PALYNO-MORPHOLOGICAL STUDY OF WEEDY MELLIFEROUS (BEE VISITED) PLANTS USING LIGHT MICROSCOPIC TECHNIQUES FROM SOUTHERN KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Pollen morphology of 10 different weedy bee foraged plants belong to 10 various families from Southern Khyber Pakhtunkhwa were collected, identified, and studied using light microscopy (LM). The plants were *Asphodelus tenuifolius*, *Euphorbia helioscopia, Parthenium hysterophorus, Rhazya stricta, Datura innoxia, Eruca sativa, Convolvulus arvensis, Anagallis arvensis, Galium aparine,* and *Anethum graveolens.* Slides for light microscopic studies were prepared using materials of acetic acid, glycerin jelly and anthers of flowers. Pollen grain recorded ranged from monocolpate to hexacolporate and from psilate to echinate which were important systematic significance. Pollen size, shape, ratio of polar to equatorial diameter exine thickness, number of colpi, number of pores, equatorial diameter, polar diameter, colpus width, colpus length, spines number, length and width of spines were examined with the help of light microscopy and all these values were analyzed statistically using software SPSS. This research provides a data to the optimal utilization of bee foraged weed plants by honeybees and identification of bee flora for the beekeeping business and honey production. Results revealed that weedy melliferous flora of study area is very helpful for botanical origin, geographical origin of bee species and adulterations found in honey.

KEYWORDS: bee foraged, weeds, light microscopy, pollen, honey, beekeeping

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INTRODUCTION

Bee foraged plants is frequently visited by honeybees for nectars and pollen collections. The weedy melliferous flora are the honey source in southern Khyber Pakhtunkhwa. The study of weedy bee forage flora not limit to the field of taxonomy but also related to the field of plant genetics, plant biotechnology, plant ecology and molecular study of plants (Ahmad *et al.* 2019). Pollen is male gametophyte of bee foraged plants. Pollen morphological characteristics compared with other morphological properties of leaves, stems, roots, etc has high genetic stability. When honeybees visit the flowers to collect pollen, normally focus on only one bee foraged plant in each trip. Honeybee used the flower of weedy bee foraged flora as a source of food (Tashev and Pancheva, 2011). At some advanced level macro and micro morphological evolution of pollen has an important role in taxonomic studies(Naz *et al.* 2019). The honeybee is the primary pollinator of weedy melliferous flora both in wild and cultivated form (Ponnuchamy *et al*. 2014). The pollen study of weedy bee foraged plants indicate about flora origin, medicinal and economics importance related to it (Saibal, 2005). Pollen study determines adulterations found in honey.

Plants is the natural source of honey, its floral parts suck by Honey bees to collect nectars and pollen (Rosdi *et al*. 2016). Honey is sweet in tests and used as a medicine from millions of years. (Crane, 1975). Melissopalynology is an applicable field of pollen study. Bees collect pollen pellets, process it, store it in combination with certain substances and deposit in the comb cells (White and Doner, 1980). These pollen pellets also called as pollen load, bee meat or bee bread. In addition, pollen analysis provides important information about honey filtration, extraction, and fermentation (Russmann, 1998). Plant origin is determined according to the relative frequency of pollen species using melissopalynology (Von Der Ohe *et al*. 2004). Weedy melliferous plants have colorful flowers, growing abundantly and blooming, which attract the bees for pollen, nectar, and honeydew for honey make up (Hrynko *et al.* 2019). Fresh supply of nectars used to build storage combs in hive (Ramanujam and Kalpana, 1994). Therefore, weedy bee foraged plants is primary source for progress and growth of apiculture industry. The Southern Khyber Pakhtunkhwa is divided into districts, for example Lakki Marwat, Karak, Bannu, D I Khan (Akhtar *et al.,* 2018).

Aim of the present study is to investigate pollen morphological study of weedy melliferous plants from southern Khyber Pakhtunkhwa while using microscopic techniques. The study also helps to examine the bee foraged plants in the study area, share the local knowledge regarding to beekeeping, availability of honey and determination of seasons for honey productions.

MATERIAL & METHODS

SAMPLE COLLECTIONS

A field survey was arranged during the start of February in Southern Khyber Pakhtunkhwa. All weedy bee foraged plants were collected from different areas of the Southern Khyber Pakhtunkhwa including Jathanbanda, Inzar, Wadinshaw, Seraj Khel, Khamedan, Amberikalai, Garang, Naraikhwara, Tati and Sabir Abad. All the 13 weedy bee foraged species collected from the study area belong to 11 different families including wild species. During the field trip before the collection of plants, we were confirmed that such weed plants is frequently visited by honeybees for pollen and nectars. After complete confirmation, plants were photographed, then collected with flowers and brought to the Lab for identification and research work.

LIGHT MICROSCOPIC STUDIES

First of all, from the anther of flower, the pollen grain was taken and then put on the slides for microscopic study. According to the techniques of acetolysis of Erdtman, the pollen grain was prepared for light microscopic study (Erdtman, 1969). Pollen was then treated

with Acetic acid and crush with the help of a metal rod. As a result of these crushing's pollen released from the sac of stamen. All the debris because of crushing were discarded with a small camel brush and pure pollen grain treated with glycerin jelly for staining purposes. Glycerin jelly were prepared through method of (Meo and Khan, 2005). Coverslip was then put on the slide; readings of pollen were taken with the help of a microscope. Voucher number and taxon name were noted on the slide and then put in the slides box. All measurement of pollens were taken within 7 days. Fertile and sterile pollen numbers were counted in the slides with the help of Light Microscope (LM). We compile quantitative and qualitative data of pollen and show in the Table 1 & 2. In qualitative characters pollen size and shape were noted.

In quantitative characters, we studied about the equatorial diameter, polar diameter, colpi width, colpi length, colpi number, exine thickness, spine width and length with the help of Light Microscopy. Micrographs of pollen captured with the assistance of Leica magnifying lens fixed with camera Meiji infinity 1.

RESULTS & DISCUSSION.

All the 13 weedy plants of 11 different families were clearly observed in the field as a melliferous character and studied its pollen with the help of light microscopic techniques. Both quantitative and qualitative character of pollen morphology including polar diameter, equatorial diameter, exine thickness, ratio of polar to equatorial diameter were showed in (Table 1 & 2).

S.No	Taxa	Collection site	Vernacular name	Family	Cultivation status/ Distribution within Pakistan	Distribution in World
1	*Anagallis arvensis* L.	Amberi kalai (karak)	Warkharai	Primulaceae	Sawabi, Charsada, Karak, Mardan, Lakki Marwat, Bannu. Islamabad, Rawalpindi, Swat, Jehlum, Kaghan, Abbottabad, Nowshera.	Mexico, Colombia, Venezuela, Brazil, Sudan, Nigeria, china, Pakistan, South east Asia, and North America.
2	*Anethum graveolens* L	Jathan banda (karak)	Kalvangi	Apiaceae	Mardan, lakki marwat, Bannu, Karak, swat, Karachi, Islamabad, Peshawar, Charsada, Lower and Upper dir.	Australia, Nepal, Bangladesh, Italy, Africa, North America, Japan, South west Asia, Afghanistan, Pakistan, India.
3	*Asphodelus tenuifolius* Cav.	Inzar (karak)	Pazakai	Asphodelaceae	Bannu, Dera Ismail Khan, Tank, Lakki Marwat, Karak, Nowshera, Rawalpindi, Jhelum, Peshawar.	North Africa, Europe, South West Asia, Pakistan, India, Somalia, Asia, Afghanistan.
4	*Convolvulus arvensis* L.	Serak (karak)	Parkhatoon	Convolvulaceae	Mardan, Sawabi, Mianwali, Karak, Islam abad, Rawalpindi, Lahore, Peshawar, Murree, Kaghan, Naran, Swat.	Asia, Europe, Maldives, Sri Lanka, India, Pakistan, Bangladesh.
5	*Datura innoxia* Mill.	Khamedan (karak)	Rhand	Solanaceae	Sawabi, lakki marwat, Karak, Mardan, Mianwali, Gujrat, Gujranwala, Karachi.	South Europe, South Africa, Japan, Afghanistan, India, South west Asia, Australia, Africa.
6	*Eruca vesicaria* (L.) Cav.	Serak (karak)	Salaad	Brassicaceae	Mardan, Sawabi, Lakki marwat, Bannu, karak, Jehlum, Charsada, Lower Dir, Rawalpindi.	Mediterranean, North east Africa, Australia, America, Singapore, china, Pakistan, Russia, India, Center Asia.
7	*Euphorbia helioscopia* L.	Seraj khel (karak)	Perparai	Euphorbiaceae	Lakki marwat, Bannu, Bahawalpur, Isa khel, Karak, Sawabi, Mianwali,and South punjab	America, Africa, Bhutan, Bangladesh, Pakistan, Afghanistan, Sri Lanka.
8	*Galium aparine* L	Tati (karak)	Awatunkai	Rubiaceae	South Punjab, Bannu, lakki marwat, Dera Ismail Khan, Karak, Rawalpindi, Jhelum, Lahore, Ziarat, Karachi, Hyderabad, Nowshera.	India Japan, South west Asia, Nepal, Bhutan, Bangladesh, North America, and Brazil.
9	*Parthenium hysterophorus* L.	Amberikalai (karak)	Spin gulai	Asteraceae	Karak, Bannu, Swabi, Lakki marwat, Dera Ismail Khan, Mardan, Mianwali, Peshawar, Hazara, Islamabad.	Africa, Australia, Ethiopia, Africa, Switzerland, Afghanistan, Pakistan, Uganda.
10	*Rhazya stricta* Decne.	Khamedan (karak)	Zara wena	Apocynaceae	Mardan, Mianwali, Karak, Lakki Marwat, Dera Ismail Khan, Hazara, Islam abad, Rawalpindi.	Saudi Arabia, japan, America, Bhutan, Maldeep, Afghanistan, Iraq, Bangladesh.

TABLE 1. Melliferous plant collection and their distribution

S.No	Taxa	Pollen shape	Colpi	Number of colpi	Spines	Shape of spines	Exine Sculpturing
1	*Anagallis arvensis* L.	Oblate -spheroidal	P	Tricolporate	A	A	-
2	*Anethum graveolens* L.	Prolate	p	p	A	A	-
3	*Asphodelus tenuifolius* Cav.	Prolate -spheroidal	P	Monocolpate	A	A	-
4	*Convolvulus arvensis* L.	Sub prolate	P	Tricolpate	A	A	Psilate
5	*Datura innoxia* Mill.	Sub oblate	P	Tricolpate	A	A	Psilate
6	*Eruca vesicaria* (L.) Cav.	Oblate-spheroidal	P	Tricolpate	A	A	-
7	*Euphorbia helioscopia* L.	Oblate-spheroidal	P	Tricolpate	A	A	-
8	*Galium aparine* L.	Prolate	P	Tricolporate	A	A	-
9	*Parthenium hysterophorus* L.	Oblate -spheroidal	P	Tricolpate	P	Small and cylindrical	Echinate
10	*Rhazya stricta* Decne.	Sub prolate	P	Tricolpate	A	A	Psilate

TABLE 2. Micro-morphological pollen-Qualitative characters of melliferous flora.
Keywords: P = Present, A = Absent

S.No	Species name	P/E ratio Mean (min-max) SE µm	Exine thickness Mean (min-max) SE µm	Polar diameter Mean (min-max) SE µm	Equatorial diameter Mean (min-max) SE µm	Colpi length Mean (min-max) SE µm	Colpi width Mean (min-max) SE µm	Spine length Mean (min-max) SE µm	Spine width Mean (min-max) SE µm
1	*Anagallis arvensis* L.	0.94	1.80(1.70-1.90) ±0.03s	12.54(10.5-14.50) ±0.75	13.22(12.50-14.00) ±0.25	3.78(1.90-6.50) ±1.11	2.00(1.90-2.10) ±0.03	A	A
2	*Anethum graveolens* L.	1.58	2.32(2.20-2.40) ±0.03	10.62(10.00-11.10) ±0.20	6.72(6.40-7.10) ±0.13	3.04(2.90-3.20) ±0.05	3.50(3.40-3.60) ±0.03	A	A
3	*Asphodelus tenuifolius* Cav.	1.02	2.22(2.10-2.22) ±0.04	27.06(26.90-27.06) ±0.07	26.52(26.00-26.52) ±0.27	27.12(26.70-27.12) ±0.16	6.60(6.40-6.60) ±0.07	A	A
4	*Convolvulus arvensis* L.	1.32	3.70(3.50-4.10) ±0.12	40.00(39.50-41.00) ±0.27	30.28(29.50-31.00) ±0.25	15.02(14.50-15.50) ±0.16	18.60(18.00-19.00) ±0.19	A	A
5	*Datura innoxia* Mill.	0.81	1.76(1.70-1.80) ±0.02	13.66(13.40-14.00) ±0.11	16.72(16.50-17.00) ±0.10	16.46(15.70-16.90) ±0.20	3.68(3.50-3.90) ±0.07	A	A
6	*Eruca vesicaria* (L.) Cav.	0.92	1.76(1.70-1.90) ±0.04	11.04(10.60-11.60) ±0.16	11.96(11.50-12.70) ±0.20	5.62(5.50-5.80) ±0.06	5.86(5.70-6.10) ±0.08	A	A

7	*Euphorbia helioscopia* L.	0.97	2.72(2.50-2.90) ±0.07	18.68(18.30-19.10) ±0.16	19.12(18.90-19.50) ±0.10	4.52(4.40-4.60) ±0.04	5.74(5.50-6.00) ±0.09	A	A
8	*Galium aparine* L.	1.35	2.10(2.00-2.30) ±0.54	14.72(14.00-15.10) ±0.19	10.90(10.50-11.20) ±0.13	6.78(6.50-7.20) ±0.15	4.66(4.50-5.00) ±0.92	A	A
9	*Parthenium hysterophorus* L.	0.96	2.40(2.30-2.50) ±0.04	7.68(7.50-7.90) ±0.08	7.96(7.70-8.20) ±0.08	A	A	2.76(2.50-3.10) ±0.10	1.80(1.60-2.10) ±0.08
10	*Rhazya stricta* Decne.	1.30	2.12(1.90-2.40) ±0.09	23.84(22.50-25.00) ±0.39	18.20(17.50-19.00) ±0.24	8.00(7.70-8.20) ±0.09	9.70(11.90-12.00) ±1.90	A	A

TABLE 3. Micro-morphological pollen-Quantitative characters of melliferous flora.
Keywords: A = absent; max = maximum; min = minimum; SE = Standard error; µm = micrometer

1. *Asphodelus tenuifolius* L
Family: Asphodelaceae
English name: Asphodelus
Local name: Pazakai
Location: Inzar
Colour of Flower: White
Pollen Morphology: Pollen is monocolporate and monad. Pollen diameter in polar case is 27.06 µm (26.90-27.06µm), equatorial diameter is 26.52µm (26.00-26.52µm), P/E 1.02 and exine thickness is 2.22 µm (2.10-2.22µm).

2. *Euphorbia helioscopia* L.
Family: Euphorbiaceae
English name: Sun spurge
Local name: Perparai
Location: Seraj Khel
Colour of Flower: Yellow
Pollen Morphology: Pollen is tricolporate and monad. Pollen diameter in polar case is 18.68µm (18.30-19.10µm), equatorial diameter is 19.12µm (18.90-19.500µm), P/E is 0.97 and exine thickness is 2.72µm (2.50-2.90µm).

3. *Parthenium hysterophorus* L
Family: Asteraceae
English name: Carrot grass
Local name: Spin gulai
Location: Amberikalai
Colour of Flower: White
Pollen Morphology: Pollen is tricolporate, echinate and monad. Polar diameter of pollen is 7.68µm (7.50-7.90µm), equatorial diameter is 7.96µm (7.70-8.20µm), P/E ratio is 0.96 and exine thickness is 7.96µm (7.70-8.20µm).

4. *Rhazya stricta* Decne
Family: Apocynaceae
English name: Harmal
Local name: Zara wena
Location: Khamedan
Colour of Flower: White
Pollen Morphology: Pollen is tricolporate and monad. Polar diameter of pollen is 23.84µm (22.50-25.00µm), equatorial diameter is 18.20µm (17.50-19.00µm), P/E is 1.30 and exine thickness is 2.12µm (1.90-2.40µm).

5. *Datura innoxia* Mill
Family: Solanaceae
English name: Hoary thorn apple
Local name: Rhand
Location: Khamedan
Colour of Flower: White
Pollen Morphology: Pollen is tricolporate and monad. Polar diameter of pollen is 13.66µm (13.40-14.00µm), equatorial diameter is 16.72µm (16.50-17.00µm), P/E is 0.81 and exine thickness is 1.98µm (1.90-2.10µm).

6. *Eruca sativa* Mill
Family: Brassicaceae
English name: Rocket salad
Local name: Salad
Location: Serak
Colour of Flower: White -Yellow
Pollen Morphology: Pollen is tricolporate and monad. Polar diameter is 11.04µm (10.60-11.60µm), equatorial diameter is 11.96µm (11.50-12.70µm), P/E is 0.92 and exine thickness is 1.76µm (1.70-1.90µm).

***7. Convolvulus arvensis* L**
Family: Convolvulaceae
English name: Chardvel
Local name: Parkhatoon
Location: Serak
Colour of Flower: White
Pollen Morphology: Pollen is tricolporate and monad. Polar diameter of pollen is 40.00µm (39.50-41.00µm), equatorial diameter is 30.28µm (29.50-31.00µm), P/E is ratio is 1.32 and exine thickness is 3.70µm (3.50-4.10µm).

8. *Anagallis arvensis* L.
Family: Primulaceae
English name: Red scarlet
Local name: Warkharai
Location: Ambari Kala
Colour of Flower: Bluish
Pollen Morphology: Pollen is monad with 9 colpi and 9 pores, Polar diameter is 14.72µm (14.00-15.10µm), equatorial diameter is 10.90µm (10.50-11.20µm), P/E is 1.35 and exine thickness is 2.10µm (2.00-2.30µm).

9*. Galium aparine* L.
Family: Rubiaceae
English name: Cleavers
Local name: Awatunkai
Location: Tati
Flower color: White
Pollen Morphology: Pollen is tricolporate and monad. Polar diameter is 12.54µm (10.5-14.50µm), equatorial diameter is 13.22µm (12.50-14.00µm), P/E is 0.94 and exine thickness is 1.80µm (1.70-1.90µm).

10. *Anethum graveolens* L
Family: Apiaceae
English name: Sanskrit
Local name: Kalvangi
Location: Jathan banda
Colour of Flower: Yellow

Pollen Morphology: Pollen is monad and dicolporate. Polar diameter is 10.62µm (10.00-11.10µm), equatorial diameter is 6.72µm (6.40-7.10µm), P/E is ratio 1.58 and exine thickness is 2.32µm (2.20-2.40µm).

In bee foraged plants we observed three groups (i) pollen source, which is visited for pollen collection (ii) nectars source, which is visited for nectars collection (iii) nectars and pollen source, which is visited for both nectars and collection for honey make up (Kalpana *et al.* 1990). These groups were confirmed from melissopalynology of different honey samples of the studied areas. Palynological study of investigated taxa was observed under the light microscope (LM); most of the pollen were tricolporate, and few are monocolporate and dicalporate. We measured different aspects of studied weedy bee foraged plant species as equatorial diameter, polar diameter, colpi length, colpi width, exine thickness, spines, and pores numbers. Differences in exine thickness of pollen was also examined in the reported study of (Asmat *et al.* 2011). The pollen and nectars collected by *Apis mellifera* from different weedy bee foraged vegetation as shrub, and herbs. The maximum P/E ratio was observed in *Anethum graveolens* was 1.58 µm. The minimum P/E ratio was noted in *Datura innoxia* Mill. 0.81 µm. Maximum exine thickness was observed in *Convolvulus arvensis* as 3.70µm while the minimum one was 1.76 µm noted in *Datura innoxia.* The *Convolvulus arvensis* was maximum polar diameter as 40.00 µm while the minimum polar diameter was noted in *Parthenium hysterophorus* as 7.68 µm. The maximum equatorial diameter was noted in Convolvulus arvensis as 30.28 µm while minimum observed in *Anethum graveolens* as 6.72 µm. The maximum colpi length was observed in *Asphodelus tenuifolius* is 27.12. µm while minimum observed in *Anethum graveolens* is 3.04 µm. The studied plants show maximum Spine length of *Sonchus asper* is 3.60 µm while minimum in *Parthenium hysterophorus* is 2.76 µm. *Convolvulus arvensis* have maximum width of colpi is 18.60 µm and *Anagallis arvensis* have minimum is 2.00 µm.

Most of the weedy bee foraged plants species origin are tropical dry ever green ; supported by the pollen used in the honey make up indicate the tropical dry Evergreen forest species (Ponnuchamy *et al.* 2014). The studied plants blooming period show variation to species. The study of weedy bee foraged plants is help full for beekeeper to manage their business according to the flowering season in different areas (Noor *et al,* 2017). The melliferous weed plants are most important because local people conserve it for honey production. During field trip and interviews from local beekeepers, it is observed that in Southern Khyber Pakhtukhwa the dominant flowering period of melliferous weeds plants in March and April. The information about bee foraged species identifies botanical source of honey. Data of bee foraged plants can encourage the beekeeper for the beekeeping and maximize the honey production for export purposes to the developed countries in many areas (Bhalchandra *et al.* 2014). Endangerments of weedy bee foraged flora may cause to decrease in nutrition and honey production which causes diseases (Basu and Cetzal-Ix., 2018). Pollination in weedy melliferous flora mostly occurred by the entomophilous. In field observation the most common pollinators were *Apis mellifera.*

CONCLUSION

Weedy Bee foraged species were identified for managing bee keeping business and honey production. Pollen morphological studies of weedy bee foraged plants in the study area showing high potential of bee keeping business. The study area showed high potential of bee keeping business. In Southern Khyber Pakhtunkhwa purchase and sale of honey needs to be expended as well as need of quality control to identify non-Southern Khyber Pakhtunkhwa honey samples. Established some

geographical limitation of best honey samples through adulteration detection. Pollen identification was possible with the help of previous published data. These results also confirm that Southern Khyber Pakhtunkhwa is main region for honey production due to presence of weedy bee foraged flora. The information's about weedy bee foraged flora may encouraged and propagate to development of bee forming in Southern Khyber Pakhtunkhwa, Pakistan.

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Figure caption 1.pollen pictures (a) *Anagallis arvensis* L. (b) *Anethum graveolens* L. (c) *Asphodelus tenuifolius* Cav. (d) *Convolvulus arvensis* L. (e) *Datura innoxia* Mill. (f) *Eruca vesicaria* (L.) Cav. (g) *Euphorbia helioscopia* L. (h) *Galium aparine* L. (i) *Parthenium hysterophorus* L. (j) *Rhazya stricta* Decne.