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Original Research

Insect Pollinators and Plant Interactions; Identification of Selected Melliferous Plants Using Pollen Morphological Features

Shabir Ahmad[1]*, Muhammad Zafar[1], Mushtaq Ahmad[1], Khalid H. Alamer[2], Aqsa Abid[1], Rozina[1], Shaista Jabeen[1], Ateef Ullah[1], Hussain Shah[1], Urwah Shafeeq[3], Muhammad Manzoor[1], Syed Waseem Gillani[1], Houneida Attia[4]

[1]Department of Plant Sciences, Quaid- i- Azam University Islamabad, 45320, Pakistan
[2]Biological Sciences Department, Faculty of Science and Arts, King Abdulaziz University, Rabigh, 21911, Saudi Arabia
[3]Department of Botany, University of Agriculture, Faisalabad, Pakistan
[4]Department of Biology, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

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Abstract

In this study, we explored the palynological features of melliferous taxa from different areas in Khyber Pakhtunkhwa. We collected and identified eight species belonging to four different families, including *Brassica campestris*, *Brassica juncea*, *Brassica oleracea*, *Eruca sativa*, *Chorispora tenella*, *Cicer arietinum*, *Saccharum spontaneum*, and *Asphodelus tenuifolius*. Under microscopy examination, pollen grains exhibit diverse shapes, including oblate-spheroidal and prolate-spheroidal shapes. With the help of statistical software IBM SPSS Statistics 20, the quantitative data of pollen grains was measured. In this study, *Asphodelus tenuifolius* were reported to have a maximum polar diameter of 46.29 µm and *Brassica oleracea* had a minimum of 17.97 µm. The apertures of these pollen grains range from monocolpate to tricolpate. Exine sculpturing exhibited psilate and reticulate patterns, with no presence of spines. Quantitative features, such as polar diameter, equatorial diameter, exine thickness, colpi length, colpi width, and P/E ratio, were studied using light microscopy. The data was statistically analyzed using IBM SPSS software. The result of the present study showed the vital role of pollen morphology in plant identification, floral calendar preparation, and honey production in Khyber Pakhtunkhwa. The study presents a novel approach and perspective on honey pollen profiles, revealing the plants visited by honeybees for food. Furthermore, the research contributes to enhancing honey production and improving pollination services.

Keywords: light microscopy, pollen, melliferous, honeybees, Khyber Pakhtunkhwa

*e-mail: shabir@bs.qau.edu.pk

Introduction

Melliferous plants include those species visited by honeybees to collect pollen and nectar for honey production and bee foraging activities [1]. Melliferous flora of an area plays an important role in maintaining ecosystems through insect pollination. In the study area, honeybees forage on different flowers based on their overall floral morphologies and produce different types of honey. The interrelationships between plants and honeybees are very important to take advantage of each other. Honeybees feed on plants for their survival and plants depend on honeybees for pollination and many other purposes [2]. Numerous characteristics like shape, size, and other morphological features are used in the description of pollen grains. Pollen morphology of melliferous species establishes evolutionary and phylogenetic linkages to the previously existing fossil records [3, 4]. Entomophilous pollination results in increasing annual yield of seeds, fruits, vegetables, and cereals [5].

Beekeeping is one of the important businesses nowadays throughout the Khyber Pakhtunkhwa. This activity is directly related to the floral and geographical conditions of an area. Three species of honeybees, i.e. *Apis dorsata, Apis florea,* and *Apis mellifera,* were observed as the dominant species in many districts of the province [6]. *Apis mellifera* were kept by beekeepers in wooden boxes while the other two species were considered wild [7]. The business of beekeeping has increased nowadays on the global level. According to field surveys and interviews with different beekeepers and local expert people, it was noted that there has been an increase in the business of beekeeping over the past few years. The study aids in determining the flowering periods of different plants [8, 9]. Such information is useful for beekeepers to start the beekeeping business in the most appropriate season for honey production.

Pollen morphological studies aid in the taxonomic and systematic identifications of species belonging to the same genera. Microscopic studies of melliferous flora from the Lakki Marwat district had been conducted previously [10]. Different morphological characters of pollen were described using the previously established terminology [11]. Melliferous species of the study area were very diverse and important from an evolutionary point of view. Exine thickness, pollen size, and shape are important features useful in taxonomic studies of different plants [12]. Palynology is an important field related to many fields of genetics, pharmacology, evolutionary biology, and plant ecology [13]. A wealth of knowledge regarding pollen analysis, climate change, allergy detection, and criminal cases can be obtained by studying pollen morphology [14].

Khyber Pakhtunkhwa is one of the northwestern provinces of Pakistan, sharing its boundaries with Afghanistan to the northwest, India to the southeast, and China to the northeast, spread over 101,741 km². Within the country, the province shares its boundaries with Punjab, Balochistan, Islamabad, Azad Kashmir, and Gilgit Baltistan. Geographically, it is the smallest province in the country, containing both hilly and plain areas [15]. It covers the green forests and vegetation, which are used for ornamental, medicinal, fuel, edible, and many other purposes, but most famously, they are used for the production of honey through honeybees, which were never reported before these studies in the study area [16]. Southern districts of the province have more business of beekeeping than any other. Flora of Khyber Pakhtunkhwa is luxuriant and important for honey production all over the country. Honeybees visit several species to collect nectar and pollen from various flowers for honey production [17]. The aim of the present study is to identify melliferous plants for the first time from Khyber Pakhtunkhwa, making its link with plant taxonomic studies, spreading its knowledge in local peoples, description of pollen morphology, its uses in honey formations, and protection of plants from over-grazing.

Materials and Methods

Plant Collection and Identification

Vegetation surveys were carried out during the period between March 2019- February 2020 in many districts of the province, i.e. Tank, Swabi, Karak, Dera Ismail Khan, Mardan, and Lakki Marwat. Selected plants that were abundantly foraged by honeybees were collected after a careful visit in the field, and interviews were conducted with different beekeepers and local people. A total of 8 melliferous species belonging to 4 families were collected and then brought to the Herbarium of Pakistan (ISL), Quaid-i-Azam University Islamabad, Pakistan for identification and analytical purposes (Table 1, Figs 1, 2).

Investigation of Morphological Features Using LM

The anthers of flowers were separated from flowers with the help of forceps to extract pollen grains. Using the previously mentioned method, pollen grains were processed through the process of acetolysis [18]. The acetolysis involves a dehydration step in glacial acetic acid and then a solution of acetic anhydride and sulphuric acid is poured on the sample in a vial immersed in a water bath at 100°C. Pollen grains were put on the slide, one to two drops of acetic acid were added to it, and then crushed with a metal rod. Using a needle, the debris was scraped, and the rest of the pollen on the slide was treated with glycerin jelly for the purpose of staining. A cover slip was placed on a slide and cleaned through the tissue paper. The slides were marked with labels and voucher numbers, and names of species were written down, and they were placed

Table 1. Botanical names, local names, family, domestication status and distribution of collected plants.

S.no.	Taxa	Local name	Family	Domestication status	Cultivation status/ distribution in Pakistan	Cultivation status/ distribution in World
1	*Brassica campestris* L.	Khoaz Saag	Brassicaceae	Cultivated	Malakand, Swabi, Peshawar, Charsada, Jhelum, Rawalpindi, and Islamabad.	China, Canada, Finland, India, Italy, and Bangladesh.
2	*Brassica juncea* (L.) Czern.	Weraye Saag	Brassicaceae	Cultivated	Kharan, Mastung, Chakwal, Malakand, Faisalabad, Chakwal, Dera Ghazi Khan, and Tank.	India, China, Russia, Canada, Burma, Australia, Mexico, Nepal, Bangladesh, and the United States.
3	*Brassica oleracea* L.	Ghobi	Brassicaceae	Cultivated	Jhelum, Karak, Faisalabad, Bannu, Peshawar, Quetta, and Rawalpindi.	United Kingdom, Spain, France, and Italy.
4	*Eruca sativa* Mill.	Tareakh Saag	Brassicaceae	Cultivated	Kharan, Mianwali, Sargodha, Bannu, Lakki Marwat, Bhakkar, and Jhelum.	Afghanistan, Pakistan, India, Nepal, Bangladesh, and Bhutan.
5	*Chorispora tenella* DC.	Ghulabi Saag	Brassicaceae	Cultivated	Dera Ismail Khan, Lakki Marwat, Kohat, Dera Ghazi Khan, and South Waziristan.	Turkey, China, Iran, Kazakhstan, Ukraine, and Afghanistan.
6	*Cicer arietinum* L.	Charnha	Fabaceae	Cultivated	Fatheh Jang, Bahawalpur, Bhakkar, Bannu, Peshawar, South Waziristan, Mardan, and Sukkur.	Iran, India, Afghanistan, Pakistan, Mexico, Bangladesh, and America.
7	*Saccharum spontaneum* L.	Khana	Poaceae	Wild	Bannu, Karak, Kohat, Rawalpindi, Attock, Jhelum, Mianwali, Lakki Marwat, and Bannu.	Egypt, Indonesia, Bangladesh, Myanmar, Japan, and Thailand.
8	*Asphodelus tenuifolius* Cav.	Piazo bhotye	Asphodelaceae	Wild	South Waziristan, Dera Ghazi Khan, Tank, Mianwali, Bannu, and Lakki Marwat.	Bangladesh, Italy, Kuwait, Yemen, Algeria, Syria, Sudan, and West Himalaya.

Fig. 1. Plants Collected- A: *Brassica campestris* L. B: *Brassica juncea* (L.) Czern C: *Brassica oleracea* L. D: *Eruca sativa* Mill.

Fig, 2. Plants Collected- A: *Chorispora tenella* DC B: *Cicer arietinum* L. C: *Saccharum spontaneum* L. D: *Asphodelus tenuifolius* Cav.

upright in a wooden box. On every slide, the number of pollen grains was quantified. Exine thickness, number of apertures (pores, colpi), polar and equatorial diameters, pollen shapes, and number of fertile and sterile pollen were examined with the help of a light microscope Meiji Techno MT4300H. Investigated slides were then stored in a wooden box in a vertical position.

Statistical Analysis

For each species, pollen grains of 35-45 morphological parameters were measured. Quantitative data of pollen were measured by following the previously established method [19, 20]. Using statistical software IBM SPSS Statistics 20, mean, min, max, and standard dev for each of the pollen grains were investigated. Values are presented in the table in the form of Mean (Mini-Max)±SE.

Results

Pollen grains of 8 selected melliferous plants collected from different regions of Khyber

Table 2. Micromorphological characteristics of pollen-quantitative

S.no.	Taxon	P/E ratio	Exine thickness Mean (Min-Max) SD (um)	Polar diameter Mean (Min-Max) SE (um)	Equatorial diameter Mean (Min-Max) SE (um)	Length of colpi Mean (Min-Max) SE (um)	Width of colpi Mean (Min-Max) SE (um)
1	*Brassica campestris* L.	0.98	1.38 (1.05-1.80)±0.13	19.89 (15.90-25.95)±1.85	20.1 (16.80-25.35)±1.42	5.94 (3.60-8.70)±0.88	4.57 (3.30-5.85)±0.62
2	*Brassica juncea* (L.) Czern.	1.04	1.62 (1.20-1.95)±0.13	21.39 (17.40-26.10)±1.67	20.46 (14.85-25.20)±1.68	6.42 (4.50-8.55)±0.76	5.10 (2.70-7.05)±0.86
3	*Brassica oleracea* L.	1.03	1.56 (1.05-2.10)±1.05	17.97 (13.20-22.05)±1.47	17.40 (14.10-21.00)±1.21	8.49 (6.90-10.65)±0.71	5.46 (3.45-7.65)±0.76
4	*Eruca sativa* Mill.	1.24	1.35 (0.90-1.80)±0.17	17.97 (13.20-22.05)±1.47	17.94 (15.45-20.40)±1.80	5.97 (4.95-7.20)±0.39	7.59 (3.75-10.95)±1.16
5	*Chorispora tenella* DC.	1.04	1.80 (1.35-2.40)±0.18	18.18 (14.85-20.70)±1.02	17.43 (14.55-21.15)±1.20	6.75 (5.10-7.95)±0.53	4.41 (4.05-4.65)±0.12
6	*Cicer arietinum* L.	1.10	1.71 (1.20-2.25)±0.18	30.03 (26.25-34.95)±1.50	27.27 (24.60-32.40)±1.36	9.33 (5.40-12.45)±1.23	8.19 (4.95-11.40)±1.17
7	*Saccharum spontaneum* L.	0.87	1.59 (1.20-2.10)±0.16	27.48 (23.70-30.75)±1.10	31.56 (27.90-38.40)±2.03	A	A
8	*Asphodelus tenuifolius* Cav.	0.92	1.74 (1.35-2.25)±0.16	46.29 (37.05-59.40)±4.11	45.96 (34.05-57.60)±3.81	32.67 (20.40-41.40)±3.67	6.45 (4.35-8.40)±0.72

Table 3. Micromorphological characteristics of pollen-qualitative

S.no.	Taxa	Pollen size	Pollen shape	Colpi P/A	Pollen class	Exine Sculpturing
1	*Brassica campestris* L.	Small	Oblate-spheroidal	P	Tricolpate	Reticulate
2	*Brassica juncea* (L.) Czern.	Small	Prolate-spheroidal	P	Tricolpate	Reticulate
3	*Brassica oleracea* L.	Small	Prolate-spheroidal	P	Tricolpate	Reticulate
4	*Eruca sativa* Mill.	Small	Subprolate	P	Tricolpate	Reticulate
5	*Chorispora tenella* DC.	Small	Prolate-spheroidal	P	Tricolpate	Reticulate
6	*Cicer arietinum* L.	Medium	Prolate-spheroidal	P	Tricolporate	Psilate
7	*Saccharum spontaneum* L.	Medium	Suboblate	A	Monoporate	Psilate
8	*Asphodelus tenuifolius* Cav.	Large	Oblate-spheroidal	P	Monocolpate	Reticulate

Pakhtunkhwa, belonging to 4 different families, were examined using light microscopy. The analyzed pollen with qualitative and quantitative features is hereafter presented in tabular form.

***Brassica campestris* L.**
English name: Field mustard
Flowering periods: January- April
Flower color: Yellow
Pollen Morphology: The pollen is tricolpate, reticulate, and monad. Its equatorial diameter is 20.1 µm (16.80 -25.35 µm), polar diameter is 19.89 µm (15.90-25.95 µm), exine thickness is 1.38 µm (1.05-1.80 µm), and P/E ratio is 0.98. Pollen shape is oblate-spheroidal.

***Brassica juncea* (L.) Czern**
English name: Brown mustard
Flowering periods: February- April
Flower color: Yellowish
Pollen Morphology: The pollen is tricolpate, reticulate, and monad. Its equatorial diameter is 20.46 µm (14.85 -25.20 µm), polar diameter is 21.39 µm (17.40-26.10 µm), exine thickness is 1.62 µm (1.20-1.95 µm), and P/E ratio is 1.04. Pollen shape is prolate-spheroidal.

***Brassica oleracea* L.**
English name: Cabbage
Flowering periods: January-March
Flower color: White
Pollen Morphology: The pollen is tricolpate, reticulate, and monad. Its equatorial diameter is 17.40 µm (14.10 -21.00 µm), polar diameter is 17.97 µm (13.20-22.05 µm), exine thickness is 1.56 µm (1.05-2.10 µm), and P/E ratio is 1.03. Pollen shape is prolate-spheroidal.

***Eruca sativa* Mill.**
English name: Garden rocket
Flowering periods: January-April
Flower color: Yellowish
Palynomorph: The pollen is tricolpate, reticulate, and monad. Its equatorial diameter is 17.94 µm (15.45 -20.40 µm), polar diameter is 17.97 µm (13.20-22.05 µm), exine thickness is 1.35 µm (0.90-1.80 µm), and P/E ratio is 1.24. Pollen shape is subprolate.

***Chorispora tenella* DC.**
English name: Blue mustard
Flowering periods: January-March
Flower color: Bluish
Pollen Morphology: The pollen is tricolpate, reticulate, and monad. Its equatorial diameter is 17.43 µm (14.55-21.15 µm), polar diameter is 18.18 µm (14.85-20.70 µm), exine thickness is 1.80 µm (1.35-2.40 µm), and P/E ratio is 1.04. Pollen shape is prolate-spheroidal.

***Cicer arietinum* L.**
English name: Chickpea
Flowering periods: January-April
Flower color: Pinkish
Pollen Morphology: The pollen is tricolporate, psilate, and monad. Its equatorial diameter is 27.27 µm (24.60 -32.40 µm), polar diameter is 30.03 µm (26.25-34.95 µm), xine thickness is 1.71 µm (1.20-2.25 µm), and P/E ratio is 1.10. Pollen shape is prolate-spheroidal.

***Saccharum spontaneum* L.**
English name: Kansgrass
Flowering periods: August-November
Flower color: Yellowish
Pollen Morphology: The pollen is tricolporate, reticulate, and monad. Its equatorial diameter is 31.56 µm (27.90 -38.40 µm), polar diameter is 27.48 µm (23.70-30.75 µm), exine thickness is 1.59 µm (1.20-2.10 µm), and P/E ratio is 0.87. Pollen shape is suboblate.

***Asphodelus tenuifolius* Cav.**
English name: Onionweed
Flowering periods: February-April
Flower color: White
Pollen Morphology: The pollen is monocolpate, psilate, and monad. Its equatorial diameter is 45.96 µm (34.05 -57.60 µm), polar diameter is 46.29 µm (37.05-59.40 µm), exine thickness is 1.74 µm (1.35-2.25 µm), and P/E ratio is 0.92. Pollen shape is oblate-spheroidal.

Discussion

Flowers from 8 selected species belonging to various localities of Khyber Pakhtunkhwa were collected, identified, and then analyzed for their pollen morphology. Brassicaceae were recorded as the dominant family represented by 5 taxa, then Fabaceae, Poaceae, and Asphodelaceae with 1 taxon. Quantitative and qualitative characters of pollen grains were investigated using light microscopy. During field surveys, it was observed that honeybees regularly visit plants for honey formations and their foraging activities. Brassicaceae, Asphodelaceae were recorded as the dominant families during spring and Poaceae in Autumn as far as the activities of honeybees are concerned. Studying pollen morphology is very useful in discovering many taxonomic problems [21]. Many studies have been performed regarding melliferous potentials of plants worldwide and Pakistan [22, 23]. Pollen morphology of melliferous species is very useful for future studies of melissopalynology and apiculture fields.

Pollen investigated through LM showed great variability in equatorial diameter, polar diameter, colpus length, colpus width, and exine thickness [24]. *Saccharum spontaneum* has psilate exine while the rest of the species contains reticulate sculpturing, which is a remarkable feature in plant identification and evolutionary studies. *Eruca sativa* had the highest P/E ratio at 1.24 and *Saccharum spontaneum* had the lowest P/E at 0.87. *Asphodelus tenuifolius* had a maximum equatorial diameter of 46.29 µm and *Brassica oleracea* had a minimum of 17.40 µm. *Asphodelus tenuifolius* had also a maximum colpus length and *Brassica campestris* had a minimum of 5.94 µm. *Cicer arietinum* had a maximum colpus width of 8.19 µm and *Chorispora tenella* had a minimum of 4.41 µm. The exine thickness of *Chorispora tenella* had a maximum value of 1.80 µm and *Eruca sativa* had a minimum of 1.35 µm. A very

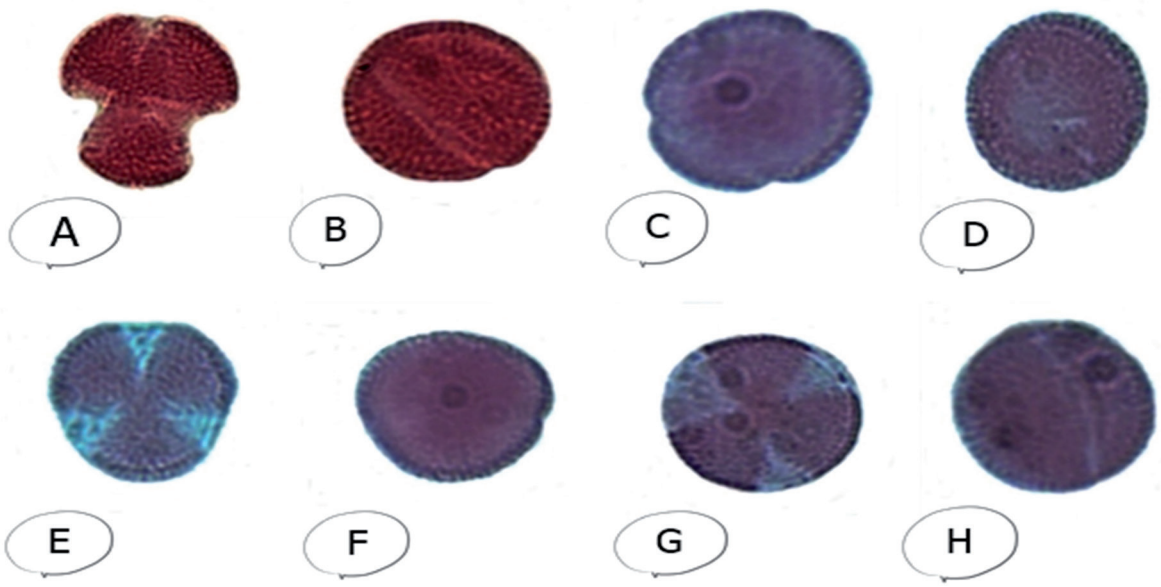

Fig. 3. LM pollen micrographs (A) *Brassica campestris* L. (B) *Brassica juncea* (L.) Czern. (C) *Brassica oleracea* L. (D) *Eruca sativa* Mill. (E) *Chorispora tenella* DC. (F) *Cicer arietinum* L. (G) *Saccharum spontaneum* L. (H) *Asphodelus tenuifolius* Cav.

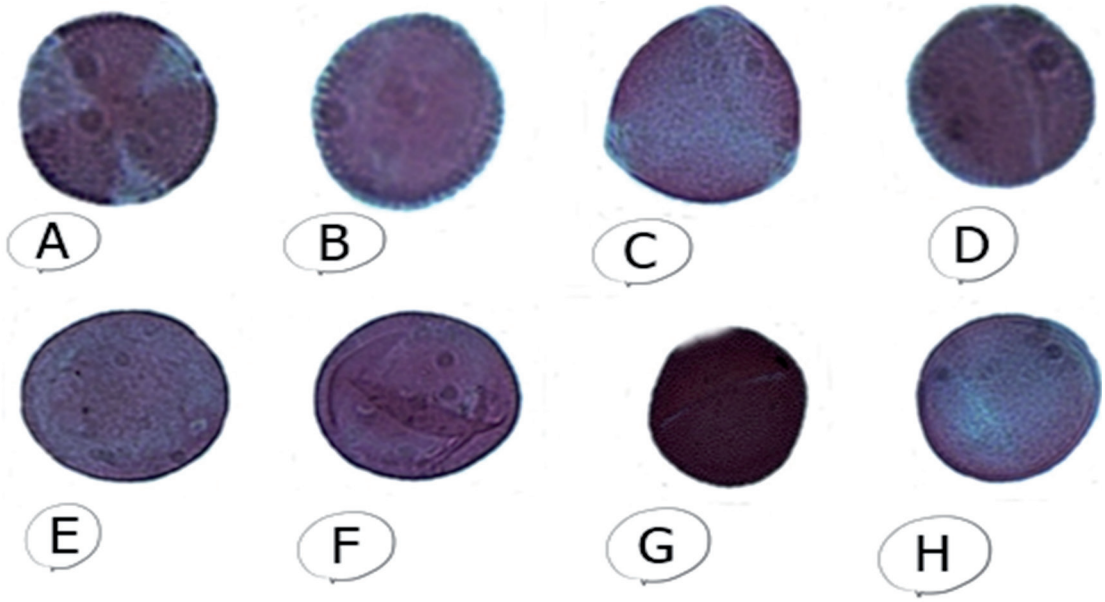

Fig. 4. LM pollen micrographs (A) *Brassica campestris* L. (B) *Brassica juncea* (L.) Czern. (C) *Brassica oleracea* L. (D) *Eruca sativa* Mill. (E) *Chorispora tenella* DC. (F) *Cicer arietinum* L. (G) *Saccharum spontaneum* L. (H) *Asphodelus tenuifolius* Cav.

wide range of variations was observed in the pollen morphology of selected species from the study area, showing that the field of palynology plays a vital role in evolutionary studies of plant species [25].

A very diverse range of pollen was observed in the plants of the study area. Pollen size varies from small to large, monocolpate to tricolporate, subprolate to oblate-spheroidal, and reticulate to psilate. Pollen morphological studies in the present study link the foraging behaviors of honeybees with specific plants [26]. Species belonging to the family Brassicaceae are tricolpate, reticulate, small, and of different shapes. *Saccharum spontaneum* pollen is medium-sized, prolate-spheroidal, monoporate, and psilate type. *Asphodelus tenuifolius* pollen was monocolpate, reticulate, large size, and oblate-spheroidal [27]. Very small differences were recorded in the plants of the family Brassicaceae [28].

Spring and fall are the two important seasons for honey production in the Khyber Pakhtunkhwa. Honeybees visit some plants abundantly in the selected season [29]. Plants belonging to the family Brassicaceae and Asphodelaceae were collected by honeybees in spring, while Fabaceae and Poaceae in fall. On the field

trip, it was noted that *Brassica* were the highly foraged species in spring and *Saccharum* in fall. It was observed that honey produced during the fall season in the study area is better in quality and quantity if compared to spring production [30]. It was also noted that during spring honeybees visit Brassicaceae, Asphodelaceae, and Euphorbiaceae while in fall they visit Fabaceae, Lamiaceae, and Poaceae. Identification of melliferous species and their cultivations helps in the production of honey on a commercial scale [31]. Beekeepers from many regions of the country organize their business of beekeeping in the study area for honey production.

Plants analyzed in the current study are typical of hot and dry regions. Melliferous plants have many useful applications in the fields of apiculture, agriculture, medicines, pollination biology, plant biodiversity, and conservation. Honeybees are regarded as one of the important sources of pollination. The study provides knowledge about the botanical and geological origins of plants and honeybees [32]. It has been recorded that the identification of melliferous species helps in developing pollen literature, which is useful for increasing honey production in the coming seasons [33].

Table 4. Botanical names, collector, locality and voucher number, Province/Country and Date of collected plants.

S.no.	Taxa	Collector	Locality/District	Voucher number	Province/Country	Date
1	*Brassica campestris* L.	Shabir Ahmad and Misal Khan	Kabal/ Swabi	ISL-SA-922	Khyber-Pakhtunkhwa/ Pakistan	03-20-2019
2	*Brassica juncea* (L.) Czern.	Shabir Ahmad and Asif Kamal	Aba Khel/ Tank	ISL-SA-925	Khyber-Pakhtunkhwa/ Pakistan	03-12-2019
3	*Brassica oleracea* L.	Shabir Ahmad and Bashir Khan	Jhang Khel/ Lakki Marwat	ISL-SA-927	Khyber-Pakhtunkhwa/ Pakistan	03-29-2019
4	*Eruca sativa* Mill.	Shabir Ahmad and Khushdil Khan	Takht Bhai/ Mardan	ISL-SA-931	Khyber-Pakhtunkhwa/ Pakistan	04-2-2019
5	*Chorispora tenella* DC.	Shabir Ahmad and Nasir Muhammad Khan	Paharpur/Dera Ismail Khan	ISL-SA-933	Khyber-Pakhtunkhwa/ Pakistan	02-15-2019
6	*Cicer arietinum* L.	Shabir Ahmad and Nasir Muhammad Khan	Takhte Nasrati/ Karak	ISL-SA-934	Khyber-Pakhtunkhwa/ Pakistan	08-25-2019
7	*Saccharum spontaneum* L.	Shabir Ahmad and Asif Kamal	Azhar Khel/ Lakki Marwat	ISL-SA-940	Khyber-Pakhtunkhwa/ Pakistan	11-13-2019
8	*Asphodelus tenuifolius* Cav.	Shabir Ahmad and Nasir Muhammad Khan	Domail/Bannu	ISL-SA-942	Khyber-Pakhtunkhwa/ Pakistan	04-02-2020

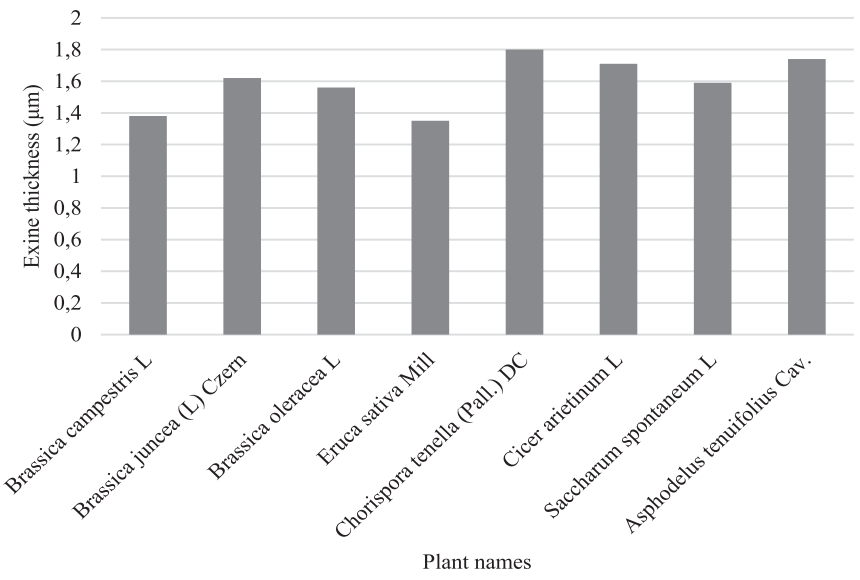

Fig. 5. Variations in exine thickness of selected melliferous species from Khyber Pakhtunkhwa, Pakistan.

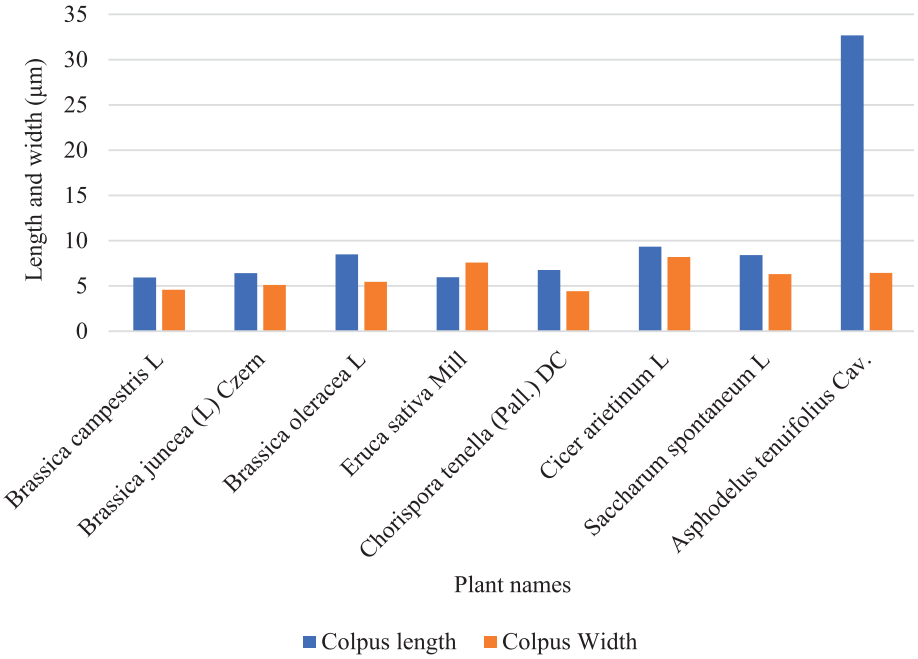

Fig. 6. Variations in colpus length and colpus width of selected melliferous species from Khyber Pakhtunkhwa, Pakistan.

Conclusions

In conclusion, our study sheds light on the morphological characteristics of pollen grains from selected melliferous species in the Khyber Pakhtunkhwa province. The diagnostic value of exine sculpturing in plant systematic studies was evident. By analyzing bee-foraged species across various localities, we contribute to local beekeepers' understanding of melliferous plants and their conservation. Our findings bridge plant ecology, aeropalynology, chemotaxonomy, molecular biology, and environmental sciences, emphasizing the importance of preserving these valuable species. Prioritizing melliferous plant species will support sustainable honey production and overall ecosystem health.

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Conflict of interest

The authors of this manuscript have no conflicts of interest to declare.

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