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Palyno-morphological assessment of Asteraceae taxa grown in Qarshi Botanical garden at Qarshi Industries (Pvt.) Ltd by using LM and SEM, Pakistan

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The present study covers the micro-morphological characteristics of 32 taxa of Asteraceae family from Qarshi Botanical Garden at Qarshi Industries (Pvt.) Ltd., Hattar Haripur, Pakistan. The morphopalynological characters studied were size, shape, polar and equatorial diameter and their exine ornamentation using light microscopy (LM) and scanning electron microscopy (SEM) for its taxonomic significance. In the present investigations the pollen apertures of examined taxa were tricolporate, tricolpate, trizonocolporate, pentaporate and tetracolporate. mesocolpium orientation according to P/E ratio was identified as reticulate, echinate, oblate-spheroidal, echino-lophate, densely perforated and echinate perforate. It was found that the following 32 types of exine sculpture occurred in the investigated species: Echinate-perforate, aerolate-echinate, echinate-microreticulate, verrucateechinate, scabrate-Echinate, reticulate and shortly spinulate, echinate, elliptic to rarely circular and hexagonal view and spinulose-verrucate. Similarly, variation in polar diameter was prominent and the largest pollen was recorded on Artemisia vulgaris, Chicorium intybus, Helianthus annuus and Carthamus oxyacantha which have polar diameter. This was found with large size of 42.65 µm while the smallest polar diameter size was found in Chrysanthemum leucanthemum, Artemisia absinthium, Stevia rebaudiana, Tanacetum vulgare, Saussurea costus and Tanacetum cinerariifolium. Exine thickness of maximum value was examined in Centratherum punctatum 1.7 µm, while the minimum one was recorded in Seriphidium kurramense 0.5 µm (0.15-0.90). Pollen fertility was estimated with the highest percentage which was counted in C. leucanthemum (89.55%), while the highest sterility percentages were observed in Lactuca sativa (63%). The lowest fertility and sterility percentages were observed in L. sativa and S. kurramense (40 and 36.80%), respectively. The findings from the analysis of pollen morphological data show species level and taxonomic importance.

Key words: Asteraceae taxa, Qarshi Botanical Garden, pollen morphology, taxonomy, light microscopy (LM), scanning electron microscopy (SEM).

INTRODUCTION

Recent phylogenetic studies of the Asteraceae have resolved many systematic issues at higher taxonomic levels in the family (Ahmed et al., 2019). Asteraceae plant family is also used to be known as the Compositae plant family, and it is known as one of the largest plant families with thousands of plant species. The Asteraceae plant family consists of24,000 accepted species. About 1,600 to 1,700 of its genera are distributed around the world, excluding Antarctica (Khan et al., 2020a, b). Asteraceae is assigned an advanced position in plant systematic. Several plants in the Asteraceae family are economically important as medicinal, ornamentals and vegetables (Joujeh et al., 2019; Ali et al., 2020).

There are many disciplines associated with taxonomy, which are used by taxonomists to improve the identification, classification, and systematic position of plant taxa (Alasbahi and Al-Hawshabi, 2020). Among these disciplines, palynology is one of the most significant tools used to identify and differentiate closely related taxa. Palynological studies are not only useful in solving many taxonomic problems; they are also useful in determining the incidence of pollen that causes pollinosis and in identifying plants through Palynotaxonomic studies. The study of pollen biology has direct relevance in agriculture, horticulture, forestry, plant breeding, and biotechnology (Zafar et al., 2020). Pollen grains have potential use in gene transfer and also studies expression gene of cloning and research on intracellular differentiation and polarity. The pollen morphology of medicinal plants was first carried out in Pakistan. In the current study, medicinal herbs from the Qarshi botanical garden were studied and certified using Qarshi products, and the same parameters were studied by Bahadur et al. (2019), who studied various aspects of plants such as morphology, palynology, and medicinal properties. Pollen morphology has provided an approach to the systematic relationships among the genera of Asteraceae.

Polynomorphological investigation, using diverse microscopic techniques is conducted as an aid to the morphological studies to provide a significant tool for plant taxonomist for species delimitation (Nazish et al., 2019). Scanning electron microscopy (SEM) has been used for the dissimilarity of species based on exine ornamentation (Bahadur et al., 2018). In this modern era, the recent use of SEM has developed essential research techniques in the micro morphological studies of pollen grains (Ahmad et al., 2019: Alamgir, 2017). Scanning electron microscope have a high resolution, which gives detail of pollen structure while under light microscope clear detail of the pollen surface is not shown. Scanning electron microscopy of pollen is also used to create new describing pollen terminology for ornamentation developing a numerical approach to pollen sculpturing and even computer analysis of the exine. They may also be useful in observing the effects of climate on the pollen characteristics of selected species by comparing their features with those existing in other regions (Nazish et al., 2019: Albert and Innes, 2020). There are many areas related to plant taxonomy used by taxonomists to support and improve the systematic identification, classification,

and placement of plants. Within these disciplines, pollen morphology research is one of the most important tools used by modern taxonomists to identify and distinguish closely related classes (Zhigila et al., 2014).

The characteristic of the pollen of different species or groups of Asteraceae taxa have been studied; previous studies have conducted on pollen morphology of the Asteraceae family, demonstrating the importance of pollen morphology in understanding the classification of the family (Zafar et al., 2020; Ahmad et al., 2019; Bahadur et al., 2019: Ajaib et al., 2016).). The pollen morphology is also considered a tool for depicting the genera and species of the Asteraceae family.

The present studies illustrate the range of variability in pollen characters that proved to be important for intrageneric taxonomy and to compare the pollen micromorphology in Asteraceae taxa using light and scanning electron microscope collected from Qarshi Herb Garden, Khyber Pakhtunkhwa, Pakistan.

MATERIALS AND METHODS

Collection and identification

During collection, 32 Asteraceae species were collected in different flowering seasons during the period of April 2021 to April 2022 (Table 1 and Plates 1-6). Samples were collected randomly and collected plant species were dried, preserved, poisoned and mounted on herbarium sheet following the previously published protocol (Khan et al., 2021; Al-Watban et al., 2013). The International Plant Name Index (IPNI) was used to get the accurate botanical names authorization for taxonomic validation. Voucher specimens were submitted in the Herbarium of Qarshi Herb Research Center (QHRC), at Qarshi Industries (Pvt.) Ltd., Hattar Haripur, Pakistan.

Preparation of glycerin jelly

Glycerin jelly is prepared for qualitative investigation of pollen under Light microscopy. Glycerin jelly was prepared according to the modified method of Zafar et al. (2020), who followed Erdtman (1960). 500 ml of distilled water was taken in a beaker and heated on a hot plate (model UELP Scientific, Germany). When temperature reached 70 to 80°C, 35 g of gelatin was added. With an increase in temperature, it became a viscous liquid of glycerin jelly. Heating was continued for 1 h and thereafter, 35 g of glycerol was mixed in it with few crystals of phenol. Subsequently, 0.1% safranin was added with 1/8th volume of the glycerin jelly and it was stirred till a uniform pink color appeared. The jelly was stabilized at room temperature.

Processing of pollen using LM

Thirty-two Asteraceae taxa were studied using light microscope. For this study, the methodology of and Erdtman (1986) was followed

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S/N	Таха	Habit	Climate	Temperature (°C)	Rainfall (mm/year)	Soil	рН	Reproduction
1	Achillea millefolium L.	Perennial herb	Temperate regions	Max: 38°C, Min: -25°C	1130-9730	The plant prefers light sandy, medium loamy, heavy clay and well-drained soil and can grow in nutritionally poor soil	5.0 -7.5	By seed and runners
2	Ageratum houstonianum Mill.	Annual herb	Tropical and sub-tropical regions	Max: 36°C, Min: -10°C	2500	The plant prefers light sandy, medium loamy and heavy clay soils	5.6-7.5	By seeds
3	Artemisia absinthium L.	Perennial herb	Tropical and sub-tropical regions	Max: 27.5°C, Min: 12.5°C	1500-2000	This plant prefers a well-drained humus rich fertile, sandy loam	4.5 - 8.0	By dividing the roots in autumn and by seeds
4	Artemisia scoparia Waldst. & Kitam.	Biennial or Perennial	Arid and low hills	Max: 40°C, Min: -4°C	800-1300	Sandy-clay soils	6.5-7.5	By seed
5	Artemisia vulgaris L.	Perennial herb	Temperate climate	Max: 27ºC, Min: 12.5ºC	586	Sandy, medium loamy, heavy clay and well- drained soil	4.6-8.2	By seed and stem cutting
6	Carthamus tinctorious L.	Annual herb	Alpine Mediterranean regions	Max: 38°C, Min: -2°C	270-750	The plant prefers light sandy, medium loamy and well-drained soil	5.4-8.2	By seeds
7	Cichorium intybus L.	Perennial herb	Temperate regions	Max: 35°C, Min: 2°C	300-4000	The plant prefers light sandy, medium loamy and heavy clay and well-drained soil	5.5-7	By seeds
8	Leucanthemum vulgare (Vaill.) Lam.	Perennial herb	Temperate regions of Asia.	Max: 35°C, Min: -5°C	1100	This plant prefers sandy loam or a clay loam soil	6.6-8.5	By seeds
9	Cynara scolymus L.	Perennial herb	Alpine and Mediterranean regions	Max: 36°C, Min: -2°C	600-700	The plant prefers light sandy, medium loamy and heavy clay and well- drained soil	6.5-8.4	By seeds
10	Helianthus tuberosus L.	Perennial herb	Temperate and tropical regions	Max: 36°C, Min: - 3.8°C	1250	The plant prefers light sandy, medium loamy and heavy clay and well-drained soil	4.5-8.2	By seed and tuber
11	Stevia rebaudiana (Bertoni) Bertoni	Perennial shrub	Semi-humid subtropical regions	Max: 43⁰C, Min: 21⁰C	1375	The plant prefers light sandy and medium loamy soils	6.5-7.5	By seeds and cuttings
12	Calendula officinalis L.	Perennial herb	Temperate region	Max: 32⁰C, Min: 17⁰C	500-800	The plant prefers light sandy to loamy soils with good drainage	6.1-7.5	By Seed
13	Eclipta prostrata (L.) L.	Annual herb	Tropical, subtropical and temperate region	Max: 35⁰C, Min: 20⁰C	600-1000	The plant prefers loamy soils with little moisture	5.6-7.4	By Seed

Table 1. List of medicinal plant species of family Asteraceae collected from Qarshi herb garden.

14	Helianthus annuus L.	Annual herb	Tropical and temperate	Max: 34°C, Min: 17°C	600-1000	It grows on dry clays or heavy sands	4.5-8.7	By seed and rhizome multiplication
15	Silybum marianum (L.) Gaertn.	Annual herb	Tropical, sub tropical and temperate region	Max: 40°C, Min:10°C	270-750	The plant prefers sandy loam, calcareous and rocky soils with good drainage	6.6-8.5	By seed
16	Tanacetum parthenium (L.) Sch.Bip.	Perennial herb	Temperate region	Max: 25°C, Min:10°C	600-800	It grows on clay, loamy and sandy soils with good drainage	6.1-7.8	By seeds and cutting
17	Tanacetum vulgare L.	Perennial herb	Temperate regions	Max: 25°C, Min:10°C	600-800	The plant prefers light sandy and sandy loam well-drained soils	6.1-7.8	By seeds and division
18	Bidens pilosa L.	Perennial herb	Tropical regions	Max: 10°C, Min:35°C	307	It can be grown on any soil (clay, sandy, loamy or stony) with low water requirements and can tolerate alkaline soils	6.8 - 7.8	By seed
19	Carthamus oxyacantha M. Bieb.	Annual herb	Sub-tropical to temperate regions	Max: 10°C, Min:30°C	300	It can survive on many soil types including alkaline and halotrophic soils and is drought tolerant plant	6.1-7.8	By seed
20	Echinops echinatus Roxb.	Annual herb	Arid regions	Max: 27°C, Min:42°C	268.4	It can survive on rocky and sandy soils with little water needs. It can also survive on sodic soils	6.5-7.8	By seed
21	Gerbera jamesonii Bolus ex Hook.f.	Perennial herb	Tropical regions	Max: 27.5°C, Min: 12.5°C	1500-2000	The plant prefers well drained stony clay soils	7.0-8.0	By seed or vegetative methods
22	Saussurea costus (Falc.) Lipsch.	Perennial herb	Temperate regions	Max: 35°C, Min: -5°C	1100	The plant grows best on humus rich well drained soils	5.5-7.0	By seed and cuttings
23	Seriphidium kurramense (Qazilb.) Y.R. Ling	Deciduous shrub	Temperate regions	Max: 35°C, Min: -5°C	1100	Gravelly, fine to coarse sandyclay soils	5.8-7.0	By seed
24	Tagetes erecta L.	Annual herb	Alpine, continental and mediterranean regions	Max: 36°C, Min: -2°C	600-700	It grows best on well drained loamy soils	6.1-7.8	By seed and layering
25	<i>Tanacetum cinerariifolium</i> (Trevir.) Sch.Bip.	Perennial herb	Temperate and tropical regions	Max: 36°C, Min: - 3.8°C	1250	The plant prefers light sandy and well drained soils	5.1-7.5	By seed and divisions
26	Xanthium strumarium L.	Annual herb	Temperate and sub-tropical regions. It is common in the temperate zone	Max: 36°C, Min: - 3.8°C	1250	It can grow in acid, neutral and alkaline soils but it prefers a loamy or sandy and moist to mesic soil.	5.2-8.0	By seed

27	Zinnia elegans L.	Annual herb	Sub-tropical regions.	Max: 35°C, Min: - 4°C	1300	It requires well drained soil	6.8-7.5	By seed and vegetative methods
28	Parthenium hysterophorus L.	Herb	Tropical and temperate regions	Max: 40°C, Min:10°C	270 -750	Mostly sandy loam and sandy clay loam for its optimum growth	2.5-10	By seed
29	Centratherum punctatum Cass.	Herb	Tropical to semi-tropical regions	Max: 40°C, Min:10°C	270 -750	Moist, fertile, well-drained soils	5.5-7.5	By seed
30	Coreopsis grandiflora Hogg ex Sweet	Herb	Temperate and tropical regions	Max: 40°C, Min:10°C	270 -750	Sandy, rocky well drained soils	5.5-7.5	By seed and division
31	Gazania rigens (L.) Gaertn.	Herb	Tropical regions	Max: 27.5°C, Min: 12.5°C	1500-2000	Sandy, well-drained soils	6.1-7.8	By seed
32	Lactuca sativa L.	Herb	Tropical and temperate regions	Max: 36°C, Min: -10°C	2500	Light sandy and medium loamy well-drained soils	6.0-8.0	By seed

Source: Authors

with slight modifications.

Pollen grains were removed with the help of tweezers from the anthers of dried and fresh flowers from the plant species. Pollen slides were prepared by the addition of few drops of acetic acid on the dried anthers and crushing them with the help of a glass rod. Furthermore, pollen debris was removed from the glass slide through brushes followed by the pouring of one or two drops of glycerin jelly on the area containing pollen grains (Sivaguru et al., 2018; Shaheen et al., 2009: Perveen and Qaiser, 2007; Amina et al., 2020). Immediately, cover slips were placed over the slides and then examined under the LM (Model 00179048, Canada). Different pollen features (polar and equatorial diameter, P/E ratio, colpus length and width,mesocolpium, exine ornamentation and thickness) were studied.

SEM analysis

Pollens belonging to the Asteraceae family have been prepared for SEM studies using the most common method of Ullah et al. (2018).

We used SEM to investigate the exine thickness and decorative coating in detail. For SEM studies, pollen grains were suspended in a drop of 40% acetic acid. Transferred to clean metallic stubs and coated with gold using a JEOL

JFC 1100 E ion sputtering device. SEM observations were carried out on a JEOL microscope JSM5910. The work was carried out in the Centralized Resource Laboratory, University of Peshawar (Pakistan). The terminology used is that of Azeez et al. (2019).

Pollen sterility and fertility

On each slide the number of fertile and sterile pollen were counted. The properly stained pollen was considered fertile while the damaged, broken or the pollen, which was not properly stained was considered sterile. Pollen fertility (viability) and sterility were calculated in percentage. On the slides, fertility of the pollen was found by the following formula:

Fertility (viability) = $F/F+S \times 100$.

where F = number of fertile pollen, and S = the number of sterile pollen. Fertility is always measured in percentage.

The partially stained pollen would be counted under sterile pollen because they are abnormal and even they take a little stain they could fail to germinate properly. The unstained would not germinate and the partially stained if germinate would produce shorter pollen tube as compared to the normal one and hence unable to fertilize the egg cell and central cell.

Qualitative features

In qualitative features, both the polar and equatorial \ diameters were found through which the size and shape of pollen were determined. In qualitative investigation, the number and type of colpi, length and width of spines, exine width and sculpturing, pore number and orientation were determined.

Quantitative features

In quantitative features, the length and width of polar and equatorial diameter of pollen, exine thickness, pore length, width and number, length and width of colpi, pore and spines were determined.

Statistical analysis

For each plant specimen, different measurements were



Plate 1. SEM micrographs showing pollen grains of family Asteraceae and also showing detailed exine ornamentation. PV: Polar view; EV: equatorial view; ES: exine sculpture. Source: Authors

inspected using SPSS 16.0 version. Pollen size includes polar axis (P) and equatorial axis or diameter (E), and pollen aperture, mesocolpium, exine (exodermises) thickness, pore width and length, width and length of spines and colpi are measured. The terminology of this study was used according to Erdtman (1966).

RESULTS

The morpho-palynological variation is described in terms of size, shape, exine ornamentation, number and size of aperture, length and width of colpi and percentage of pollen fertility. The representative pollen traits of all the species studied were summarized in Tables 1 to 3.

Medicinal plants cultivation

To promote cultivation of medicinal plants, herb research center of Qarshi Industry developed a number of high yielding varieties, worked out agro-technologies and processing technologies for them. Profitable cultivation of medicinal plants practiced can be by farmers/companies/entrepreneurs along with traditional agricultural horticultural crops as sole crops, intercrops, sequential crops, etc. They can be profitably intercropped in plantations. List of some medicinal herbs are used in Qarshi product present in Qarshi herb garden having detailed information like correct botanical name, plant name, part/s used, habit, climate, temperature, rainfall, soil and



Plate 2. SEM micrographs showing pollen grains of family Asteraceae and also showing detailed exine ornamentation. PV: Polar view; EV: equatorial view; ES: exine sculpture. Source: Authors

pH (Table 1 and Plates 7 to 10).

Size

The size of the pollen grain of polar and equatorial diameter ranged from 17.85 to 33.55 μ m and 15.8 to 42.65 μ m by using SPSS version 16.0. *Artemisia vulgaris* Burm.f., *Cichorium intybus* L., *Helianthus annuus* L. and

Carthamus oxyacantha M. Bieb. have polar diameter found with large size of 42.65 µm while smallest size polar diameter are found in *Chrysanthemum leucanthemum* L., *Artemisia absinthium* L., *Stevia rebaudiana* (Bertoni) Bertoni, *Tanacetum vulgare* L., *Saussurea costus* (Falc.) Lipsch and *Tanacetum cinerariifolium* (Trevir.) Sch.Bip.; while in 23 taxa, namely *Achillea millefolium* L., *Ageratum houstonianum* Mill.,



Plate 3. SEM micrographs showing pollen grains of family Asteraceae and also showing detailed exine ornamentation. PV: polar view; EV: equatorial view; ES: exine sculpture. Source: Authors

Artemisia scoparia Waldst., Carthamus tinctorius L., Cynara scolymus L., Helianthus tuberosus L., Calendula officinalis L., Eclipta prostrata L., Leucanthemum vulgare (Vaill.) Lam., *Silybum marianum* Gaertn., *Tanacetum parthenium* (L.) Sch.Bip, *Bidens pilosa* L., *C. oxyacantha* M.Bieb., *Echinops echinatus* Roxb., *Gerbera jamesonii*

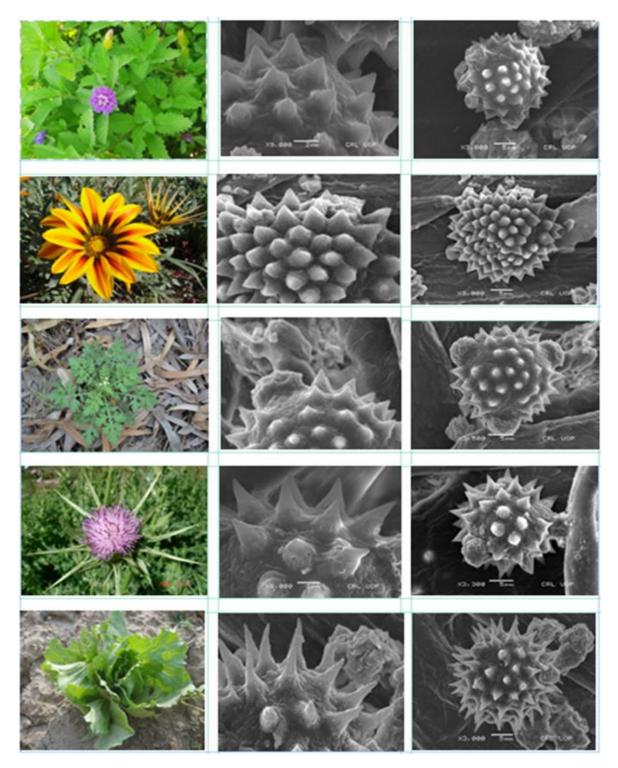


Plate 4. SEM micrographs showing pollen grains of family Asteraceae and also showing detailed exine ornamentation. PV: Polar view; EV: equatorial view; ES: exine sculpture. Source:Authors

Bolus ex Hook.f., *Seriphidium kurramense* (Qazilb.) Y.R.Ling, *Tagetes erecta* L., *Xanthium strumarium* L., *Zinnia elegans* L., *Parthenium hysterophorus* L.,

Centratherum punctatum Cass., *Coreopsis grandiflora* Hogg ex Sweet and *Lactuca sativa* L. were medium size (Table 2 and Figure 2).



Plate 5. SEM micrographs showing pollen grains of family Asteraceae and also showing detailed exine ornamentation. PV: Polar view; EV: equatorial view; ES: exine sculpture Source: Authors.

Pollen shape and exine sculpturing

The relationship between the polar axis and the equatorial diameter was as follows: P/E = <0.50 was peroblate, 0.50-0.75 was oblate, 0.76-0.88 was suboblate,

0.89-0.99 was oblate-spheroidal, 1.00 was spherical, 1.01-1.14 was prolate-spheroidal, 1.15-1.33 is subprolate, 1.34-2.00 was prolate and >2.00 was perprolate (Figure 1). While in the present finding, six taxa namely *C. intybus, C. scolymus, S. rebaudiana, C. officinalis*



Plate 6. SEM micrographs showing pollen grains of family Asteraceae and also showing detailed exine ornamentation. PV: Polar view; EV: equatorial view; ES: exine sculpture. Source: Authors

X. strumarium and C. grandiflora have echinate category and five taxa (A. millefolium, A. vulgaris, T. parthenium, T. vulgare and B. pilosa) in the Echinate-perforate category. Aerolate and echinate category were found in three taxa (A. houstonianum, L. vulgare, Τ. cinerariifolium) while in A. absinthium, A. scoparia, C. tinctorious, C. scolymus, H. tuberosus, E. prostrata, S. marianum, G. jamesonii, S. costus, S. kurramense, T. erecta, Z. elegans, P. hysterophorus, C. punctatum, Gazania rigens and L. sativa have reticulate- scabrate and echinate, reticulate-scabrate and echinate, verrucate and echinate, scabrate-echinate, echinate (macro-size thorn), echinate, spine broad, porous at the base with mucronate tip, spinate and elliptic to rarely circular and hexagonal view, conical pointed and reticulate and shortly spinulate are found. The exine thickness value was observed to be maximum in *C. punctatum*, that is, 1.7 μ m, while minimum in *S. kurramense* 0.5 μ m (0.15-0.90), respectively (Table 2 and 3 and Figure 4).

Aperture types

The size and number of pollen grain openings were observed; 17 species exhibited tricolporate: *Cichorium intybus, C. scolymus, S. rebaudiana, C. officinalis, H. annuus, S. marianum, T. parthenium, T. vulgare, Bidens pilosa, C. oxyacantha, E. echinatus, G. jamesonii, S. costus, T. erecta, T. cinerariifolium, C. punctatum and C.*

S/N	Taxa/family	Part used	Folk uses	Tib uses	Culinary uses	Qarshi uses
1	Achillea millefolium	Leaves, flowers, roots	A wound healing, regulates menstrual cycle, reduces heavy bleeding and eases menstrual pain.	Treat wounds, menstrual ailments, and bleeding Hemorrhoids, flue, fevers, Colds and relief of stomach and intestinal upset	Yarrow soup is prepared. Finely chopped leaves can also be added to salads and soft cheese dips	It is basic ingredient of herbal products Khatooni, Hepta care (Tab & Cap) and Madamol Syrup
2	Ageratum houstonianum	Whole plant	Used as a bacteriocide, antidysentery and antilithic	Treat cuts and wounds, cure fever, diarrhea, rheumatism, headache and colic	Culinary uses are not known	
3	Artemisia absinthium	Leaves and flowers	Help in gastrointestinal digestion, promote menstruation and heal bruising	For gastric insufficiency, intestinal disorder, gastritis, stomachache, liver disorders, bloating, anemia, irregular menstruation and intermittent	Extract of wormwood containing absinthe is found in a few European alcoholic beverages (notably Strega). Today, very small amounts may be found in vermouth, as a flavoring which goes a long way	It is used in Musafeen tablets and syrup
4	Artemisia scoparia	Leaves	Used for removing intestinal parasites	Used as purgative and cure for earache	Not edible	-
5	Artemisia vulgaris	Leaves and flowers	Used as insecticide, used for sore feet, moxibustion and help to manage the menstrual pain	Used for digestive system, menstrual complaints, treatment of worms, treatment of nervous and spasmodic affections, sterility, functional bleeding of uterus, dysmenorrheal and asthma	Fresh leaves make a horrible tasting beer. Leaves – raw or cooked, aromatic and somewhat bitter, their addition to the diet aids the digestion and so they are often used in small quantities as a flavoring, especially with fatty food. They are also used to give color and flavor to glutinous- rice dumpling. The young shoots are used in spring as food	It is basic ingredient of Musafeen (Syr & Tab)
6	Carthamus tinctorious	Seed and flowers	Flowering heads are used for fever, Cough, in domestic practice, externally, they are applied to bruising, sprains, skin inflammations and wounds	Seed and honey effective against respiratory catarrhal affections, keep the throat clear of phlegm and mucus, also helpful for relieving hoarseness of voice, Oil is useful remedy in application for itch, also applied on painful joints and to heal sore.	It is used mainly as cooking oil, in salad dressing, and for the production of margarine. Safflower flowers are occasionally used in cooking as a cheaper substitute for saffron. Tender shoots eaten as a salad and potherb. Seeds are both edible and nutritious, are eaten roasted or fried and used in chutney	-
7	Chicorium intybus	Roots and leaves	Used as a health tonic, anti-nauseating, anti-bilious, stomachic, jaundice, liver enlargement, gout and rheumatism	Great value for its tonic affect upon the liver and digestive tract and also applied externally in inflammatory infections due to its cooling properties	Leaves and flowers are eaten raw or cooked. The blanched leaves are often used in winter salads (they are known as chicons) and are also cooked. Roots are cooked like parsnip. The boiled young roots form a very palatable vegetable. Chicory- root is used in seasoning soups, sauces and gravies, and to impart a rich deep color. The roasted root is used as a caffeine-free coffee adulterant or substitute.	It is basic ingredient of herbal products Iksir-e-Jiger (Syr), Majoon Dabeed-ul-ward, Khatooni, Renex powder, Thandak, Sharbat bazoori and Heptacare (Tab & Cap)
8	Chrysanthemum Leucanthemum	Leaves, flowers and roots	It is used as fodder. The plant is also used as insecticide and pesticide	It is used medicinally as an antispasmodic, diuretic, and a treatment for coughs. A lotion made from the plant was applied to wounds, bruises, and ulcers	The Ox-eye Daisy is edible. Its young shoots are often enjoyed in salads, and its youthful leaves can be cooked as pot herbs	-

Table 2. The information collected from traditional health practioner and local informant as well as obtained from Qarshi Natural Medicine Department (NMD).

9	Cynara scolymus	Leaves, flowers	Uses for high cholesterol, digestive problems, gallstones, irritable bowel syndrome and support liver function	Assists in the treatment of jaundice, hepatitis, diabetes, helps lower blood sugar, increase the flow of bile from the gallbladder, and to increases the contractive power of the bile duct	Flower buds, stems and leaves are edible raw or cooked. The buds are usually boiled before being eaten. Lateral stems can be pickled or used in soups and stews. Young leaf stem is used as celery substitute	-
10	Helianthus tuberosus	Root, tuber, stem and seed	It is a folk remedy for diabetes, rheumatism, Diuretic and may be used in many febrile and inflammatory forms of disease	The oil obtained from the seeds by expression, has been employed with benefit in cough, in dysentery, in inflammation of the mucous coat of the bladder, and in disease of the kidney	The edible tubers produced by are delicious and nutritious. They are especially good grated into fresh salads, and are a perfect snack for dieters. Boiled and mashed they are rather similar to potatoes, and can be used like potatoes in most recipes. It makes delicious French fries. The British make a creamy soup from it. It is used as excellent pickled. The roasted tubers are coffee substitute	-
11	Stevia rebaudiana	Leaves	It is used as natural sweetener	Not available	Leaves are eaten raw or cooked. It has very sweet liquorices like flavor. The dried leaves can be ground and used as a sweetener or soaked in water and the liquid used in making preserves. The powdered leaves are also added to herb teas. The leaves are sometimes chewed by those wishing to reduce their sugar intake. The leaves can also be cooked and eaten as a vegetable	-
12	Calendula officinalis	Flowers and Leaves	Used to treat stomach upset and ulcers, used to relieve menstrual cramps, it shows to speed healing of wounds, and prevent the infection of severe wounds on the body, treatment of problems of the gallbladder and stimulate the flow of bile, it accelerates the healing of topical eye ailments, conjunctivitis and associated with acne without any scarring and help to prevent and treat dermatitis	Used in tinctures, ointments and washes and are commonly used to speed the healing of burns, bruises and cuts as well, treat hemorrhoids, it is also used as a constituent of mouthwashes, tooth pastes to alleviate the inflammation mouth ulcers	Leaves are eaten raw. Fresh petals are chopped and added to salads. The dried petals have a more concentrated flavor and are used as a seasoning in soups and cakes. An edible yellow dye is obtained from the petals. It is used as saffron substitute to color and flavor rice and soups etc	-
13	Eclipta prostata	Whole plant	The expressed leaf juice is Plant is rubbed on the gums in toothache and applied with a little oil for relieving headache. It also has traditional external uses like athlete foot, eczema and dermatitis, on the scalp to address hair loss	The leaf extract is considered a powerful liver tonic, rejuvenative and especially good for hair. It is useful in inflammations, hernia, eye diseases, bronchitis, asthma, leucoderma, heart and skin diseases, right blindness and syphilis	Tender leaves and young shoots are cooked and used as a vegetable	-
14	Helianthus annuus	Seeds, Flowers, roots and bark	Seeds are diuretic, expectorant, and used for colds, coughs, throat, and lung ailments. It is reported to be anodyne,	Used for pulmonary troubles, rattlesnake bites, lung ailments, malaria, high fevers. Seeds and leaves said to be diuretic and	Sunflower seeds are popular in breads, cereals, salads and many other dishes. The bright yellow florets make a colorful salad garnish. Roasted	-

			antiseptic, aphrodisiac, bactericidal,			
			diuretic, emollient, expectorant, insecticidal and malaria preventative. It is a folk remedy for blindness, bronchitis, carbuncles, catarrh, cold, colic, cough, diarrhea, dysentery, dysuria, fever, flu, fractures, inflammations, malaria, menorrhagia, pleuritis, rheumatism and urogenital ailments	expectorant	seeds are a substitute of coffee	
15	Silybum marianum	Whole plant	Milk thistle is reported to have protective effects on the liver and to improve its function. It is typically used to treat liver cirrhosis, chronic hepatitis and gallbladder disorders	It has been considered helpful in jaundice, colitis, pleurisy and diseases of the spleen, Fruit is used for the treatment of dyspeptic symptoms, loss of appetite, liver and gall bladder complaints including inflammation of the gall bladder duct, toxic liver disease and hepatic cirrhosis	The leaves and flower buds are edible when cooked; they make an acceptable spinach substitute. Roots and stems are edible either raw or cooked. They can be used like asparagus or rhubarb or added to salads. Good quality oil is obtained from the seeds. The roasted seed is a coffee substitute	It is basic ingredient of herbal product Heptacare Tablets
16	Tanacetum parthenium	Leaves, flowers and stem	Chewing 1 - 4 leaves per day has proven to be effective in the treatment of some migraine headaches. It is used for reducing fever, arthritis and digestive problems. It has also been used for the treatment of psoriasis, allergies, asthma, tinnitus, dizziness, nausea and vomiting	Feverfew has proved to be of special benefit in the treatment of certain types of migraine headaches and rheumatism. The leaves and flowering heads are anti- inflammatory, antispasmodic, aperient, carminative, emmenagogue, sedative, stimulant, stings, stomachic and vermifuge	The dried flowers are used as a flavoring in cooking certain pastries. The plant is used in cooking to impart a deliciously aromatic bitter taste to certain foods. A tea is made from the dried flowers	-
17	Tanacetum vulgare	Flowers and leaves	It is used externally as a poultice on swelling and eruptive skin diseases. It is also used externally to kill the lice fleas and scabies. Bath in a solution of tansy and salt is beneficial to cure joint pain	The leaves and flower tops work to expel intestinal worms from the body. It is used to treat menstrual irregularities and induce menstrual bleeding. An infusion of the leaves or whole plant is used to treat menstrual irregularities and as an anthelmintic, especially for children	Seeds were traditionally used in biscuits served at funerals. Young leaflets are edible either raw or cooked. Leaves can be added in small quantities to salads. The plant is also used as a flavoring as a substitute for cinnamon. A bitter, somewhat lemon-flavored tea is made from the leaves and flowering stems	-
18	Bidens pilosa	Leaf, flower and root	In the old medicine use of the Red Indians of America, it was used to treat eye and ear infections, applied to skin affections in general, as haemostatic on wounds and leaves wrapped around the umbilical cord of babies to protect against diseases	The juice of the leaves used against coughs, angina, headache, fever, diabetes, constipation, diarrhea, intestinal worms, stomachache, toothache, poisoning, muscular pains and as a bath to treat itching and rheumatic pains. Crushed leaves applied on the skin to treat inflammations, burns, on wounds to stop bleeding and on ulcers. Flowers paste used externally to extract pus from boils. Decoction of roots applied on eyelids to treat eye infections and roots are chewed for toothache	In old days, fresh leaves were used for eating during food scarcity	-

19	Carthamus oxyacantha	Flower and Seed (oil)	Folk medicines indicate its use as having wound healing properties. Its flowers decoction was traditionally used an anthelmintic for children. Oil extracted from seeds has been used as a dressing for bad ulcers, itch and skin allergic reactions and pain of the joints. Flowers have been used largely for yellow dye preparations	The seeds are used for treatment of diabetes in some parts of Pakistan. It is recommended for sores and pain related to rheumatism (seed oil); jaundice and related liver ailments (flower); laxative (seed); boils, candida infections, dysmenorrhea, rashes, fever, gout, gynecopathy, lack of apetite, measles andtumors. Powdered seed poultice used to aid inflammation of the womb after child birth	Not known	-
20	Echinops echinatus	Whole plant parts	Traditionally in the subcontinent, the whole plant has been used against skin itching i.e., the plant was left submerged in water and then bathed with that water. It was also used for wounds and scorpion stings. It has been considered magical and used for contact therapy i.e., root pieces tied round the neck of the children suffering from cough, fever and cold.	It is commonly used in Ayurveda, Unani and Sidha medication. Whole plant decoction used indyspepsia, malaria and hysteria. Cough suppressant, as a tonic for increasing nerve strength and in indigestion, premature ejaculation and in diabetes. Pounded roots given for diarrhea and dysentery and roots decoction given for easy childbirth. The roots are boiled with milk and taken for sexual vigor, impotency and hysteria. Root and leaves paste mixed in water and applied on scorpion stings and given as antidote to snakebite and for head lice. In veterinary medicine, powdered root applied to wounds in cattle to destroy maggots.	Not known	-
21	Gerbera jamesonii	Flowers	No report of folkloric medicinal use	It is used for curing colds and treating rheumatism	Not known	
22	Saussurea costus	Roots and flower	It is effective in general weakness after diarrhea and cholera. The plant is used for the treatment of asthma, bronchitis and nausea. Roots are useful in chronic and foul ulcers, flatulence, leprosy, leucoderma, jaundice, dysentery, hiccough, hysteria, gout and general debility. It is also beneficial for liver problems, skin diseases and cardiac disorders	It is a good remedy for freckles and chloasma if applied on face; with vinegar it is effective in ringworm. It is a blood purifier and a good insect repellent. The flowers are used in Chinese medicine as remedy for rheumatoid arthritis, cold, cough, feverand stomach ache. Internally, it is a good expectorant, antispasmodic and neurotoxin. Oil from the root is very beneficial in the treatment of rheumatism. It is used in the treatment of swelling and fullness of the stomach	It is used as a spice. In foods and beverages, oil is used as a flavoring component	-
23	Seriphidium kurramense	Leaves, flower and seed	The seeds are bitter, hot, with a sharp taste; stomachic, appetizer, and aphrodisiac, anthelmintic; cure indigestion, abdominal pain, and mucus	Powder made from flower-heads, stalks and leaves mixed with castor oil is used for the treatment of roundworms. The floral tops are vermifuge. Seeds are used for	Not known	-

			diarrhea. The plant is given to children for stomachache; it is a cure for jaundice. Powder consisting of the flower heads with an admixture of small stalks and leaves has been found to be efficacious in expelling round worms with the aid of castor oil. The juice of flower heads is applied to the part affected in snake-bite and scorpion sting. Wormseed is principally used as a vernifuge against ascarides (not tapeworm); whooping cough, anorexia and bed wetting	indigestion, stomach complaints, loss of appetite and abdominal pain. Herb is febrifuge and used for jaundice and asthma, a poultice to provide relief from pain when stung by scorpions		
24	Tagetes erecta	Whole plant	The flowers are used to cure colds, coughs and ailments. Decoction of flowers is used to relieve flatulence. It is used for treatment of indigestion, colic, severe constipation, coughs and dysentery. Externally, it is used for sore eyes and rheumatism. It repels insects	The roots and seeds are used as purgative. Flowers are considered carminative, aromatic, digestive, diuretic and sedative. The oil has antifungal property and is used to treat wounds, parasites and other ailments	The flowers are used in refreshing drinks. The leaves and essential oil is used as food flavoring	
25	Tanacetum cinerariifolium	Flower	It is effective for head lice and crab lice infestations	It has sometimes been used as a vermifuge in China	Not known	-
26	Xanthium strumarium	Root, fruit and seed	Cocklebur was once used for rabies, fevers, malaria, inusitis, and allergic rhinitis with headaches, chronic lumbago, leprosy, and pruritis (severe itching) of the skin. Native Americans used the leaf tea for kidney diseases, rheumatism, arthritis, tuberculosis (TB), colds, as a blood tonic, and diarrhea. The Chinese had similar uses. It is a very valuable therapeutic medicine used by the Chinese for rheumatic pains and aches as well as sinus blockage. Also used as a yellow dye	The plant is considered to be useful in treating long-standing cases of malaria and is used as an adulterant for Datura stramonium. An infusion of the plant has been used in the treatment of rheumatism, diseased kidneys and tuberculosis. It has also been used as a liniment on the armpits to reduce perspiration. A decoction of the seeds has been used in the treatment of bladder complaints. A poultice of the powdered seed has been applied as a salve on open sores	Leaves and young plants are cooked. They must be thoroughly boiled and then washed. Caution is advised, the plant is probably poisonous. Seed is used as raw or cooked. It can be used as a pinole. The seed can be ground into a powder and mixed with flour for making bread, cakes etc. The seed contains protein, fat and ash. It also contains a glycoside and is probably poisonous	-
27	Zinnia elegans	Flower and leaves	Flowers are used for constipation	Leaves extracts used as a wound healer	Used as salads and teas	
28	Parthenium hysterophorus	Root, stem, leaves	Root decoction is useful in amoebic dysentery. It is applied externally on skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments. In Jamaica the decoction is used as a flea-repellent both for dogs and other animals	The decoction of P. hysterophorus has been used to treat fever, diarrhea, neurologic disorders, urinary tract infections, dysentery, and malaria and also used as emmenagogue	Not known	-

29	Centratherum punctatum	Leaves, flower and oil	In Brazil, it is used as a relaxative and for the treatment of heart problems	It is used for wound healing and to increase libido, used as pain killer and as an antidote for snake bite and tiger bite.	Not known -
30	Coreopsis grandiflora	Root, leaves and floral tissues	A tea made from the roots is emetic and is also used in the treatment of diarrhea. An infusion of the whole plant without the root has been used by women desiring a female baby	The roots are used to cure dysentery and diarrhea	Not known -
31	Gazania rigens	Root, rhizome, leaves and flowers	In folk medicine used to prevent toothache and relieve earache	Not known	Not known -
32	Lactuca sativa	Whole plant	The seed is said to be used to make hair grow on scar tissue. Lettuce has acquired a folk reputation as an anaphrodisiac, anodyne, carminative and diuretic	It is taken internally in the treatment of insomnia, anxiety, neuroses, hyperactivity in children, dry coughs, whooping cough, and rheumatic pain	Leaves are used as raw or cooked. It has a mild slightly sweet flavor with a crisp texture; lettuce is a very commonly used salad leaf and can also be cooked as a pot herb or be added to soups etc. Edible oil is obtained from the seed

Source: Authors

grandiflora while seven taxa were tricolpate: *A. millefolium, A. houstonianum, A. absinthium, C. tinctorious, H. tuberosus, E. prostrata* and *S. kurramense.*

Four taxa were trizonocolporate (*L. vulgare, Z. elegans, P. hysterophorus* and *L. sativa*); tetracolporate and pentaporate were found in *X. strumarium* and *L. sativa*, respectively (Table 3).

Colpi size

The light and SEM studies of pollen showed a rich diversity in the ornamentation of the colpus surface, as well as in size and width. The largest colpus length and width were found in *L. vulgare* (6.15 μ m) and the maximum colpus length was observed in *L.*

vulgare (6.15 μ m) and the minimum value was observed in *C. officinalis* (1.28 μ m). The largest

width of colpi was examined in *A. absinthium* (9.85 μ m) and the smallest in *L. sativa,* respectively (Table 4 and Figure 3).

Mesocolpium

Mesocolpium ornamentation is significant taxonomically as it can aid in plant identification. Most of pollen has same mesocolpium ornamentation as exine but some pollen has shown variations as they exhibit differences between the sculpturing of exine and mesocolpium. Thirteen species have echinate mesocolpium; but the colpi orientation of the 13 species is sunken, slit like split or margins, tapering at the tip, angled, sharp-edged, protruding and rounded at the end, tinged, elongated, ellipsoidal ovate to globose, concave, and their base broad, elongated-spherical and

circular in polar view, semi-oblong and flattened in orientation. Four species show an echino-porous mesocolpium pattern while the rest of the species in Table 3 show reticulate, scabrous and echinate, oblate-spheroidal, spheroidal, echino- lophate, circular-semi-circular, perforated and echinate, densely perforated, slightly elongated and perforated pattern of the mesocolpium while the orientation of the colpi is prominent and rounded at the ends, rounded, sunken, oblate-spheroidal and medium, radially symmetrical, oblate spheroidal and suboblate orientation of the colpus (Table 3).

Pollen fertility and sterility

The fertility and sterility percentages have been determined for each species (Table 2). Maximum

Table 3. Qualitative palyno-morphological characters of Asteraceae taxa observed under light microcope.

S/N	Taxon	Exine sculpturing	Aperture condition	Colpi orientation	Mesocolpium
1	Achillea millefolium L.	Echinate-perforate	Tricolpate	Prominent and rounded at ends	Reticulate
2	Ageratum houstonianum Mill.	Aerolate and echinate	Tricolpate	Sunken, slit like margins and end tapering	Echinate
3	Artemisia absinthium L.	Reticulate – scabrate and echinate	Tricolpate	Prominent and round at end	Scabrate and echinate
4	Artemisia scoparia Waldst. & Kitam.	Echinate-microreticulate	Tricolpate	Sunken	Oblate-spheroidal
5	Artemisia vulgaris L.	Echinate-perforate	Tricolpate	Sunken	Spheroidal
6	Carthamus tinctorious L.	Verrucate and echinate	Tricolpate	Sunken, angular, margins distinct	Echinate
7	Cichorium intybus L.	Echinate	Tricolporate	Rounded, sunken	Echino-lophate
8	Leucanthemum vulgare (Vaill.) Lam.	Aerolate and echinate	Trizonocolporate	Sunken, slit like margins	Echinate
9	Cynara scolymus L.	Scabrate - Echinate	Tricolporate	Sunken, angular	Echinate
10	Helianthus tuberosus L.	Verrucate and echinate	Tricolpate	Prominent and rounded at ends	Echinate
11	Stevia rebaudiana (Bertoni) Bertoni	Echinate	Tricolporate	Sunken	Echinate
12	Calendula officinalis L.	Echinate	Tricolporate	Sunken	circular-semicircular
13	Eclipta prostrata (L.) L.	Echinate (macro-size thorn)	Tricolpate	Sunken, Prolate spheroidal	Echinate
14	Helianthus annuus L.	Echinate and Verrucate	Tricolporate	Prominent and rounded ends	Echinate
15	Silybum marianum (L.) Gaertn.	Scabrate - Echinate	Tricolporate	Prolate spheroidal	Perforate and echinate
16	Tanacetum parthenium (L.) Sch.Bip.	Circular perforated	Tricolporate	Oval to spherical	Echinate
17	Tanacetum vulgare L.	Echinate-perforate	Tricolporate	Concave and their bases are broad	Echinate
18	Bidens pilosa L.	Echinate-perforate	Tricolporate	Oblate-spheroidal and medium	Densely perforated
19	Carthamus oxyacantha M. Bieb.	Echinate	Tricolporate	Prolate-spheroidal and circular in polar view	Echinate
20	Echinops echinatus Roxb.	Echinate	Tricolporate	Radially symmetrical, Oblate Spheroidal	Slightly Elongate
21	Gerbera jamesonii Bolus ex Hook.f.	Verrucate and echinate	Tricolporate	Sunken, angular, margins distinct	Echinate
22	Saussurea costus (Falc.) Lipsch.	Reticulate and shortly spinulate	Tricolporate	Spheroidal	Echinate
23	Seriphidium kurramense (Qazilb.) Y.R. Ling	Reticulate and echinate	Tricolpate	Prominent and round at end	Echinate
24	Tagetes erecta L.	Spinate	Tricolporate	Suboblate	Echinate perforate
25	Tanacetum cinerariifolium (Trevir.) Sch.Bip.	Aerolate and echinate	Tricolporate	Spheroidal	Perforate
26	Xanthium strumarium L.	Echinate	Tetracolporate	Prolate and spheroidal	Echinate perforate
27	Zinnia elegans L.	Echinate, spine broad, porous at the base with mucronate tip	Trizonocolporate	Spheroidal	Echinate perforate
28	Parthenium hysterophorus L.	Spinate with small rudimentary base, mucronata tip	Trizonocolporate	Subprolate and Suboblate	Echinate perforate
29	Centratherum punctatum Cass.	Conical pointed	Tricolporate	Prolate spheroidal	Reticulate
30	Coreopsis grandiflora Hogg ex Sweet	Echinate	Tricolporate	Suboblate and Subprolate	Echinate perforate
31	Gazania rigens (L.) Gaertn.	Spinate	Pentaporate	Subprolate and oblate	Echinate
32	Lactuca sativa L.	Elliptic to rarely circular and hexagonal view	Trizonocolporate	Spheroidal to oblate-spheroidal	Echinate perforate

Source: Authors.



Plate 7. The family Asteraceae plants used in the following Product of Qarshi Industries (Pvt.) Ltd., Hattar, Harpur, Pakistan. Source:Authors



Plate 8. The family Asteraceae plants used in the following Product of Qarshi Industries (Pvt.) Ltd Hattar, Harpur, Pakistan. Source: Authors.



Plate 9. The family Asteraceae plants used in the following Product of Qarshi Industries (Pvt.) Ltd Hattar, Harpur, Pakistan. Source: Authors.



Plate 10. The family Asteraceae plants used in the following Product of Qarshi Industries (Pvt.) Ltd Hattar, Harpur, Pakistan. Source: Authors.

number of fertile pollen percentage was counted in *C. leucanthemum* (89.55%); while the highest sterility percentages were observed in *L. sativa* (63%). Lowest fertility and sterility percentage are observed in *L. sativa* and *S. kurramense* (40 and 36.80%), respectively.

DISCUSSION

The Asteraceae pollen described in this article showed differences in size, shape, number of colpi, number of spines, exine decoration, and pollen shape in polar view. A similar difference has been observed in other members of the <u>A</u>steraceae family by several authors worldwide

(Sivaguru et al., 2018; Shaheen et al., 2009; Makra et al., 2005; Coutinho et al., 2021; Almeida et al., 2019: Da Silva et al., 2017). Pollen morphological parameters play a very important role in the field of taxonomy, and most botanists, especially taxonomists, rely heavily on these traits for accurate identification at various taxonomic ranks (Irfan et al., 2019; Birjees et al., 2021). SEM has been shown to be essential for surface decoration and species identification (Almeida et al., 2019 Anwer et al., 2020).

The taxonomic relevance of palynology has value in other interdisciplinary sciences, such as melisparinology, aeropollinology and pharmacology. According to Ullah et al. (2018), pollen micromorphological features are of particular importance in the study of plant species evolution and phylogeny. Pollen morphological features

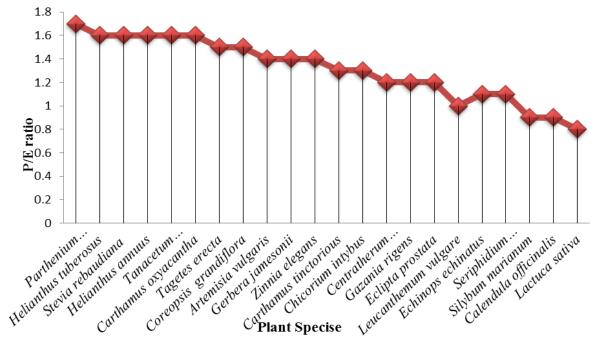


Figure 1. P/E ratio. Source: Authors.

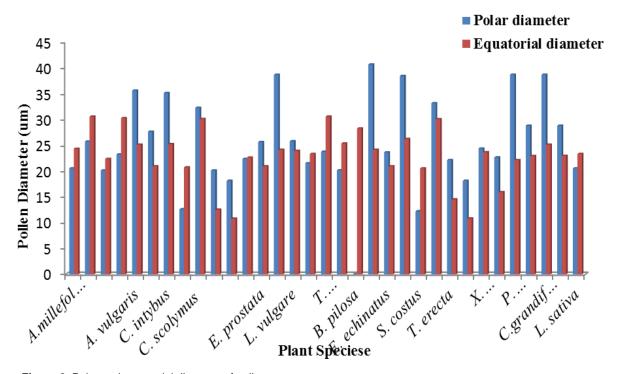


Figure 2. Polar and equatorial diameter of pollen. Source: Authors

of selected plant species in the Asteraceae family, which play important roles in several drugs, are studied in this work (Almeida et al., 2019). Pollen characters are useful in solving complicated problems of interrelationships between various taxa and assessment of their status in the classification, Table 4. For statistical analysis used SPSS version 16.0 for quantitative palyno-morphological characters of Asteraceae taxa collected from Qarshi herb garden.

S/N	Taxon	P/E ratio	Polar diameter Mean (Min-Max) SE (μm)	Equatorial diameter Mean (Min-Max) SE (μm)	Length of Colpi Mean (Min-Max) SE (µm)	Width of Colpi Mean (Min-Max) SE (µm)	Size in μm) Exine Mean (Min-Max) SE (μm)	Length of spine Mean (Min-Max) SE (µm)	Width of spine Mean (Min-Max) SE (μm)	Viability %age
1	Achillea millefolium L.	0.84	20.52(20.71-21.48) ±0.23	24.30(22.71-30.72) ±0.52	2.1(1.3-0) ±0.21	2.3(1.75-5.15) ±0.23	1.4(1.5-2.5) ±2.3	2.6(3-4.1) ±0.22	2.25(1.65-3.20) ±0.20	75
2	Ageratum houstonianum Mill.	0.84	25.71(27.72-28.746±0.32	30.52(30.20-32.20) ±0.45	2.14(0.68-28) ±0.34	3.25(6.75-9.55) ±0.12	2.25(1.40-3.15) ±0.20	3.45(3-2.20) ±0.15	1.10(0.55-1,65) ±0.14	89
3	Artemisia absinthium L.	0.89	20.11(14.73-20.71)0.50	22.35(21.72-30.20) ±0.90	3.45(3.40-4.20)0.23	4.85(7.25-10.40)0.20	1.25(1.20-3)0.25808	5.45(6.30-8.20) ±0.28	1.65(2-3.20)0.230	63
4	Artemisia scoparia Waldst. & Kitam.	0.76	23.20(23.21-23.42) ±0.34	30.22(25.70-30.745) ±0.50	2.20(1.45-2.45) ±0.12	2.94(3.25-2) ±0.28	1.7(1.20-2.55) ±0.15	2.81(3-4.2) ±.20	2.10(0.40-11.40) ±2.30	78
5	Artemisia vulgaris L.	1.4	35.55(38-38.21) ± 0.20	25.10(22.23-24.22) ± 0.52	4.35(1.20-3.45) ±0.20	4.15(1.70-9) ± 1.30	3.45(1.40-10.00)± 3.50	2.75(2.20-3.20) ± 0.12	1.25(1.00-1.35) ± 0.11	81
6	Carthamus tinctorious L.	1.3	27.61(22.71-28.71) ±0.32	20.91(20.22-21.46) ±0.20	4.35(2.65-3.20) ±0.20	4.80(6-7.55) ±0.28	1.4(1-1.55) ±0.25	2.20(3.40-5.20) ±0.25	0.44(.20-1) ±0.12	88
7	Cichorium intybus L.	1.3	35.10(36.46-40.46) ±0.31	25.20(25.4-25.6) ±0.20	2.10(1.65-3.4) ±0.11	4.45(2.55-4) ±0.20	2.3(2.55-4) ±0.20	2.1(10.01-12.10) ±0.52	6.1(4.52-8.20) ±0.71	68
8	Leucanthemum vulgare (Vaill.) Lam.	0.6	12.60(11.70-15.00) ±0.69	20.70(15.70-22.72) ±1.14	2.40(2-4.3) ±0.51	2.05(2.35-6.55) ±0.93	1.0(1.15-3.00) ±0.30	2.1(2.55-5.65) ±0.52	2.10(1.5-5.60) ±0.50	93
9	Cynara scolymus L.	1.0	32.23(31.22-34.22) ±0.32	30.05(22-30.70) ±1.01	2.3(3.55-4.65) ±0.13	4.8(5.55-8.15) ±0.40	1.55(2-3.20) ±.21	2.36(10.5-15.21) ±0.85	2.5(5.20-8.35) ±0.50	70
10	Helianthus tuberosus L.	1.6	20.10(21.73-22.21) ±0.41	12.50(12.20-18.45) ±0.45	1.2(2.15-4.20) ±0.30	5.4(3.55-14.40) ±2.2	1.55(1.20-2.15) ±0.12	2.25(1.55-2.40) ±0.28	1.65(1-2.55) ±0.25	88
11	Stevia rebaudiana (Bertoni) Bertoni	1.6	18.10(15.71-18.00) ±0.36	10.80(12.70-14.43) ±0.33	3.10(3.20-5.24) ±0.25	2.35(3.55-5.20) ±0.20	1.35(0.20-2.15) ±0.25	1.5(1.65-3.15) ±0.22	0.3(0.20-1) ± 0.11	79
12	Calendula officinalis L.	0.9	22.35(21.24-31.71) ±1.20	22.60(22.20-28.46) ±0.92	1.18(0.49-1.92) ±0.21	2.04(3.45-6.35) ±0.45	2.64(2.00-2.20) ±0.13	2.25(2.25-3.50) ±0.20	0.56(0.2-1.20) ±0.20	69
13	Eclipta prostrata (L.) L.	1.2	25.61(24.72-27.70) ±0.32	20.92(20.22-21.45) ±0.20	2.50(3.55-4.20) ±0.20	4.20(6.0-7.40) ±0.25	1.2(1-2.45) ±0.25	2.30(3.40-5.20) ±0.25	0.35(0.20-1) ±0.10	88
14	Helianthus annuus L.	1.6	38.62(38-40.20) ±0.20	24.10(23.21-25.20) ±0.54	2.90(2.10-3.40) ±0.20	5.10(1.55-9) ±1.30	3.5(1.5-10) ±3.2	1.7(2.3-3.1) ±0.13	1.2(1-1.55) ±0.12	54
15	Silybum marianum (L.) Gaertn.	1.0	25.75(24.43-31)0.91	23.90(3.23-5.01) ±0.30	4.15(2.25-5.01) ±0.28	2.10(1.20-2.55) ±0.20	1.20(20.3-20.40) ± 0.82	2.2(1.4-3.00) ±1.20	1.5(0.00-10) ±1.91	66
16	Tanacetum parthenium (L.) Sch.Bip.	0.9	21.52(20.70-21.50) ±0.24	23.30(22.70-25.70) ±0.52	1.30(1.5-1) ±0.24	3.40(3.55-4.20) ±0.20	2.5(1.6-3.2) ±2.4	2.5(3-4.3) ±0.25	1.25(1.75-3.25) ±0.20	45
17	Tanacetum vulgare L.	0.7	23.72(22.71-24.743±0.30	30.52(30.22-33.24) ±0.45	2.15(0.35-30) ±0.35	6.20(3.55-9.70) ±0.12	2.25(1.30-3.15) ±0.20	2.45(3-4.20) ±0.20	1.20(0.65-1,65) ±0.14	49
18	Bidens pilosa L.	0.7	20.13(17.71-20.72)0.52	25.35(20.72-30.22)0.92720	2.45(1.50-5.20)0.20	5.55(9.15-10.40)0.20	2.15(1.20-1)0.20810	5.45(6.45-8.20) ±0.28	1.55(2-3.15)0.223	73
19	Carthamus oxyacantha M.Bieb.	0.7	2120(23.21-22.46) ±0.35	28.22(25.70-30.745) ±0.50	1.25(2.65-3.55) ±0.14	2.39(2.25-6) ±0.30	1.20(1.20-2.55) ±0.20	2.81(3-4.5) ±.20	2.1(0.40-12.30) ±2.30	53
20	Echinops echinatus Roxb.	1.6	40.61(38-39.21) ± 0.20	24.1(22.21-23.20) ± 0.52	1.35(2.15-3.40) ±0.12	5.10(1.55-9) ± 1.35	1.60(1.30-10.00) ± 3.29	1.50(2.20-2.15) ± 0.15	1.20(1.00-1.65) ± 0.11	64
21	Gerbera jamesonii Bolus ex Hook.f.	1.1	23.61(21.72-27.72) ±0.34	20.91(20.21-20.45) ±0.20	2.45(3.70-5.22) ±0.20	3.80(6-7.55) ±0.22	1.4(1-2.70) ±0.31	2.30(3.40-5.20) ±0.20	0.45(.20-1) ±0.10	60
22	Saussurea costus (Falc.) Lipsch.	1.4	38.40(38.46-40.46) ±0.33	26.20(25.5-25.4) ±0.21	2.10(2.65-2.5) ±0.12	4.25(4.55-6) ±0.18	2.3(2.55-2) ±0.20	2.2(10.01-12.11) ±0.52	5.1(4.70-8.20) ±0.71	88
23	Seriphidium kurramense (Qazilb.) Y.R.Ling	0.5	12.20(11.72-14.00) ±0.72	20.50(13.70-20.71) ±1.12	1.60(2-5.2) ±0.55	2.05(2.15-6.15) ±0.53	1.1(1.25-3.00) ±0.30	2.1(2.70-5.70) ±0.52	3.15(1.5-5.50) ±0.70	91
24	Tagetes erecta L.	1.1	33.12 (31.23-32.20) ±0.30	30.02(23-33.70) ±1.01	2.30(3.65-4.70) ±0.14	5.80(5.65-8.20) ±0.40	1.35(2-3.20) ±.21	5.36(10.5-17.20) ±0.87	4.7(6.20-8.42) ±0.51	89
25	<i>Tanacetum cinerariifolium</i> (Trevir.) Sch.Bip.	1.5	22.10(21.72-24.21) ±0.41	14.50 (12.20-20.45) ±0.50	2.10(2.21-4.20) ±0.31	3.40(3.70-17.40) ±2.1	1.55(1.20-1.25) ±0.16	1.25(1.65-3.40) ±0.25	1.70(1-2.75) ±0.30	86
26	Xanthium strumarium L.	1.6	18.10(15.72-20.00) ±0.35	10.82(10.7112.64) ±0.32	4.10(3.20-5.30) ±0.35	4.25(3.70-5.20) ±0.22	1.30(0.21-2.20) ±0.30	2.2(1.65-3.15) ±0.25	0.3(0.20-1) ± 0.11	95
27	Zinnia elegans L.	1.0	24.35(24.22-31.70) ±1.20	23.64(21.20-31.45) ±0.93	1.25(0.40-1.92) ±0.23	3.40(5.40-8.20) ±0.45	2.50(2.00-3.20) ±0.20	2.50(2.25-3.50) ±0.22	0.57(0.2-1.25) ±0.25	87
28	Parthenium hysterophorus L.	1.4	22.63(25.71-28.72) ±0.35	15.90(15.20-20.45) ±0.20	2.50(2.65-4.15) ±0.20	3.67(6.0-7.25) ±0.25	1.4(1-2.55) ±0.25	4.40(3.50-5.15) ±0.30	0.45(0.20-1) ±0.12	76
29	Centratherum punctatum Cass.	1.7	38.62(40-41.22) ±0.20	22.10(20.21-21.20) ±0.55	1.95(2.15-3.40) ±0.20	2.45(1.55-9) ±1.30	2.7(1.5-20) ±3.3	2.4(2.1-3.1) ±0.15	1.2(1-1.73) ±0.10	77
30	Coreopsis grandiflora Hogg ex Sweet	1.2	28.76(25.47-31)0.91	22.90(3.21-5.00) ±0.30	2.15(2.25-4.02) ±0.25	3.30(1.20-2.65) ±0.20	1.20(10.4-22.40) ± 0.62	2.1(1.2-3.00) ±3.20	2.9(0.00-14) ±1.99	83
31	Gazania rigens (L.) Gaertn.	1.5	38.62(38-40.21) ±0.20	25.10(20.21-25.20) ±0.50	2.90(2.21-3.45) ±0.20	4.10(1.70-9) ±1.30	3.2(1.5-10) ±3.4	2.5(2.3-2.3) ±0.15	1.2(1-1.70) ±0.12	90
32	Lactuca sativa L.	1.2	28.75(22.46-31)0.92	22.94(3.23-5.00) ±0.30	4.12(3.23-5.01) ±0.25	2.30(1.20-2.55) ±0.20	2.22(20.3-23.40) ± 0.72	2.1(1.2-3.00) ±3.20	2.9(0.00-14) ±1.99	93

Source: Authors.

particularly with reference to the families, subfamilies, tribes, genera, species, and subspecies. Pollen morphology is conducted as an aid to the morphological study and a significant tool for modern taxonomist for the delimitation of species. Mature pollen grain size, exine sculpturing, and number of pores are the most distinctive features. Palynological data has been useful at generic and specific level (Figueredo-Urbina et al., 2017; Joujeh et al., 2019: Amjad, 2015). The morphological characteristics of pollen grains have been found to be useful in Asteraceae taxonomy (Joujeh et al., 2019).

The largest value of pollen diameter via polarity was observed in *E. echinatus* (40.61 (38-39.21) \pm 0.20), that is, dissimilar to the finding of Qureshi et al. (2002); while the smallest polar diameter was observed in *S. kurramense* (12.20 (11.72-14.00)

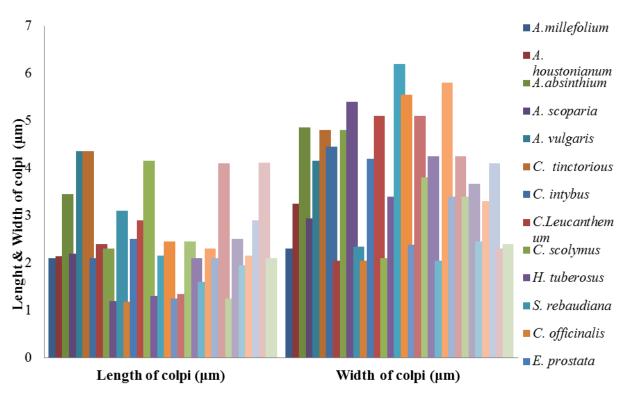


Figure 3. Length and width of colpi. Source: Authors.

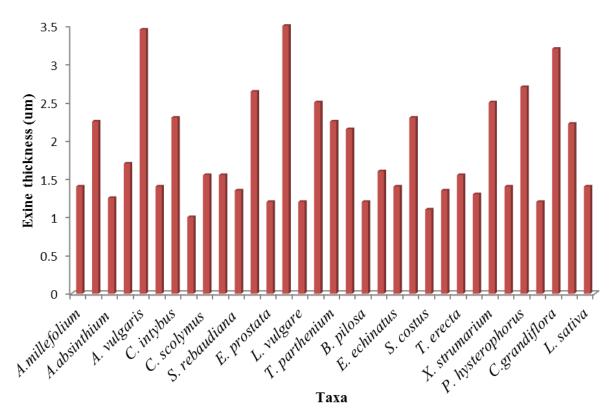


Figure 4. Exine thickness. Source: Authors.

±0.72). In the present studies, maximum colpus length was observed in L. vulgare (6.15 µm) and the minimum value is observed in C. officinalis (1.28 µm). Similarly, the largest width of colpus examined in A. absinthium (9.85 µm) and the smallest in L. sativa, results are similar to the results of Siniscalchi et al. (2017). Mesocolpium of most of the pollen of the collected Asteraceae species were echinate, echinate perforate, reticulate and perforate. Ratio of equatorial diameter to polar diameter is also very important. In our results variations were observed in P/E ratio. P/E value is also important taxonomic character. The P/E value can be calculated from the ratio of the average of pollen polar diameter and the equatorial diameter. The P/E value was observed highest in C. punctatum (1.7 μ m), and the lowest value was found in T. vulgare (0.7 µm). In our present research studies, most of the pollen are echinate that is why our result links similarities with the work of Zafar et al. (2007).

Exine sculpturing is one of the most significant features in pollen identification. In our results *S. costus*, having reticulate and shortly spinulate exine ornamentation is similar to the finding of Salamah et al. (2019) and Syamsuardi and Nurainas (2018). Similarly, reticulate, scabrate and echinate sculpturing were noticed in *A. absinthium* and *S. kurramense* which is dissimilar to Umber et al. (2022) findings, who reported echinate reticulate ornamentation in *P. hysterophorus* and Scabrate echinate exine sculpturing in *X. strumarium*. According to Mazari et al. (2017), pollen grain reported is circular to sub angular and echinate in *X. strumarium*. Attique, et al. (2022): recently, described that *A. vulgaris* have echinate perforate exine ornamentation, which is dissimilar to the finding of the current study.

The findings of this study were mostly similar with the previous studies on palynological studies of Asteraceae flora with little differences. These differences might be due to the climatic changes (Khan et al., 2021: Ashfaq et al., (2020). Documented quantitative and qualitative palynological characters are useful for taxonomic studies within the species of family Asteraceae. This research work will help to shown the authentication and correct identifications of herbs used in Qarshi products using in various palynological features.

Conclusion

The goal of this study was to use light and SEM to look at pollen characters in order to discriminate between the family of Asteraceae species. All 32 spineless species of family showed little variations in both qualitative and quantitative pollen attributes.

Both SEM and LM studies of pollen grains have played vital roles in identifying the plants up to genus and species level. Based on the present findings, it is concluded that LM and SEM can be used as tools to identify the species at micro level. This present study along with previous study comparison reveals palynological characters under light and SEM is of immense value to correctly identify the species. The results of the study show clear difference in pollen diameter, exine thickness, sculpturing and the shape of pollens and pollen fertility. It is therefore recommended to study pollen micro morphological features to identify taxonomically problematic taxa. This research is to expand the morphological knowledge of the species, thus contributing to taxonomic knowledge and subsequently conservation of species. However, it is also suggested that researchers should investigate the remaining species of family Asteraceae utilizing palynological qualities for identification, which could enable the identification of unique species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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