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MORPHO-PALYNOLOGICAL INVESTIGATIONS OF MELLIFEROUS PLANTS USING MICROSCOPIC TECHNIQUES FROM KHYBER PAKHTUNKHWA, PAKISTAN

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Pollen morphology of 16 species belonging to 8 different families; Apocynaceae, Brassicaceae, Capparaceae, Euphorbiaceae, Fabaceae, Poaceae, Solanaceae and Zygophyllaceae were analysed from Khyber Pakhtunkhwa with the help of microscopic techniques. Both qualitative and quantitative features of pollen were examined including polar and equatorial diameter, colpus length and width, exine sculpturing, pores number, pollen shape, number of sterile and fertile pollen using Leica microscope (D1000) fitted with camera Meiji Infinity 1 and examined statistically by software IBM SPSS Statistics 20. Pollen observed were small to large with suboblate, oblate-spheroidal, prolate-spheroidal and subprolate shape. Exine ornamentations were reticulate and psilate type in all the studied plants. Colpi and pores of the selected plants observed are tricolporate, tricolpate and monoporate. The present study showed that both spring and autumn seasons are the prominent seasons for honey production and beekeeping industries in Khyber Pakhtunkhwa. *Brassica campestris* is the most visited species by honeybees in the study area. Melliferous plants gave knowledge about botanical origin of honey and geographical origin of honeybees. The current study identified numerous bee forage plants which may help to raise the concept of cultivation of melliferous herbaceous plants by the local people, to be used for honey production. The identification of these potential sources may help the beekeepers to increase the honey production and increase in agricultural yields through pollinations.

Key words: honeybee, Khyber Pakhtunkhwa, melliferous, microscopy, palynology, pollen

INTRODUCTION

Melliferous flora of an area plays an important role in maintenance of ecosystem through insect pollination. Honeybees forage different flowers on the basis of their structure and shape, and produce different types of honey. Melliferous plants are those plants which are visited by honeybees to collect pollen and nectars for honey formation and bee foraging activities (Nguemo *et al*. 2016). The honeybees and other insects' population, which helps in pollination has decreased in the present years due to destruction of the bees dwelling places and loss of flora (Goulson 2003, Kremen *et al*. 2002). Pollination caused by honeybees in plants has more advantages over the others as these plants

display more resistance against diseases, more chances of survival in the environments and variations occurs within the species (Huryn 1997).

The palyno-morphological characteristics varies among different plant families (Mignot *et al*. 1994). Various taxonomists contributed to the knowledge of palynology and its relationship with other disciplines. Study of pollen morphology plays an important role as taxonomic tool in the accurate identification of plants species (Ullah *et al*. 2018).

The melliferous potential of plants varies according to flowering period and environmental conditions of an area. The melliferous plants posses certain important features relating to their coloured flowers, edibility, and some other ethnobotanical uses. The melliferous potentials of perennial plants are different from annual by producing more nectars in the blooming season (Ion *et al*. 2007). The palynological studies help in solving the taxonomic problems in the transgenic plants (Ahmad *et al*. 2010). Melissopalynological studies are very important for determining the adulterations in honey. The flora of a specific region is determined by honey analysis of the collected samples from that area (Ebenezer and Olugbenga 2010). The present research work aimed to report the melliferous herbaceous plants from Khyber Pakhtunkhwa province, Pakistan. The knowledge regarding the spring melliferous plants were first time reported from the study area, helps in the identification and delimitation of existing among the plant species and bee floral calendar preparation. The present study also aimed to explore the flora, identify melliferous species used by *Apis mellifera* in honey formations.

MATERIALS AND METHODS

A survey was carried out to confirm that bees forage the herbaceous plants. The plants were collected from different regions of the district Dera Ismail Khan, Kohat, Karak, Mardan, Peshawar, Lakki Marwat and Bannu. A total of 16 plant species were collected during the months of March–April 2019 from the above-mentioned localities. The plants collected were photographed in the field (Figs 3–4), pressed in newspapers, and dried. The plants were then deposited in the Herbarium of Pakistan (ISL), Quaid-i-Azam University Islamabad. The general description of the collected plant species is presented in Table 1.

Fresh flowers were collected, and their anthers were dissected with the help of needle on clean glass slides. Erdtman (1952) acetolysed protocol was followed to crush the anthers in 1–2 drops of acetic acid so that pollen can be released. Debris were removed and pollen released and stained with glycerin jelly according to the method used by (Ahmad *et al*. 2008). The pollens were then studied using Leica D1000 microscope fitted with camera Meiji Infinity 1.

Quantitative characteristics of the 10 randomly selected pollen grains, i.e., polar axis, equatorial diameter, colpus/pore length, colpus/pore width,

Table 1
Botanical names, family, locality, flower colour, flowering periods and domestication status (DoSt) of collected plants

Taxon	Family	Locality	Flower colour	Flowering periods	DoSt
Zea mays	Poaceae	Taji kai/Bannu	yellowish	Sept.–Jan.	cultivated
Cenchrus biflorus	Poaceae	Dowa/Bannu	pale green	Aug.–March	natural
Eruca sativa	Brassicaceae	Lachi/Kohat	white yellow	Nov.–Apr.	cultivated
Saccharum bengalense	Poaceae	Taji kali/Bannu	light yellow	Dec.–March	natural
Withania somnifera	Solanaceae	Bank of River Kurram/Bannu	greenish	Nov.–Apr.	natural
Brassica campestris	Brassicaceae	Lachi/Kohat	yellow	Nov.–March	cultivated
Rhazya stricta	Apocynaceae	Bank of River Kurram/Bannu	white	Oct.–May	natural
Fagonia indica	Zygophyllaceae	Amberi Kali/ Karak	purple	Sept.–Apr.	natural
Cleome brachycarpa	Capparaceae	Dowa/Bannu	yellow	Nov.–March	natural
Cymbopogon jwarancusa	Poaceae	Domail/Bannu	purple	July–Apr.	natural
Ricinus communis	Euphorbiaceae	Taji kali/Bannu	whitish-green	Sept.–March	natural
Brassica juncea	Brassicaceae	Paharpur/Dera Ismail Khan	yellow	Nov.–March	cultivated
Cicer arietinum	Fabaceae	Naurang/Lakki Marwat	pink	Nov.–April	cultivated
Brassica oleracea	Brassicaceae	Hayatabad/Peshawar	white	Oct.–April	cultivated
Raphanus sativus	Brassicaceae	Taji kali/Bannu	white	Dec.–March	cultivated
Physalis minima	Solanaceae	River Kurram/ Lakki Marwat	yellow	Sept.–March	natural

exine thickness and P/E ratio were investigated along with the mean value and standard error by using software IBM SPSS Statistics 20. The pollen fertility, shapes and size were also noted showing morphological characters of pollen (Tables 2–3).

RESULTS

The melliferous plants of Khyber Pakhtunkhwa were examined under light microscope exhibiting a wide range of differences in pollen morphology. The plants were *Zea mays* L., *Cenchrus biflorus* Roxb., *Eruca sativa* Mill., *Saccharum bengalense* Retz., *Withania somnifera* (L.) Dunal, *Brassica campestris* L., *Rhazya stricta* Decne., *Fagonia indica* Burm. f., *Cleome brachycarpa* (Forssk.)

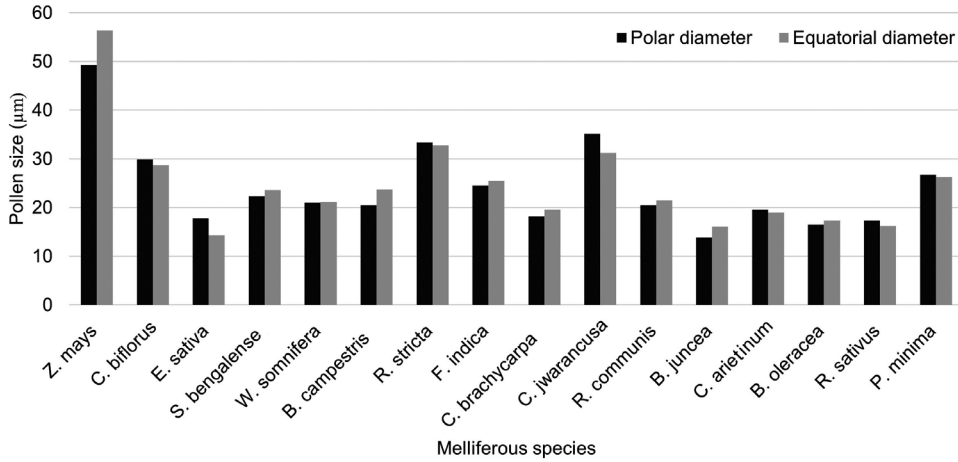

Fig. 1. Variations in polar and equatorial diameter of spring melliferous plants from Khyber Pakhtunkhwa, Pakistan

Vahl ex DC., *Cymbopogon jwarancusa* (Jones) Schult., *Ricinus communis* L., *Brassica juncea* (L.) Czern., *Cicer arietinum* L., *Brassica oleracea* L., *Raphanus sativus* L. and *Physalis minima* L. Both the qualitative and quantitative features of the melliferous herbaceous plants were studied and presented in Tables 2 and 3. Results showed that most of pollen observed were tricolporate and monoporate. A very wide range of variations was observed in exine sculpturing, pollen shape and size. Apertures of pollen observed were monoporate, tricol-

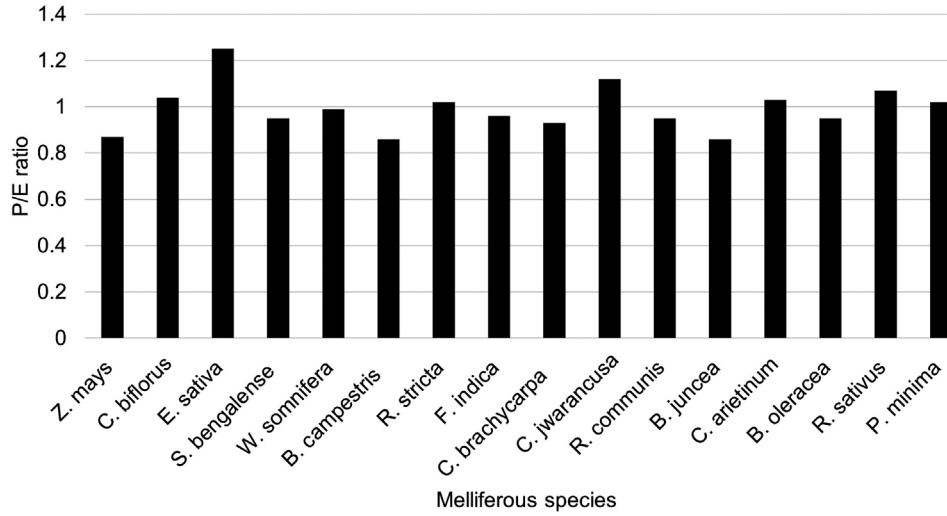

Fig. 2. P/E ratio variations of spring melliferous plants from Khyber Pakhtunkhwa, Pakistan

Table 2
Quantitative pollen micromorphological features of melliferous species

Taxon	P/E ratio	Exine thickness (μm) mean ± SE (min–max)	Polar axis (μm) mean ± SE (min–max)	Equatorial diameter (μm) mean ± SE (min–max)	Length of colpi/pores (μm) mean ± SE (min–max)	Width of colpi/pores mean ± SE (μm) (min-max)
Zea mays	0.87	1.11±0.23 (0.60–1.80)	49.29 ±5.51 (32.55–62.70	56.40 ±4.08 (42.00–65.10)	2.4±0.64 (1.30–2.85)	1.5±0.71 (0.8–1.90)
Cenchrus biflorus	1.04	1.14±0.16 (0.75–1.65)	29.85±1.56 (24.45–33.75)	28.74±2.14 (25.50–37.20)	1.4±0.42 (0.60–1.75)	1.1±0.3 (0.5–1.30)
Eruca sativa	1.25	1.26±0.20 (0.75–1.80)	17.82±0.59 (16.20–19.65)	14.28±1.41 (11.55–18.00)	6.54±1.00 (4.05–9.60)	4.41±0.48 (3.30–6.15)
Saccharum bengalense	0.95	1.38±0.13 (1.05–1.80)	22.32±1.37 (18.75–26.55)	23.58±1.92 (18.30–28.80)	2.8±1.2 (1.45–3.05)	2.1±0.91 (0.95–3.20)
Withania somnifera	0.99	1.35±1.90 (0.90–1.80)	21.03±0.47 (19.95–22.50)	21.18±0.57 (20.10–22.80)	9.39±0.73 (7.50–12.60)	5.88±0.73 (4.05–7.95)
Brassica campestris	0.86	1.05±0.11 (0.75–1.35)	20.46±2.27 (12.60–25.65)	23.67±0.22 (22.95–24.30)	8.28±1.07 (4.95–11.55)	9.60±2.08 (4.05–14.70)
Rhazya stricta	1.02	1.20±0.11 (0.90–1.50)	33.36±1.64 (27.00–35.85)	32.76±1.14 (28.35–34.80)	8.07±1.07 (5.85–12.15)	12.66±2.05 (6.00–18.00)
Fagonia indica	0.96	0.96±0.15 (0.60–1.50)	24.48±0.73 (22.50–27.00)	25.47±0.84 (22.80–28.05)	13.23±1.00 (10.65–16.80)	8.34±2.03 (4.95–16.20)
Cleome brachycarpa	0.93	1.32±0.12 (0.90–1.65)	18.21±0.54 (16.50–19.80)	19.57±0.96 (16.95–22.20)	7.50±0.79 (4.80–9.15)	4.38±1.16 (2.85–9.00)
Cymbopogon jwarancusa	1.12	0.84±0.07 (0.60–1.05)	35.10±1.25 (31.65–39.00)	31.23±2.90 (24.60–41.85)	3.2±0.8 (2.25–5.50)	2.8±0.75 (1.95–3.85)
Ricinus communis	0.95	1.50±0.11 (1.20–1.80)	20.49±0.70 (18.75–22.50)	21.48±0.45 (19.80–22.50)	8.58±0.61 (6.60–10.05)	2.22±0.21 (1.80–3.00)
Brassica juncea	0.86	1.41±0.76 (1.20–1.65)	13.83±0.75 (11.55–16.05)	16.08±0.38 (15.15–17.10)	4.41±0.74 (2.55–6.15)	2.31±0.27 (1.80–3.30)
Cicer arietinum	1.03	1.14±0.17 (0.60–1.50)	19.56±0.18 (19.05–20.10)	18.96±0.50 (18.00–20.85)	7.80±1.92 (3.45–14.55)	4.02±0.86 (1.50–6.45)
Brassica oleracea	0.95	1.20 ±0.15 (0.75–1.65)	16.50±0.92 (14.70–19.80)	17.34±0.24 (16.80–18.00)	6.75±0.44 (5.55–7.65)	4.80±0.53 (3.60–6.60)
Raphanus sativus	1.07	1.53±0.13 (1.05–1.80)	17.31±1.09 (14.40–19.80)	16.20±0.69 (14.70–18.00)	8.82±0.82 (6.60–11.10)	4.47±0.6 (3.45–6.75)
Physalis minima	1.02	1.11±0.08 (0.90–1.35)	26.76±0.57 (25.35–28.20)	26.28±0.58 (25.20–28.50)	4.08±0.41 (3.00–5.25)	7.08±0.97 (4.95–10.65)

Table 3
Pollen qualitative morphological characters of studied melliferous taxa

Taxon	Pollen size	Pollen shape	Colpi/ pore	Number of colpi/pore	Exine sculpturing
Zea mays	large	sub-oblate	present	monoporate	psilate
Cenchrus biflorus	medium	prolate-spheroidal	present	monoporate	reticulate
Eruca sativa	small	sub-prolate	present	tricolpate	reticulate
Saccharum bengalense	small	oblate-spheroidal	present	monoporate	psilate
Withania somnifera	small	oblate-spheroidal	present	tricolporate	rugulate
Brassica campestris	small	sub-oblate	present	tricolpate	reticulate
Rhazya stricta	medium	prolate-spheroidal	present	tricolporate	psilate
Fagonia indica	small	oblate-spheroidal	present	tricolporate	reticulate
Cleome brachycarpa	small	oblate-spheroidal	present	tricolporate	verrucate
Cymbopogon jwarancusa	medium	prolate-spheroidal	present	monoporate	reticulate
Ricinus communis	small	oblate-spheroidal	present	tricolporate	reticulate
Brassica juncea	small	sub-oblate	present	tricolpate	reticulate
Cicer arietinum	small	prolate-spheroidal	present	tricolporate	psilate
Brassica oleracea	small	oblate-spheroidal	present	tricolpate	reticulate
Raphanus sativus	small	prolate-spheroidal	present	tricolporate	reticulate
Physalis minima	medium	prolate-spheroidal	present	tricolporate	scabrate

porate and triporate. Fertility and sterility of pollen were determined with highest percentage (92%), observed in *Ricinus communis* L., while lowest in *Zea mays* L. (71%). Exine sculpturing was psilate or reticulate.

Size and pollen shape

Pollen sizes noted were small, large, and medium. *Brassica campestris*, *B. juncea*, *B. oleracea*, *Cicer arietinum*, *Cleome branchycarpa*, *Eruca sativa*, *Fagonia indica*, *Raphanus sativus*, *Ricinus communis*, *Saccharum bengalense*, *Withania somnifera* have small sized pollen. *Cenchrus biflorus*, *Cymbopogon jwarancusa*, *Physalis minima* and *Rhazya stricta* have medium sized pollen, while *Zea mays* has large sized pollen. Number of pores observed ranged from monoporate in *Cenchrus biflorus* to 3-colporate in *Cicer arietinum*. Pollen investigated were suboblate, oblate-spheroidal, subprolate, prolate-spheroidal and oblate-spheroidal shaped. Dominant pollen shape was oblate spheroidal. *Zea mays* had maximum polar diameter (49.29 μm) and *Brassica juncea* had minimum (13.83 μm). Similarly, *Zea mays* had maximum equatorial diameter (56.40 μm), while *Eruca sativa* had minimum (14.28 μm). Maximum P/E ratio was observed in *Eruca sativa* (1.25), while *Brassica campestris* and minimum in *B. juncea* (0.86).

Qualitative and quantitative features of apertures, exine thickness and P/E ratio

Qualitative and quantitative features were recorded using light microscopy. *Fagonia indica* had maximum length of colpi (13.23 μm), while *Physalis minima* had minimum (4.08 μm). Maximum width of colpi was recorded in *Rhazya stricta* (12.66 μm) and minimum in *Ricinus communis* (2.22 μm). Colpi were present in all the studied melliferous species except *Cenchrus biflorus*

Fig. 3. Plants collected: A = *Zea mays*, B = *Cenchrus biflorus*, C = *Eruca sativa*, D = *Saccharum bengalense*, E = *Withania somnifera*, F = *Brassica campestris*, G = *Rhazya stricta*, H = *Fagonia indica*

Table 4
Fertility percentage of selected melliferous plants from Khyber Pakhtunkhwa, Pakistan

Species	Fertile pollen	Sterile pollen	Fertility %
Zea mays	27	11	71
Cenchrus biflorus	63	13	83
Eruca sativa	548	78	88
Saccharum bengalense	38	9	81
Withania somnifera	131	16	89
Brassica campestris	294	40	88
Rhazya stricta	367	36	91
Fagonia indica	147	29	84
Cleome brachycarpa	232	30	89
Cymbopogon jwarancusa	54	18	75
Ricinus communis	174	15	92
Brassica juncea	118	29	80
Cicer arietinum	228	27	89
Brassica oleracea	445	43	91
Raphanus sativus	385	51	88
Physalis minima	289	37	89

Roxb., *Cleome branchycarpa*, and *Zea mays* L. *Raphanus sativus* had the highest exine thickness (1.53 μm) and *Cymbopogon jwarancusa* had minimum (0.84 μm). Tricolporate type of pollen were studied in majority of the species.

Exine sculpturing

Psilate, reticulate, verrucate, regulate and scabrate type of exine sculpturing was observed in the reported species. *Brassica campestris*, *B. oleracea*, *B. juncea*, *Cenchrus biflorus*, *Cymbopogon jwarancusa Eruca sativa*, *Fagonia indica*, *Raphanus sativus*, and *Ricinus communis*, had reticulate type of exine surface. Psilate type exine sculpture were examined in *Cicer arietinum*, *Rhazya stricta*, *Saccharum bengalense* and *Zea mays.* In *Cleome brachycarpa*, *Physalis minima* and *Withania somnifera* regulate, verrucate and scabrate type ornamentation were observed respectively (Figs 5–6).

DISCUSSION

The flora of Khyber Pakhtunkhwa is very diverse, and first time explored as spring melliferous plants. The spring melliferous pollen species of the study

Fig. 4. Plants collected: A = *Cleome brachycarpa*, B = *Cymbopogon jwarancusa*, C = *Ricinus communis*, D = *Brassica juncea*, F = *B. oleracea*, E = *Cicer arietinum*, G = *Raphanus sativus*, H = *Physalis minima*

area were examined through the Light Microscopy exhibiting a large range of difference in pollen morphological features. Both qualitative and quantitative characters of the pollen were studied (Tables 2–3). A total of 16 melliferous

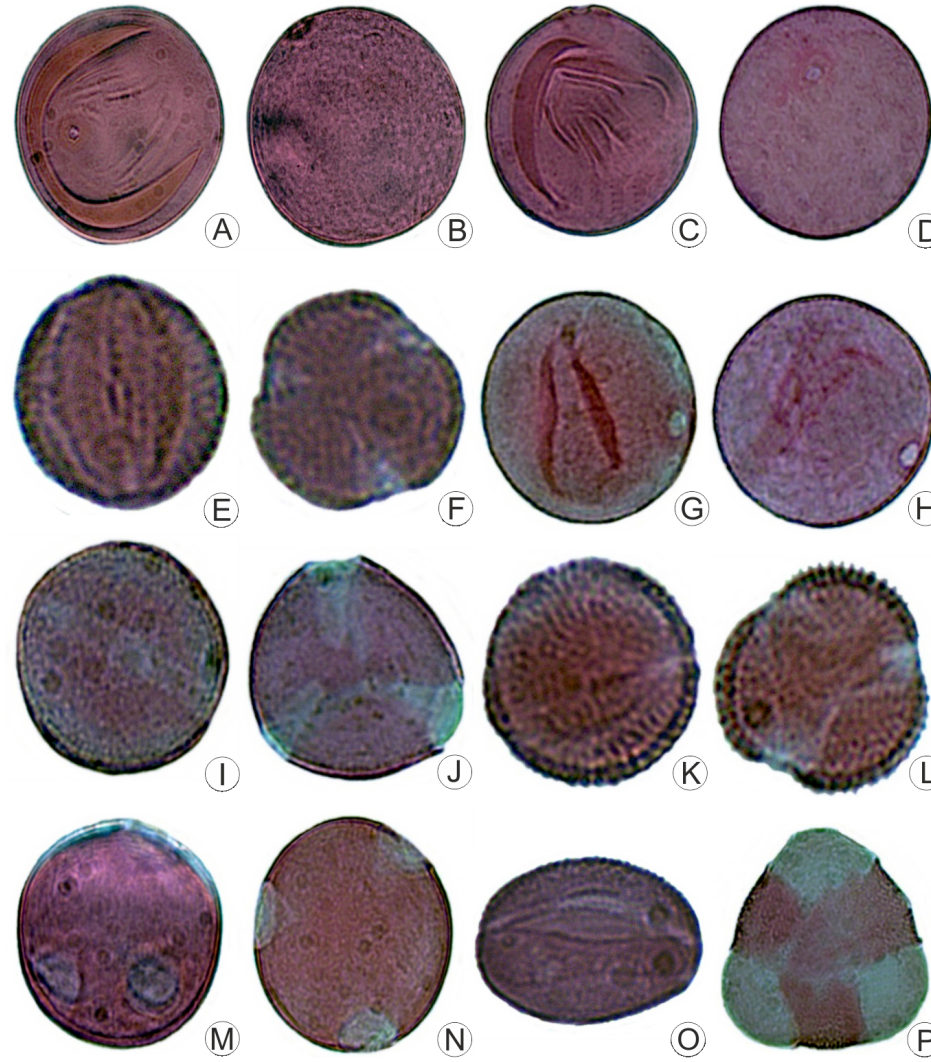

Fig. 5. Light microscopy pollen micrographs taken at (40×). *Zea mays*: A = equatorial view, B = polar view; *Cenchrus biflorus*: C = equatorial view, D = polar view; *Eruca sativa*: E = equatorial view, F = polar view; *Saccharum bengalense*: G = equatorial view, H = polar view; *Withania somnifera*: I = equatorial view, J = polar view; *Brassica campestris*: K = equatorial view, L = polar view; *Rhazya stricta*: M = equatorial view, N = polar view; *Fagonia indica*: O = equatorial view, p = polar view

plants from 8 different families were collected, identified, and examined for pollen morphology. Pollen were photographed to observe the microscopic features (Figs 5–6). Variations in polar diameter, equatorial diameter and p/e

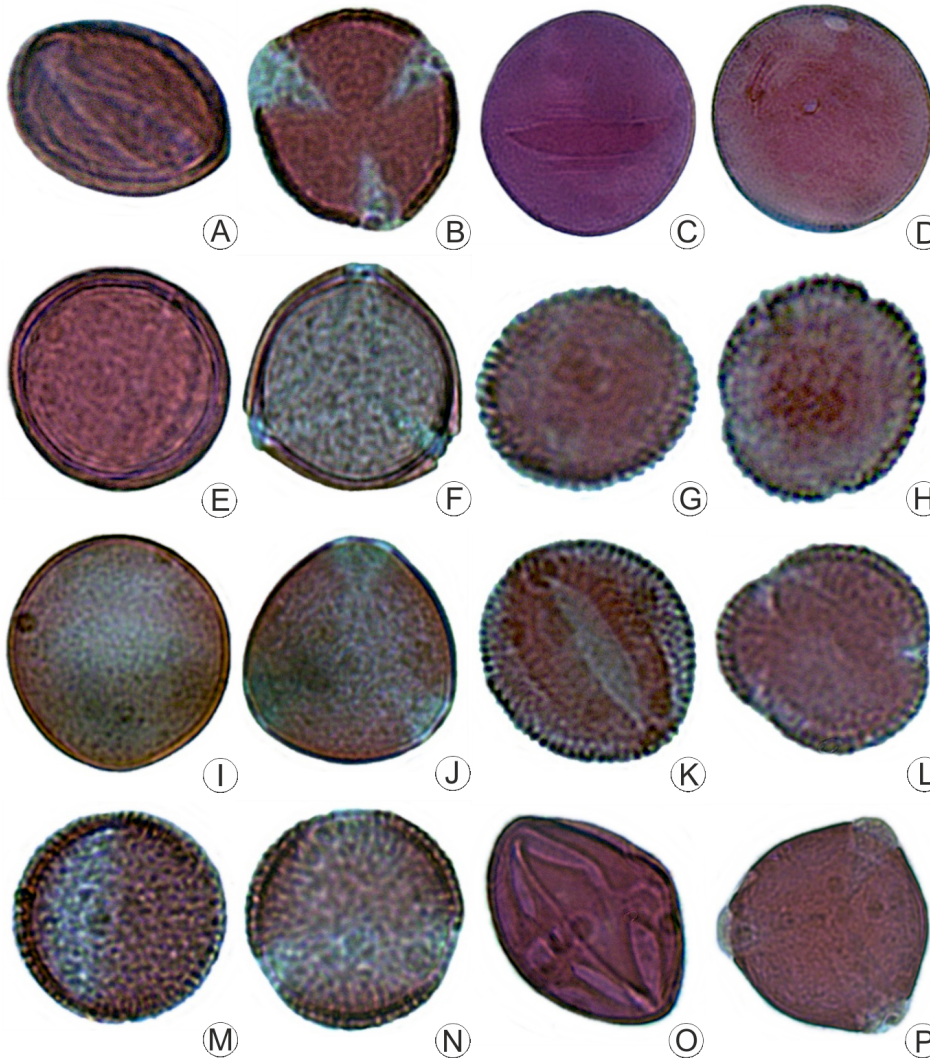

Fig. 6. Light microscopy pollen micrographs taken at (40×): *Cleome branchycarpa*: A = equatorial view, B = polar view; *Cymbopogon jwarancusa*: C = equatorial view, D = polar view; *Ricinus communis*: E = equatorial view, F = polar view; *Brassica juncea*: G = equatorial view, H = polar view; *Cicer arientum*: I = equatorial view, J = polar view; *Brassica oleracea*: K = equatorial view, L = polar view; *Raphanus sativus*: M = equatorial view, N = polar view; *Physalis minima*: O = equatorial view, P = polar view

ratio were observed (Figs 1–2). Brassicaceae was recorded as the dominant family having five species followed by four species of Poaceae, Solanaceae two species, while rest of the families Apocynaceae, Capparaceae, Euphorbiaceae, Fabaceae and Zygophyllaceae with one species each. Pollen grains are monoporate with psilate sculpturing and were observed in *Saccharum bengalense* and *Zea mays*, tricolpate with reticulate surface in *Brassica campestris* and *B. juncea* and tricolporate with reticulate, rugulate and psilate surface pattern.

Various taxonomic characteristics of the pollen were reported by (Punt *et al*. 1994). The plants belonging to family Brassicaceae were commonly visited by honeybees during the month of March. It was also observed that honeybees visit herbaceous plants more abundantly than others in spring and visit plants for foraging activities and honey production in mid part of the day. Pollen morphology study is a very helpful tool from taxonomic point of view (Clark *et al*. 1980). The present study deals with the surface patterns, size, shapes, and all features of pollen. The pollen of Brassicaceae were radially symmetrical, tricolpate, reticulated surface pattern, sub-oblate, sub-prolate ranging from prolate-spheroidal to oblate-spheroidal in shape. Poaceae species were usually monoporate, psilate to reticulate surface pattern, prolate spheroidal, oblate spheroidal and suboblate in shape. Pollen morphology of Poaceae has been reported in previous studies (Chaturvedi *et al*. 1994, Köhler and Lange 1979). Pollen of Solanaceae were tricolporate, reticulated surface pattern with prolate spheroidal to oblate spheroidal shape. Apart from these families the pollen belonging to family Fabaceae, Apocynaceae, Capparaceae, Euphorbiaceae and Zygophyllaceae were tricolporate and with different shapes and surface patterns of exines. The exine thickness ranges from thin to thick. Polar diameter, equatorial diameter and p/e ratio varied, and these variations may help in the taxonomic identification and diversity existing among the species. Pollen morphology helps in determining the diversity of melliferous flora which are threatened by environmental changes, forest cuttings and habitat loss which are the main issues nowadays in district Bannu (Noor *et al*. 2017).

The palynological features not only describe the taxonomic features of a plant but also help to determine the evolutionary relationship among the plants (Qureshi *et al*. 2002). Morphological studies of pollen study help in identification of plants species. The spring melliferous plants of the study area are diversified based on pollen morphology of each species. Pollen fertility recorded in herbaceous flora of Bannu ranges from 71–92% and average fertility percentage of all pollen is 85.5% which revealed that plants are well established in the study area as shown in Table 4. This study is very helpful in determining favourable and unfavourable conditions for a specific plant. According to the interviews taken from local people and beekeepers of that area, there are two main seasons of honey production in which beekeepers arrange their business, one in February–March and another one in August–

September. In February–March the honeybees mostly visit plants of *Brassica campestris*, *B. juncea*, *B. oleracea* and *Eruca sativa*.

The branch of palynology is not only limited to taxonomy but also makes link directly with other branches of science (Zafar *et al.* 2007). Apart from taxonomic studies, it describes the evolutionary relationship between plants. Morpho-palynological characteristics describes the systematic study of vascular plants (Ahmad *et al*. 2013). In plants, pollination occurred through insects which are very useful in agriculture. It has been reported that closely related flora of a region forage by honeybees having environmental conditions match with each other (Carpes *et al.* 2007). Due to existence of melliferous species the beekeepers can arrange their own business annually and so easily earn their income (Ahmad *et al*. 2019). Planting of bee forage species enhances the health of honeybee and increases the honey production by collecting more pollen and nectars from flowers. The knowledge regarding the melliferous flora can solve problems relating to agriculture and honey quality with their production (Farkas and Zajácz 2007). The palynological study of spring melliferous herbaceous plants gave attention to growing more herbaceous plants in spring season and arrange business of beekeeping in coming season for more honey production.

It was observed during field trips that honeybees collect nectars and pollen from both wild and cultivated species. After autumn, spring season is considered as the most favourable season for honey productions not only in the study area but also throughout southern Khyber Pakhtunkhwa. While visiting various flowers by honeybees cross pollination occurred in plants which results in increase of annual yields of crop. The present study realises the importance of melliferous plants, honey production and enhances sustainable development of rural area. In Khyber Pakhtunkhwa flora is nowadays very threatened and endangered. So, it is very necessary to conserve melliferous plants so that with increase in honey yield other advantages could be obtained.

CONCLUSIONS

The present study aims to explore and identify spring melliferous plants from Khyber Pakhtunkhwa. Variations found in exine sculpturing, diameters of pollen, number of pores, colpi, presence and absence of spines are very significant characteristics for future systematic studies and solving taxonomic problems of vascular plants. The most abundant and attractive plant for honeybees recorded in the study area was *Brassica campestris*. This study may help to expand knowledge of pollen morphology, making its relationship with apiculture and highlighting conservation of melliferous plants for honey production in Khyber Pakhtunkhwa.

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