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Original article

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

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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.

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 Crailsheim 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

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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 (Adekanmbi 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 corbicular 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. (Balasubramanya 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	Apicultural value (references + personal observations)	Habit
1.	*Abelmoschus esculentus* L.	Bhindi/ Okra	P (Marwat S. K et al., 2013), N (Mishra et al., 1987), NP[2] (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), NP[1] (PO)	Shrub
4.	*Acacia nilotica* L.	Kikar	N (Marwat, S.K. et al., 2013), N(PO)	Tree
5.	*Albizia lebbeck* L.	Sareen	Pn (Bista, S., & Shivakoti, G.P.,. 2001), P[2](PO)	Tree
6.	*Allium cepa* L.	Vasa or Piazl/Onion	N (McGregor, 1976; Kumar & Gupta, 1993), P (McGregor, 1976; Bhuiyan et al., 2002), NP (Layek et al., 2015), NP[2] (PO)	Herb
7.	*Brassica napus* L.	Canola	N (McGregor, 1976), PN (Bista, S., and Shivakoti, G.P., 2001), NP[1] (PO)	Herb
8.	*Brassica campestris var toria* L.	Rapeseed	PN(Bista & Shivakoti, 2001; Rijjal et al., 2018), N P[1](PO)	Herb
9.	*Brassica rapa* L.	Ghongloo/shalgam/ Turnip	P (McGregor, 1976) PN (Bista & Shivakoti, 2001; Bhuiyan et al., 2002; Taha and Bayoumi,2009) , NP[1] (PO)	Herb
10.	*Bauhinias variegate Linn.*	Kachnar	NP (Bista & Shivakoti, 2001), NP[2] (PO)	Tree
11.	*Calendula officinalis* L.	Marigold	NP[2] (PO)	Herb
12.	*Callistemon lanceolatus* L.	Bottle brush	Np (Bista & Shivakoti, 2001; Bareke, et al.,2017; Rijal et al., 2018), NP[3] (PO)	Tree
13.	*Ixora coccinea* L.	—————————	P[1](PO)	Tree
14.	*Capsicum annum* L.	Sabz mirch/Chilli	P[3](PO)	Herb
15.	*Cassia fistula* L.	Ambal tas/Golden Shower	N (Layek, U., Bhakat, R. K., & Karmakar, P., 2015), N P[2] (PO)	Tree
16.	*Citrullus lanatus (Thunb.) Matsum. & Nakai*	Tarbooz/watermelon	N (Morton, 1964; Taha & Bayoumi., 2009), P (McGregor, 1976), PN (Taha and Bayoumi, 2009); Layek, et al., 2015), NP[2](PO)	Prostrate or Climber
17.	Cicer arietinum L.	Channa/Chick peas	N (Marwat et al.,2013), NP[3](PO)	Herb
18.	*Citrus aurantium* L.	Khatta/Bitter or sour orange	P[1]N (Alghoson, 2004), NP[1] (PO)	Small Tree
19.	*Citrus sinensis* L.	Malta/Orange	P (Ismail et al., 2013) N (Morton, 1964; Adjaloo and Yeboah-Gyan, 2003), NP[1](PO)	Small tree
20.	*Citrus limon* L.	Lemon	N (Morton, 1964), NP[2] (PO)	Shrub
21.	*Coriandrum sativum* L.	Dahnia/ Coriander	P (McGregor, 1976; Ismail et al., 2013), N (McGregor, 1976), NP(Bhuiyan et al., 2002; Layek et al., 2015), NP[1] (PO)	Herb
22.	*Chrysanthemum indicum* L.	Guldaudi	PN(Layek, et al., 2015), N P[2] (PO)	Herb
23.	*Cucurbita maxima Duchesne*	Pumpkin	N(Layek, et al., 2015), N(PO)	Climber/Prostrate
24.	*Combretum indicum* L.	Har Singhar	Np (Ahmad et al., 2019), np(PO)	Shrub
25.	*Conocorpus erectus* L.	Cono	NP[3](PO)	Shrub or small to medium size tree
26.	*Cucumis sativus* L.	Khhira/Cucumber	pn (Morton, 1964; McGregor, 1976), Np (Hossain et al., 2018), N(PO)	Herb
27.	*Cucumis melo* L.	Kharbooza/ Muskmelon	PN (McGregor, 1976), N(Ribeiro et al., 2015; Ahmad et al., 2019), N(PO)	Creeper
28.	*Dahlia variabilis* L.	Dahlia/ Fireworks Mixed	N P[2] (PO)	Herb
29.	*Dalbergia sissoo Roxb.*	Shisham, Tali	N(Rijal et al., 2018., Layek et al., 2015) P (Ahmad et al., 2019), N(PO)	Tree
30.	*Daucus carota* L.	Gajar/Carrot	NP (McGregor, 1976), NP[2] (PO)	Herb
31.	*Euclyptus* spp.	Sufaida/Kafour	P (Morton, 1964; Ismail et al., 2013; Sivaram, P. 2013), N (Morton, 1964; Bhuiyan et al., 2002; Alghoson, 2004), NP[1] (PO)	Tree
32.	*Foeniculum vulgare* L.	Saunf	NP[1] (PO)	Herb
33.	*Gardenia jasminoides* L.	Jasmine	P(Ahmad et al., 2019), P[2](PO)	Shrub
34.	*Grewia asiatica* L.	Phalsa	N(PO)	Shrub
35.	*Gailardia aristata* L.	Blanket flower	N(PO)	Shrub
36.	*Gossypium herbaceum* L.	Kappas/ Cotton	Np (McGregor, 1976; Hussein, 2001) NP[3](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), NP[1] (PO)	Shrub
38.	*Hibiscus sabadirifa* L.	Roselle	NP[3](PO)	Shrub
39.	*Lagerstroemia indica* L.	Bouganvilla	N(PO)	Shrub
40.	*Leucaena leucocephala (Lam.) de Wit*	Sareen/ river tamarind	NP(Layek, et al., 2015), NP[3](PO)	Tree
41.	*Luffa cylindrica* L.	Tori/Sponge gourd	NP(Layek, et al., 2015), NP[2](PO)	Vines
42.	*Medicago sativa* L.	Alfalfa	N (McGregor, 1976; Alghoson, 2004), P (Alghoson, 2004), NP[2](PO)	Herb
43.	*Mangifera indica* L.	Aam/ Mango	N (Layek, et al., 2015; Chauhan et al., 2018), N(PO)	Tree
44.	*Melia azedarach* L.	Bkayan	N (Bista & Shivakoti, 2001) N(Layek, et al., 2015), P (Rijal et al., 2018), N(PO)	Tree
45.	*Mimosa pudica* L.	Lajvanti/ chhui mui	P(Ahmad et al., 2019), PN(Bista & Shivakoti, 2001; Layek et al., 2015), NP[3] (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)	Climber or prostrater
47.	*Moringa oleifera Lam.*	Sohanjna/Moringa	PN(Layek, et al., 2015), NP[2] (PO)	Tree
48.	*Morus australis* L.	Shehtoot	P[1](PO)	Tree
49.	*Morus alba* L.	Mulberry/Toot	P[1](Rijal et al., 2018), P[1](PO)	Tree

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[3](PO)	Shrub
51.	*Ocium tenuiflorum* L.	Tulsi/ Niaz bo	NP[3](PO)	Herb
52.	*Phoenix sylvestris* L.	Khaji /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)	Herb
54.	*Portulaca grandiflora* Hook.	Luni	P (Wongpiyasatid and Hormchan, 2001), NP[3](PO)	Herb
55.	*Punica granatum* L.	Anar/Pomegranate	NP (Rijal et al., 2018), NP[3](PO)	Tree
56.	*Psidium guajava* L.	Amrood/Guava	PN(Bista and Shivakoti, 2001), Pollen (Adjaloo and YeboahGyan, 2003; Anita et al., 2012; Shubharani et al., 2013) N (Adjaloo and YeboahGyan, 2003), NP[2] (PO)	Tree
57.	*Raphanus sativus* L.	Mooli/Radish	P (McGregor, 1976) P[2](PO)	Herb
58.	*Rosa indica* L.	Gulab/Rose	P (Morton, 1964; Noor et al., 2009; Shubharani et al., 2013) NP (Bista and Shivakoti, 2001; Rijal et al., 2018), NP[3](PO)	Shrub
59.	*Ruta graveolens* L.	Kaner/Zehr Booti	N (Ren and Tang., 2012), N(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.	Tamatar/Tomato	Pollen little (McGregor, 1976), N (Adjaloo and YeboahGyan, 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[1](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	NP (Bista and Shivakoti 2001; Rijal et al., 2018), NP[2](PO)	Herb
66.	*Trifolium alaxandrinum* L.	Barseem/Egyptian clover	PN (Shawer, 1987; Hussein, 2001; Bista and Shivakoti, 2001; Ismail et al., 2013), NP[1] (PO)	Annual Herb
67.	*Trifolium. Resupinatum* L.	Shaftal	PN (Bista and Shivakoti, 2001), NP[1] (PO)	Annual Herb
68.	*Zea mays* L.	Makai/Maize	P (Morton, 1964; Shawer, 1987; Adjaloo and Yeboah-Gyan, 2003; Ismail et al., 2013; Bista and Shivakoti, 2001), P[1](PO)	Herb
69.	*Zizyphus* Spp.	Ber	NP (Bista, S., and Shivakoti, G.P., 2001; Marwat et al., 2013), N (Alghoson, 2004), N(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[1]n**: Mainly attractive for pollen with little attraction for nectar; **NP[2]**: Mainly attractive for nectar with little attraction for pollen; **NP[3]**: mainly attractive for nectar with least attraction for pollen; **P**: Only attractive for pollen; **N**: Only attractive for nectar (P[1], P[2] and P[3] indicate major, Intermediate and minor pollen sources respectively).

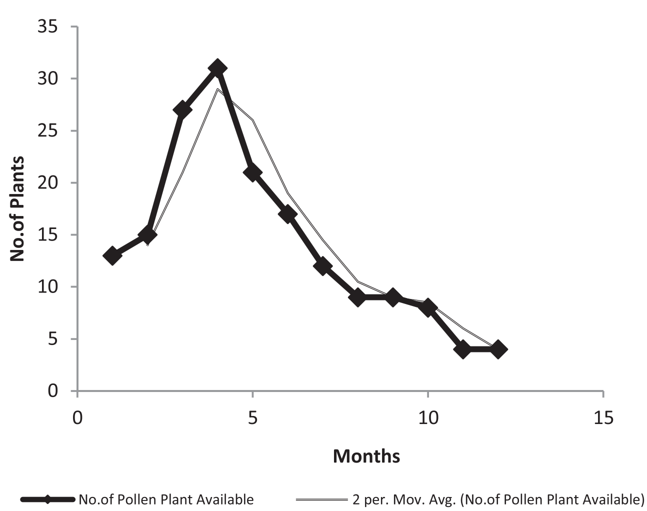

Fig. 1. Month-wise pollen resources availability in DIK.

removed. The trapped pollen loads were weighed, segregated based on colours, put in plastic bags and stored in a freezer at −20 °C. These loads were then used to make slides for identification of botanical origin through light microscopy and also sent to Centralise Resource Laboratory (CRL), the University of Peshawar for Scanning Electron Microscope (SEM) images (Fig. 4) for identification purposes.

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

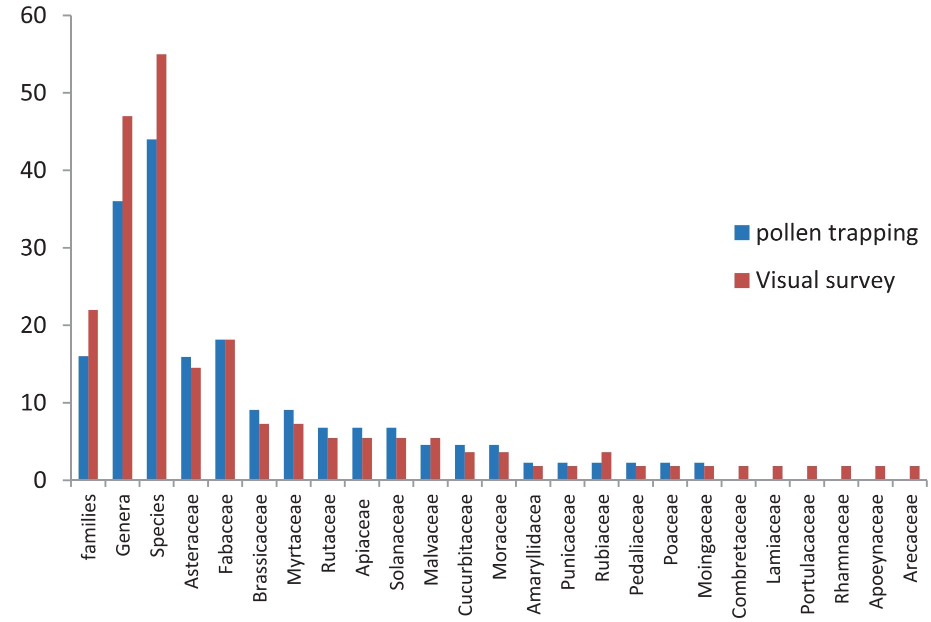

Fig. 2. Result comparison of both methodologies.

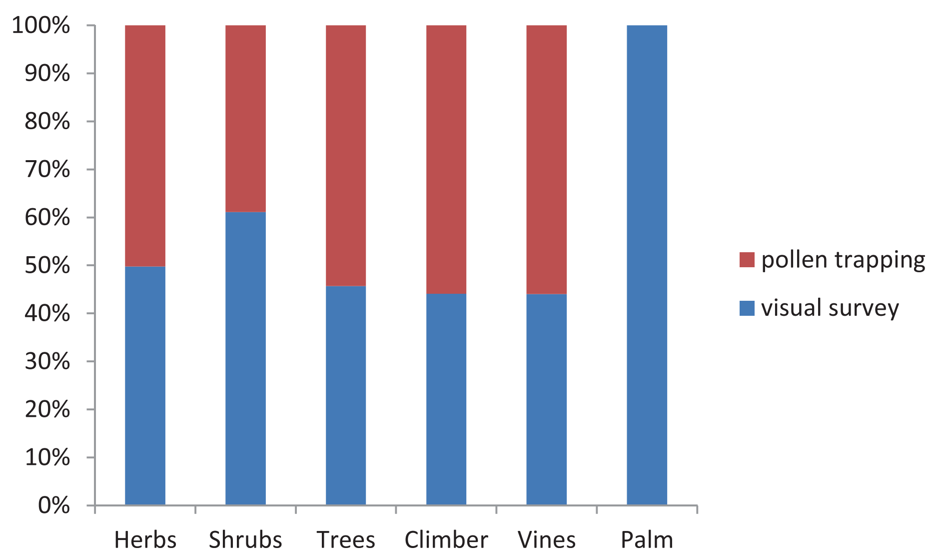

Fig. 3. Contribution of different categories of flora in pollen production in DIKhan.

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^n ($P^{n=1,\ 2,\ 3}$), 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, P^1 and NP^1 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 *Brassica napus*[1] L., *Brassica campestris*[2] var toria L., *Brassica rapa*[3] L., *Foeniculum vulgare*[4] L., *Trifolium alaxandrinum*[5] L., *Trifolium Resupinatum*[6] L., Taxacum officinale[7] L., *Coriandrum sativum*[8] L., *Acacia modesta*[9] L., *Helianthus annuus*[10] L., *Citrus aurantium*[11] L., *Citrus sinensis*[12] L., *Euclyptus spp*[13]., L., *Syzygium cumini*[14] L.. *Ixora coccinea* L[15]., *Morus australis*[16] L., *Morus alba*[17] L., *Zea mays*[18]L., were found to be major pollen sources. Herbs were serving the most abundant source(50%) fol-

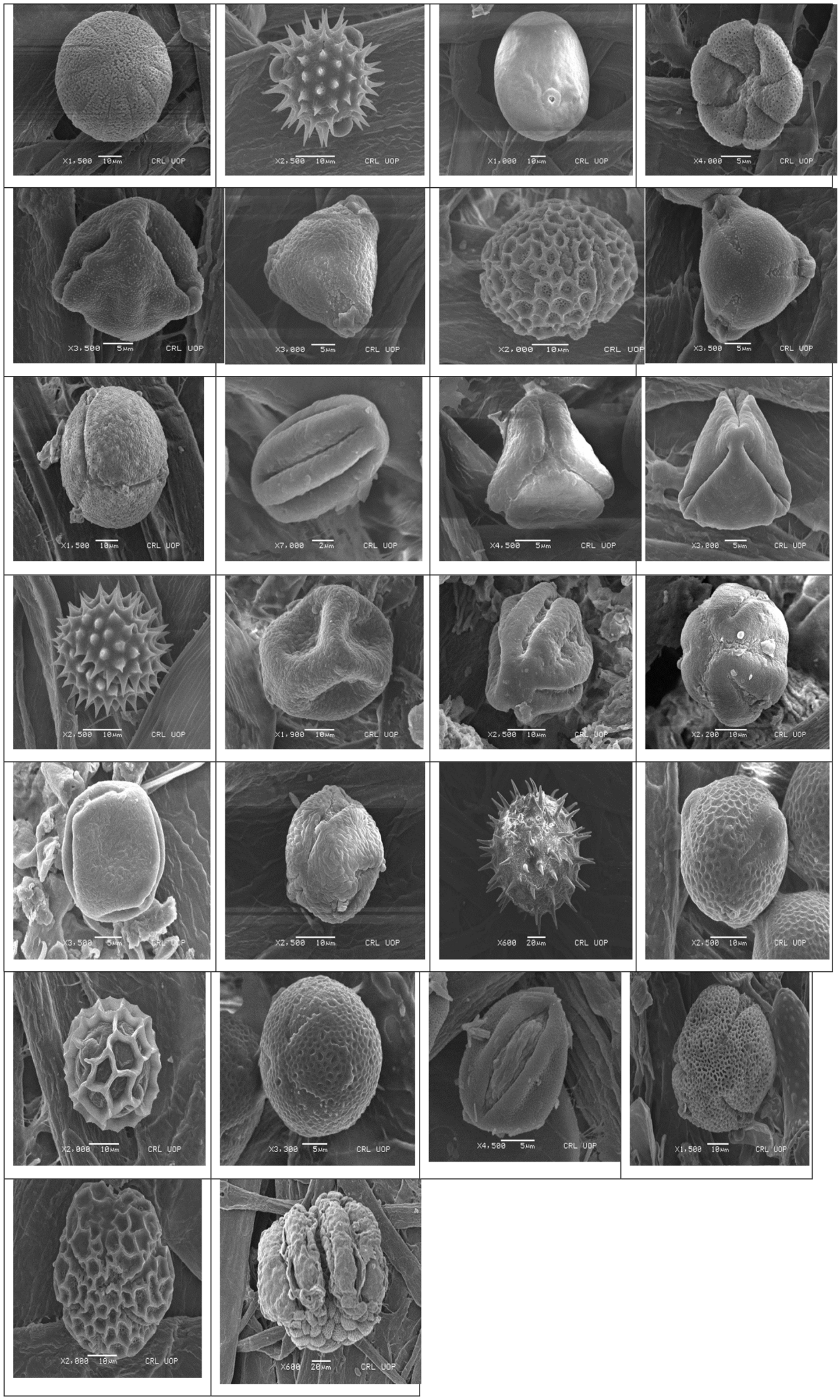

Fig. 4. Scanning electron micrographs of pollen grain1 of some melliferous plants (from left to right). 1. *Acacia nilotica* L. 2. *Helianthus annus* L. 3. *Zea mays* L. 4. *Luffa cylindrica* L. 5. *Gardenia jasminoides* L. 6. *Capsicum annum* L. 7. *Ocium tenuiflorum* L. 8. *Momordica charantia* L.9. *Cucurbita maxima* Duchesne 10. *Conocorpus erectus* L. 11. *Callistemon lanceolatus* L. 12. *Cassia fistula* L. 13. *Tagetes erecta* L. 14. *Lagerstroemia indica* L. 15. *Punica granatum* L.16. *Combretum indicum* L. 17. *Zizyphus Jujuba* L.18. *Rosa indica* L. 19. *Hibiscus sabadirifa* L. 20. *Trifolium alaxandrinum* L. 21. *Daucus carota* L. 22. *Brassica napus* L. 23. *Phoenix sylvestris* L. 24. *Ixorea coccinea* L. 25. *Gossypium herbaceum* L. 26. *Sasemum indicum* L.

lowed by trees (33.33%) and shrub (16.66%) for major pollen flora. Twenty-two plant species categorized as P^2 and NP^2 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,

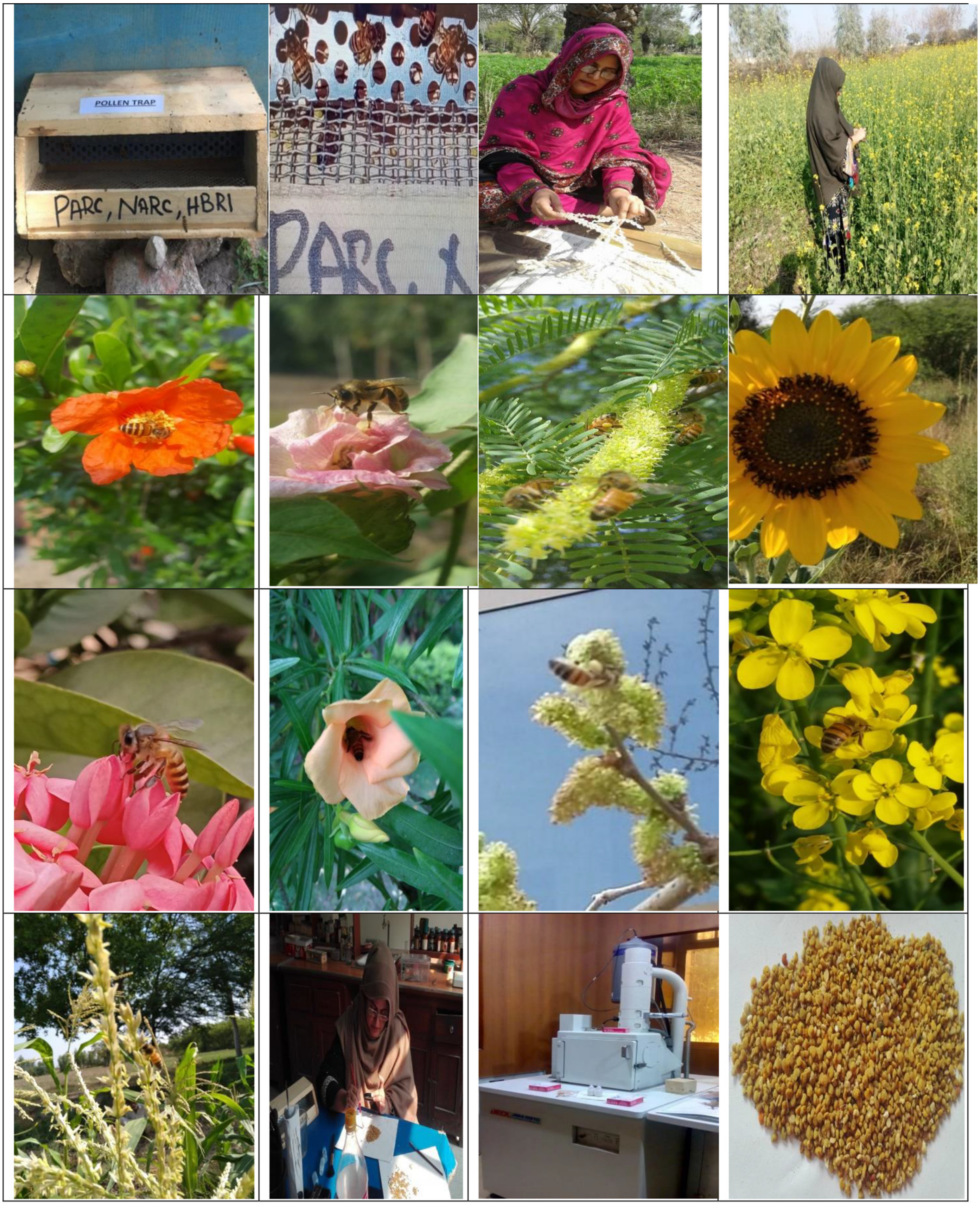

Fig. 5. Few glimpses of research work.

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 The honey bee visited *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; Amaryllidacea, Apiaceae, Apoeynaceae, Arecaceae, Asteraceae, Brassicaceae, Combretaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Malvaceae, Moingaceae, Moraceae, Myrtaceae, Pedaliaceae, Poaceae, Portulacaceae, Punicaceae, Rahmnaceae 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 bee-keepers 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 D_2 of March 2018 and 2019: 20 types) and the smallest in the sample 23 & 24 (pooled sample D_1 & D_2 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, Punicaceae, Compositae, Moingaceae, Amaryllidaceae, Poaceae were represented by the lowest number of pollen types i.e., one (2.3% each). Botanical affinity 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 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., *Pisum sativum* L., *Trifolium resusipinatum, Syzygium cumini* L, *Helianthus annuus* L, *Brassica rapa* L., *Morus australis* L.) appeared as Secondary pollen (16–45%) in ensnared pollens. *Tagetes 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 Moingaceae.

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, Arecaceae, Asteraceae, 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 Asteraceae.

Our findings of *Acacia modesta* L., *Brassica compestris* L., citrus spp., *Dalbergia sissoo*, *Phoneix dactylifera* L., *Psidium guajava* L., Trifolium spp., *Zea mays* L. and Ziziphus spp. as primary pollen sources 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. (Sarsoon), *Trifolium alexandrinum* L. (Berseem), *T. resusipinatum* L. (Shaftal) and *Zea mays* L. (Maki) 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 *Euclyptus* spp., *Moringa olifera* L., *Azadirachta indica* L., *Dalbergia sissoo L., Acacia nilotika* 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*, *Pisum 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 Bhalchandra 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 *Combrtum 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	Pollen types			
			Predominant pollen (≥45%)	Secondary pollen (16–45%)	Important Minor Pollen (3–15%)	Minor pollen (<3%)
1.	D_1:PLS-1 (1026)	15.01.2018 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. *Chrysanthemum indicum* L., *Pisum sativum*
2.	D_2:PLS-2 (960)	30.01.2018 30.01.2019	*Brassica napus* L.	*Moringa oleifera*, L,	*Coriandrum sativum* L., *Brassica compestris* L.	*Morus australis, Morus alba* , *Solanum lycopersicum* ,*Citrullus lanatus (Thunb.) Matsum. &Nakai* *Chrysanthemum indicum* L. *Brassica rapa* L., *Tagetes erecta* L.
3.	D_1:PLS-3 (880)	15.02.2018 15.02.2019	*Brassica napus* L.	*Morus alba*	*Morus australis* L., *Morus alba* L., *Pisum sativum* L., *Moringa oleifera* L., *Brassica rapa* L.	*Raphanus sativus, Tagetes erecta* L, *Citrullus lanatus*,., *Solanum lycopersicum* L.,, *Chrysanthemum indicum* L, *Bauhinia variegate* L., *Brassica compestris* L., *Coriandrum sativum* L., *Citrus aurantium* L.
4.	D_2:PLS-4 (3002)	28.02.2018 28.02.2019	*Morus alba* L.	*Brassica napus* L., *Pisum sativum* L., *Morus australis* L., *Brassica rapa* L.	, *Pisum sativum* L., *Eucalyptus globulus, Citrus aurantium* L.	*Raphanus sativus, Tagetes erecta* L, *Citrullus lanatus (Thunb.) Matsum. &Nakai.*, *Solanum lycopersicum* L., *Chrysanthemum indicum* L, *Bauhinia variegate* L., *Moringa oleifera*, L., *Brassica compestris* L.
5.	D_1:PLS-5 (4120)	15.03.2018 15.03.2019		*Eucalyptus globulus* L., *Morus australis* L., *Citrus aurantium* L.	*Citrullus lanatus, Citrus sinensis* L. *Citrus limon L., Syzygium cumini* L., *Brassica napus* L., *Brassica rapa* L.	*Allium cepa, Morus alba*, L., *Melia azedarach* L.,, *Foeniculum valgarre* L, *Calendula officinalis* L., *Cassia fistula* L, *Foeniculum vulgare* L., *Combretum indicum* L. *Pisum sativum* L., *Moringa oleifera* L, *Tagetes erecta* L.
6.	D_2:PLS-6 (5290)	30.03.2018 30.03.2019	*Citrus aurantium* L.	*Eucalyptus globule*,	*Foeniculum vulgare* L., *Syzygium cumini* L, *Citrus aurantium* L. *Citrus sinensis* L, *Cassia fistula* L.	*Solanum nigrum(mako), Pisum sativum* L.,, *Combretum indicum* L., *Brassica napus* L., *Brassica rapa* L.,*Tagetes erecta* L.
7.	D_1:PLS-7 (2950)	15.04.2018 15.04.2019	*Eucalyptus globulus* L.	*T. alaxandrinum* L., *T. resusipinatum* L.*Syzium cumini* L.*Citrus aurantinum* L.	*Foeniculum vulgare* L., *Cassia fistula* L. *Citrus sinensis* L.	*Punica granatum*,, *Daucus carota, Capsicum annum* L., *Foeniculum vulgare, Dahlia variabilis, Cassia fistula* L. *Eucalyptus globulus, Tagetes erecta* L.
8.	D_2:PLS-8 (955)	30.04.2018 30.04.2019	*Trifolium alexandrinum* L.	*Acacia modesta* L.	*Syzgium cumini* L., *T. resusipinatum* L. *Citrus arietinum* L.	*Punica granatum, Mimosa pudica* L, *Eucalyptus globulus*., *Tagetes erecta* L.
9.	D_1:PLS-9 (750)	15.05.2018 15.05.2019	*Acacia modesta* L.	*Syzgium cumini* L.,	*Psidium guajava*, L. *Eucalyptus globulus* L.	*Punica granatum, Mimosa pudica* L., *Tagetes erecta* L.
10.	D_2:PLS-10(684)	30.05.2018 30.05.2019	*Acacia modesta* L.		*Psidium guajava*, L. *Syzygium cumini* L.	*Punica granatum, Mimosa pudica* L, *Abelmoschus esculentus* L.,, *Luffa cylindrica* L., *Eucalyptus globulus*,. *Tagetes erecta* L.
11.	D_1:PLS-11(695)	15.06.2018 15.06.2019		*Acacia modesta* L.	*Psidium guajava, Momordica charantia* L., *Abelmoschus esculentus s* L. *Trifolium alexandrinum* L.	*Mimosa pudica* L, *Luffa cylindrica* L, *Syzygium cumini* L., *Tagetes erecta* L.
12.	D_2:PLS-12(540)	30.06.2018 30.06.2019		*Acacia modesta* L	*Psidium guajava, Momordica charantia* L., *Abelmoschus esculentus* L., *Acacia modesta* L.	*Zea mays* L., *Mimosa pudica* L, *Luffa cylindrica* L., *Conocorpus erectus Acacia modesta Trifolium alexandrinum* L., *Tagetes erecta* L.
13.	D_1:PLS-13(555)	15.07.2018 15.07.2019			*Zea mays* L., *Luffa cylindrica* L. *Helianthus annuus* L.	*Sesamum indicum* L.,*Mimosa pudica* L, *Abelmoschus esculentus, Gossypium herbaceum, Acacia modesta* L., *Tagetes erecta* L.
14.	D_2:PLS-14(420)	30.07.2018 30.07.2019		*Zea may L. Helianthus annuus* L.	*Luffa cylindrica* L.	*Sesamum indicum* L., *Gossypium herbaceum* L., *Acacia modesta* L.
15.	D_1:PLS-15(220)	15.08.2018 15.08.2019	*Zea mays* L.	*Helianthus annuus* L.	*Sesamum indicum* L. *Luffa cylindrica* L.	*Gossypium herbaceum* L., *Luffa cylindrica* L. *Tagetes erecta L.*
16.	D_2:PLS-16(210)	30.08.2018 30.08.2019	*Zea mays* L.	*Helianthus annuus* L.	*Sesamum indicum* L.	*Gossypium herbaceum* L., *Tagetes erecta* L.
17.	D_1:PLS-17(130)	15.09.2018 15.09.2019		*Helianthus annuus* L.		*Gossypium herbaceum, Sesamum indicum, Tagetes erecta* L.
18.	D_2:PLS-18(150)	30.09.2018 30.09.2019			*Helianthus annuus* L.	*Gossypium herbaceum* L., *Tagetes erecta* L.
19.	D_1:PLS-19(140)	14.10.2018 14.10.2019				*Albizia lebbeck*,L, *Gossypium herbaceum, Helianthus annuus* L, *Tagetes erecta* L.
20.	D_2:PLS-20(120)	27.10.2018 27.10.2019				*Gossypium herbaceum, Helianthus annuus* L., *Tagetes erecta* L. *Acacia nilotica* L.
21.	D_1:PLS-21(480)	15.11.2018 15.11.2019	*Brassica compestris* L.			*Acacia nilotica, Gossypium herbaceum* L., *Tagetes erecta* L.
22.	D_2:PLS-22(526)	30.11.2018 30.11.2019	*Brassica compestris* L.			*Tagetes erecta* L.
23.	D_1:PLS-23(240)	15.12.2018 15.12.2019	*Brassica compestris* L.		*Brassica rapa* L.	*Tagetes erecta* L.
24.	D_2:PLS-24(350)	30.12.2018 30.12.2019	*Brassica compestris* L.		*Brassica rapa* L.	*Tagetes erecta* L.

D_1=First collection of the month D_2=Second Collection of the month PLS= Pollen Load Sample.

helpful for the planning and establishment of forage plantings in billion tree Tsunami project in KPK 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 DIKhan. 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 Billion 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.

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