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Palyno-morphological diversity of Asteraceous and Poaceous allergenic plant using microscopic techniques in lesser Himalaya-Pakistan

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Abstract

In classification and identification of plant species, palyno morphological character has remain helpful for plant taxonomist. The aim to investigation this study was to identify the characteristic features of Asteraceae and Poaceae family member collected from lower Himalayas of Pakistan. Light microscope and Scanning electron microscope were used for feature study of allergenic pollen. Our study includes 10 species of Asteraceae and Poaceae family. The most dominant shape of pollen was Oblate-spheroidal, Prolate-Spheroidal, Prolate and Suboblate shape of pollen. Quantitative character of pollen includes maximum pollen diameter found in *Dichanthium annulatum* (46.0 µm) and minimum polar axis pollen diameter found in *Parthenium hysterophorous* (16.0 µm). Similarly highest value of P/E ratio was noted in *Cenchrus echinatus* (1.05) and smallest value of P/E ratio was observed in *Erigeron bonariensis* (0.88). Poaceae family member were monoporate. Character feature of exine sculpturing include Microechinate, granulate, echinate, conical base, lacunae Nanogammate, Aerolate-Scabratre, Verrucate, like variation found in exine of both family member. Highest value of pollen fertility was observed in *P. hysterophorous* 93%, Similarly maximum value of pollen sterility was noted in *E. bonariensis* and *Brachiaria reptans* 24%. This investigation provides the characteristic feature of allergenic pollen species and help to arrange them in define class.

KEYWORDS

Asteraceae, LM, palyno-morph, Poaceae, SEM

Research Highlights

- Pollen has affected many people around the world.
- Using microscopic techniques, morpho-palynological analysis of Asteraceae and Poaceae families were recognized.
- LM and SEM study show both qualitative and quantitative character of pollen.
- Identification of allergenic pollen and microscopic identified character play important role for proper guideline of identification of pollen having allergenic potential.

1 | INTRODUCTION

Clemens von Pirquet in 1906 introduced the “allergy” term (Greek-*allos* *ergon*) an Austrian pediatrician, Wrocław, and Vienna university professor. Initially, this term was referred to altered organism which response to antigen and it has been repeatedly used. Its synonyms were used for allergic reaction with time (Żukiewicz-Sobczak et al., 2012). Naturally developed and man-made products can be source of allergy. Any constituent which can permit through the respiratory tract moving through air are minor enough so they cannot be seen with eye familiarize to immunological system can elicit allergy (e.g., pollen, spores, dust particulates etc.) (Diette et al., 2008). Allergies are not only public health problem but also effecting socioeconomic impact recognized by WHO World Allergy Organization (Bastl et al., 2018). Aeroallergens are major contributor of biological pollution which include both airborne pollen and fungal spores. These allergens are present in the environment and are common source of inhalant allergies. About 20–30% of population in Ukraine various regions has affected by pollinosis (Melnychenko et al., 2020). In Australia almost 1 million inhabitants have affected by pollen allergy out of 8 million population (Bastl et al., 2018).

From last few decades respiratory allergy reaction increasing and prevalence in Europe showing increase of pollen allergy almost 40% and it also has clinical impact all over Europe (D'amato et al., 2007). Information about quantitative dynamics of allergy causing pollen from any area for predicting an also for assessing situation of aero allergenic pollen (Melnychenko et al., 2020). From industrialized countries almost 20% inhabitant are suffering from pollen allergy of flowering plants and in many areas of world it is affecting human health. Researchers are working to identify the allergy causing pollen and the component of pollen which cause allergy (De Weerd et al., 2002).

Pollen is chief source of allergy, and they are in broader of respiratory health issue and work in amalgamation with air pollution, chronic obstructive disease, asthma, and cause complex breathing related health issue (Desjardins et al., 2017). Pollen a male gametophyte fragment of flowering plant and is most leading cause of allergy in exterior atmosphere. During dispersion time of pollination, the pollen grains release by anther in dehydrate form and when they contact with wet surface, water absorbed by pollen grains and pollen undergo metabolic changes rapidly (Beggs, 2004).

The outer wall of pollen grains is made up of complex pigments, lipids and aromatic compounds which fill the pollen wall cavities of exine, in higher plants production proteins and proteolytic enzymes is vital. Pollen grains produce cytoplasmic content of pollen and also intine of pollen which is rich in cellulose. Tapetum is the outer floral layer of cell help in synthesis of pollen coat and outer layer of pollen called exine. This outer layer of pollen grain surround pollen during process of development (Hameed et al., 2020). Subsequently, this is not surprising that identified allergens through cDNA library of pollen are present inside the cytoplasm of pollen. Allergens from the outer wall of pollen coat are not recognized

because it is possible during pollen extraction the allergens can be extracted away, for example, during washing with organic solvent, for testing of skin prickle test and for immunotherapy (Bashir et al., 2013).

Study of pollen grains and spores called “Palynology” (palynos and dust). Ultrastructural and morphological features of pollen grains provide a wealth characteristic to identify a specific plant taxon (Ahmad et al., 2018; Ahmed et al., 2019; Bano et al., 2012, 2020; Simpson, 2019). To place taxa in correct taxonomic rank pollen morphological features are used by taxonomists for identification of flora (Khan et al., 2021). Mostly airborne pollen size ranges from 12 to 70 μm . Certain characteristics have made detection easier like air bladders present on cone-bearing pollens. Smooth exine of a few grains (psilate) while others have features like projections called spines (echinate). Pores most display apertures of pollen rounded and furrows like when elongated. If pollen grains have only pores called porate, and if only furrows present called colpate, or with both pores and furrows called colpiate. Pores capped by an operculum mostly in grass pollens or surrounded by a refractile shield “aspis,” for example, birch (Bush, 1989). Morphology of pollen is of massive importance for the interpretation and documentation of relationship among different species at several different taxonomic levels (Khan et al., 2018). Palynologist investigated the significance of palynological features in different group of plants from time to time and inaugurate it highly effective in the process of identification of plant species (Ashfaq et al., 2020; Jamil Noor et al., 2016; Khan et al., 2018; Mir et al., 2019; Noor et al., 2017). Palynology of medicinally important plants has been carried out in Pakistan which inspected pollen morphology and medicinal properties of these plants (Qureshi et al., 2019).

Taxonomists employ a variety of tools linked to plant taxonomy to aid in the identification systematically, classification, and positioning of plants (Bano et al., 2012, 2020). Morphology of pollen have paid crucial role in classification and identification of plants. Pollen morphological features have been used by taxonomist, recognized in flora in order to arrange taxa in proper taxonomic rank (Ahmad et al., 2020; Kayani et al., 2019; Khan et al., 2021). Morpho palynological research is one of the significant tools used by modern taxonomists to distinguish closely related species (Ahmad et al., 2018). Recently, the most requisite approach for micromorphological studies of pollen is scanning electron microscopy. The study of pollens through SEM is consequential due to its high resolving power (Khan et al., 2018; Ullah, Ahmad, et al., 2021; Ullah, Zafar, et al., 2021; ur Rahman et al., 2019). The pollen characteristics were employed by many researchers for systematic objectives (Ashfaq et al., 2018).

Investigator are working from all over globe on the palynomorphological structures of the several different plants species using light microscopy and scanning electron microscopy. While from study of Palazzesi et al. (2007), Anacardiaceae, Asteraceae, Celtidaceae, and Phytolaccaceae pollen grains were examined and these four species are unrelated and have different characters. Sixty-one allergenic plants pollens were investigated, with transmission and scanning

TABLE 1 List of allergenic plant species of Astraceae and Poaceae family of Islamabad-Pakistan

S.no	Botanical name	Synonyms	Family	English name	Habit	Habitat	Flower color	Flowering period	Distribution in Pakistan	Global distribution	Voucher number	Accession number	Coordinates	Collector	Collection date
1.	<i>A. scoparia</i> waldst. & Kitam.	<i>Artemisia capillaris</i> Miq.	Astraceae	Virgate wormwood, redstem wormwood	Herb	Sandy soil, low hills, wasteland, roadside,	Greenish white	Jul-Nov	Baluchistan, Punjab, KPK, Islamabad	Europe, Turkey, Iran, Iraq, Afghanistan, Pakistan, India, China, Russia, Mongolia,	MA-3	131,316	33°44'38.00"N 73°01'22.00"E	Maryam Anar	12-Sep-2020
2.	<i>E. bonariensis</i> L.	<i>Conyza bonariensis</i> (L.) Cronquist	Asteraceae	Flax-leaf fleabane, hairy horseweed, asthma weed	Herb	Warm-temperate, tropical	Gray, white	May-Oct	Islamabad, Rawalpindi,	Afghanistan, Egypt, Malaya, Iran, Iraq, India, Palestine, New Zealand, Sir Lanka, Tonga, Turkey	MA-13	131,317	33°45'40.28"N 73°08'03.95"E	Maryam Anar	12-sep-2020
3.	<i>P. hysterocephorus</i> L.	<i>Parthenium glomeratum</i> Rollins.	Asteraceae	Weed grass, congress weed,	herb	Subtropical, tropical, warmer area	white	Mar-oct	Islamabad, Rawalpindi, Talagang, Mandi Baha Uddin, Gujrat, Lahore	Uganda, Argentina, Brazil, Zimbabwe	MA-20	131,318	33°45'51.55"N 73°08'14.95"E	Maryam Anar	March 13, 2021
4.	<i>S. asper</i> (L.) Hill	<i>S. asper</i> All.	Asteraceae	Spiny Sow thistle	Herb	Terrestrial, Tropical, Wet, Dry, Saline soil, field margins, Desert, upland, Mountains.	Yellow - orange flower	March- Nov	Swat, Islamabad, Rawalpindi,	Europe, North Africa, Asia, Australia, Russia, Spain, Sudan, Europe, Norway, Poland, Romania, Turkey,	MA-27	131,319	33°45'01.35"N 73°08'06.12"E	Maryam Anar	12-Sep-2020
5.	<i>S. oleraceus</i> (L.) L.	<i>S. angustissimus</i> Hook.f.	Asteraceae	Common sowthistle, milk thistle,	Herb	Roadsides, waste places, fields,	Yellow orange	Jul-Oct	Islamabad, Rawalpindi, Peshawar,	Albania, Bulgaria, Greece, Somalia, Sicilia, Morocco, Libya, Palestine, Poland, Ukraine, Yemen, Turkey,	MA-28	131,320	33°44'38.00"N 73°01'22.00"E	Maryam Anar	12-Sep-2020
6.	<i>B. reptans</i> (L.) C.A.	<i>Bracharia balansae</i> Henrad	Poaceae	Panic grass	Herb	Tropical, Subtropical	green	Aug-oct	Punjab, Sindh, Baluchistan	Africa, Australia, Europe, Mexico, Florida	MA-05	131,332	33°44'40.00"N 73°01'33.00"E	Maryam Anar	April 2, 2021
7.	<i>C. echinatus</i> L.	<i>Cenchrus ciliaris</i> Mez	Poaceae	Southern sandbur, southern sandspur	Herb	Moist area, foot path, tropical, subtropical, semiarid,	Green-pale yellow	Sep-Oct	Islamabad, Mandi Baha Uddin, Chakwal,	Argentina, Belize, Bermuda, Brazil, Colombia, California, Florida, Mexico, Bangladesh	MA-08	131,333	33°44'44.71"N 73°9'26.55"E	Maryam Anar	12-sep-2020
8.	<i>Cymbopogon cearius</i> (Hook. & Arn.) Stapf	<i>C. caesi</i> (Hook. & Arn.) Stapf	Poaceae	Malabar grass, Lemon grass	Herb	Roadsides, field, tropical and subtropical	Yellowish green	Oct-Nov	Sindh, Baluchistan, Islamabad, Punjab	India, Pakistan, Sri Lanka, Sudan, South Africa, Eastern Africa, Arabia,	MA-09	131,334	33°44'45.02"N 73°07'42.47"E	Maryam Anar	April 28, 2021
9.	<i>D. annulatum</i> (Forsk.) Stapf	<i>Andropogon annulatus</i> Forsk.	Poaceae	Marvel grass	Herb	Urban areas, Arid wet land, Fertile soil,	Purple	April-Nov	Sindh, Baluchistan, Punjab, Kashmir, KPK,	Africa, Asia, Malaysia, Argentina, Belize, Burma, Egypt, India, Mexico, Iraq, Iran, Queensland, Palestine,	MA-10	131,335	33°44'48.00"N 73°01'32.00"E	Maryam Anar	April 8, 2021
10.	<i>S. spontaneum</i> L.	<i>Imperata spontanea</i> (L.) P.Beauv.	Poaceae	Kans grass	Herb	Swamps, Saline swamps, Sandy River flats,	White	July-Sept	Sindh, Punjab, KPK, Gilgit, Kashmir, Jhelum valley, karakar,	Nepal, India, Bangladesh, Afghanistan, Egypt, Thailand, Taiwan,	MA-23	131,336	33°44'51.55"N 73°08'13.95"E	Maryam Anar	18-sep-2020

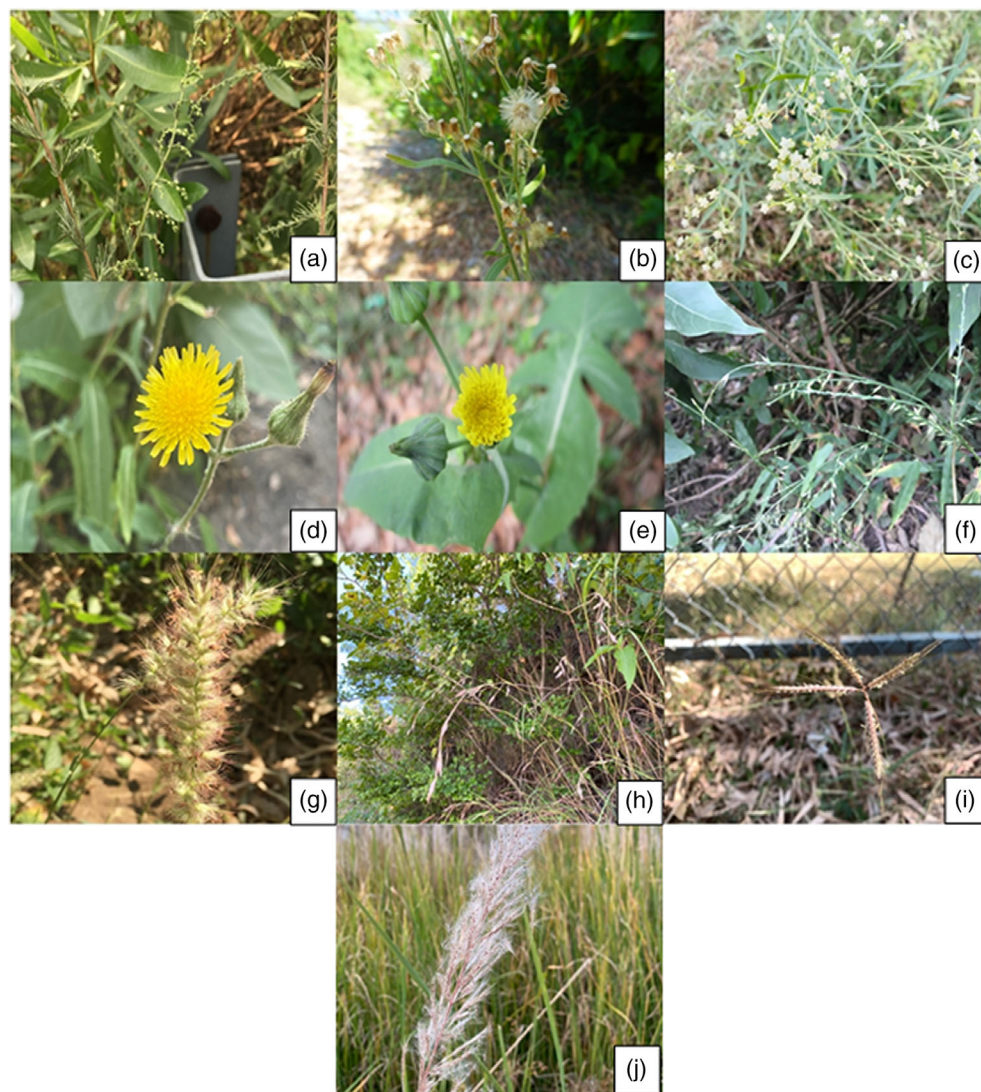


FIGURE 1 Species collected of family Astraceae and Poaceae, that is, (a) *A. scoparia*, (b) *E. bonariensis*, (c) *P. hystrophorous*, (d) *S. asper*, (e) *S. oleraceus*, (f) *B. reptans*, (g) *C. echinatus*, (h) *C. ceasius*, (i) *D. annulatum*, and (j) *S. spontaneum*

electron microscopy, minor allergenic plants species like families of Fagaceae, Salicaceae, or Ulmaceae showing typical pollen wall organization with compact endexine, intine, and ectexine while majority of allergenic plants species like Poaceae and Betulaceae the endexine is not detectable (Diethart et al., 2007). Using microscopic techniques of LM and SEM Pollen morphology of allergenic plant family Poaceae was taken out in Pakistan first time which show pollen were porate and their exine ornamentation vary (Ullah, Ahmad, et al., 2021; Ullah, Zafar, et al., 2021). Noor and Ahmad (2021) studied Palyno-morphic character of 16 allergenic flora of Northern and Arid irrigated agro-ecological zones of Pakistan.

Micro-morphological attributes examined via SEM is less costly, most reliable, easy, and time saving as compare to other modern techniques (Ayaz et al., 2020; Rashid et al., 2021). The surface morphology has been evaluated utilizing SEM approach has been proved very effective field because it is used to explore the surface peculiarities of plants organs (Ahmad et al., 2020, 2021; Usma et al., 2019). For quantitative examination SEM is a powerful technique which explained morphological parameters such as sculpturing in depth, which could not be explained through typical light microscopy (Arshad et al., 2019;

Rashid et al., 2018). Due to the recent development of SEM visualization, extensive exploration in various fields has emerged during the last decade (Asif et al., 2019; Luqman et al., 2019).

The purpose of this study was to explore pollen morphology of Astraceae and Poaceae allergenic family from capital-Islamabad, Pakistan. Member of Poaceae, and Astraceae belong to main allergenic families. For this purpose, Light microscope and Scanning electron microscopic techniques were used to explore their palyno-morphological character.

2 | MATERIAL AND METHODS

2.1 | Sampling area and climatic condition

The study was conducted on lesser Himalayas, have diversity of plant species. Capital Islamabad of Pakistan bounded by district Haripur to North and district Rawalpindi of Punjab from all other sides (33°28'N-33°48'N and 72°48' E-73°22'E) (Abbas et al., 2012). altitude 500–600 m above sea level surrounded by Margalla hills to the northeast.

The area is characterized by its subtropical climate and semi-arid zone with cold temperature from October to February, hot and humid summers from May to June, While a monsoon season from July to August (Abeer et al., 2020). The average rainfall recorded is around 1150 mm each year and average humidity level is about 55%. Predominantly winds blow from the southeast and north side. Because of High quality planning, lesser Himalayas of Islamabad has a well-developed flora that is diverse and abundant. It has been further enhanced by the addition of a large number and variety of ornamentals, particularly trees (Khan et al., 2010).

2.2 | Collection, identification, and preservation of plants

For the collection of allergenic flora of lesser Himalayas-Pakistan a field trip was planned. First trip arranged to those areas from where

we can get maximum number of plants specimen, which include Margalla Hills, Shadra Mountains, Ramli, PIDE (Pakistan Institute of Development Economics Islamabad) and Quaid-I-Azam university, Islamabad which have diversity of flora. Plants were collected using the required materials for collection of allergenic flora which includes cutter, scissors, shoppers, zipper bags, pressor, and tags. Mask and surgical gloves were used for the collection of allergenic species. The collected plant specimens belong to Asteraceae and Poaceae family were taken to the Herbarium of Pakistan (ISL) for identification by expert taxonomist and allergenic plants are conformed through previous literature on allergy and allergy causing flora. The identified plant conformed through different plant identification source, for example, e Flora of Pakistan, The Plant List, Herbarium of Pakistan (ISL), The plants of the World Online, International plant name Index, and Medicinal Plant Names Services. Correct identified allergenic plants are dried properly, preserve with chemicals, and submitted to herbarium (Table 1; Figure 1).

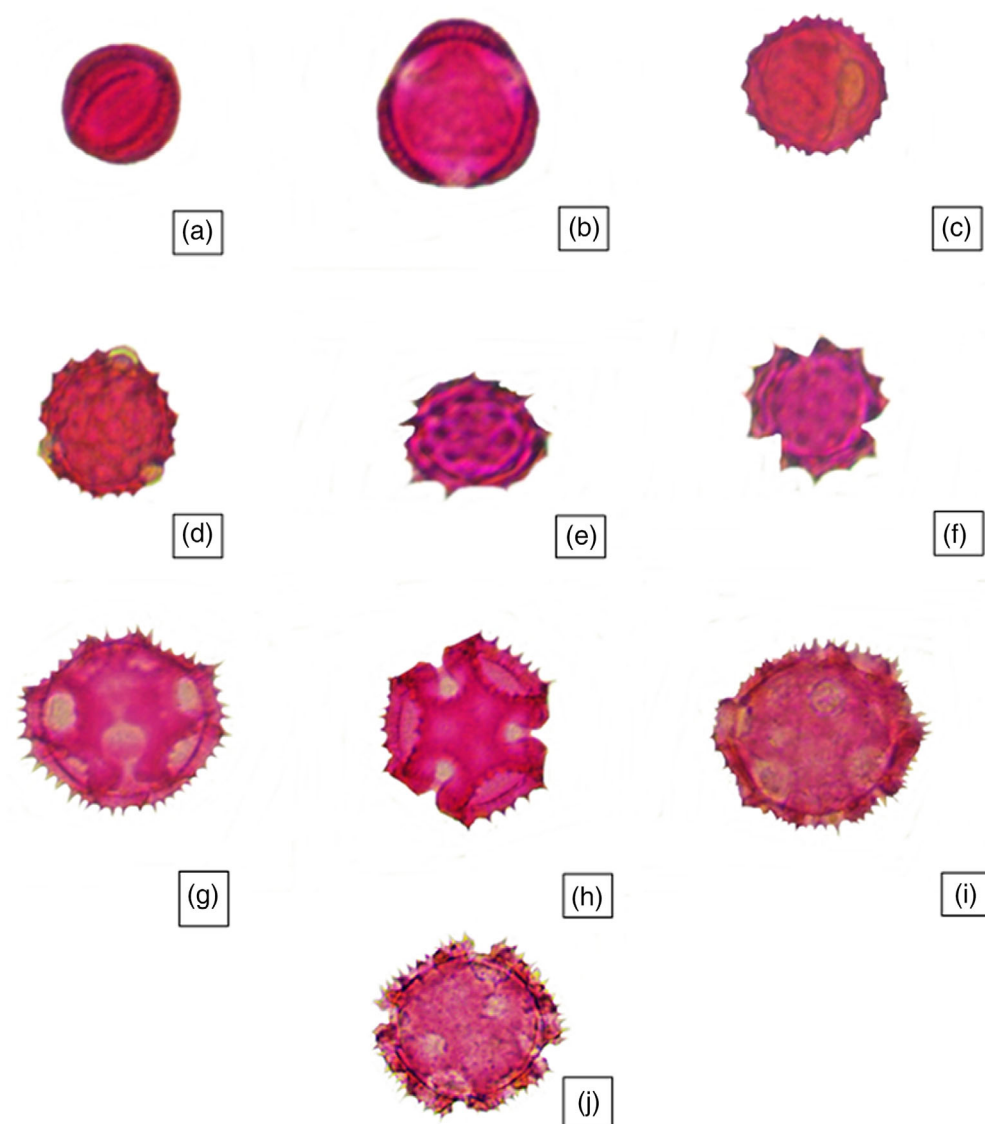


FIGURE 2 LM micrographs of Astraceae family showing polar view (PV) and equatorial view (EV); (a) *A. scoparia* (EV), (b) *A. scoparia* (PV), (c) *E. bonariensis* (EV), (d) *E. bonariensis* (PV), (e) *P. hystrophorus* (EV), (f) *P. hystrophorus* (PV), (g) *S. asper* (EV), (h) *S. asper* (PV), (i) *S. oleraceus* (EV), and (j) *S. oleraceus* (PV)

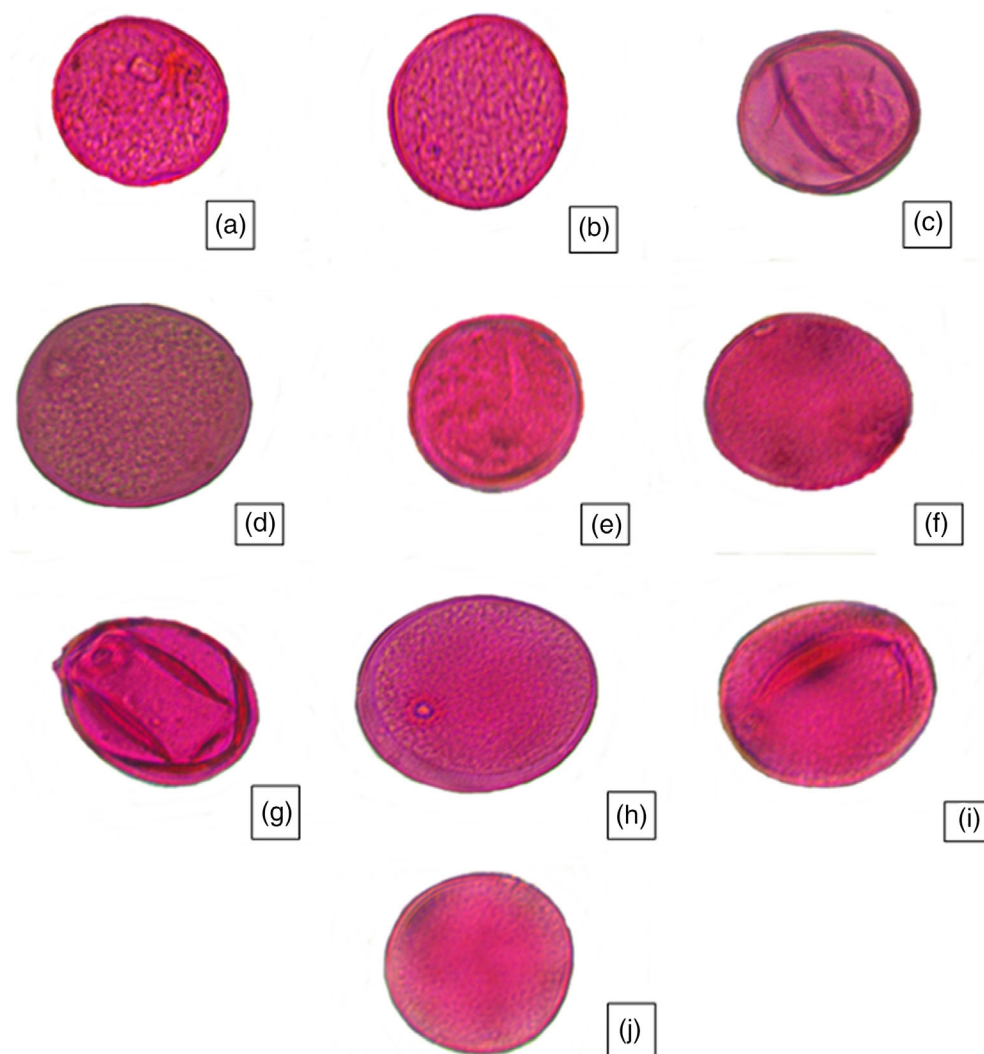


FIGURE 3 LM micrographs of Poaceae family showing polar view (PV) and equatorial view (EV); (a) *B. reptans* (EV), (b) *B. reptans* (PV), (c) *C. echinatus* (EV), (d) *C. echinatus* (PV), (e) *C. ceasius* (EV), (f) *C. ceasius* (PV), (g) *D. annulatum* (EV), (h) *D. annulatum* (PV), (i) *S. spontaneum* (EV), and (j) *S. spontaneum* (PV)

2.3 | Morphometric analysis of pollen grains (LM)

Slides were prepared for microscopic investigation with the help of forceps, the anthers are separated from collected sample of dried flower on slide. A drop of acetic acid with the help of dropper put over the anthers and the allow to evaporate. Debris of flower were removed with the help of brush for clear study of pollen. Then we add glycerin jelly for staining of pollen on slide and add a cover slip. After visualizing pollen presence under light microscope Slide were made permanent with the help transparent nail paint. Tagging tape were used for giving slide number or name according to choose (Khan et al., 2020).

Pollen slides were studied under the light microscope (MEIJI TECHNO) (Model: MT4300H). Pollen samples were examined under 100 $\times$, 400 $\times$ magnification. Measurement of intact pollen were carried out. Morpho palynological character Qualitatively and quantitatively were recorded which include diameter of Polar and equatorial view, colpi no, colpi length, colpi width, Mesocolpium, P/E ratio and Exine thickness, no of pores, Pore's length, and Width, no of spines b/w colpi, length, and width of colpi and total no of colpi also polar view, equatorial view, pollen shape, pollen type, size, spines presence,

or spines absence and exine sculpturing (SEM) (Ahmad et al., 2013; Bahadur et al., 2018; Khan et al., 2021).

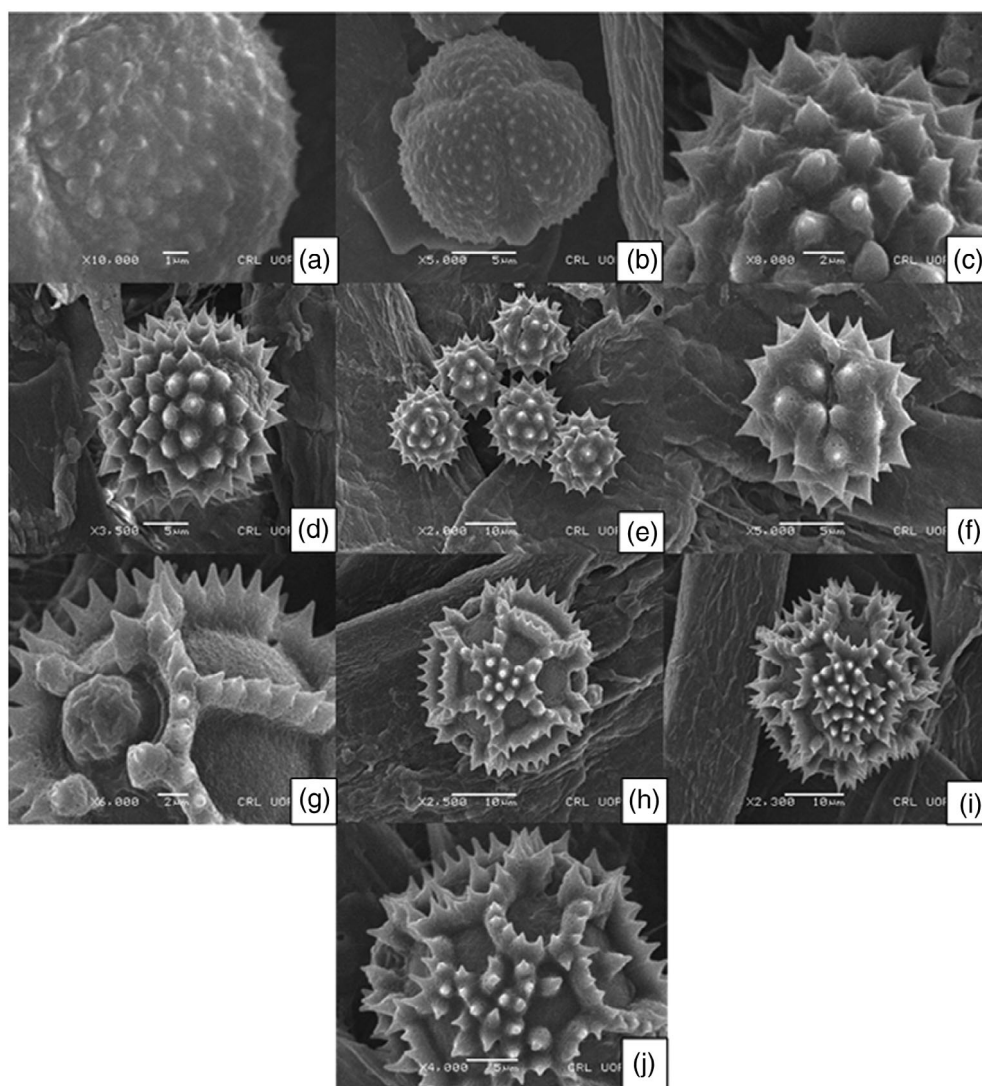
2.4 | Scanning electron microscopy (SEM)

SEM slides were prepared by separating anther from the flower handled with 45% acetic acid. Anther were squashed and then acetolyzed pollen grains were suspended in 90% ethanol and then mounted on prepared stubs. These samples were sputter coated with gold palladium and observed under Scanning electron microscope JEOL JSM5910 Model (Butt et al., 2018; Hussain et al., 2019). Pollen exine ornamentation was studied following the terminology of Bahadur et al. (2018).

2.5 | P/E ratio

P/E ratio was calculated using the formula of Ullah et al., (2018) for determination of pollen shape.

FIGURE 4 SEMM micrographs of Astraceae family showing Exine sculpturing (ES), pollen view (PV); (a) *A. scoparia* (ES), (b) *A. scoparia* (PV), (c) *E. bonariensis* (ES), (d) *E. bonariensis* (PV), (e) *P. hystrophorus* (PV), (f) *P. hystrophorus* (ES), (g) *S. asper* (ES), (h) *S. asper* (PV), (i) *S. oleraceus* (PV), and (j) *S. oleraceus* (ES)



$$P/E = PA/ED \times 100$$

(PA = polar axis, ED = equatorial diameter of the same pollen)

2.6 | Pollen fertility and sterility

Estimation of pollen sterility and fertility percentage was calculated by using the formula adapted by (Ullah et al., 2018).

$$\text{Percentage of Fertility} = \text{Fertility}/F + S \times 100$$

where F showing the fertile pollen number and S showing total sterile pollen. Present in slide.

$$\text{Percentage of Sterility} = \text{Sterility}/S + F \times 100$$

Sterility rate showing by S while number of fertile pollens is showing by F on slide.

2.7 | Data analysis

Data was investigated to find out the mean, maximum, minimum, and standard error using IBM SPSS 16.00 statistical software. For statistical analysis of Mean (minimum–maximum) $\pm$ SE, almost 10 readings were taken (ur Rahman et al., 2019). The Photoscape and Adobe Photoshop software used for the quantification and analysis of images.

3 | RESULTS

Pollen grain morphological character such as shape of pollen, size of pollen, number of pores, number of spines, presences of colpi, exine sculpturing are very important character for correct identification of plant species at family and generic level (Ullah, Ahmad, et al., 2021; Ullah et al., 2018, 2019; Ullah, Zafar, et al., 2021). Qualitative and quantitative character of pollen were studied using light microscope and scanning electron microscope. In this study 10 allergenic plant species were studied which belong to Asteraceae ($n = 5$) and Poaceae ($n = 5$) family member. During investigation pollen morphological

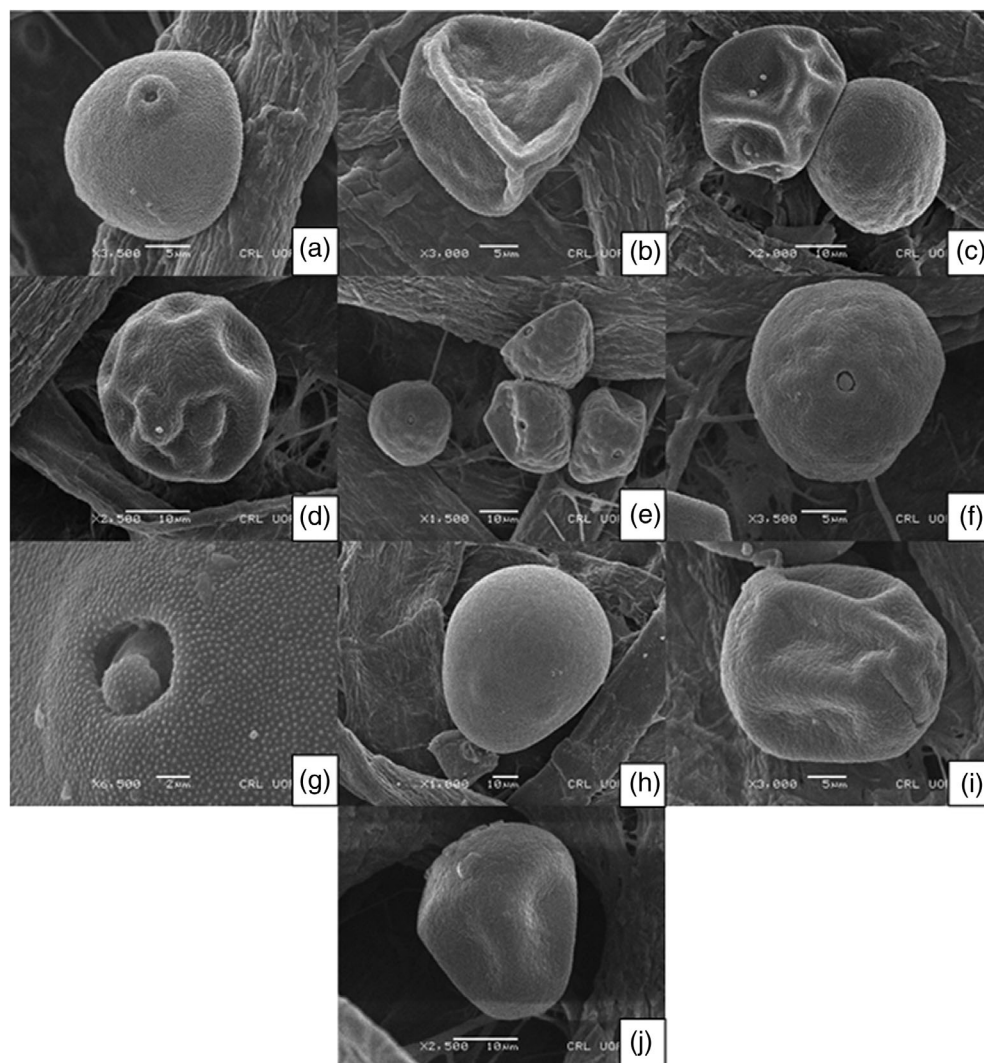


FIGURE 5 SEM micrographs of Poaceae family showing Exine sculpturing (ES), pollen view (PV); (a) *B. reptans* (ES), (b) *B. reptans* (PV), (c) *C. echinatus* (PV), (d) *C. echinatus* (ES), (e) *C. ceasius* (PV), (f) *C. ceasius* (ES), (g) *D. annulatum* (ES), (h) *D. annulatum* (PV), (i) *S. spontaneum* (ES), and (j) *S. spontaneum* (PV)

character were identified and morphological changes were noted in Asteraceae and Poaceae family. Such morphological character are main features for accurate identification. Light microscope and Scanning electron microscope used for correct indentation of allergenic plant of capital-Islamabad. Data about pollen character summarized in Figures 2–5.

3.1 | Pollen shape and size

In this study the most dominant shape of pollen in Asteraceae and Poaceae family member was Oblate-spheroidal (*Parthenium hysterophorous*, *Sonchus asper*, *Brachiaria reptans*, *Dichanthium annulatum*, and *Saccharum spontaneum*), Prolate-Spheroidal (*Artemisia scoparia* and *Cymbopogon ceasius*), Prolate (*Sonchus oleraceus* and *Cenchrus echinatus*), and Suboblate (*E. bonariensis*) shape of pollen. Quantitative character of pollen includes pollen diameter and equatorial size which show size of pollen, maximum pollen diameter of polar axis found in *D. annulatum* (46.0 μm) and minimum polar axis pollen diameter found in *P. hysterophorous* (16.0 μm). similarly maximum pollen diameter of

equatorial axis found in *D. annulatum* (48.5 μm) and minimum equatorial axis pollen diameter found in *P. hysterophorous* (17.6 μm). pollen sizes determine based on P/E ratio and different variation in size of Astraceae and Poaceae family was observed. Highest value of P/E ratio was noted in *C. echinatus* (1.05) and smallest value of P/E ratio was observed in *E. bonariensis* (0.88) (Tables 2 and 3; Figures 6 and 7).

3.2 | Spines, Colpi, and Mesocolpium

Asteraceae family was characterized by the presences of spines. This character was absent in Poaceae family. Spine's lengths vary in different species. *E. bonariensis* show maximum length of spines (4.35 μm). *A. scoparia* show minimum spines length. Similarly, colpi was observed in Asteraceae family member, highest value of colpi length was noted in *S. oleraceus* (8.1 μm) and minimum value of colpi length was observed in *P. hysterophorous* (1.2 μm). Maximum colpi width was character of *S. oleraceus* (6.80) and minimum colpi width was noted in *P. hysterophorous* (1.0 μm). Mesocolpium is distance between two colpi and the characteristic feature of Asteraceae family highest value

TABLE 2 Quantitative pollen characteristic of taxa in allergenic family Astraceae and Poaceae

Sno	Botanical name	Polar Diameter (µm)	Equatorial Diameter (µm)	P/E ratio	Colpi length (µm)	Colpi width (µm)	Spines length (µm)	Mesocolpium (µm)	Exine size (µm)
Mean (Min–Max) ± SE									
1.	<i>A. scoparia</i> waldst. & Kitam.	20.7(18.75–22.75) ± 1.7	20.3(19.75–21.25) ± 0.53	1.01	2.25(30–55) ± 21.25	4.00(2.75–4.75) ± 0.7	Reduce spines	14.2(13.0–15.75) ± 1.28	4.05(3.00–4.75) ± 0.69
2.	<i>E. bonariensis</i> L.	22.55(19.75–24.50) ± 2.55	25.35(20.25–30.25) ± 4.54	0.88	3.55(2.75–4.75) ± 0.90	4.85(3.25–5.75) ± 0.9	4.35(2.25–6.25) ± 1.74	14.30(8.75–20.25) ± 4.7	1.95(0.25–2.75) ± 1.03
3.	<i>P. hystrophorous</i> L.	16.0(12.0–20.0) ± 2.75	17.6(15.0–20.0) ± 1.66	0.90	1.2(1.0–1.5) ± 0.20	1.0(0.75–1.25) ± 0.2	1.80(1.50–2.25) ± 0.32	12.5(11.25–13.0) ± 0.72	1.7(1.2–0.20) ± 0.32
4.	<i>S. asper</i> (L.) Hill	32.0(27.5–35.0) ± 2.73	34.0(30.0–40.0) ± 4.18	0.94	4.15(2.0–7.50) ± 2.05	6.45(1.75–10.0) ± 3.0	2.25(1.75–2.50) ± 0.3	20.0(15.0–25.0) ± 3.9	5.20(5.0–5.50) ± 0.20
5.	<i>S. oleraceus</i> (L.) L.	43.15(38.25–50.25) ± 4.47	41.75(38.25–46.25) ± 3.27	1.03	8.17(7.5–8.75) ± 0.37	6.80(4.25–10.50) ± 2.28	2.45(1.75–3.00) ± 0.48	22.4(19.75–26.2) ± 2.78	5.80(4.25–8.0) ± 1.41
6.	<i>B. reptans</i> (L.) C.A.	34.85(27.75–40.25) ± 5.5	35.65(32.0–37.75) ± 2.30	0.97	A	A	A	A	1.95(1.75–2.25) ± 0.20
7.	<i>C. echinatus</i> L.	41.20(32.75–48.75) ± 6.52	39.05(30.25–45.75) ± 7.1	1.05	A	A	A	A	3.05(2.25–4.25) ± 0.37
8.	<i>Cymbopogon ceasius</i> (Hook. & Arn.) Stapf	42.90(35.50–47.75) ± 5.1	41.95(37.75–50.25) ± 5.7	1.02	A	A	A	A	2.35(1.75–3.0) ± 0.51
9.	<i>D. annulatum</i> (Forsk.) Stapf	45.0(32.5–50.5) ± 8.4	48.5(36.0–51.0) ± 9.35	0.92	A	A	A	A	3.30(2.25–5.0) ± 1.1
10.	<i>S. spontaneum</i> L.	35.8(30.0–40.7) ± 4.26	39.9(32.5–45.25) ± 5.64	0.89	A	A	A	A	1.85(1.50–2.25) ± 0.28

of Mesocolpium was noted in *S. oleraceus* (22.4 µm) and smallest value of mesocolpium was observed in *P. hystrophorous* (12.5 µm) (Table 2; Figures 8 and 9).

3.3 | Exine ornamentation

The main character in identification of pollen is pollen aperture. Species of Asteraceae family pollen grain *A. scoparia*, *E. bonariensis*, *P. hystrophorous*, *S. asper*, and *S. oleraceus* were tricolporate and tetracolporate. While poaceae family member *B. reptans*, *C. echinatus*, *C. ceasius*, *D. annulatum*, and *S. spontaneum* were monoporate and heteroporate type pollen. Tricolporate pollen were (*A. scoparia*, *E. bonariensis*, *P. hystrophorous*, and *S. asper*) and tetracolporate present in *S. oleraceus*.

Exine ornamentation show variation in Asteraceae and Poaceae species. Both qualitative and quantitative character in exine ornamentation were observed. Character feature likes Microechinate, granulate, echinate, conical base, lacunae Nanogamete, Aerolate-Scabrate, like variation found in exine of both family member. Microechinate and granulate type character were present in *A. scoparia* echinate, conical base was character of *E. bonariensis* and *P. hystrophorous*, echinate and lacunae were main features of *S. asper* and *S. oleraceus*. Poaceae family member show different exine ornamentation because spines are absent and exine was smooth Nanogamete and Aerolate-Scabrate were dominant character of exine ornamentation of Poaceae family (Table 3; Figure 10).

3.4 | Percentage of fertility and sterility

Fertility and Sterility Percentage estimated figure number was now mentioned in relevant place of (*A. scoparia*, *E. bonariensis*, *P. hystrophorous*, *S. asper*, *S. oleraceus*, *B. reptans*, *C. echinatus*, *C. ceasius*, *D. annulatum*, and *S. spontaneum*) as shown in Table 3; Figure 11. Highest value of pollen fertility was observed in *P. hystrophorous* 93% and smallest value of pollen fertility was noted in *C. ceasius* 74%. Similarly maximum value of pollen sterility was noted in *E. bonariensis* and *B. reptans* 24% and minimum value of pollen sterility was character of *P. hystrophorous* 6% (Table 3; Figure 11).

4 | DISCUSSION

The results of present investigation were collected using technique of Light microscope and scanning electron microscope to study micro-morphological character of Asteraceae and Poaceae family member collected from lower Himalaya, Pakistan. At different level of taxonomy palyno morphological character play important for correct identification of different species of Asteraceae and Poaceae family member. In our present study we investigated five members of Asteraceae family and five members of Poaceae family. Both these families belong to allergenic families (Denisow-Pietrzyk et al., 2019;

TABLE 3 Qualitative pollen characteristic of taxa in allergenic family Astraceae and Poaceae

S.no:	Taxa	Pollen shape	Pollen size	No of colpi	Colpus ornamentation	Mesocolpium	No of spines	No of pores	Type and polarity	Aperture condition	Exine ornamentation	Fertility %	Sterility %
1.	<i>A. scopooria</i> waldst. & Kitam.	Prolate-Spheroidal	small	3	Raised and prominent	Granulate	Many	A	Monad	Tricolporate,	Microechinate, granulate,	87	12
2.	<i>E. bonariensis</i> L.	Suboblate	Small-medium	3	Sunkun, slit like, distinct margins,	Echinate,	29	A	Monad, spheroidal	Tricolporate, spines present	Equinate, echinate, conical base,	75	24
3.	<i>P. hysterophorous</i> L.	Oblate-spheroidal	Small-medium	3	slit like margins distinct,	echinate	32	A	Spheroidal,	Tricolporate, spines present	echinate, conical base,	93	6
4.	<i>S. asper</i> (L.) Hill	Oblate-Spheroidal	Medium	3	Sunkun area	Pslate, echinate	40	A	Monad	Tricolpate	Echinate, lacunae,	88	11
5.	<i>S. oleraceus</i> (L.) L.	Prolate	Medium-Large	4	Sunkun area	Pslate, echinate	45	A	Monad	Tetracolpate	Echinate, lacunae,	91	8
6.	<i>B. reptans</i> (L.) C.A.	Oblate-Spheroidal	Medium	A	A	A	A	1	Heteropolar, Monad, Monoporate	Ulcerate, Irregular folding	Nanogammate, Aerolate-Scabratre,	75	24
7.	<i>C. echinatus</i> L.	Prolate	Medium	A	A	A	A	1	Heteropolar, Monad, Monoporate, circular	Ulcerate, Irregular folding,	Nanogammate, Aerolate-Scabratre,	81	18
8.	<i>C. ceasius</i>	Prolate-Spheroidal	Medium-Large	A	A	A	A	1	Heteropolar, Monad, Monoporate	Ulcerate, Irregular folding	Nanogammate, Aerolate-Scabratre,	74	23
9.	<i>D. annulatum</i> (Forsk.) Stapf	Oblate-spheroidal	Medium	A	A	A	A	1	Heteropolar, Monad, Monoporate	Ulcerate, Irregular folding, heteropolar,	Nanogammate, Verrucate, Aerolate-Scabratre,	85	14
10.	<i>S. spontaneum</i> L.	Oblate-spheroidal	Medium	A	A	A	A	1	Heteropolar, Monad, Monoporate	ulcerate, Irregular folding, heteropolar, circular	Nanogammate, verrucate Aerolate-Scabratre,	78	21

FIGURE 6 Pollen size variation of allergenic family Astraceae and Poaceae

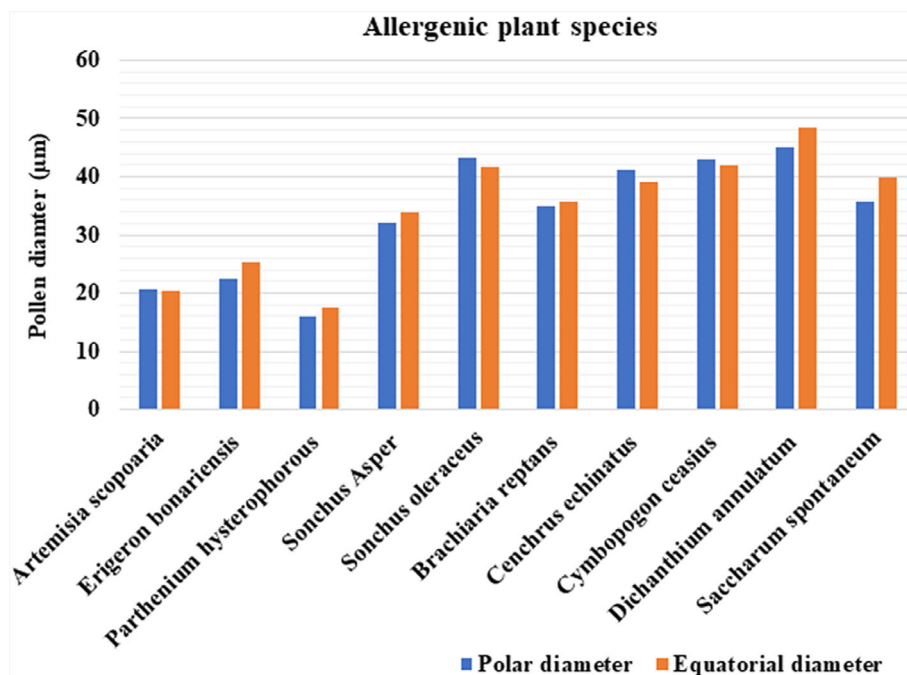
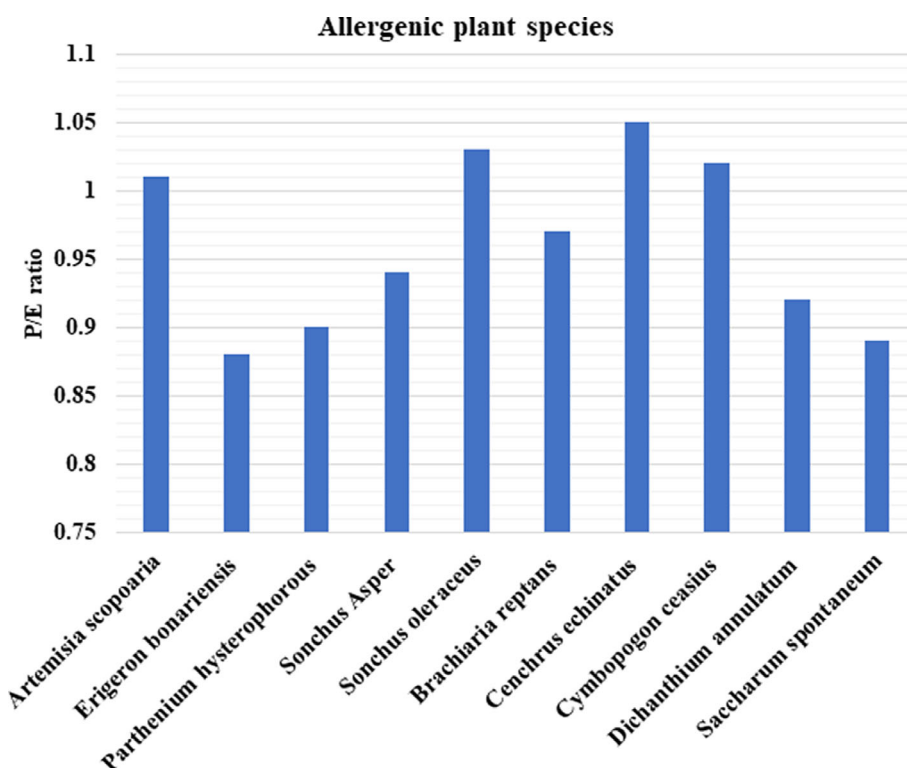


FIGURE 7 P/E ratio of allergenic family Astraceae and Poaceae



García-Mozo, 2017; Noor & Ahmad, 2021; Sapiña et al., 2021). LM and SEM analysis has been used for study of pollen exine sculpturing (Bahadur et al., 2018; Guimarães et al., 2018).

In current investigation 10 species were investigation which belong to Asteraceae and Poaceae family. Our study about Asteraceae family show pollen were monad, tricolporate and tetracolporate, echinate and have conical base. Our findings were like (Kodak et al., 2012), who found Asteraceae family were tricolporate,

tetracolporate, pollen was echinate, and shape of pollen were oblate spheroidal. In our finding *A. scoparia* spines were small and reduce in size, similarly another study show pollen of *Artemisia* specie were tricolporate, echinate and spine of pollen were reduced (Ahmad et al., 2013).

During study of Poaceae family the pollen was monad, monoporate, and surface ornamentation of pollen was psilate. Similarly, Guimarães et al. (2018), study shows finding of Poaceae species,

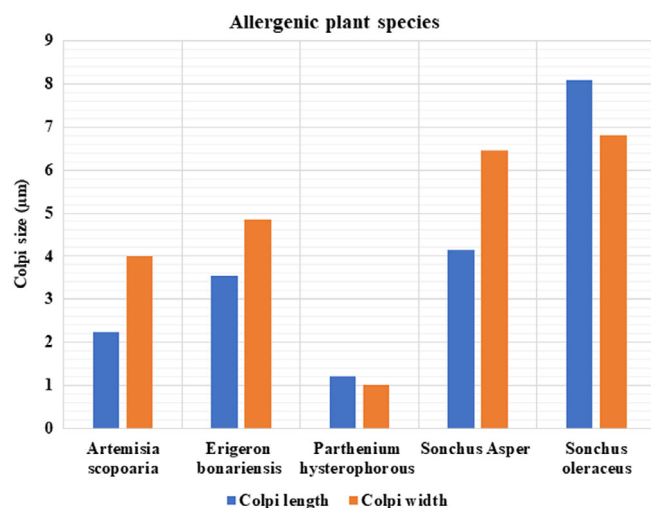


FIGURE 8 Graphical representation of colpi length and colpi width of allergenic family Astraceae

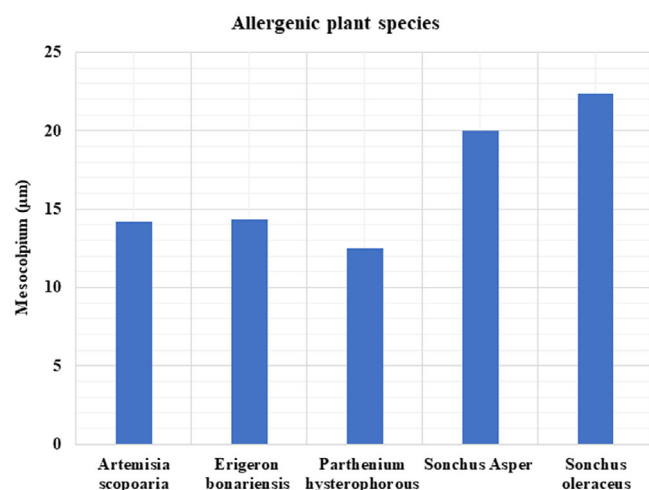


FIGURE 9 Mesocolpium variation of allergenic family Astraceae

pollen have single pore and sealed at end. The surface ornamentation of pollen was psilate type. In this study the most dominant shape of pollen in Astraceae and Poaceae family member was Oblate-spheroidal (*P. hysterophorus*, *S. asper*, *B. reptans*, *D. annulatum*, *S. spontaneum*), Prolate-Spheroidal (*A. scoparia*, *C. ceasius*), Prolate (*S. oleraceus*, *C. echinatus*) and Suboblate (*E. bonariensis*) shape of pollen which were similar to the findings of (Ahmad et al., 2013; Guimarães et al., 2018; Meo & Khan, 2005; Qureshi et al., 2002).

Among the study of Asteraceae family the highest polar and equatorial diameter was found of *S. oleraceus* 43.15 µm and 41.75 µm minimum polar and equatorial diameter was found in *P. hysterophorus* 16.0 µm and 17.6 µm. Maximum P/E ratio was observed in *S. oleraceus* 1.03 and minimum P/E ratio was character of *E. bonariensis* 0.88. Similarly maximum colpi length and width was noted in *S. oleraceus* 8.1 µm and 6.80 µm and minimum value of colpi length and width was observed on *P. hysterophorus* 1.2 µm and

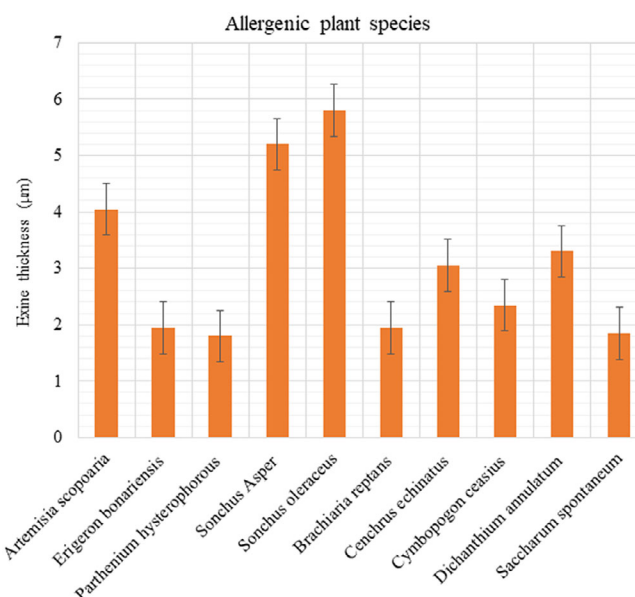


FIGURE 10 Exine thickness of allergenic family Astraceae and Poaceae

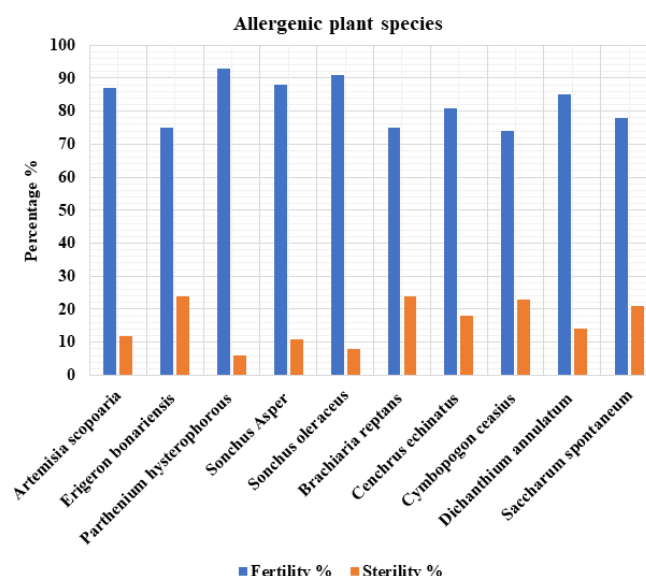


FIGURE 11 Fertility and sterility percentage variation of allergenic family Astraceae and Poaceae

1.0 µm. Spines are the main character of this family. The highest value of spines was observed in *E. bonariensis* 4.35 µm and *A. scoparia* show reduce spines. Mesocolpium is the distance between two colpi, maximum value of Mesocolpium was main character of *S. oleraceus* 22.4 µm and minimum value was noted in *P. hysterophorus* 12.5 µm. *S. oleraceus* show the highest value of exine thickness 5.80 µm and *P. hysterophorus* show the minimum value of exine thickness 1.7 µm. Exine ornamentation show pollen were micro-echinate, granulate, and lacunae were present. Our findings are similar to Diez et al. (1999), Perveen (1999), Ghahraman et al. (2007), Ahmad et al. (2013),

Shaheen et al. (2018), Hussain et al. (2019), Alam et al. (2020) Khan et al. (2020), and Ali et al. (2021).

During investigation of Poaceae family *D. annulatum* show maximum polar and equatorial diameter 45.0 μm and 48.5 μm . Highest value of P/E ratio was observed in *C. echinatus* 1.05 and smallest value of P/E ratio was noted in *D. annulatum* 0.92. P/E ratio was important in determining the shape of pollen. Spines are absent in Poaceae family the exine of Poaceae family was psilate. Main character feature of this family was monoporate. Maximum exine thickness was noted in *D. annulatum* 3.30 μm and minimum value of exine thickness was observed in *S. spontaneum* 1.85 μm . Exine ornamentation show pollen were Nanogammate, Verrucate, and Aerolate-Scabratre type. Our results are coinciding with the findings of Chaturvedi et al. (1998), Mishra et al. (2002), Shaheen et al. (2013), Ghosh and Karmakar (2017), Guimarães et al. (2018), Ullah, Ahmad, et al. (2021), and Ullah, Zafar, et al. (2021).

For correct identification of pollen palynologist using light microscope and scanning electron microscope for fast and accurate identification of pollen to develop allergenic pollen atlas for Asteraceae and Poaceae family. Microscopic techniques playing important role in many fields include mellisoplainology, aeropalynology and geopalynology.

Potent aeroallergens are of Poaceae family (Puc & Puc, 2004). Study of allergenic families which show the highest number of allergens has identified and belong to Poaceae family 15 species have 71 allergens, 5 species Asteraceae have 13 allergens (Radauer & Breiteneder, 2006). Survey of central India was carried out also showed that the dominant pollen categories belong to Poaceae and Asteraceae (Singh & Kumar, 2002).

This investigation provides us better understanding of pollen morphology of Asteraceae and Poaceae taxa which belong to allergenic family. This identification helps in allocation of species to different taxonomic rank. The use of Light microscope and scanning electron microscope help in correct identification of taxa and help to solve phylogenetic linkages of numerous problematic taxa. Previous study has also reported the importance the Scanning electron microscope and Light microscope (Khan et al., 2018, 2019, 2021; Majeed et al., 2020; Ullah, Ahmad, et al., 2021; Ullah, Zafar, et al., 2021).

5 | CONCLUSION

In this study we investigated allergenic family Asteraceae and Poaceae member collected from lower Himalayas of Pakistan. Qualitative and quantitative character were measured using microscopic techniques for morpho palynological study of pollen. Key finding includes maximum polar and equatorial diameter was noted in *D. annulatum* (46.0 μm and 48.5 μm). Highest value of P/E ratio was noted in *C. echinatus* (1.05) and smallest value of P/E ratio was observed in *E. bonariensis* (0.88). *E. bonariensis* show maximum length of spines (4.35 μm) *A. scoparia* show reduced spines also highest value of Mesocolpium was noted in *S. oleraceus* (22.4 μm) and smallest value of mesocolpium was observed in *P. hysterothorax* (12.5 μm). These

were dominant character of Astraceae family. Poaceae member were monoporate. Character feature likes Microechinate, granulate, echinate, conical base, lacunae Nanogammate, Aerolate-Scabratre, Verrucate, like variation found in exine of both family member. Highest value of pollen fertility was observed in *P. hysterothorax* 93%. Characteristic descriptions of pollen wall were renowned with the help of SEM. Identification of allergenic pollen and microscopic identified character play important role for proper guideline for identification of pollen have allergenic potential and help in pollen literature establishment.

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CONFLICT OF INTEREST

All the authors declared that they have no competing interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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