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POLLEN ANALYSIS: USING MELISSOPALYNOLOGY TO DETERMINE THE BEE-FORAGED SOURCES FROM TANDO ALLAH YAR AND TANDO MUHAMMAD KHAN, TWO AGRICULTURALLY IMPORTANT DISTRICTS OF SINDH, PAKISTAN

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Abstract

Present work involved the pollen investigation of 16 honey samples collected from different localities of district *Tando Allah Yar* and *Tando Muhammad Khan*, Sindh-Pakistan. Out of the total samples analyzed, 06 (37%) samples were graded as unifloral, which contained *Ziziphus jujuba*, *Brassica campestris, Azadirachta indica, Mangifera indica* and *Terminalia catapa* as predominant pollen. The remaining 10 (63%) samples were graded as multifloral, consisting of two or more than two (mixed) pollen types. With respect to the frequency distribution classes, pollen taxa were graded as very frequent (> 50%), frequent (20–50%), infrequent (10–20%) and rare (< 10%). The chief source of pollen as revealed by the current study were found to be *Mangifera indica, Ziziphus jujuba, Brassica campestris, Azadirachta indica, Delonix regia, Brassica nigra, Terminalia catapa, Syzygium cumini, Moringa oleifera* and *Raphanus sativus*. Pollenomorphic analysis revealed that 45 different plant species were source of pollen. The amount of pollen from each of the honey samples varied from 15000 to 75000. Out of total 24 recognized families, *Caesalpiniaceae, Brassicaceae* and *Fabaceae* were graded as the most leading families of the area. Identification of 45 plant species from 24 different families from the sampled area is an open indication that the area is richly covered with dense vegetation, eventually that will support the apiaries and bee keeping practice in the locality. This information is also very valued and meaningful for the beekeepers, in the selection of suitable sites for apiaries, in order to promote the apiculture industries and the yield of natural honey production in area.

Key words: Mellissopalynology, Pollen, Bees, Forage, Sindh, Pakistan.

Introduction

Honey is the natural sweet substance, mainly used as a food supplement in daily diet and beverages of infants and adults (Al-Jabri, 2005). Honey and other bee products have been extensively used in the preparation of traditional medicine in many parts of the world since prehistoric times, as it is considered the most valuable food offered by nature to human. Resultantly, the demand for honey has been increased around the globe over the past few years. Honey is known as a best anti-oxidant (Dżugan *et al*., 2018) and anti-microbial agent (Kacániová *et al*., 2012). Honey, the liquid gold of nature, is concentrated water solution of two sugars, lavulose and dextrose with small amount of at least 22 other more complex and natural ingredients, mainly prepared by the little friends of human "the bees" (*Apis mellifera* and *A.dorsata*) from floral and other vegetative parts of the plants. Many other important substances also occur in honey, but the sugars are the most important components. Approximately 95% of total substances are sugars, whereas the remaining 5% includes all ingredients like minerals (0.02-0.45%), amino acids (ca 0.05%) and very small amounts of vitamins, aroma compounds and organic acids (Bogdanov, 2016). Among all the creatures on earth, the bees are perhaps the most beloved as diligent, devoted and disciplined beings. When the bees collect nectar from the flowers, they also carry some quantity of pollen from the flowers. Therefore, some pollen remains in the honey when nectar is converted into honey in the hive (Lopez-Portillo *et al*., 1993; Morse & Calderone, 2000; Tan, Fuchs, & Engel, 2008). The variance in the nectar source of honey is due to the plenteous floristic diversity fencing the hives (Tiwari *et al*., 2010). The first scientific information on botanical and geographical origin of honey was reported by Pfister in (1895), since then various other researchers have devoted themselves to this subject and have shown their keen interest and curiosity in this field in order to understand the bee-plant relationship. There are many set procedures and laboratory suggestive measures for the study of pollen in honey samples and their connection with floral resources (Siska *et al.*, 2001). The simple and easy approach to explore the floral source of honey is assessing the pollen loads of honey samples (Kilic *et al.*, 2016). The investigation of the resource value of different plant species to bees is a magnum opus aim of mellissopalynology (Lieux, 1981 and Louveaux *et al.*, 1978).

For proper growth and development of bee colonies, the knowledge of bee plant resources and their blooming period in an area is essential and constitutes a basis for beekeeping management (Sivaram *et al*., 2012). The ascendancy of particular pollen in a honey is commonly used to specify the preference of bees for particular taxa (Schulz & Lueke, 1994). Therefore, the pollen examination of a honey is an unavoidable devise used to determine plant preferences of bees in an area which in turn helps to understand the botanical and geographical origin of honey (Louveaux *et al*., 1978; Oddo *et al*., 2004). Earlier several studies have been reported on different aspects of honey and pollen worldwide (Faegri *et al.*, 1989; Mangi *et al*., 2018; Nair & Kapoor, 1974; Khan *et al.*, 2019). However, from this part of Sindh-Pakistan no any significant attempt has been made. So the present investigation is aimed at finding out the bee-forage source. In spite of the most methods used to understand the foraging preferences of the bees, the mellissopalynology is more reliable and effective method in the recognition of bee loving taxa of particular area (Letsyo & Ameka, 2019). Thus, the study was designed to assess the potential floral sources of honey bee in area that would be very effective and helpful for beekeeping practice for sustaining the growth and development of the honey industry.

Study area: The area of district Tando Allah Yar and Tando Muhammad Khan has been bestowed with a green cover of dense vegetation, which serves as reserve stock of unconventional fruits, cereal crops, ornamental and medicinal plants. The region represents a rich diversity of herbage. Broadly, these are categorized into herbs, shrubs, creepers, climbers and trees. Due to thick diversity, the region is an excellent source of pollen and nectar. Therefore, the area is taken under investigation to explore the major and minor forage sources for honey bees. To understand the dependence and preference of the bees for their forage sources, we carried out a study to analyze the distribution of different types of pollen loads from various honey samples of this region (Fig. 1).

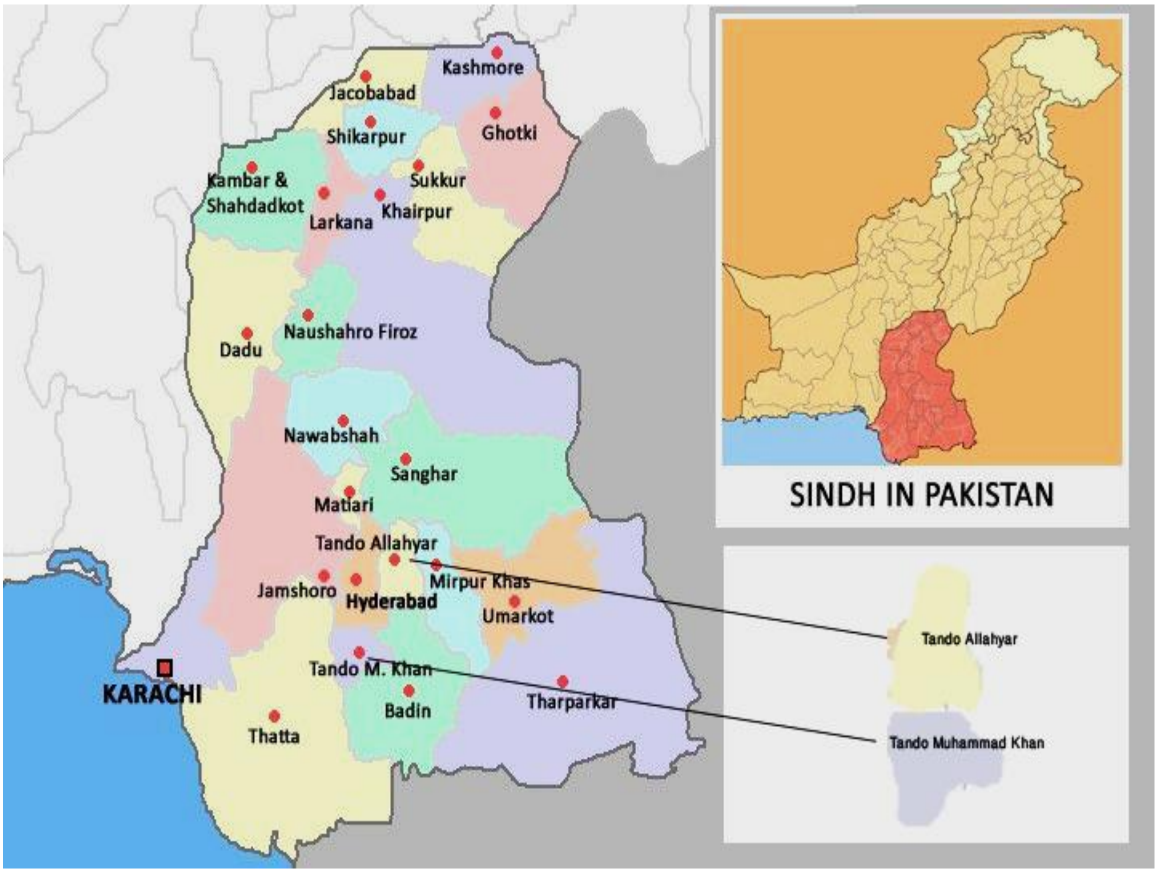

Fig. 1. Map of the site area.

Material and Method

Sampling and pollen analysis: From February to April 2017, 16 honey samples were collected from different localities of the region. The collection was made directly from bee hives of wild and gardening plants. All samples were collected with the intention of understanding the pollen preferences of bees in the area. After collection, the procured samples were stored in containers. For pollen investigation, honey samples were followed by the method adopted by international Commission for Bee Botany (Louveaux *et al.*, 1978) with minor modifications (Seeley, 1989). For pollen analysis, 5g of honey sample was dissolved in 10 ml of distilled water and then centrifuged at 2,500 rpm for 10 min. After centrifugation, the obtained sediment was processed with 5ml glacial acetic acid. Thereafter, the glacial acetic acid was removed and the material was subjected to acetolysed as per Erdtman, (1969). This solution was again centrifuged and the supernatant solution was discarded; the sediment containing pollen was taken and glycerin jelly was added and transferred to a glass slide. To melt the jelly, the glass slide was slightly heated and cover slip was applied. To secure the cover slip, colorless nail polish was applied to its edges (Ahn *et al.*, 2012). To analyze the pollen present in the honey samples qualitatively and quantitatively, five pollen slides were prepared for each of the sample. All slides were examined using Olympus BX 50 microscope at magnification of 40x and 100x and microphotographs of pollen from each sample were taken with the help of an Olympus camera model U-CMAD2. Pollen identification was based on relevant literature (Cayenne Engel & Irwin, 2003; Kaya & Kutluk, 2007; Mbagwu *et al.*, 1992) and comparison with reference pollen slides available at the Palynology Research Laboratory, Institute of plant Sciences, University of Sindh-Jamshoro, Pakistan (Al-Ghamadi *et al.*, 2019). Absolute pollen count in 05g of honey sample was also made with the help of haemocytometer. Pollen grains were observed using light microscope and their measurement was recorded by using the micrometer eyepiece and the micrometer slide. "Magnus Pro" software was also used to verify the exact length and breadth of pollen.

In order to understand the relative frequency of nectariferous species, a total of 500 pollen were counted for each slide and classified, according to their percentages, as predominant/unifloral pollen (> 45% of the counted pollen grains), secondary pollen type (16–44%), important minor pollen type(3–15%), and minor pollen type (< 3%) (Louveaux *et al*., 1978).

$$\% \text{ Frequency} = \frac{\text{Number of particular pollen type counted}}{\text{Total number of pollen counted (500)}} \times 100$$

For the determination of frequency distribution classes of pollen types from various honey samples, very frequent (> 50%), frequent (20–50%), infrequent (10–20%) and rare (<10%) distribution method was followed. Frequency of occurrence was calculated by dividing the sum of one pollen type by the total number of samples and then multiplying the quotient by 100 (Von Der Ohe *et. al.*, 2004).

$$\text{I-e frequency distribution occurrence} = \frac{\text{Number of occurance of each pollen type}}{\text{Total number of samples (16)}} \times 100$$

Results and Discussion

The pollen count, pollen percentage, pollen frequencies and pollen morphology from various honey samples of study area have been given in the tables and charts below (Fig. 2a,b).

Frequency occurrence of pollen type in various honey samples: With respect to the frequency distribution classes in honey samples, pollen types represented as 'very frequent' (i.e.> 50%) were *Mangifera indicia* (i.e. 10 out of 16; 62%) and *Ziziphus jujuba* (i.e 09 out of 16; 56%). While *Azadirachta indica* (i.e 03 out of 16; 19%), *Delonix regia* (i.e 03 out of 16; 19%), *Brassica nigra* (i.e 02 out of 16; 12%), *Brassica campestris* (i.e 02 out of 16; 12%), *Cassia fistula* (i.e 02 out of 16; 12%), *Syzygium cumini* (i.e 02 out of 16; 12%), *Gossypium herbaceum (*i.e 02 out of 16; 12%), *Moringa oleifera (*i.e 02 out of 16; 12%), *Pongamia pinnata* (i.e 02 out of 16; 12%), *Acacia nilotica*(i.e. 02 out of 16; 12%) *Tamarindus indica* (i.e. 02 out of 16; 12%), *Bombax ceiba* (i.e. 02 out of 16; 12%), *Terminalia catapa* (i.e. 02 out of 16; 12%), *Raphanus sativus* (i.e. 02 out of 16; 12%) and *Vitex diversifolia* (i.e. 02 out of 16; 12%) were graded as infrequent (10–20%) pollen class. Whereas, pollen of *Averrhoa carambola, Bauhinia purpurea, Bauhinia variegata, Cassia alata, Capparis decidua, Catharanthus ruseus Dodonaea viscosa, Abutilon grandifolium, Albizia amara, Barleria cristata, Cannabis sativa, Acacia mellifera, Capparis zeylanica, Chenopodium album, Justica diffusa, Lantana camara, Terminalia arjuna and Cassia senna* were recovered from only one sample (i.e. 01 out of 16; 06.25%). Therefore, they are classified as rare (<10%) frequency class (Fig. 3). And none of the taxan was ranked between the ranges of (20-50%). Hence, no frequent pollen class was graded in the study. Even though the pollen of *Delonix regia* were found in 03 out of 16 honey samples, they were however not found in highest proportion. Meanwhile, the pollen of *Terminalia catapa* were detected from two samples but their percentage frequency was more (>45%.). However, the pollen of *Mangifera indica and Ziziphus jujuba* were recovered from 10 and 09 samples respectively, at the same time, these taxa were found loaded with highest proportion of pollen content as well.

The pollen frequency of *Mangifera indica, Ziziphus jujuba, Azadirachta indica, Delonix regia, Brassica campestris, Brassica nigra, Terminalia catapa, Syzygium cumini Moringa oleifera and Raphanus sativus* was very high. It was concluded that these taxa were the principal pollen and nectar source for bee in the area. Therefore, these species were graded as the most important bee forage sources in the study area. On the account of the high proportion of pollen from these species, it could be inferred that these plants play important role in honey production. Conversely, plant species with low frequencies of pollen and nectar were ranked as minor bee forage source in the study area. The presence of lesser types of pollen from various taxa might be due to presence of pyrrolizidine alkaloids which furthermore prohibits honey bees to visit those taxa (Letsyo & Ameka, 2019).

Percentage frequency of nectariferous pollen (relative frequency) in each honey samples: Through common agreement, honey is characterized in terms of the number of grains per 5gm of honey (Ashman *et al*., 2004; Louveaux *et al.*, 1978). According to palynological assessment of honey samples of researched area, 06 (37%) were unifloral, having a predominant pollen type (i.e. >45%) belonging to *Ziziphus jujuba, Mangifera indica, Azadirachta indica, Terminalia catapa* and *Brassica campestris*. The remaining 10 (63%) samples were declared as multifloral consisting of two or more secondary pollen types. Dealing with frequency class of pollen in honey samples, the study revealed that in sample (05 Tando Soomro), *Ziziphus jujuba* were the predominant pollen ranging from 45% to 50%. In the sample (06 Dhegano Buzdar), *Mangifera indica* were graded as predominant pollen ranging from 45% to 48%. *Azadirachta indica pollen* were predominant pollen in sample (08 Massan), ranging from 45% to 50%. In sample (11 Saeed Khan Lund), *Terminalia catapa* was predominant with 52%. In sample (12 Wassi Malooq Shah), *Brassica campestris* was found as predominant pollen with 46%. Sample (13 Hassan Khan Laghari), was found loaded with predominant pollen of *Mangifera indica*. Therefore, honey samples of these 06 localities were recognized as unifloral honeys, and the taxa recovered from these sites were considered as important source of forage for honey bees in the area. However, the most important secondary pollen class (i.e. 16-45%) was represented by *Pongamia pinnata, Litchi chinensis, Ziziphus jujuba, Delonix regia, Acacia nilotica, Cassia alata, Moringa oleifera, Barleria cristata, Chenopodium album, Cassia senna, Heteropogon contortus, Tamarindus indica* and *Justicia diffusa*. Furthermore, the pollen spectra like *Cassia fistula, Syzygium cumini, Terminalia catapa, Tinospora cordifolia, Raphanus sativus, Vigna radiata, Albizia amara, Capparis zeylanica, Brassica nigra* and *Datura metel* were detected in important minor (i.e.3-15%) pollen class. Whereas, minor pollen class (< 3%) was recorded in honey sample numbers, 01, 04, 08 and 11 respectively, and the valuable species were *Capparis decidua, Cannabis sativa, Moringa oleifera* and *Averrhoa carambola* (Table 2).

Fig. 2(a). Microphotographs of pollen taxa identified from various honey samples.

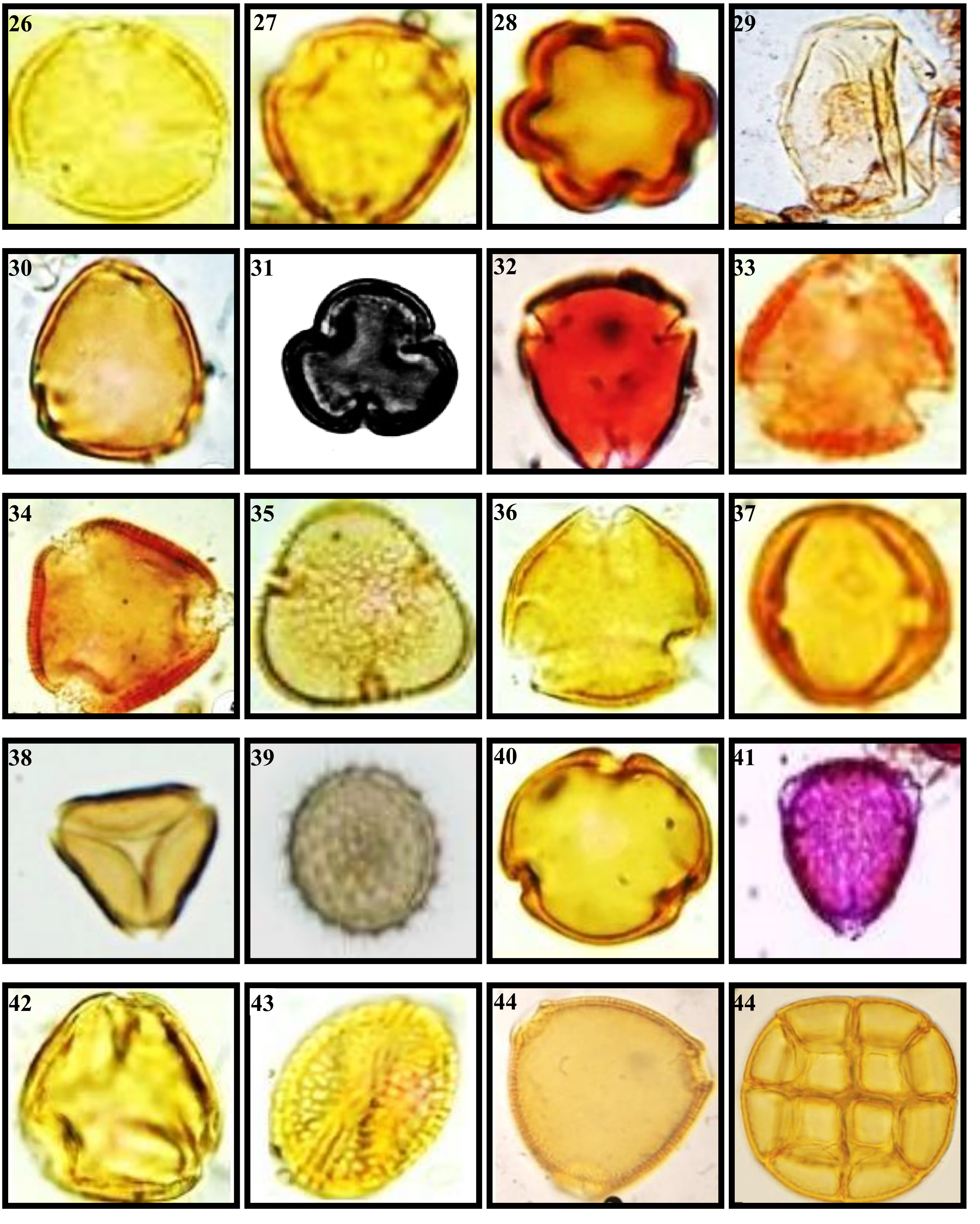

Fig. 2(b). Microphotographs of pollen taxa identified from various honey samples.
All pictures were taken at 40x and 100x. For the pollen measurement *Magnus Pro Image analysis software* was used.
1=*Abutilon grandifolium*; 2=*Acacia mellifera*; 3=*Albizia amara;* 4=*Azadirachta indica*; 5=*Barleria cristata*; 6=*Brassica campestris;* 7=*Brassica nigra*; 8=*Cannabis sativa*; 9=*Capparis zeylanica;* 10=*Cassia fistula;* 11=*Chenopodium album*; 12=*Convolvulus microphylius*; 13=*Datura metel*; 14=*Delonix regia*; 15=*Eruca sativa;*16=*Gossypium herbaceum*; 17=*Heteropogon contortus*; 18=*Hygrophila ringens*; 19=*Justica diffusa*; 20=*Lantana camara*; 21=*Lobelia alsinoides*; 22=*Mangifera indica;* 23=*Pongamia pinnata*; 24=*Raphanus sativus;* 25=*Cassia senna;* 26=*Tamarindus indica;* 27=*Terminalia catapa*; 28=*Terminalia arjuna;* 29=*Vitex trifolia;* 30=*Ziziphus jujuba;* 31=*Capparis decidua;* 32=*Averrhoa carambola;* 33=*Bauhinia purpurea;* 34=*Bauhinia variegata;* 35=*Bombax ceiba;* 36=*Cassia alata;* 37=*Dodonaea viscosa;* 38=*Syzygium cumini;* 39=*Ipomoea purpurea;* 40=*Moringa oleifera;* 41=*Litchi chinensis;* 42=*Plumeria alba;* 43=*Tinospora cordifolia;* 44=*Vigna radiata;* 45=*Acacia nilotica.*

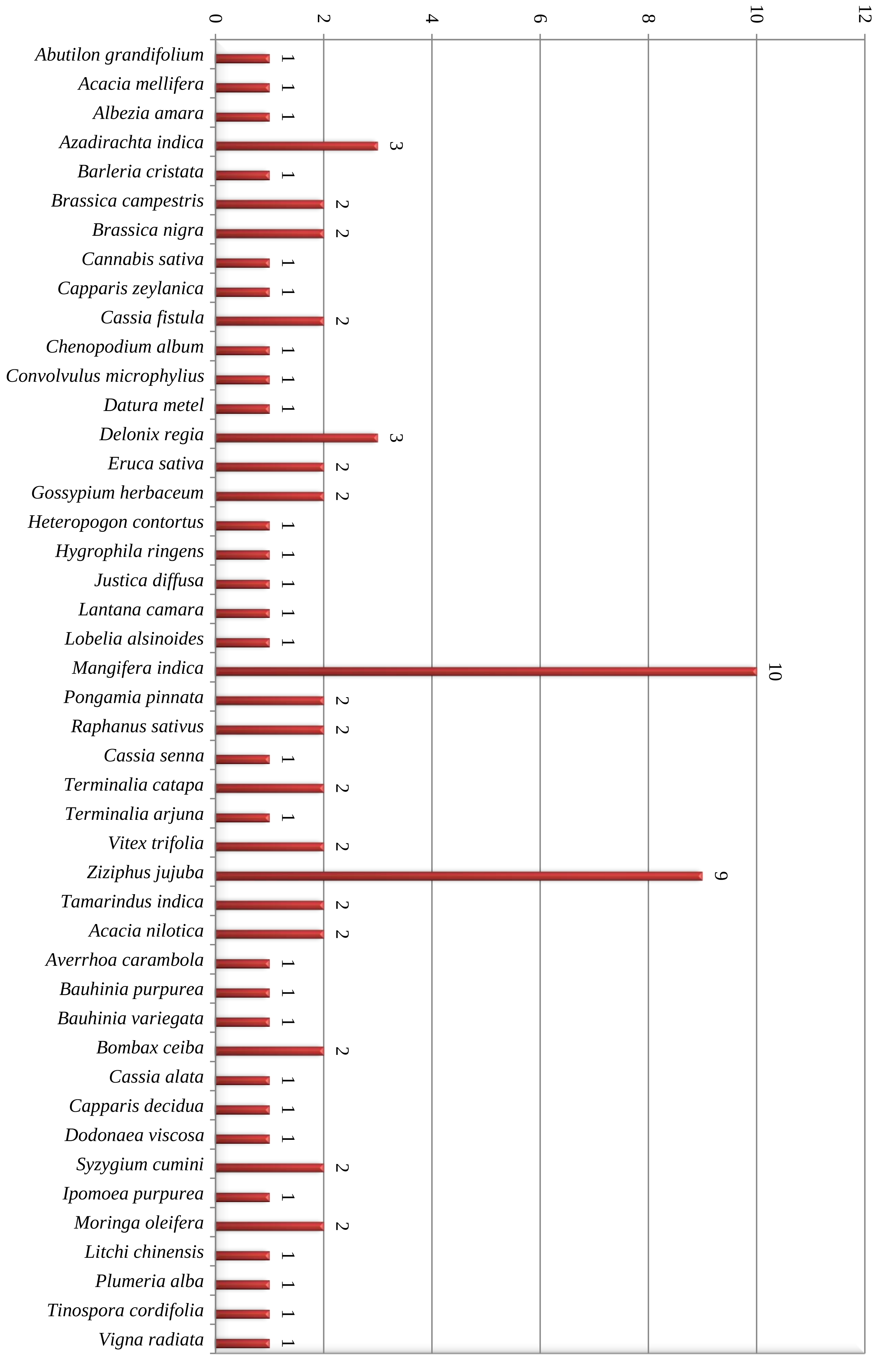

Fig. 3. Frequency occurrence of various pollen types found in 16 different honey samples.

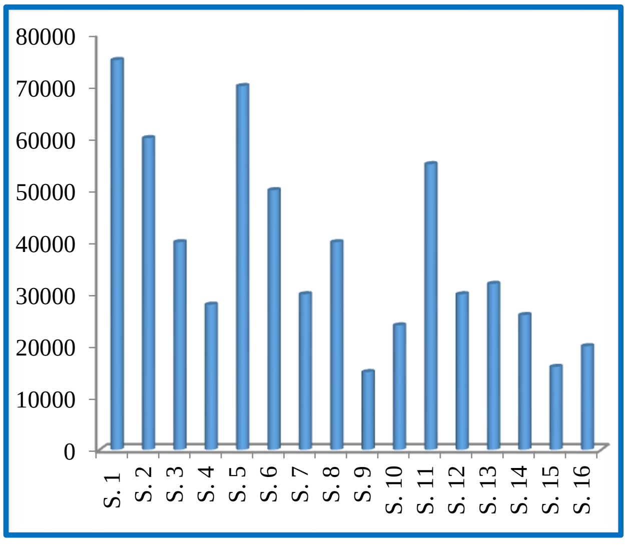

Fig. 4. Comparision of pollen contents in 16 different honey samples. Note: S= Sample

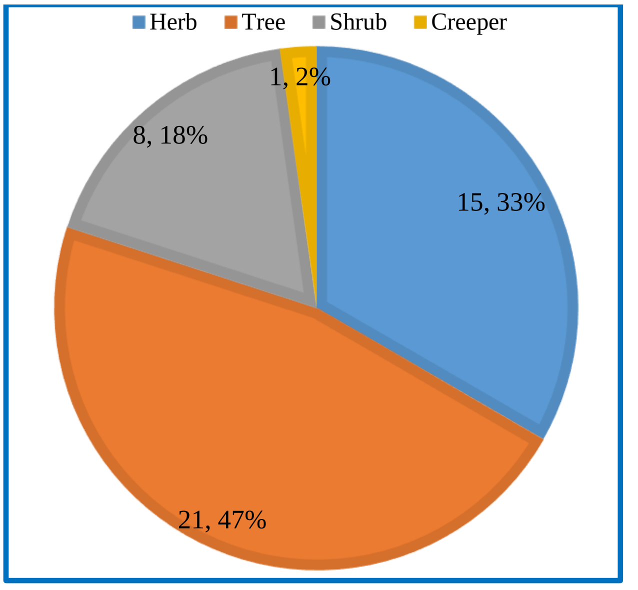

Fig. 5. Diversity in the habit of taxa.

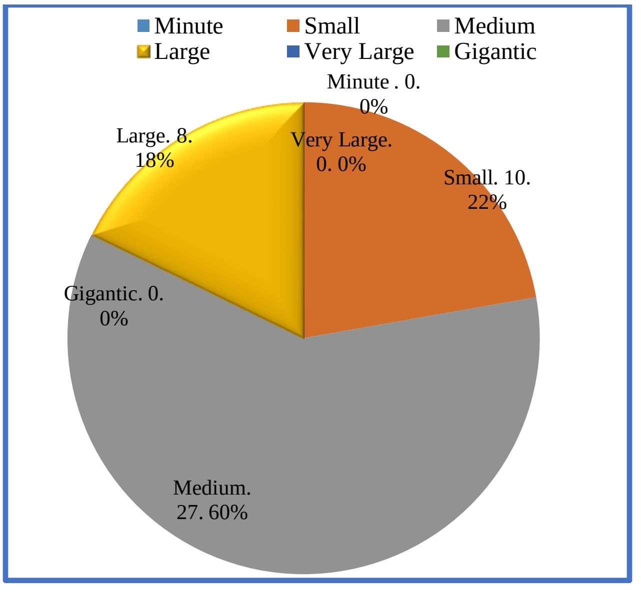

Fig. 6. Diversity in Pollen size.

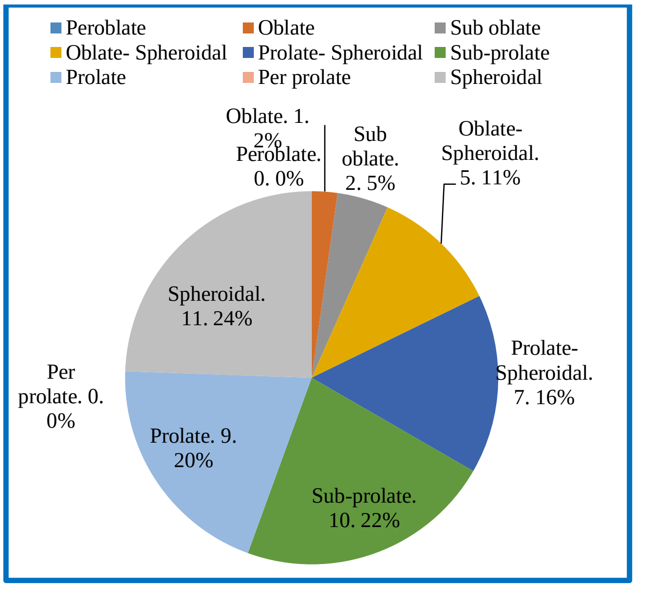

Fig. 7. Diversity in Pollen shape.

Comparison of pollen contents in various honey samples of study area: This study reveals the significant variation in the amount and type of pollen contents of various experimented honey samples. An absolute pollen count was measured 5/gm of honey samples; minimum number of absolute pollen count was recorded in sample number 09 (*Bulri Shah Karim*) with 15000 pollen grains and maximum in sample number 01, (*Tando Allah Yar*) with 75000 pollen grains (Table 1). Pollen richness in the honey sample depends on the pollen production by the parent plant. Several uncontrolled factors affect the presence of pollen content in honey samples; some of them are imbalance in the abundance of flowers of different plant species, availability of pollen and honey bees' collection preferences. The distance from the beehive to the flower field, the amount of pollen filtered out in the bee's honey sac cause the fluctuation in pollen contents of honey samples. More specifically the climatic condition, affects the nectarous and pollenic properties of the local flora. Chaturvadi (1983) proved that the pollen flora of honey samples is also influenced by the crops which are cultivated in the localities. The pollen types and percentage of pollen in honey does not represent genuinely the same proportion of quantity of nectar gathered from these plants, because some pollen types are over-represented and some underrepresented.

Apart from the bees visit to entomophilous plants for nectar, and on such visits, some quantity of pollen grains is carried away. This subsequently reduces the amount of pollen grains present for foraging bees, assuming they visit and feed on both entomophilous and anemophilous plants at the same proportion. Moreover; there is a necessity of more studies addressing these questions more specifically with controlled experiments (Oliveira *et al.*, 2010). However, from the pollen spectra, it is apparent that honey bees eagerly visit the honey sources for nectar and pollen to form honey (Iwama & Melhem, 1979; Louveaux *et al.*, 1978) (Fig. 4).

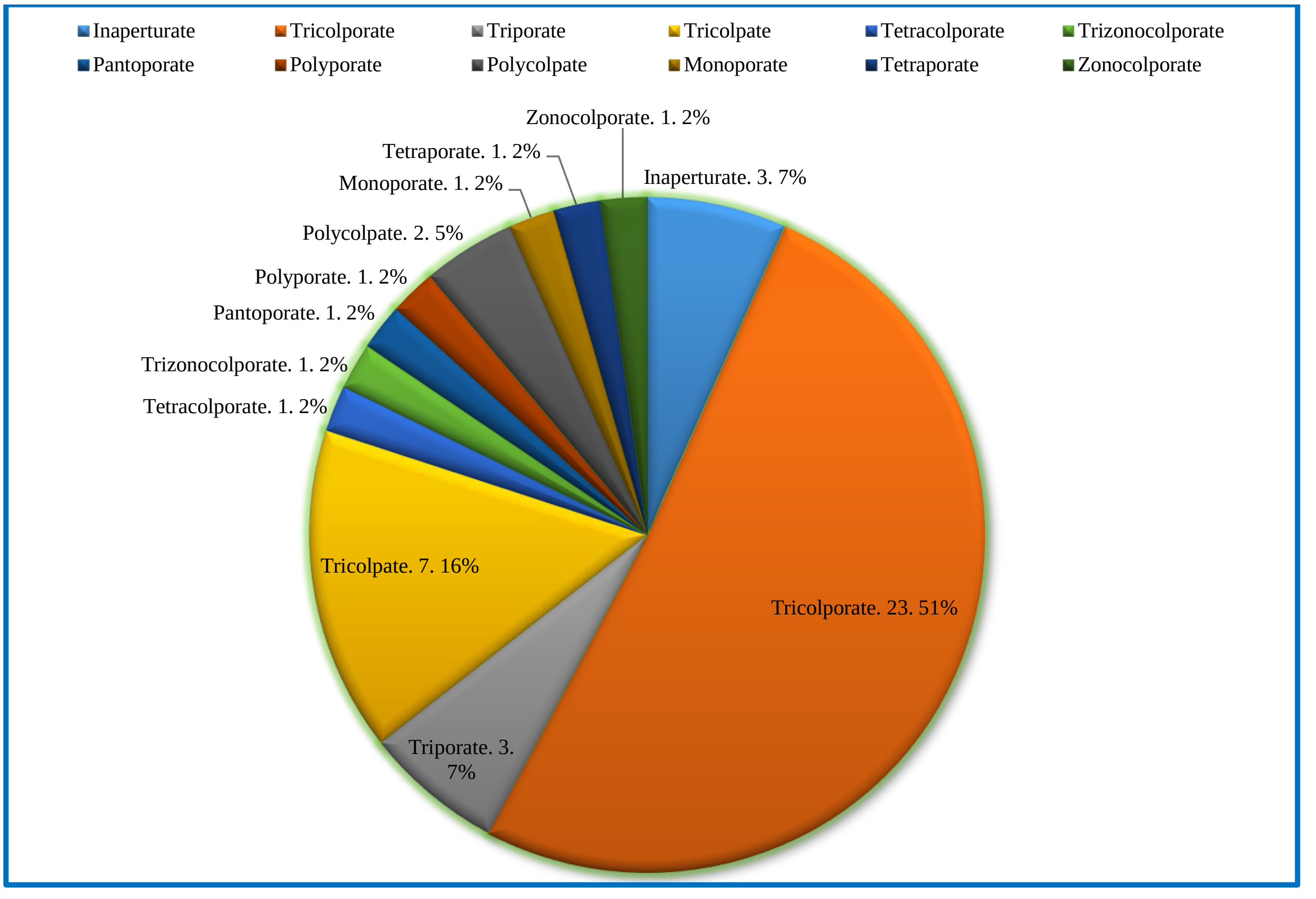

Fig. 8. Diversity in Pollen aperture.

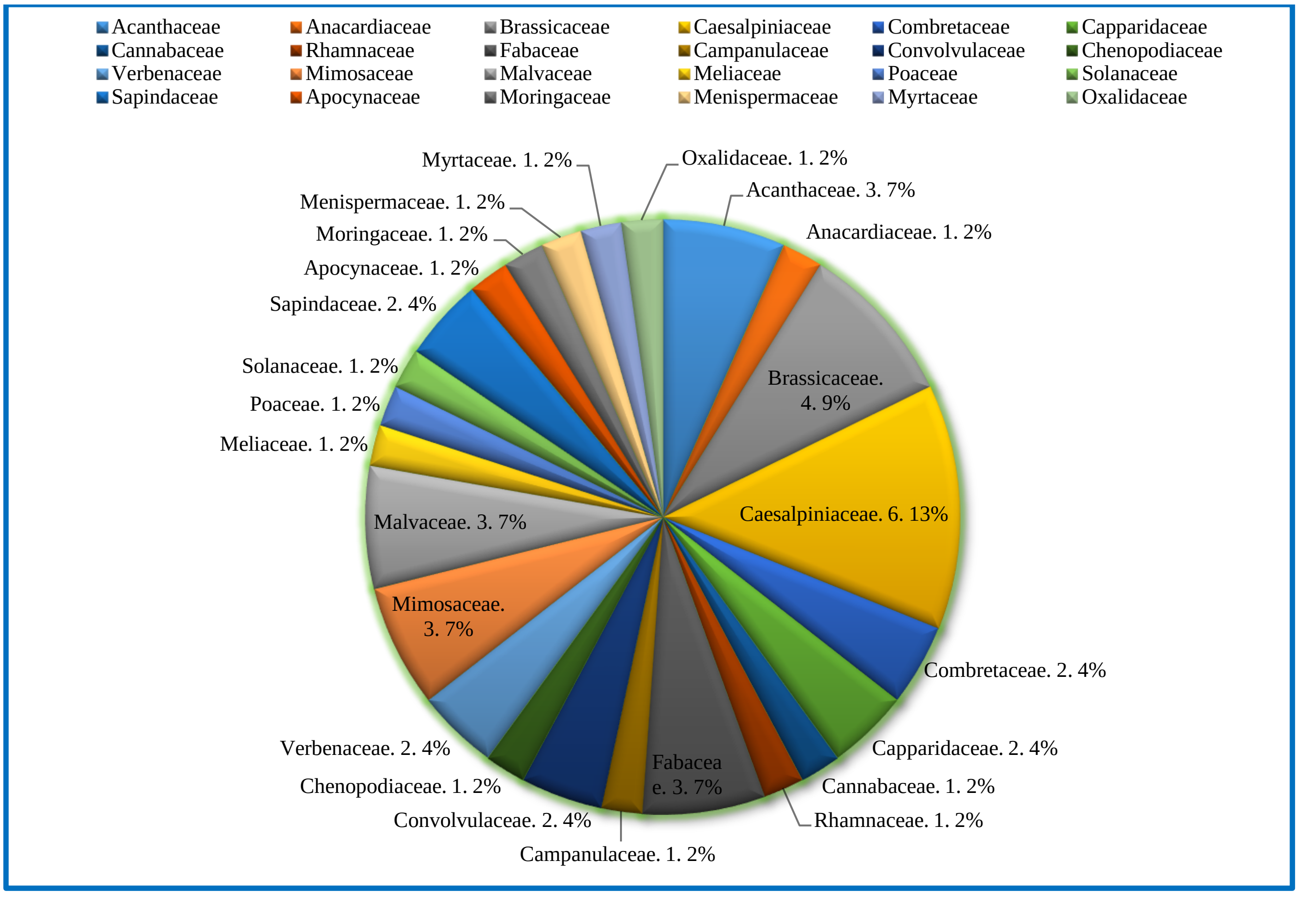

Fig. 9. Family diversity.

Table 1. Morphological characteristics of various pollen taxa found in 16 different honey samples.

Sample No.	Total pollen count per 5ml.	Pollen characteristics			Name of taxa	Family	Habit
		Size(μ)	Shape	Aperture			
01	75000	24–25 L 18– 19 B	Sub prolate	Tricolporate	*Averrhoa carambola* Linn.	Oxalidaceae	Tree
		33–37 L 22– 26 B	Sub prolate	Tricolporate	*Pongamia pinnata* (Linn) Pierre	Fabaceae	Tree
		57–63 L 37– 43 B	Prolate spheroidal	Tricolporate	*Cassia fistula* Linn.	Caesalpiniaceae	Tree
		21–27 L 13– 18 B	Oblate	Tricolporate	*Syzygium cumini* (L.)Skeels	Myrtaceae	Tree
		37–42 L 17 –23 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
02	60000	37–42 L 17 –23 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
		33–39 L 15 –21 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
		21–27 L 13 –18 B	Oblate	Tricolporate	*Syzygium cumini* (L.)Skeels	Myrtaceae	Tree
		28–29 L 28–29 B	Spheroidal	Tricolpate	*Bombax ceiba* Linn.	Malvaceae	Tree
		45–47 L 45–47 B	Spheroidal	Tricolporate	*Bauhinia variegata* Linn.	Caesalpiniaceae	Tree
03	40000	58–61 L 54– 57 B	Sub oblate	Tricolporate	*Delonix regia* (Bojer) Rafin.	Caesalpiniaceae	Tree
		52–58 L 42 –48 B	Sub prolate	Inaperturate	*Acacia nilotica* (Linn.) Delile	Mimosaceae	Tree
		33–39 L 15 –21 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
		21–26 L 19–25 B	Oblate spheroidal	Tricolporate	*Terminalia catapa* Linn.	Combretaceae	Tree
		26–30 B 20 –24 L	Sub prolate	Tricolporate	*Plumeria alba* L.	Apocynaceae	Shrub
04	28000	13–19 L 9 –14 B	Sub prolate	Triporate	*Brassica campestris* Linn.	Brassicaceae	Herb
		24–26 L 22 –24 B	Prolate spheroidal	Tricolporate.	*Cassia alata* Linn.	Caesalpiniaceae	Tree
		35–41 L 16 –22 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
		16–18 L 2–14 B	Prolate	Tricolporate	*Tinospora cardifolia* (Thoms.) Miers	Menispermaceae	Shrub
		30–33 L 29–34 B	Sub prolate	Tricolporate	*Moringa oleifera* Lam.	Moringaceae	Tree
05	70000	15–20 L 10 –15 B	Sub prolate	Trizono colporate	*Brassica nigra* (Linn.)	Brassicaceae	Herb
		40–46 L 38 –44 B	Prolate spheroidal	Tricolporate	*Bauhinia pupurea* Linn.	Fabaceae	Tree
		35–41 L 16 –22 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
		32–38 L 15–19 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
		34–38 L 34 –38 B	Spheroidal	Pantoporate	*Gossypium herbaceum* Linn.	Malvaceae	Herb
06	50000	58–61 L 54 –57 B	Sub oblate	Tricolporate	*Delonix regia* (Bojer) Rafin.	Caesalpiniaceae	Tree
		84–86 L 84 –86 B	Spheroidal	Polyporate	*Ipomoea purpurea* (Linn.) Roth.	Convolvulaceae	Creeper
		22–26 L 20 –22 B	Prolate spheroidal	Tricolporate	*Dodonaea viscosa* (Linn.) Jacq.	Sapindaceae	Shrub
		35–41 L 16 –22 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
		32–38 L 22– 28 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
07	30000	30–33 L 29– 34 B	Sub prolate	Tricolporate	*Moringa oleifera* Lam.	Moringaceae	Tree
		24–26 L 20– 22 B	Prolate	Tricolpate	*Vitex trifolia* Linn.	Verbenaceae	Herb
		27–33 L 25–31 B	Oblate spheroidal	Tricolporate	*Tamarindus indica* Linn.	Caeselpiniaceae	Tree
		33–39 L 24–28 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
		39–45 L 38– 42 B	Prolate spheroidal	Tetracolporate	*Azadirachta indica* Adr. Juss.	Meliaceae	Tree
08	40000	25–30 L 18– 24 B	Prolate	Tricolporate	*Litchi chinensis* Soon.	*Sapindaceae*	Tree
		18–24 L 14– 18 B	Prolate	Tricolporate	*Capparis decidua* (Forssk.) Edgew.	Capparidaceae	Tree
		39–45 L 38– 42 B	Prolate spheroidal	Tetracolporate	*Azadirachta indica* Adr. Juss.	Meliaceae	Tree
		22–24 L 18–20 B	Sub prolate	Tricolpate	*Raphanus sativus* Linn.	Brassicaceae	Herb
		27–33 L 25–31 B	Prolate	Zonocolporate	*Vigna radiata* (L.) Wilczek.	Fabaceae	Herb

Table 1. (Cont'd.).

Sample No.	Total pollen count per 5ml.	Pollen characteristics			Name of taxa	Family	Habit
		Size(µ)	Shape	Aperture			
09	15000	93−96 L 93− 96 B	Spheroidal	Tricolporate	*Barleria cristata* L.	Acantheceae	Shrub
		33–37 L 22– 26 B	Sub prolate	Tricolporate	*Pongamia pinnata* (L.) Pierre	Fabaceae	Tree
		57–63 L 37– 43 B	Prolate spheroidal	Tricolporate	*Cassia fistula* Linn.	Caesalpiniaceae	Tree
		73−76 L 73− 75 B	Spheroidal	Inaperturate, polyad type	*Albizia amara* (Roxb.)Boiv.	Mimosaceae	Tree
		37–42 L 17 –23 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
10	24000	37–42 L 17 –23 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
		33–39 L 15 –21 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
		26–28 L 22 –24 B	Prolate	Tricolpate	*Eruca sativa* Mill.	Brassicaceae	Herb
		14−18 L 11−14 B	Sub prolate	Tricolporate	*Terminalia arjuna* (Roxb. ex DC.)	Combritaceae	Tree
		14−16 L 14−16 B	Spheroidal	Triporate	*Capparis zeylanica* Linn.	Capparidaceae	Shrub
11	55000	58−61 L 54− 57 B	Sub oblate	Tricolporate	*Delonix regia* (Bojer) Rafin.	Caesalpiniaceae	Tree
		28–30 L 26 –28 B	Oblate spheroidal	Polycolpate	*Hygrophila ringens* (L.)R.Br.ex stued.	Acantheceae	Tall herb
		33–39 L 15 –21 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
		21–26 L 19–25 B	Oblate spheroidal	Tricolporate	*Terminalia catapa* Linn.	Combritaceae	Tree
		20−23 B 20 −23 L	Spheroidal	Triporate	*Cannabis sativa* Linn.	Cannabaceae	Herb
12	30000	13–19 L 9 –14 B	Sub prolate	Triporate,trilobed	*Brassica campestris* Linn.	Brassicaceae	Herb
		51–57 L 41 –47 B	Sub prolate	Inaperturate	*Acacia nilotica* (Linn.) Delile	Mimosaceae	Tree
		35–41 L 16 –22 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
		22–24 L 18–20 B	Oblate spheroidal	Polyporate	*Chenopodium album* L.	Chenopodiaceae	Herb
		30–32 L 30–32 B	Spheroidal	Tricolpate	*Lantana camara* Linn.	Verbenaceae	Shrub
13	32000	15–20 L 10 –15 B	Sub prolate	Trizonocolporate	*Brassica nigra* (Linn.)	Brassicaceae	Herb
		60–65 L 56 –61 B	Oblate spheroidal	Tetraporate	*Abutilon grandifolium* (Wild.)Swet	Malvaceae	Shrub
		35–41 L 16 –22 B	Prolate	Tricolporate	*Mangifera indica* L.	Anacardaceae	Tree
		23–26 L 18 –22 B	Prolate	Tricolporate	*Lobelia alsinoides* Lam.	Campanulaceae	Herb
		34–38 L 34 –38 B	Spheroidal	Pantoporate	*Gossypium herbaceum* Linn.	Malvaceae	Herb
14	26000	24–26 L 22 –24 B	Sub-prolate	Tricolpate	*Cassia senna* Linn.	Caesalpinioceae	Shrub
		46–48 L 46 –48 B	Spheroidal	Monoporate	*Heteropogon contortus* (L.) P.Beauv.ex Roem	Poaceae	Herb
		26–30 L 26 –30 B	Spheroidal	Tricolpate	*Bombax ceiba* Linn.	Malvaceae	Tree
		35–41 L 16 –22 B	Prolate	Tricolporate	*Magifera indica* L.	Anacardaceae	Tree
		32–38 L 22– 28 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
15	16000	30–34 L 30– 34 B	Spheroidal	Tricolpate	*Convolvulus microphylus* Siebex. Spring.	Convolvulaceae	Herb
		24–26 L 20– 22 B	Prolate	Tricolpate	*Vitex trifolia* Linn.	Verbenaceae	Herb
		27–33 L 25–31 B	Oblate spheroidal	Tricolporate	*Tamarindus indica* Linn.	Caeselpiniaceae	Tree
		33–39 L 24–28 B	Sub oblate	Tricolporate	*Ziziphus jujuba* Mill.	Rhamnaceae	Tree
		34–38 L 33– 36 B	Prolate spheroidal	Tricolporate	*Datura metel* L.	Solanaceae	Herb
16	20000	30–33 L 22– 24 B	Prolate	Tricolporate	*Justicia diffusa* Wild.	Acanthaceae	Herb
		25–27 L 21– 23 B	Prolate	Tricolpate	*Eruca sativa* Mill.	Brassicaceae	Herb
		39–45 L 38– 42 B	Prolate spheroidal	Tetracolporate	*Azadirachta indica* Adr.Juss.	Meliaceae	Tree
		22–24 L 18–20 B	Sub prolate	Tricolpate	*Raphanus sativus* Linn.	Brassicaceae	Herb
		75–77 L 71–75 B	Prolate spheroidal	Inaperturate	*Acacia mellifera* (M.vahl) Benth.	Mimosaceae	Tree

Table 2. Investigation of 16 honey samples together with their floral sources and percentage of pollen content per 5 ml of honey (relative frequency).

Sample No.	Collection area	Percentage of pollen content (per 5 ml of honey)	Predominant pollen	Floral type (i.e. unifloral or multifloral honey)
01	Tando Allahyar city	42% *Syzygium cumini*, 30%, *Pongamia pinnata*, 12% *Cassia fistula*,08% *Mangifera indica*, 02% *Averrhoa carambola*	---	Multifloral
02	Chamber	40% *Mangifera indica*, 31% *Ziziphus jujuba*, 14% *Syzygium cumini*, 08% *Bombax ceiba*, 4% *Bauhinia variegata*,	---	Multifloral
03	Sultanabad	35% *Delonix regia*, 28% *Acacia nilotica*, 15% *Ziziphus jujuba*, 09% *Terminalia catapa*, 02% *Plumeria alba*	---	Multifloral
04	Piyaro Lund	40% *Brassica campestris*, 22% *cassia alata*, 15% *Mangifera indica*, 14% *Tinospora cardifolia*, 2% *Moringa oleifera*	---	Multifloral
05	Tando Soomro	50% *Ziziphus jujuba*, 18% *Bauhinia purpurea*, 16% *Brassica nigra*, 11% *Mangifera indica*, *03% Gossypium herbaceum*	*Ziziphus jujuba*	Unifloral
06	Dhegano Buzdar	48% *Mangifera indica*, 21% *Ipomoea purpurea*, 20% *Dodonaea viscosa*, 07% *Delonix regia*, 03% *Ziziphus jujuba*	*Mangifera indica*	Unifloral
07	Rashida Abad	42% *Moringa oleifera*, 24% *Vitex diversifolia*, 14% *Tamarindus indica*, 10% *Azadirachta indica*, 3% *Ziziphus jujuba*	---	Multifloral
08	Massan	50% *Azadirachta indica*, 20% *Litchi chinensis*, 14% *Raphanus sativus*, 09% *Vigna radiata*, 02% *Capparis decidua*	*Azadirachta indica*	Unifloral
09	Bulri Shah Kareem	26% *Barleria cristata*, 19% *Pongamia pinnata*, 15% *Cassia fistula*, 10% *Albizia amara*, 05% *Mangifera indica*	---	Multifloral
10	Tando Ghulam Hyder	42% *Ziziphus jujuba*, 20% *Eruca sativa*, 12% *Mangifera indica*, 13% *Capparis zeylanica*, 03 % *Terminalia arjuna*	---	Multifloral
11	Saeed Khan Lund	52% *Terminalia catapa*, 22% *Hygrophila ringens*, 10% *Ziziphus jujuba*, 06% *Delonix regia*, 2% *Canabis sativa*	*Terminalia catapa*	Unifloral
12	Wassi Malooq Shah	46% *Brassica campestris*, 22% *Chenopodium album*, 18% *Lantana camara*, 03% *Acacia nilotica*, 03% *Mangifera indica*	*Brassica campestris*	Unifloral
13	Hassan Khan Laghari	50% *Mangifera indica*, 20% *Abutilon grandifolium*, 10% *Brassica nigra*, 07% *Lobelia alsinoides*, 05% *Gossypium herbaceum*	*Mangifera indica*	Unifloral
14	Mula Katiyar	35% *Cassia senna*, 22% *Heteropogon contortus*, 15% *Bombax ceiba*, 13% *Mangifera indica*, 03% *Ziziphus jujuba*	---	Multifloral
15	Wahid Dino Jagsi	30% *Convolvulus microphylius*, 22% *Vitex trifolia*, 15% *Tamarindus indica*, 15% *Ziziphus jujuba*, 3% *Datura metel*	---	Multifloral
16	Sobho Chandio	33% *Justicia diffusa*, 19% *Eruca sativa* ,14% *Azadirachta indica*,11% *Raphanus sativus* ,5% *Acacia mellifera*	---	Multifloral

Note: Samples containing > 45% of a single pollen type were declared as unifloral honey

Diversity in plant habit: Height of trees is no barrier for bees in the collection of pollen, because this study suggested that bees visited all kind of plants in order to collect nectar and pollen e.g., *Ipomoea purpurea* were a few inches high are equally visited the tall trees such as *Bombax ceiba, Acacia nilotica, Mangifera indica* and *Azadirachta indica*. However, according to some researchers perennials are better bee forage sources than annuals (Dibble *et al*., 2020). Although some annuals are rich source of nectar and pollen for bees because they provide quick and relatively abundant bee forages. While the reviewed literature is advocating that perennials are generally richer source of nectar and pollen as compared to annuals because of their long life they provide more-or-less dependable food source for honey bee population year after year and encourage repeated nesting in the area (Delaplane *et al.*, 2013). According to the result of the study, forages sources are characterized as trees, herbs and shrubs. Nevertheless, they are superior bee forage plants and deserve special attention of bee conservationists to save them for better bee management. Among all 45 taxa; trees were (21 out of 45; 47%), shrubs (08 out of 45; 18%), herbs (15 out of 45; 33%) and only one pollen from creeper species was identified that makes 02% of the total taxa (Fig. 5).

Diversity in pollen size: The pollen types recovered from various honey samples, exhibited different sizes. Out of 45 identified pollen types, 27 (60%) pollen taxa were recorded as medium in size, (10 out of 45; 22%) pollen was found small in size. However, (08 out of 45; 18%) pollen were of large in size, none of the pollen was recorded as gigantic or minute in size. According to the measurement of various pollen taxa, pollen of *Ipomoea purpurae* and *Barleria cristata* were classified as the bigger pollen compared to all other pollen types. Whereas, the pollen of *Tinospora cordifolia* and *Capparis zeylanica* were found to be the smallest among all identified taxa for pollen percentage (Fig. 6) and for pollen size (Table 1).

Diversity in pollen shape: According to the findings of the study, diverse pollen shapes were identified, 09 pollen were as prolate, 10 sub-prolate, 13 spheroidal, 07 prolate spheroidal, 01 oblate, 02 sub oblate and 05 pollen were recorded as oblate spheroidal in shape. Spheroidal structure was found as most dominating form (i.e. 11 out of 45; 24%) of all existing pollen shapes, the spheroidal grains were of *Albizia amara, Capparis zeylanica, Barleria cristata, Canabis sativa, Lantana camara, Gossypium herbaceum, Heteropogon contortus, Convolvulus microphylius, Bombax ceiba, Bauhinia variegata* and *Ipomoea purpurea.* It was followed by sub-prolate structure (i.e. 10 out of 45; 22%) of all identified pollen types (Fig. 7). The main taxa found with sub-prolate structure were of *Pongamia pinnata, Averrhoa carambola, Brassica campestris, Plumeria alba, Delonix regia, Moringa oleifera, Raphanus sativus* and *Brassica nigra.*

Diversity in pollen aperture: The study revealed the different apertural pattern and ornamentations. All pollen apertural forms are recorded in the present study, tricolporate model was found more advanced in comparison to all other apertural structures as it occupied more prevalent position (i.e. 23 out of 45; 51% of all recovered pollen types. Tricolpate structure was found in 07 pollen taxa with 16% of all pollen types. However; the other registered apertural pattern were found in 3, 2 or just in one (Fig. 8).

Family representation with respect to their taxa: According to findings of the study, large number of the species belonging to different families is known to be visited by bees. However, the majority of visits was restricted to a few families such as *Caesalpiniaceae, Brassicaceae, Fabaceae, Malvaceae, Mimosaceae, Verbenaceae, Convolvulaceae, Capparidaceae* and *Combretaceae*. Out of total (24) recognized families *Caesalpiniaceae, Fabaceae* and *Brasssicaceae* are graded as the most dominating families of the area. *Caesalpiniaceae* sharing (i.e. 06 species out of 45; 13%), *Fabaceae* (i.e. 03 species out of 45; 07%) and *Brasssicaceae* (i.e.04 out of 48; 09%) of total taxa (Fig. 9).

The plant species representing to these families are considered as good source of pollen and nectar for honey bees in the area sampled.

Conclusion

Based on designed research, 45 plant species belonging to 24 different families have been identified. Primarily the study was aimed to recognize the important nectariferous and polleniferous bee forage sources in the area. To explore the major and minor bee forage sources of sampled area, the mellissopalynological method was adopted. As it is globally recognized and accepted method of exploring the floral sources on which the bees feed on to produce honey. The proposed research furnishes a new insight into our knowledge that area is very imperative, efficiently and profitably can be managed for apiaries to increase natural honey production. Mellissopalynological analysis of *Tando Allah Yar* and *Tando Muhammad Khan* offer huge potential for beekeeping with its vast bee-forage resource which could assist beekeepers in the selection of suitable sites as well as preserving these forages around apiaries in order to maximize honey production. The information incorporates 45 species belonging to 24 families, as useful source of forage to honey bees. The important forage sources are *Mangifera indica, Ziziphus jujuba, Azadirachta indica, Delonix regia, Brassica campestris, Brassica nigra, Terminalia catapa, Syzygium cumini, Moringa oleifera, Litchi chinensis, Acacia mellifera* and *Raphanus sativus*. While working on comparative pollen morphology and pollen count, it was observed that the bees had an impressive flight range in the area. It is also concurred that the propagation of these taxa could improve the efficiency of apiculture industries and commercial honey production. The study will be useful in the identification of flora used by honeybees and to improve the conservation status of economically important plants. Based on all the above observations, conservation of bee loving taxa is crucial and much needed to increase honey production in the commercial market. Additionally, the mellissopalynological investigation has shown a striking diversity among all pollenomorphic characteristics such as; pollen size, shape, apertural pattern and pollen count. Therefore, this study provides base line information about honey pollen flora of the region in turn which will be very helpful in compiling of honey pollen flora of researched area. This information can be used for further confirmation of plant species i.e. authentic identification and classification of taxa recovered from the area under investigation. Furthermore, the better understanding of all these parameters can help us to present a fairly perfect picture of bee foraged sources, predominant, dominant, very frequent and frequent taxa of the researched area.

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