | *Salvadora persica* | Salvadoraceae | |
| **05 Phulji Station** | 20000 | 37 – 43 L 23 – 29 B | Prolate | Tricolporate, colpi long | *Dalbergiasisso* | Fabaceae | Multi-floral |
| | | 32 – 38 L 22– 28 B | Sub oblate | Tricolporate | *Zizyphusjujube* | Rhamnaceae | |
| | | 19 – 25 L 11 – 17 B | Prolate | Tricolporate | *Rosa sp.* | Rosaceae | |
| | | 32 – 38 L 22 – 28 B | Oblate spheroidal | Colporate | *Eucalyptiscamaldulensis* | Myrtaceae | |
| | | 25 – 30 L 14 – 18 B | Perprolate | Tricolpate Colpi long | *Coriandrum sativum* | Apiaceae | |
| **06 Tharri Mohabbat** | 12000 | 25 – 31 L 25 – 31 B | Spheroidal | Inaperturate | *Albizzialebbeck* | Mimosaceae | Multi-floral |
| | | 19 – 24 L 14 – 18 B | Prolate | Tricolporate | *Capparis decidua* | Capparidaceae | |
| | | 47 – 53 L 37 – 43 B | Sub prolate | Inaperturate | *Acacianilotica* | Mimosaceae | |
| | | 28 – 34 L 25 – 31 B | Sub prolate | Tricolporate | *Capsicum annum* | Solanaceae | |
| | | 38 – 44 L 38 – 44 B | Spheroidal | Tricolporate | *Moringaoleifera* | Moringaceae | |
| **07 Khan Pur** | 44000 | 37 – 43 L 23 – 29 B | Prolate | Tricolporate, Colpi long | *Dalbergiasissos* | Fabaceae | Unifloral |
| | | 17 – 23 L 13 – 21 B | Prolate, Spheroidal | Tricolporate | *Lawsonia alba* | Lythraceae | |
| | | 32 – 38 L 22– 28 B | Sub oblate | Tricolporate | *Zizyphusjujube* | Rhamnaceae | |
| | | 32 – 38 L 28 – 33 B | Sub oblate | Tricolporate | *Carica papaya* | Caricaceae | |
| | | 15 – 20 L 10 – 16 B | Prolate to sub prolate | Triporate | *Brassica campestris* | Brassicaceae | |
| **08 Beeto Jatoi** | 28000 | 32 – 38 L 31 – 37 B | Oblate spheroidal | Tetracolporate, colpi linear | *Manilkarazapota* | Sapotaceae | Multi-floral |
| | | 15 – 20 L 10 – 16 B | Prolate to sub prolate | Triporate | *Brassica campestris* | Brassicaceae | |
| | | 38 – 44 L 36 – 42 B | Prolate spheroidal | Tetracolporate | *Azadirachtaindica* | Meliaceae | |
| | | 37 – 43 L 28 – 35 B | Prolate | Tricolporate | *Citrus limon* | Rutaceae | |
| | | 19 – 25 L 12 – 18 B | Prolate | Tricolporate | *Rosa sp.* | Rosaceae | |

**Note: ML= *Mile liter*; µ= *Micron*; L= *Length*; B= *Breadth***

**Plate. 1: Photomicrographs of pollen taxa identified from various honey samples of district Dadu, Sindh, Pakistan**


**Note: 1.All figures were taken at40x and 100x**

**Note: 2. 1=Acacia nilotica; 2= Albizzia lebbeck;3= Azadirachta indica;4= Brassica campestris; 5= Citrus limon; 6= Carica papaya; 7= Capsicum annum; 8= Coriandrum sativum; 9= Capparis decidua; 10= Dalbergia sissoo; 11= Eucalyptus camaldulensis; 12= Ficus religiosa; 13= Ficus bengalensis; 14= Lathyrus sativus; 15= Lawsonia alba; 16= Mangifera indica; 17= Manilkara zapota; 18= Moringa oleifera; 19= Prosopis glandulosa; 20= Psidium guajava; 21= Rosa sp.; 22= Salvadora persica; 23= Solanum melongena; 24= Syzygium cumini; 25= Zizyphus jujuba**

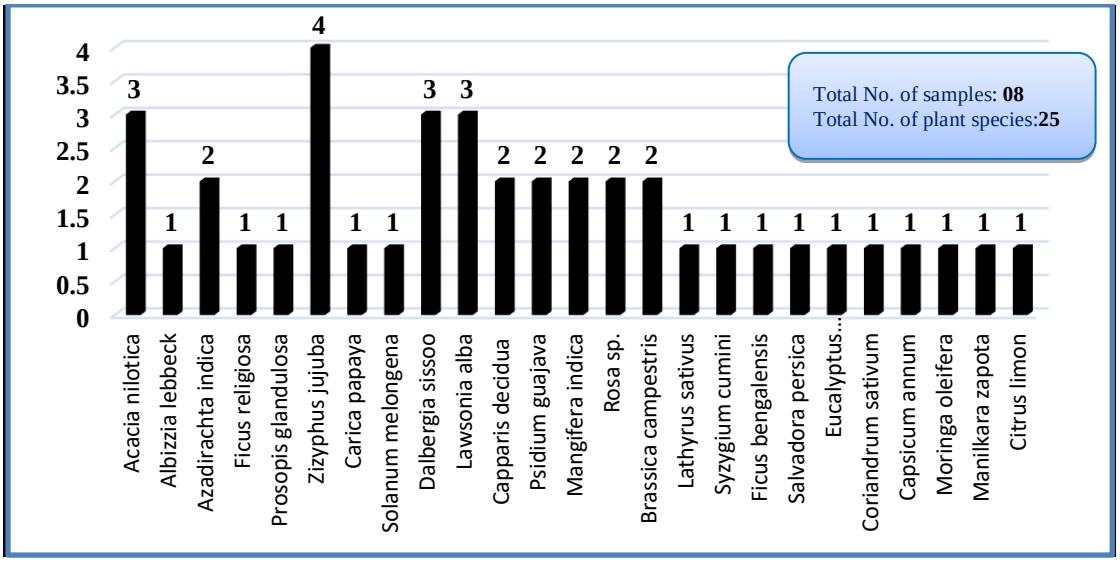


**Fig.1. Representation of variousplant taxa in eight different honey samples, collected from district Dadu, Sindh, Pakistan**

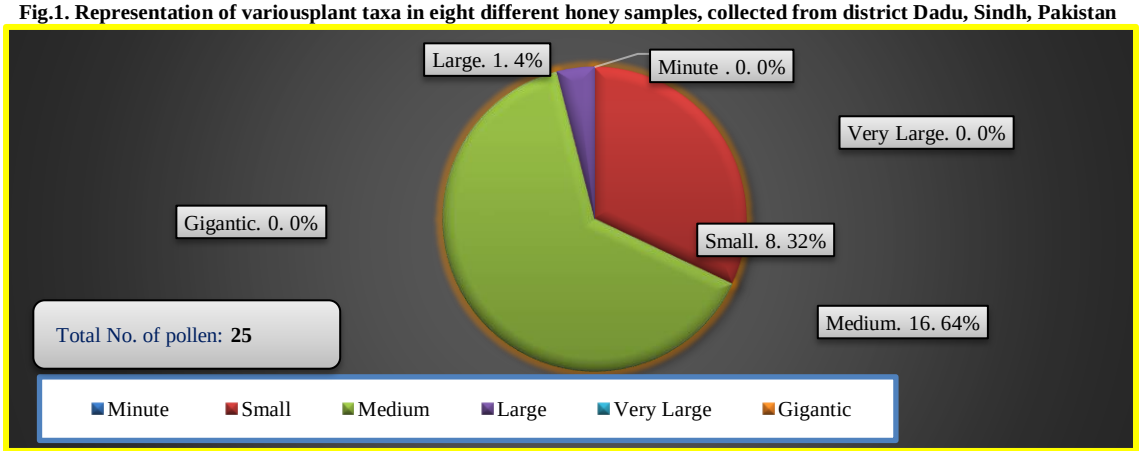


**Fig.2. Diversity in pollen size**

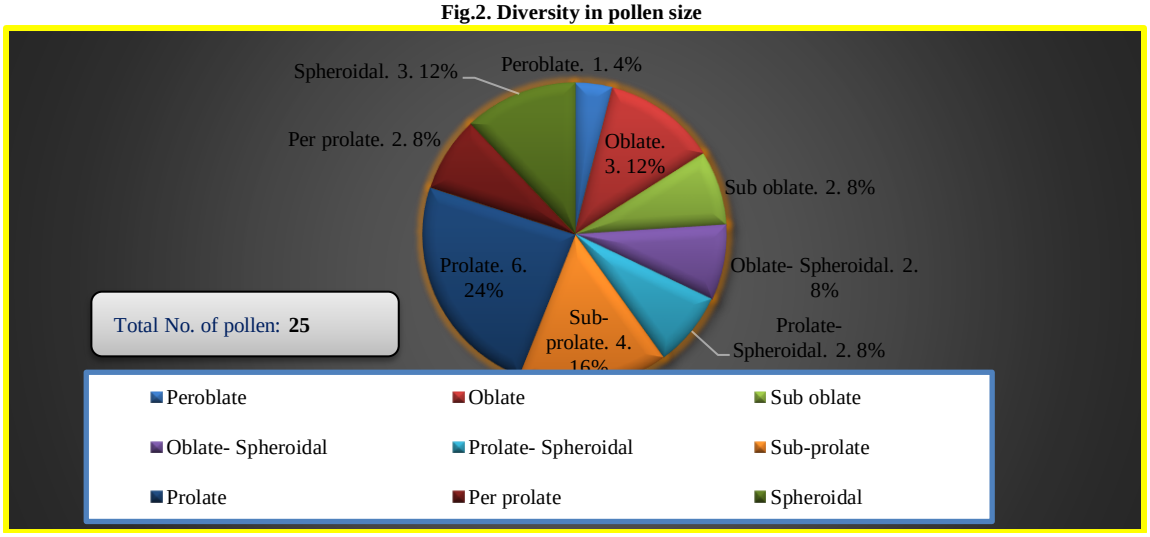


**Fig.3. Diversity in pollen shape**

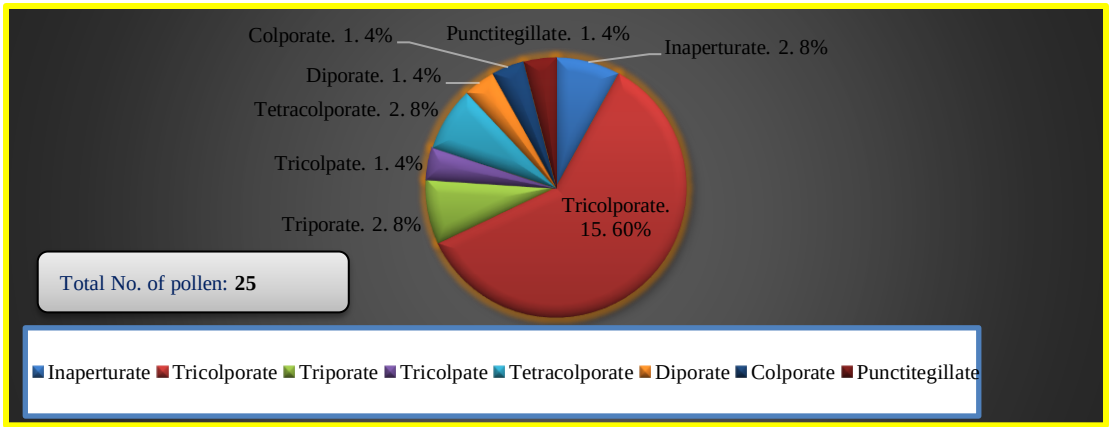


**Fig.4. Diversity in pollen aperture**

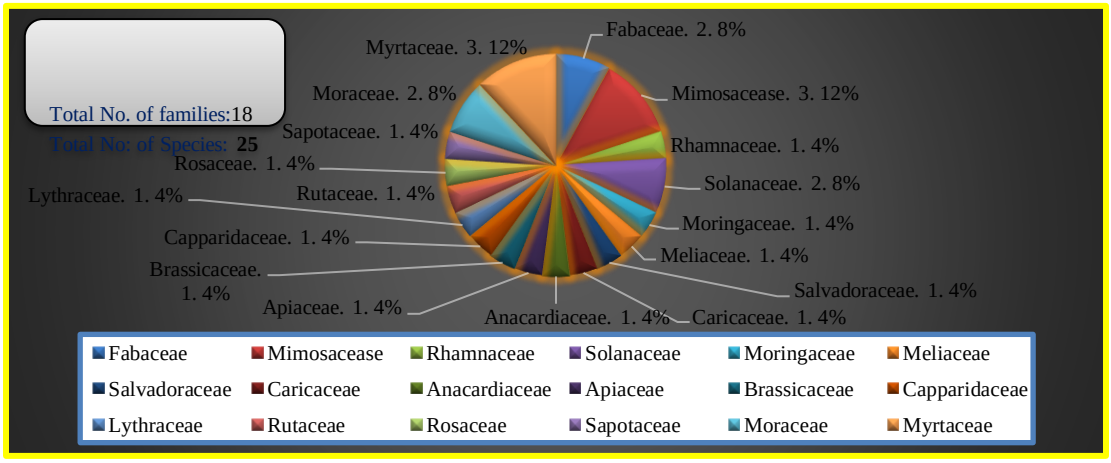


**Fig. 5. Families' diversity and their representation based on their specific taxa**

## 3. DISCUSSION AND CONCLUSION

Pollen analysis from honey samples provides very valuable information and interesting knowledge about the vegetation of that particular area from where honey samples were collected. From trade and commercial point of view, correctly labeling of the honey was very important before marketing because different honey samples have different rates and values based upon the quality, quantity, origin and nature of the honey. This area has a good potential for apiculture development and beekeeping management due to dense vegetation and highly diversified nectar and pollen source. In addition to destitute taxa, few exotic species have been identified from honey samples of this district, this indicates that the bees have travelled much in order to collect pollen and nectar. The findings of this study reveal that honeybees have intensively used native plant species. Due to the presence of the most attractive and eye-catching plant species for honey bees in this area. Whenturns out that to be the region may have proven to be more facilitating, specifically in the management of bees and in promoting the production of natural honey.

It has also been observed that the species belongs to family Fabaceae the pollen are tricolporate with long colpi and prolate in shape. In Moraceae pollen were heteropolar and spheroidal in shape. In Myrtaceae pollen are calporate and prolate in shape. However; great pollenomorphic diversity has been observed in the family of Mimosaceae because in some of taxa the pollen were in a perturate while in most of the species the pollen were found with aperture and prolate to spheroidal in shape. It is also investigated that the pollen of *Zizyphus jujuba* are tri-colporate and sub-oblate in shape in *Dalbergia sissoo* pollen were tricolpate with long colpi and prolate in shape. In *Lawsonai alba* pollen were tricolpate, prolate to spheroidal in shape. According to the frequencies of the major and minor pollen, only two samples of unifloral honey were found, one sample of honey from the Khanpur area and the second was collected from the town of Johi, while the rest of the six samples were recorded as multifloral. The highest pollen count in sample number 07 was observed and the lowest in sample number 06. Pollens of all these plants have shown significant variations in their

morphological characteristics viz. size, shape, similarity, sculpture, aperture and polarity; as it can be seen in figures no. 2, 3, and 4 respectively. All these pollen parameters are very useful in the authentic and very convincing identification of several taxa. Therefore, this study may help to identify several species of plants in their respective families, so that the obtained information is very useful in taxonomy for systematic botanists.

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