Characterization of pollen profile of *Apis mellifera* L. in arid region of Pakistan

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**Article info**

**Article history:**
Received 9 December 2020
Revised 6 February 2021
Accepted 7 February 2021
Available online 17 February 2021

**Keywords:**
*Apis mellifera* L.
DIKhan
Pollen profile
Visual survey
Loads ensnaring

**Abstract**

Honeybees rely exclusively on pollen and nectar-producing plants for strengthening their colonies and manufacturing honey. Little is known about the indigenous melliferous flora of arid zones of Khyber Pakhtunkhwa (KPK) which is crucial for honey production and how different pollen assessment techniques effect the identification of indigenous melliferous pollen flora. Visual survey and loads ensnaring through pollen traps were used to identify the botanical profile of melliferous pollen flora of Dera Ismail Khan (DIKhan), Khyber Pakhtunkhwa. The test time extended for two consecutive years 2018 and 2019. The study revealed 56 plant species as pollen flora with 18 significant pollen producing species in visual survey technique while 8 species as predominant flora in pollen trapping technique. The major pollen species found common in both the techniques were *Brassica napus* L., *Brassica campestris* L., *Trifolium alaxandrinum* L., *Zea mays* L., *Acacia modesta* L., *Citrus aurantium* L., *Euclyptus spp.*, and *Morus alba* L. Pollen interception and palynological analysis of pollen were found to be more reliable techniques as compared to focal observations. More than fifty % differences were found by comparing the results of the visual survey and pollen trapping technique in major flora of DIKhan. Based on the availability, utility status and flowering duration of apiphilic flora, mid-February to mid-May was found to be a significant pollen flow period in the study area. Maximum benefit can be taken in this period through trapping ample amount of pollen and stored for using in artificial diets, selling and feeding bees during dearth period.

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1. Introduction

Honey bees forage for pollen and nectar as their primary source of nutrition (Donkersley *et al.*, 2017). Almost all bee species lap nectar for energy and pollen to fulfil their protein and other dietary requirements (Brodschneider R and Craisleheim K., 2010). In fact, honey bees (*Apis mellifera* L.) harvest 10–1000 times more pollen than other insects. During a single foraging trip, foragers visit anywhere between 1 and 500 flowers to gather sufficient quantities of pollen and make an average of 10 to 15 visits per day (Winston, 2016).

For top-notch colony development, the foragers’ supply of pollen is crucial. Scout bees and reticent bees are the two main types of foragers. These bees are confronted with the most exigent task of searching and gathering appropriate nutriments from their surroundings for individuals residing in the hive like queen, nurse bees and developing larvae (Michener, 2007). The major bulk (40–90%) of total forager population is comprised of reticent bees (Van Nest and Moore, 2012) of which 19% of very active bees, also known as elite bees, perform 50% of the colony’s total foraging trips, contributing to both pollen and nectar collection (Klein *et al.*, 2019).

Pollen grains are harvested by the bees as loads, transported to hives and then stored as bee bread in cells. This bee bread is fed to the developing larvae other than queen and thus needed for colony population buildup. Moreover, this stored pollen is consumed by nurse bees to produce highly proteinaceous royal jelly to feed the queen and developing queen larvae (Dorea *et al.*, 2010). Although pollen grains are generally collected from a large variety of plant species (Schmidt and Johnson., 1984), honey bees focus only on a few plant patches within their foraging area (Visscher...
and Seeley, 1982). Analysis of trapped pollen reveals flora frequently foraged by honey bees (Apis mellifera L.) and their liking for pollen in that area (Olsen et al., 1979; Webster et al., 1985; Cortopassi-Laurino and Ramahlo, 1988; Pearson and Braiden, 1990; Biesmeijer et al., 1992; Goodwin and Perry, 1992; Coffey and Breen 1997; Seijo and Jato., 1998; Nagamitsu et al., 1999; Andrada and Telleria, 2005).

The composition of pollen loads found on bees varies with the geographical origin of the pollen loads (Diaz-Losada et al., 1998), botanical source, flowering season and reflects the variation in local flora (Barth, 2004).

Quantitative identification of pollen is indispensable for investigating the link between forage and foragers, especially those of pollinators (Richardson et al., 2015). Melliferous flora can be identified either by primary or secondary sources of information (Hepburn and Radloff, 1998). Primary sources include analysis of pollen stored in nests or hives (Ramanujam and Kalpana, 1992); analysis of pollen loads removed from returning foragers (Köppler et al., 2007); palynological analysis of honey (Adekammbi and Ogundipe, 2009); and identification through direct observation of foragers in field (Ayansola and Davies, 2012). On the contrary, experienced beekeepers are also an important, albeit secondary source of information on local floral resources (Teklay, 2011). Most methods used to understand the foraging preferences of bees are based on direct field observations and/or surveys by the beekeepers, and video monitoring (Richard et al., 2011). However, analyses of pollen pellets are considered to be the most suitable means to understand the forage sources of a bee species. Most palynological studies are focused on honey analyses and pollen characterisation of nectariferous plants. Literature provides insufficient evidence of studies on the palynological characterization of pollen loads carried by honeybees as Ramalho et al. (2007) and De Novais et al. (2009). Our attempt is to add later research category.

DIKhan is located in the south of Khyber Pakhtun Khwa of Pakistan between 31°15’ and 32°32’ north latitude and 70°11’ and 71°20’ east longitude with an elevation of 173 m from the sea level (Khan, 2003). The climate is continental with marked temperature fluctuations both seasonal and diurnal, with significant aridity (Title referenced). Despite its aridity, this area is rich in flora and can favor the cottage industry apiculture. A host of wild and cultivated plants grow abundantly in the area yielding nectar and pollen for honey bees (Marwat et al., 2013).

Though DIKhan is an established area for beekeepers, yet no work has been done to identify the melliferous pollen flora in DIKhan and to probe whether the bee (Apis mellifera L.) has specialist or generalist behaviour in visiting the flora. The present study was designed to identify foraging resources available for Apis mellifera L. throughout a calendar year visually as well as through analysis of intercepted pollen pellets. The major pollen yielding melliferous plants were identified by comparing results of both methodologies to guide the beekeepers for apiary management, as well as preservation and multiplication of plant species that are most important for apiculture in the region of DIKhan, KPK, Pakistan.

2. Materials and methods

2.1. Visual survey of the area

A visual survey was conducted to score the bee flora for pollen and nectar sources (Table 1) plants. The foraging behaviour of Apis mellifera L. was observed and plants were scored as P (major Pollen plant sources), N (major plant sources for nectar), NP (plants having greater attraction both for nectar and pollen) and np/pn (plants having little attraction or under certain conditions for pollen and nectar). Honeybees with their activity of extending their proboscis into the flowers and remaining calm during lapping (concentrated nectar) /sucking (watery nectar) were considered as N plant sources. In contrast, plants with bees showing hyperactivity along with corbicula loads were categorized as P plants. Honeybees with their activity of extending their proboscis into the flowers and also collecting pollen on their hind legs were determined as Np and Pn plants subject to the extent of their respective activity. Most of the plants observed were rich exclusively in pollen. The bees remain in high-strung on such plant flowers while collecting pollen.

During the same period, pollen grain references were prepared by directly collecting pollen from flowers. The plants were photographed (Fig. 5) when bees were visiting flowers, and then taken to the Plant Biodiversity Laboratory of Gomal University, DIK, for identification and further experimentation.

2.2. Preparation of reference slides

Pollen grains from the identified bee flora of DIKhan were gathered in the stage of dehiscence in field as well as from anthers brought in the lab for slides preparation. Pollen grains were washed twice with absolute ethyl alcohol to remove the debris in Petri dishes. A drop was taken after ten minutes on a glass slide, and absolute alcohol was allowed to evaporate. After that, a drop of glycerin jelly was affixed by heating the slide on a spirit lamp. A coverslip was placed over the glycerin jelly and slightly pressed so that the coverslip was uniformly placed over the glass slide.

When the glycerin jelly was set entirely, the edges of the coverslip were cleaned using a blade to remove the excess jelly, then nail polish was used to seal the edges. Usually, two coats of the nail polish were done. First, a single coat was done; later on, after the nail polish had dried a second coat on the first coat was done. To avoid seepage of colour into the glycerin jelly and interference with the pollen colour, transparent nail polish was used. (Balasubramanyam and Bhat 2015).

2.3. Method of trapping pollen loads

An easy way to obtain pollen sample collected by honey bee foragers is through pollen trapping (Dimou et al., 2006). Twelve typical Langstroth hives also known as Afghani hives in Pakistan, of relatively uniform colonies of Apis mellifera L. were selected to intercept the pollen loads, four at each location mentioned below.

- Faculty of Agriculture, Gomal University, DIKhan
- Bashir Farms near Qureshi More
- Agriculture Research Institute Ratta Kulachi (ARI), DIKhan

Standard pollen traps purchased from National Agriculture Research Center (Fig. 5) Islamabad with 50% extraction efficiency (Nagamitsu et al., 1999) were used for the determination and quantification of melliferous food sources in the area. Pollen load samples were obtained from the hives installed at three different locations of DIKhan starting with the mid-January when the pollen loads were accumulated until the end of December for two succeeding years. Pollen collected at two consecutive days on a fortnightly basis was considered one sample and all the loads collected from three sites were pooled. Starting from mid-January to the end of December total 24 pooled samples were collected in two years.

Pollen-loads were trapped from returning foragers for 2 days on a fortnightly basis, allowing 13 days of incoming pollen for the colonies. These pollen samples were removed from corbiculae of honey bees on a rack fitted in a trap, as foragers pass through the trap the loads get knocked off their pollen baskets and drop down in the collection chamber below. This harvesting of pollen loads was conducted for two hours intervals after which traps were
Table 1
Botanical profile of honey bee (Apis mellifera L.) in District Dera Ismail Khan.

| S. No | Preferred Scientific name | Vernacular/preferred common name | Agricultural value (references + personal observations) | Habit |
|-------|---------------------------|----------------------------------|--------------------------------------------------------|-------|
| 1.    | Abelmoschus esculentus L. | Bhindi / Okra                     | P (Marwat S. K et al., 2013), N (Mishra et al., 1987), NP² (PO) | Herb |
| 2.    | Azadirachta indica L.     | Neem                              | PN (Bhuiyan et al., 2002; [Rijal et al., 2018], N(PO)         | Tree  |
| 3.    | Acacia modesta L.         | Pulai                             | P (Morton, 1964; Gorain et al., 2012; Ismail et al., 2013; Sivaram, P.; 2013; N (Izhar-ul-Haq et al., 2010; Marwat et al., 2013), NP² (PO) | Tree  |
| 4.    | Acacia nilotica L.        | Kikar                             | N (Marwat, S.K, et al., 2013), N(PO)                      | Shrub |
| 5.    | Albizia lebbeck L.        | Sareen                            | PN (Bista, S., & Shivakoti, G.P., 2001), P²(PO)           | Tree  |
| 6.    | Allium cepa L.            | Vasa or Pizil/Onion               | N (McGregor, 1976; Kumar & Gupta, 1993), P (McGregor, 1976; Bhuiyan et al., 2002), NP² (Layek et al., 2015), NP² (PO) | Tree  |
| 7.    | Brassica napus L.         | Canola                            | N (McGregor, 1976), PN (Bista, S., and Shivakoti, G.P., 2001), NP² (PO) | Herb  |
| 8.    | Brassica campestris varatoria L. | Rapeseed                     | PN(Bista & Shivakoti, 2001; Rijal et al., 2018), N P²(PO) | Herb  |
| 9.    | Brassica rapa L.          | Ghonglool/shalgam/Turnup          | P (McGregor, 1976) PN (Bista & Shivakoti, 2001; Bhuiyan et al., 2002; Taha and Bayoumi, 2009) , NP² (PO) | Herb  |
| 10.   | Bauhinias variegata Linn. | Kachnar                          | NP (Bista & Shivakoti, 2001), NP² (PO)                    | Tree  |
| 11.   | Calendula officinalis L.  | Marigold                          | NP² (PO)                                                 | Herb  |
| 12.   | Callistemon lanceolatus L. | Bottle brush                      | Np (Bista & Shivakoti, 2001; Bareke, et al., 2017; Rijal et al., 2018), NP² (PO) | Tree  |
| 13.   | Inora cocinea L.          | ————                              | P²(PO)                                                   | Herb  |
| 14.   | Capsicum annum L.         | Sabz mirch/Chilli                 | P²(PO)                                                   | Herb  |
| 15.   | Cassia fistula L.         | Ambal tas/Golden Shower           | N (Layek, U., Bhakat, R. K., & Karmakar, P., 2015) N P² (PO) | Tree  |
| 16.   | Citrullus lanatus (Thunb.) Mutsum. or Nakai | Tarbuzi/watermelon               | N (Morton, 1964; Taha & Bayoumi, 2009), P (McGregor, 1976), PN (Taha and Bayoumi, 2009); Layek et al., 2015, NP²(PO) | Prostrate or Climber |
| 17.   | Cicer arietinum L.         | Channa/Chick peas                 | N (Marwat et al., 2013), NP²(PO)                         | Herb  |
| 18.   | Citrus aurantum L.        | Khattha/Bitter or sour orange     | P²N (Alghoson, 2004), NP² (PO)                            | Small Tree |
| 19.   | Citrus sinensis L.        | Malta/Orange                      | P (Ismail et al., 2013) (N Morton, 1964; Adjaloo and Yeboah-Gyan, 2003), NP²(PO) | Small tree |
| 20.   | Citrus limon L.           | Lemon                             | N (Morton, 1964), NP² (PO)                               | Shrub  |
| 21.   | Coriandrum sativum L.     | Dahnia/Coriander                  | P (McGregor, 1976; Ismail et al., 2013), N (McGregor, 1976), NP² (Bhuiyan et al., 2002; Layek et al., 2015), NP² (PO) | Tree  |
| 22.   | Chrysanthemum indicum L.  | Guldaudi                          | PN (Layek, et al., 2015), N P² (PO)                      | Herb  |
| 23.   | Cucurbita maxima Duchesnes | Pumpkine                          | N(Layek, et al., 2015), NP(PO)                           | Climber/Prostrate |
| 24.   | Combrum indicum L.        | Har Singhwar                      | Np (Ahmad et al., 2019), np(PO)                          | Shrub  |
| 25.   | Conocarpus erectus L.     | Cono                              | NP²(PO)                                                  | Shrub or small to medium size tree |
| 26.   | Cucumis sativus L.        | Khira/Cucumber                    | PN (Morton, 1964; McGregor, 1976), Np (Hossain et al., 2018), N(PO) | Herb  |
| 27.   | Cucumis melo L.           | Kharooza/ Muskmelon               | PN (McGregor, 1976), N (Ribeiro et al., 2015; Ahmad et al., 2019), N(PO) | Creeper |
| 28.   | Dahlia variabilis L.      | Dahlia/ Fireworks Mixed           | N P² (PO)                                                | Herb  |
| 29.   | Dalbergia sisoo Roxb.     | Shisham, Tali                     | N(Rijal et al., 2018, Layek et al., 2015) P (Ahmad et al., 2019), N(PO) | Tree  |
| 30.   | Dacus carota L.           | Gajar/Carrot                      | NP (McGregor, 1976), NP² (PO)                            | Herb  |
| 31.   | Eucalyptus ssp.           | Sulada/Kafour                     | P (Morton, 1964; Ismail et al., 2013; Sivaram, P. 2013), N (Morton, 1964; Bhuiyan et al., 2002; Alghoson, 2004), NP² (PO) | Tree  |
| 32.   | Foeniculum vulgare L.     | Sauf                              | NP² (PO)                                                 | Herb  |
| 33.   | Gardenia jasminoides L.   | Jasmine                           | P(Ahmad et al., 2019), P²(PO)                            | Shrub  |
| 34.   | Gewia asiatica L.         | Phalsia                           | N(PO)                                                    | Shrub  |
| 35.   | Gaurdia aristata L.       | Blanket flower                    | N(PO)                                                    | Shrub  |
| 36.   | Gossypium herbaceum L.    | Kappas/ Cotton                    | Np (McGregor, 1976; Hussein, 2001) NP²(PO)               | Herb  |
| 37.   | Helianthus annuus L.      | Suraj mukhi/ Sunflower             | P (Morton 1964; Bhuiyan et al., 2002; Ismail et al., 2013), N (Morton, 1964; Bhuiyan et al., 2002), NP²(PO) | Shrub  |
| 38.   | Hibiscus sabdariffa L.    | Roselle                           | NP²(PO)                                                  | Shrub  |
| 39.   | Lagerstroemia indica L.   | Bouganvilla                       | N(PO)                                                    | Shrub  |
| 40.   | Leucaena leucocephala (Lam.) de Wit | Sareen/ river tamarind         | N(Layek, et al., 2015), NP²(PO)                           | Tree  |
| 41.   | Luffa cylindrica L.       | Tori/Sponge gourd                 | NP(Layek, et al., 2015), NP²(PO)                         | Tree  |
| 42.   | Medicago sativa L.        | Alfalfa                           | N (McGregor, 1976; Alghoson, 2004), P (Alghoson, 2004), NP²(PO) | Herb  |
| 43.   | Mangifera indica L.       | Aam/ Mango                        | N (Layek, et al., 2015; Chauhan et al., 2018), N(PO)     | Tree  |
| 44.   | Melia azedarach L.        | Bkayyan                           | N (Bista & Shivakoti, 2001)                              | Tree  |
| 45.   | Mimosa pudica L.          | Lajyanti/ chhui mui               | P(Ahmad et al., 2019), PN (Bista & Shivakoti, 2001; Layek et al., 2015), NP² (PO) | Herb  |
| 46.   | Momordica charantia L.    | Karela/Bittergourd                | P (Bhuiyan et al., 2002; Ahmad et al, 2019) PN (Deyto and Cervancia, 2009; Layek, et al., 2015), N(PO) | Tree  |
| 47.   | Moringa oleifer L.        | Sohanjna/Moringa                  | PN(Layek, et al., 2015), NP² (PO)                        | Tree  |
| 48.   | Morus australis L.        | Sheltoot                          | P²(PO)                                                   | Tree  |
| 49.   | Morus alba L.             | Mulberry/Toot                     | P²(Rijal et al., 2018), P²(PO)                          | Tree  |
removed. The trapped pollen loads were weighed, segregated based on colours, put in plastic bags and stored in a freezer at \(-20\, ^\circ\text{C}\). These loads were then used to make slides for identification of botanical origin through light microscopy and also sent to Centralised Resource Laboratory (CRL), the University of Peshawar for Scanning Electron Microscope (SEM) images (Fig. 4) for identification purposes.

![Month-wise pollen resources availability in DIK.](image)

**Fig. 1.** Month-wise pollen resources availability in DIK.

### Table 1 (continued)

| S. No | Preferred Scientific name | Vernacular/ preferred common name | Apicultural value (references + personal observations) | Habit |
|-------|---------------------------|-----------------------------------|--------------------------------------------------------|-------|
| 50.   | Nerium oleander L.        | China gandari                     | NP\(^5\)(PO)                                           | Shrub |
| 51.   | Ocimum tenuiflorum L.      | Tulsi/ Niaz bo                     | NP\(^5\)(PO)                                           | Herb  |
| 52.   | Phoenix sylvestris L.      | Khajji (Date Palm)                | P (Morton, 1964; Alghoson, 2004; Ismail et al., 2013), N (Morton, 1964; Alghoson, 2004), NP\(^2\)(PO) | Palm  |
| 53.   | Pisum sativum             | Mattar/Peas                       | P (Ismail et al., 2013), NP\(^2\)(PO)                  | Tree  |
| 54.   | Portulaca grandiflora L.  | Luni                              | P (Wongpiyasatid and Hornchan, 2001), NP\(^5\)(PO)     | Herb  |
| 55.   | Panica granatum L.         | Anar/Pomegranate                  | NP (Rijal et al., 2018), NP\(^2\)(PO)                  | Tree  |
| 56.   | Psidium guajava L.         | Amidood/Guava                     | PN (Bista and Shivakoti, 2001), Pollen (Adjaloo and Yeboah-Gyan, 2003; Anita et al., 2012; Shubharani et al., 2013) N (Adjaloo and Yeboah-Gyan, 2003), NP\(^2\)(PO) | Tree  |
| 57.   | Raphanus sativus L.        | Mooli/Radish                      | P (McGregor, 1976)                                     | Herb  |
| 58.   | Rosa indica L.             | Gulab/Rose                        | N (Morton, 1964; Noor et al., 2009; Shubharani et al., 2013) | Shrub |
| 59.   | Bata graveolens L.         | Kiner/Zehr Booti                   | N (Ren and Tang, 2012), NP(PO)                         | Herb  |
| 60.   | Sesamum indicum L.         | Sesame                            | NP (Bista and Shivakoti, 2001; Layek et al., 2015; Taha, 2017), NP\(^2\)(PO) | Herb  |
| 61.   | Solanum lycopersicum L.    | Tomatar/ Tomato                   | Pollen little (McGregor, 1976), N (Adjaloo and Yeboah-Gyan, 2003), NP\(^3\)(PO) | Herb  |
| 62.   | Solanum nigrum L.          | Mako                              | NP\(^2\)(PO)                                           | Herb  |
| 63.   | Syzygium cumini L.         | Jamu/Jaman/Black plum             | NP\(^2\) (Layek et al., 2015), NP\(^3\)(PO)            | Tree  |
| 64.   | Tagetes erecta L.          | Ganday ka phool/ Marigold         | NP\(^2\) (Bista and Shivakoti, 2001; Layek, et al., 2015), NP\(^3\)(PO) | Herb  |
| 65.   | Tagetes patula L.          | Marigold                          | PN (Shawer, 1987; Hussein, 2001; Bista and Shivakoti, 2001; Ismail et al., 2013), NP\(^1\)(PO) | Herb  |
| 66.   | Trifolium alexandrinum L.  | Barseen/Egyptian clover           | PN (Adjaloo and Yeboah-Gyan, 2003), NP\(^3\)(PO)        | Shrub |
| 67.   | Trifolium. Resupinatum L.  | Shaftal                           | PN (Bista and Shivakoti, 2001), NP\(^3\)(PO)           | Herb  |
| 68.   | Zea mays L.                | Makai/Maize                       | P (Morton, 1964; Shawer, 1987; Adjaloo and Yeboah-Gyan, 2003; Ismail et al., 2013) | Herb  |
| 69.   | Zizyphus Spp.              | Ber                               | NP (Bista, S., and Shivakoti, G.P., 2001; Marwat et al., 2013), N (Alghoson, 2004), NP(PO) | Tree  |
| 70.   | Taraxacum officinale L.    | Dandelion                          | NP\(^1\)(PO)                                           | Herb  |

PO: Personal Observations of author
PN: equally attractive for pollen and nectar; pn: attractive for little pollen and nectar under certain conditions
P\(^n\): Mainly attractive for pollen with little attraction for nectar; NP\(^n\): Mainly attractive for nectar with little attraction for pollen; P\(^n\): Only attractive for pollen; N\(^n\): Only attractive for nectar (P\(^1\), P\(^2\) and P\(^3\) indicate major, Intermediate and minor pollen sources respectively).

### 2.4. Identification of pollen loads

Altogether, 12,696 pairs of corbicular pollen pellets of *A. mellifera* L. were collected from the said areas in two years. Palynological preparations of samples were done using methods recommended by Maurizio (1951) and the International Commission for Bee Botany (Louveaux et al., 1978). Twenty four dehydrated bee pollen samples were analyzed. Each 5-gram sample was dissolved in 25 mL of distilled water, following the methodology of Alvarado and Rueda (1985). After complete homogenization of the mixture, 2 mL from each sample suspension were taken and submitted to the acetolysis method (Erdtman 1960). The sediment was mounted on slides in glycerin jelly (stained with safranine) and sealed with paraffin wax. Pollen grains thus prepared from pollen pellets were examined under a Leica DML 1000 (Germany) bright-field trinocular light microscope with 40x and 100x (oil) apochromatic objectives. Identification was done with the help of reference slides prepared from the local flora as well as published accounts (Ahmad et al., 2019). At least 500 pollen grains were counted and identified using light microscopy at 400x magnification. Pollen classes usually followed those established by Zander (Louveaux et al., 1978) meaning honey analysis PP (Predominant Pollen > 45%), AP (Accessory Pollen: 16–45%), IP (Important Minor Pollen 3–15%) and Minor Pollen (m: <3%). These classes were used for qualitative and quantitative analyses of bee pollen loads in the present paper.

Samples were sent to Centralized Resource Laboratory (CRL), the University of Peshawar for Scanning Electron Microscope (SEM) images for further confirmation of forage resources. JEOL made in Japan model JSM 5910 Fig. 5 was used for the purpose. Preparation of reference slides was done by analyzing pollen taxa...
collected from the sampling sites throughout two calendar years. Descriptive statistic was used, and the raw pollen data was processed using the Microsoft Office Excel 2013.

3. Results

3.1. Melliferous resources in Dera Ismail Khan through visual survey

Focal observations of vegetation survey revealed seventy plant species belonging to 26 families as bee flora of DIKhan (Table 1). Flowering species offering pollen (P) or both nectar and pollen (PN, np) were considered as pollen sources. Nectariferous plants were found to be 20% while polleniferous flora was found to be 11%. 68% melliferous flora was found to be a source of both pollen and nectar. Forage source (pollen/nectar) was confirmed with published accounts (Table 1) but preference was given to the personal observations recorded during field investigation for categorization of flora into P, N, NP (Pn=1, 2, 3), np plants and for other calculations. These 56 plants species belonging to 22 families and 48 genera comprised of cultivated herbs (29 plant species), shrubs (8 plant species), trees (15 Plant species), climbers (1 plant species), vines (2 Plant species), and palm (1 Plant species). Out of total 70 floral resources, P1 and NP1 categories were regarded as major pollen-producing flora for Apis mellifera in Dera Ismail Khan. This major pollen flora visited by the honey bee most frequently and comprises 18 plant species including

![Fig. 2. Result comparison of both methodologies.](image1)

![Fig. 3. Contribution of different categories of flora in pollen production in DIKhan.](image2)
allowed by trees (33.33%) and shrub (16.66%) for major pollen flora. Twenty-two plant species categorized as P2 and NP2 namely, were found to be an intermediate source of pollen for *Apis mellifera* L. in DI Khan Table 1. Out of these intermediate sources, 54.54% were found to be herbs, 9.09% shrubs, 22.72% trees and rest belonged to climber, palm and vine categories each 4.54%. Categories P3,
NP3 and np Table 1 were regarded as minor sources as these were least frequently visited by the honey bee (*Apis mellifera* L.). These included *Combretum indicum* L only during dearth period when there was no major and intermediate flora available. Fifty per cent of minor flora was coming from herbs followed by trees (25%) and shrubs (18.75%) and vines (6.25%). After compiling the data, it was found herbs were the major source with 51.79% followed by trees 26.78% and shrubs 14.28% and vines 3.57%. In contrast, climbers and palm participated equally with 1.78% each in the provision of pollen to *Apis mellifera* L. in DIKhan (Fig. 3).

The blooming times of the plants differed with the species. However, there was no month that a plant was not in flowering. Based on the results obtained from the present study, twenty-two families viz; Amaryllidaceae, Apiaceae, Apoeynaceae, Are-caceae, Asteraceae, Brassicaceae, Combretaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Malvaceae, Moingaceae, Moraceae, Myr-taceae, Pedaliaceae, Poaceae, Portulacaceae, Punicaceae, Rahm-
naceae Rosaceae, Rubiaceae, Rutaceae and Solanaceae served to offer pollen to *Apis mellifera* L. Month-wise analysis of flora revealed that first quarter of the year is normally a hectic period for the *Apis mellifera* L. in DIKhan when most of the plant species are in full blooming. The major pollen flow period comprises mid-February to mid-May (Fig. 1). During this period temperature remains mild with little rainfall, and bees’ visitation to the field for enhancing colony reserves reaches its peak.

Trend line Fig. 1 shows a gradual increase in the availability of food sources to honey bee from mid-February to the end of May. A constant trend for melliferous plants availability was noted from mid-April to mid-May. Highest Average Directional Index (ADX) value, i.e., 31 was found in April constituting the main pollen flow period in DIKhan. Then a gradual decrease occurs with minor honey flow period in October and November due to the presence of some highly attractive melliferous plants like Ziziphus spp. Still, the only experienced can yield honey during this period if someone has taken proper maximum measures during the main pollen flow period in March, April and May.

The study disclosed this period as the most suitable time for most of the management activities to be carried out by the beekeepers to enhance their colony strength and honey production. During this period, apiculturists can install hives, re-queen colonies, harvest honey, royal jelly, beeswax, propolis, divide and reunite their colonies depending on the condition of the boxes and trap the maximum amount of pollen.

3.2. Melliferous resources in Dera Ismail Khan through pollen traps

Pollen analysis through traps can be used for establishing a trophic link of managed bee *Apis mellifera* L. with available flora in its surroundings. To verify the results of a focal survey regarding primary pollen resources in DIKhan, pollen trapping methodology and their botanical identification was used. Pollen trapping conducted for two consecutive years 2018–19 (Table 2). We found 44 pollen types belonging to 16 families and 36 Genera in contrast to the visual survey, which revealed 56 pollen types belonging to 22 families and 48 genera (Fig. 2). The highest richness of pollen types was observed in sample 5 (pooled sample D2 of March 2018 and 2019: 20 types) and the smallest in the sample 23 & 24 (pooled sample D1 & D2 of December 2018 and 2019: 3 types each). The families with the most significant representation of pollen types were Fabaceae with 8 types (18.6%), Asteraceae with six pollen types (13.9%), Myrtaceae and Brassicaceae with four types (9.1% each), Rutaceae, Solanaceae, Apiaceae with three pollen types (9.1% each), Malvaceae, Cucurbitaceae, Moraceae with two pollen types (4.5% each). In contrast, Combretaceae, Pedaliaceae, Punicaeae, Compositae, Moringaceae, Amaryllidaceae, Poaceae were represented by the lowest number of pollen types i.e., one (2.3% each). Botanical affinities of pollens revealed during pollen trapping technique was established at least to the family level. This methodology revealed 8 major pollen types as Predominant pollen flora of DIKhan with more than 45% occurrence in the samples. These eight types were in accordance with the results of a visual survey. Most of the remaining pollen flora identified through the visual survey as major flora (*Moringa oleifera* L., *Pism sativum* L, *Trifolium resuspimate*, *Zyzygium cumini* L, *Helianthus annuus* L, *Brassica rapa* L, *Morus australis* L.) appeared as Secondary pollen (16–45%) in ensnared pollens. *Tugates erecta* L. was found to occur in each sample though in minor amounts (<3%). Exceptions may be due to bee inclination for nectar collection from these species, presence of some other most preferable forage during the same period or due to decreased land cultivation of these species or some other disturbance during the collection period. The maximum number of pollen was harvested through traps in February, March and April (Spring Season in DIKhan) and minimum in autumn. In particular, “predominant” and “secondary” pollen types belonged to the families Brassicaceae, Fabaceae, Poaceae, Moraceae, Rutaceae, Asteraceae, Myrtaceae, and Moringaceae.

4. Discussion

This study investigated the pollen profile at the first step and then major, intermediate and minor pollen resources employing direct observation of bees and plants relationships in the fields (Siviram, 2013) and pollen traps, designed to remove corbicular loads from the legs of forager bees entering the hive (Koppler et al., 2007). Ramalho et al. (1990) reviewing trophic resources of Neotropical regions authenticated the families Anacardiaceae, Areaceae, Asteraeaceae, Balsaminaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Leguminosae, Moraceae, Proteaceae, Rubiaceae and Sterculiaceae as important melliferous resources. Our study also depicted many of these families with large presentation, especially Fabaceae and Asteraeaceae.

Our findings of *Acacia modesta* L., *Brassica campestris* L, *citrus* spp., *Dalbergia sissoo*, *Phonex dactylifera*, *Psidium guajava* L., *Trifolium spp.*, *Zea mays* L. and *Ziziphus* spp. as primary pollen resources were also in accordance with the results of Shahid and Qayyum (1977) who stated these plants as major sources of nectar and pollen in NWFP (Now known as KPK). Major sources in agricultural crops viz: *Brassica campestris* L. (Sarson), *Trifolium alexandrinum* L. (Berseem), *T. resuspinate* L. (Shaftal) and *Zea mays* L. (Makri) were found same as indicated by Chaudhry (1985) in Pakistan. *Zea mays* L., *Trifolium alexandrinum* L. *Citrus* spp., and *Eucalyptus* spp. were also found as main pollen flora investigated by El-Bassiouny (1989). Mehta et al. (2012) recommended the plantation of trees like *Eucyptus spp.*, *Moringa olferia* L., *Azadiraicha indica* L., *Dalbergia sissoo* L., *Acacia nilotica* L., to enhance nectar and pollen sources for honey bees matched our finding of apiphilic trees. Most of the major flora found in this investigation was in agreement with the results of Marwat et al. (2013) though five other resources were also found to be major ones in DIKhan through focal observation in our study. Contrary to the findings of Marwat et al. (2013) in our results *Phonex dactylifera* was found to be a minor source of pollen in palynological analysis of pollens which may be due to availability of other resources in sufficient amounts at the same time like *Brassica spp* in this region. Our findings of maize, sunflower and mustard as primary pollen sources in both techniques are also rectified by the results of Ismail et al. (2013). They found these three species along with sesame and clover as main pollen sources in Fayoum Governorate, Egypt. *Cucumis melo*, *Allium cepa*, *Pism sativum*, *Helianthus annuus*, *Citrullus lanatus*, *Moringa oleifera*, *Zea mays*, *Punica granatum*, *Ziziphus* spp., *Citrus limon* were found as pollen-producing forage for bees in the investigations of Bhalachandra et al. (2014) through focal observations which are similar to the result of our visual survey results. Similar results were obtained by Pande and Ramkrushna (2018) in Maharashtra, India, indicating 22 polleniferous plant species. The results of this study revealed that honeybee collects pollen and nectar from herbs, shrubs, and trees which is in accordance of the result obtained by Chauhan et al. (2018) in Pakistan. The results of the present study highlighted the significance of pollen plant sources for strengthening bee colonies and harvesting maximum pollen for commercial purposes. *Combretum indicum* was the only plant among pollen flora which was visited one year of study but no other. This may be more indicative of the lack of available forage for honeybees than their actual interest in *Combretum indicum* L.

Information on floral pollen preferences of foraging honeybees is imperative to increase the number of strong colonies in area which will ultimately cause high production of honey and other bee products for commercial purposes. The findings can also be
Table 2
Quantitative analysis of pollen samples of *A. mellifera* L. As per *Louveaux et al.* (1978).

| S. No | Sample No. with no. of pellets | Date of collection | Predominant pollen (≥45%) | Secondary pollen (16–45%) | Important Minor Pollen (3–15%) | Minor pollen (<3%) |
|-------|--------------------------------|-------------------|-----------------------------|---------------------------|--------------------------------|------------------|
| 1     | D1: PLS-1 (1026)               | 15.01.2019        | Brassica napus L            | Brassica compestris L     | Coriandrum sativum L, Moringa oleifera L | Raphanus sativus L, Callistemon lanceolatus, Tagetes erecta L, Solanum lycopersicum L |
|       |                                | 15.01.2019        |                             |                           |                                | Chrysanthemum indicum L, Pisum sativum L |
| 2     | D1: PLS-2 (960)                | 30.01.2019        | Brassica napus L            | Moringa oleifera L        | Coriandrum sativum L, Brassica compestris L | Raphanus sativus, Tagetes erecta L, Citrus lanatus, Solanum lycopersicum L, Chrysanthemum indicum L, Brassica rapa L, Bauhinia variegate L, Brassica compestris L |
|       |                                | 30.01.2019        |                             |                           |                                | Morus australis, Morus alba, Solanum lycopersicum L, Bauhinia variegate L, Brassica compestris L |
| 3     | D1: PLS-3 (880)                | 15.02.2019        | Brassica napus L            |                            | Morus australis L, Morus alba L, Pium sativum L, Moringa oleifera L, Brassica rapa L | Raphanus sativus, Tagetes erecta L, Citrus lanatus, Solanum lycopersicum L, Bauhinia variegate L, Brassica compestris L |
|       |                                | 15.02.2019        |                             |                           |                                | Morus australis, Morus alba L, Brassica rapa L, Bauhinia variegate L, Brassica compestris L |
| 4     | D1: PLS-4 (3002)               | 28.02.2019        | Brassica napus L, Pium sativum L, Morus australis L, Brassica rapa L | Brassica globulus, Citrus aurantium L |                            |                                |
|       |                                | 28.02.2019        |                             |                           |                                |                                |
| 5     | D1: PLS-5 (4120)               | 15.03.2019        | Eucalyptus globulus L, Morus australis L, Citrus aurantium L |                            | Citrus limon L, Syzygium cumini L, Brassica rapa L |                                |
|       |                                | 15.03.2019        |                             |                           |                                | Morus australis L, Morus alba L, Solanum nigrum (maize), Brassica rapa L |
| 6     | D1: PLS-6 (5290)               | 30.03.2019        | Citrus aurantium L          |                            | Foeniculum vulgare L, Syzygium cumini L, Citrus aurantium L |                                |
|       |                                | 30.03.2019        |                             |                           |                                | Morus australis L, Morus alba L |
| 7     | D1: PLS-7 (2950)               | 15.04.2019        | Eucalyptus globulus L, T. alaxandrinum L, T. resupinatum L, Syzygium cumini L, Citrus aurantium L |                            |                                |                                |
|       |                                | 15.04.2019        |                             |                           |                                | Foeniculum vulgare L, Syzygium cumini L, Citrus aurantium L |
| 8     | D1: PLS-8 (955)                | 30.04.2019        | Trifolium alexandrinum L    | Acacia modesta L, Syzygium cumini L, T. resupinatum L |                            |                                |
|       |                                | 30.04.2019        |                             |                           |                                | Morus australis L, Morus alba L, Solanum nigrum (maize) |
| 9     | D1: PLS-9 (750)                | 15.05.2019        | Acacia modesta L, Syzygium cumini L |                            | Psidium guajava, L. Eucalyptus globulus L |                                |
|       |                                | 15.05.2019        |                             |                           |                                | Morus australis L, Morus alba L, Solanum nigrum (maize) |
| 10    | D1: PLS-10 (684)               | 30.05.2019        | Acacia modesta L, Syzygium cumini L |                            | Psidium guajava, L. Eucalyptus globulus L |                                |
|       |                                | 30.05.2019        |                             |                           |                                | Morus australis L, Morus alba L, Solanum nigrum (maize) |
| 11    | D1: PLS-11 (695)               | 15.06.2019        | Acacia modesta L            |                            | Psidium guajava, Momordica charantia L, Abelmoschus esculentus s. L |                                |
|       |                                | 15.06.2019        |                             |                           |                                | Morus australis L, Morus alba L, Solanum nigrum (maize) |
| 12    | D1: PLS-12 (540)               | 30.06.2019        | Acacia modesta L, Psidium guajava, Momordica charantia L, Abelmoschus esculentus s. L |                            |                                |                                |
|       |                                | 30.06.2019        |                             |                           |                                | Morus australis L, Morus alba L, Solanum nigrum (maize) |
| 13    | D1: PLS-13 (555)               | 15.07.2019        | Zea mays L, Luffa cylindrica L, Helianthus annuus L |                            |                                |                                |
|       |                                | 15.07.2019        |                             |                           |                                | Morus australis L, Morus alba L, Solanum nigrum (maize) |
| 14    | D1: PLS-14(420)                | 30.07.2019        | Zea may L, Helianthus annuus L |                            |                                |                                |
|       |                                | 30.07.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 15    | D1: PLS-15 (220)               | 15.08.2019        | Zea may L, Helianthus annuus L |                            |                                |                                |
|       |                                | 15.08.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 16    | D1: PLS-16 (210)               | 30.08.2019        | Zea may L, Helianthus annuus L |                            |                                |                                |
|       |                                | 30.08.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 17    | D1: PLS-17 (130)               | 15.09.2019        | Helianthus annuus L          |                            |                                |                                |
|       |                                | 15.09.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 18    | D1: PLS-18 (150)               | 30.09.2019        | Helianthus annuus L          |                            |                                |                                |
|       |                                | 30.09.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 19    | D1: PLS-19 (140)               | 14.10.2019        |                                |                            |                                |                                |
|       |                                | 14.10.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 20    | D1: PLS-20 (120)               | 27.10.2019        |                                |                            |                                |                                |
|       |                                | 27.10.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 21    | D1: PLS-21 (480)               | 15.11.2019        |                                |                            |                                |                                |
|       |                                | 15.11.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 22    | D1: PLS-22 (526)               | 30.11.2019        |                                |                            |                                |                                |
|       |                                | 30.11.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 23    | D1: PLS-23 (240)               | 15.12.2019        |                                |                            |                                |                                |
|       |                                | 15.12.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |
| 24    | D1: PLS-24 (350)               | 30.12.2019        |                                |                            |                                |                                |
|       |                                | 30.12.2019        |                             |                           |                                | Morus australis L, Solanum nigrum (maize) |

D1=First collection of the month, D2=Second Collection of the month, PLS= Pollen Load Sample
helpful for the planning and establishment of forage plantings in billion tree Tsunami project in PKP to sustain honey bees, and in the development of seasonal nutritional supplements fed to colonies when pollen is unavailable. Albeit area’s potential to produce honey, commercial beekeeping is relatively underdeveloped as very little is known about bee forage resources and their contribution to apicultural activities. In fact, our review of the literature revealed only one study carried out by Marwat et al. (2013) on bee flora through the traditional method of only visual observation in DI Khan. Mostly migratory beekeeping is practiced in area and beekeepers of surrounding areas dump their colonies here when brassica species are near to bloom. Non-migratory beekeeping is possible by identifying the pollen sources and cultivating those resources near apiaries. Spring and early summer were found to be the prime time for massive pollen collection while autumn and late winter were the least one in the study area. Relative variations were also noticed in pollen amounts for the same source in the two studied years and were attributed to fluctuations in cultivated areas plus bees’ activity restricted due to change in seasonal and diurnal temperature.

5. Conclusion

This study is a pioneer and can be a baseline study for commencing beekeeping on a commercial scale in DIKhan. Melliferous pollen produced in the region of DIKhan is constituted by the association of many pollen types, most of which are related to common species from the tropical areas. The pollen spectrum outcome through Palynological analysis indicates specialist foraging behavior of Apis mellifera L. as compared to other species like dorsata and florea. In this study comparison of results of both methodologies showed more than 50% differences in major bee flora (18 species through visual survey and 8 species through palynological pollen analysis). This indicates that Palynological study of pollen loads is more authentic for investigating bee pasturage of any area than visual survey and can serve as an important tool for the development of the regional apiculture. As in Pakistan, mostly migratory beekeeping is practiced, so knowledge of this study can assist migratory beekeepers when to visit this area as a suitable site for honey production and pollen interception.

Moreover, local beekeepers and persons adopting honey bee as a hobby can also take benefit by planting the honey bee loving forage. This area is also an integral part for plantation in Tsunami Billlion Tree Project for combating climate change. The insight provided in the knowledge of bee flora can be useful for incorporating of a plantation of apiphilic trees to conserve the species of Apis mellifera L. The only difficulty lies with the management of bee colonies in summer season (especially in July and August) when the temperature rises to 46 °C and bees are prone to wax moth attack.

Based on this information, DIKhan, which is already an established area for migratory beekeeping can be considered as the potential area for non-migratory beekeeping as well. Therefore, attention must be given to the cultivation of existing bee flora to increase harvesting of bee products and development of apiculture industry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors express their sincere appreciation to the lab and field staff of faculty of Agriculture, Gomal University DIKhan for their invaluable assistance. We also acknowledge migratory beekeepers regarding their permission for installing traps on their colonies. We are highly indebted to the staff of Centralized Resource Laboratory, the University of Peshawar for Scanning Electron Microscopy and pollen grains identification, and Dr. Sundas Batool, Assistant Professor in the Department of Plant Breeding and Genetics in Faculty of Agriculture, Gomal University DIKhan for light microscopy work.

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