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Scanning electron imaging of mellitophilous and allergenic pollen grain of arid and northern irrigated agroecological zones of Pakistan

Mehwish Jamil Noor¹  | Mushtaq Ahmad² 

¹Department of Environmental Sciences,
Fatima Jinnah Women University, Rawalpindi,
Pakistan

²Department of Plant Sciences, Quaid i Azam
University, Islamabad, Pakistan

Correspondence

Mehwish Jamil Noor, Department of
Environmental Sciences, Fatima Jinnah
Women University, The Mall, Rawalpindi,
Pakistan.

Email: mehwish.jamil@fjwu.edu.pk

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Abstract

Palynomorphic studies of 65 common mellitophilous and 16 allergenic flora of Arid and Northern irrigated agroecological zones of Pakistan are carried out in this study by using field emission scanning electron microscope (FESEM). Mellitophilous pollen were extracted from honey samples of selected sites. For collection of local allergenic pollen, previously identified allergy-causing plants were selected. Pollen morphological examination was carried out under FESEM. Diverse range of pollen shapes ranging from monad to polyad and sculpturing ranging from psilate to echinate, scabrate to reticulate, biretulate, or echinolophate were observed. Brassicaceae, Myrtaceae, Asteraceae, Rosaceae, and Poaceae were observed to be dominant allergenic and mellitophilous families of the selected sites.

KEYWORDS

allergenic flora, honey plants, Mellitophilous plants

1 | INTRODUCTION

Microscopic studies support in developing taxonomic terminology of pollen ornamentation and their identification (Hameed et al., 2020) to lower taxonomical level (Ferguson, Zetter, & Paudyal, 2007) and can be used to differentiate between taxonomic characters of closely related genera (Hameed et al., 2020). Scanning electron microscopy served as an efficient mean of exploring pollen exine and sculpturing as compared to light microscopy.

Pollen morphological features, viz., shape, size, sculpturing, etc., serve as a useful identification tool for floral taxa, whereas exine sculpturing is an attractive feature in taxonomy and systematic. Moreover, pollen apertures are also observed as critical section of pollen sculpturing. They both play the basic criterion to identify flora after pollen apertures and shapes. Palynology had wider application and served as a tool in the past for identifying related taxa and various major plant families (Bahadur et al., 2018).

Pollen spectra of honey are a tool for estimating the interaction between bees and vegetation (Paudyal & Gautam, 2015). Both are communally reliant on one another (Decourtye, Mader, & Desneux, 2010). Pollen assemblage reflects floral situation of a specific site (Louveaux, Maurizio, & Vorwohl, 1978); therefore, honey's pollen content analysis is a standard and an effective tool for understanding and determining the bee floral resources, quality, authenticity, and adulteration (Barth, 2004) in honey.

Major pollen allergy-causing plants reported from the studied sites include *Acacia* sp., *Amaranthus viridis*, *Parthenium hysterophorus*, *Chenopodium album*, *Pinus roxburghii*, *Eucalyptus globulus*, *Callistemon citrinus*, *Cyperus* sp. *Parthenium hysterophorus*, *Artemisia dubia*, *Alternaria* (Anjum Perveen & Sad-ul-Islam, 2007; Ghufuran, Hamid, Ali, & Ali, 2013; Hasnain, Akhter, & Waqar, 2012; Parveen, Khan, & Zeb, 2012; Perveen, Zeb, Khan, & Qaiser, 2014; Raza, Khan, Rashid, & Abbas, 2012).

The present study is aimed at exploring the role and effectiveness of scanning electron microscopy in developing pollen spectra of mellitophilous and allergenic pollen of the selected sites.

2 | METHODOLOGY

2.1 | Sites for mellitophilous and allergenic pollen flora collection

For collection of mellitophilous pollen, apiaries placed in different urban and rural areas of arid zones and northern irrigated agroecological zones of Pakistan were surveyed and honey samples were collected from the zones. Collection sites included Balakot, Barakoh, Chakwal, DG Khan, Fort Abbass, Gali jageer, Ghumri, Gilgit, Gujar Khan, Hazara, Jabba Distt., Jhelum, Kaghan Valley, Kalar Kahar, Karak, Kashmir, khand, Attock, Khore, Kohat, Lahore, Lakimarwat, Lillah, Mansehra, Mardan, Mianwali, Morgah Biodiversity Park, Mulhal Mughlan, Murree, Mushkpuri, Nathia Gali, Pind Begwal, Sahiwal, Sargodha, Soha Swat and some Pakistani commercial honey samples were bought from the markets of Islamabad. Sampling sites included forests, roadsides, gardens, pastures, and agricultural cultivations fields. Allergenic flora of the same site was also collected in parallel.

In the present work, pollen spectrum of allergenic and Mellitophilous flora is developed. Pollen identification at species, genus, or family level is carried out by using systematic value of pollen sculpturing through microscopic techniques. Detailed morphological features (taxonomic, morphological, and sculpturing) presented in the present paper are carried out through field emission scanning electron microscope (FESEM).

2.2 | Mellitophilous pollen study

For pollen analysis, honey sample preparation was carried out followed (Von Der Ohe, Oddo, Piana, Morlot, & Martin, 2004) with little modification, for achieving better results. For FESEM analysis, pollen were fixed with gold-palladium coating under BIORAD Sputter Coater. After gold plating, the samples were shifted to FESEM for

image capturing. Most of the extraction was carried out under a fume hood to avoid contamination.

2.3 | Allergenic pollen analysis

For palynological analysis of allergenic pollen, previously identified allergenic plants' pollen were collected and slides were prepared using acetolysis method. Pollen were fixed with gold-palladium coating under BIORAD Sputter Coater. After gold plating, sample FESEM images were captured. The morphology of pollen terminology was explained next (Gosling, Miller, & Livingstone, 2013; Punt, Hoen, Blackmore, Nilsson, & Le Thomas, 2007).

3 | RESULTS AND DISCUSSION

Forty-one species of plant taxa belonging to 26 families (Table 1) were studied palynologically. Major percentages of pollen studied belong to angiosperms while only one species of gymnosperm was studied. Species representing each family include Asteraceae = 6, Poaceae = 5, Fabaceae = 4, Acanthaceae, Amaranthaceae, and Myrtaceae = 2 while Apiaceae, Apocynaceae, Betulaceae, Brassicaceae, Cannabaceae, Caryophyllaceae, Chenopodiaceae, Cyperaceae, Euphorbiaceae, Lamiaceae, Liliaceae, Malvaceae, Meliaceae, Oleaceae, Pinaceae, Plantaginaceae, Rhamnaceae, Rosaceae, and Rutaceae are represented by one taxon each. Identification of pollen was carried out with the help of a well identified pollen data, pollen bank, or published literature (Perveen & Qaiser, 1998, 2002, 2006; Perveen, Siddiqui, Fatima, & Qaiser, 1994). In the present study, binomial is identified only in cases where well-identified comparative literature exists or reference pollen is available. Various articles (Bera, Swati, Basumatary, & Sarma, 2007; Koçyiğit, Keskin, & Daştan, 2013; Perveen & Qaiser, 2002, 2006) were consulted for pollen

	Family	No. of representative genera		Family	No. of representative genera
1	Acanthaceae	2	14	Fabaceae	4
2	Apocynaceae	1	15	Betulaceae	1
3	Brassicaceae	1	16	Brassicaceae	1
4	Chenopodiaceae	1	17	Meliaceae	1
5	Amaranthaceae	2	18	Poaceae	5
6	Plantaginaceae	1	19	Cyperaceae	1
7	Caryophyllaceae	1	20	Rosaceae	1
8	Apiaceae	1	21	Lamiaceae	1
9	Liliaceae	1	22	Rhamnaceae	1
10	Asteraceae	6	23	Cannabaceae	1
11	Myrtaceae	2	24	Euphorbiaceae	1
12	Pinaceae	1	25	Oleaceae	1
13	Rutaceae	1	26	Malvaceae	1

TABLE 1 Pollen representation of different plant families

identification. Mellitophilous and allergenic spectra of the selected site were not reported from Pakistan previously. Taxonomic details of the pollen are given in Table 2. Punt et al.'s (2007) glossary and spore terminology were followed for morphological description.

Mellitophilous and allergenic pollen taxonomic descriptions were carried out with the help of morphological feature, size and shape of pollen, number and arrangement of pore or colpi, etc. Pollen was identified at family, genus, or species level, whichever was possible. Palynological key was published earlier in Noor, Ahmad, Zafar, and Ashraf (2017).

Pollen extracted were observed mostly from angiosperms and only *Pinus* sp. pollen from gymnosperms were found. Monoporate pollen was observed in the members of Poaceae only, no pollinia were identified. Monad pollen was dominant while polyad and pseudomonad were observed only in members of Fabaceae (Mimosoideae) and Cyperaceae.

Diversity in pollen shape and sculpturing ranged from psilate, scabrate, reticulate to bireticulate, and echinolphate conditions. Sculpturing is reported responsible for attaching to vectors and served as lock and key with stigma. There are certain views and theories regarding sculpturing of pollen. Grayum (1986) is of view that psilate pollen are pollinated by beetles and echinate by the fly, whereas spiny grains are considered the best adaptor of attachment to hairy insects, like honeybees as they are fast moving, whereas the psilate grains attach to slow-moving beetles.

The pollen classified as wind borne in past shows less modified of exine sculpturing (e.g., Poaceae and Cyperaceae) as compared to entomophilous pollen that have diverse feature of ornamentation like in *Taraxicum officinalis*, *C. album*, *Artemesia dubia*, *P. hystrophorus*, *Trifolium alexandrium*, *Rubinia pseudoacacia*, *Alnus nitida*, etc. In this study, all the pollen were aperturate, and monoporate apertures were found in members of Poaceae only. No monocolpate or pollinia was observed. Monad pollen are generally dominant while polyad (Faegri & Iversen, 1989) are observed in members of Fabaceae (Mimosoideae) and pseudomonad (Selling, 1947) in pollen of family Cyperaceae. Detailed description has been given in results with special emphasis on sculpturing. Pollen allergy causing pollen has been pointed among these pollen. Pollen are categorized on the basis of families. Detailed pollen sculpturing has been explained later.

3.1 | Family: Acanthaceae

In the present study, two types of pollen have been identified belonging to family Acanthaceae. Pollen shape range (Figure 1a,b) is prolate to subprolate, heterocolporate with fine reticulate tectum. Reticulate sexine remnants can be seen in colpi, therefore it is termed as heterocolporate. In the middle of colpi is pori, which is studded with areoli or insulae. Colpi in Figure 2a,b has smooth margocolpi on either side of colpi. Whereas in *Justicia*, margocolpi bands are broken into circular areas termed as areoli. Those areolae are in two rows. Both figures show granular stopple projecting out of the colpi. Colpi is also alongate, reticulated, long, extended, and elliptical.

Present findings agree with previous studies. Pollen features are typical representative of Subtribe *Justicine* (Rueangsawang,

Chantaranothai, & Simpson, 2013). Tectate and reticulate sexine were also reported by Praglowski and Punt (1973). Based on pollen diversity, Acanthaceae pollen are divided into eight pollen types, *Justicia*-type is one of them with heterocolpate pollen, reticulate sexine, and centripetal reticulum pattern. This genus presents diversity in shapes of pollen, ranging from preprolate to prolate. The present results agree with the previous work, showing reticulate sculpturing with heterocolpate and prolate shape. Immelman (1989) differentiated *Justicia* and *Siphonoglossa* pollen gross morphology and identified 2–3 colporate, areoli, or broken areoli within margocolpus of prolate *Justicia* sp. pollen. He proposed the term “stopple” for notch studded in pori. The present pollen has also notch in the middle, and psilate surface is present on either side of false colpi (margocolpi). Margocolpi contain few bands of ruminant exine in broken areoli form, in *Justicia* pollen the areolae are in two rows. In *Peristrophe penniculata*, pollen shape appears similar to Acanthaceae pollen, as both are heterocolpate but the difference is: colpi margin form, presence and absence of areoli, and sexine thickness as proposed by Perveen and Qaiser (2010). Its allergenic significance is not reported from Pakistan. A detailed study of Acanthaceae pollen can be found in the study by Raza et al. (2020).

Acanthaceae has been considered as a eurypalynous family because of remarkable diversity in palynomorphs feature (Paudyal & Gautam, 2012; Perveen & Qaiser, 2010). This diversity could serve as taxonomic key for delimiting taxa at family, genus, or even species level. However, Lippi and Rossi (1999) are of view that sometimes this diversity gives rise to conflict in taxonomic approach.

3.2 | Family: Apocynaceae

Pollen in Figure 3a,b is spheroidal to prolate. Colpi are narrow and extend to both ends of pollen. Pori protrudes out of sexine and without perforation. Pores in exine are less than 1 μm in diameter. Colpi are joined in the middle to give a syncolpate appearance. Ahmed (2015) worked on pollen analysis of *Carissa edulis* and observed the tricolporate, oblate spheroidal pollen with dimensions ranging from 42.7 to 43.7 μm . In the current analysis, species is *C. opaca* with size range of 40 μm . Pollen grains in Apocynaceae are uniform, generally spheroidal to prolate, tricolporate or tetracolporate, monad with porate exine (Erdtman, 1952; Nilsson, 1990; Nilsson, Endress, & Grafström, 1993; van de Ven & van der Ham, 2006). Apocynaceae pollen size, shape, aperture number, and type have proved to be important in recognizing pollen at genus level. Apocynaceae has eurypalynous nature with diverse aperture features ranging from porate to compound colporate, hence useful for identification at genus level (Chatterjee, Sarkar, & Rao, 2014).

3.3 | Family: Amaranthaceae, Chenopodiaceae, Caryophyllaceae, and Plantaginaceae

Amaranthaceae, Chenopodiaceae, and Caryophyllaceae families are members of order Caryophyllales. The members of these families are

TABLE 2 Morpho-taxonomic studies of allergenic and melitophilous pollen under LM

	Plants found	Family	Type/shape/class	Size (μm)		Exine (μm)	
				P	X $\pm$ SD	P/E	Aperture
1	<i>Justicia adhatoda</i> L.	Acanthaceae	Monad/symmetrical/prolate-subprolate	35–38–40 (37.7 $\pm$ 3.5)	26–30 (28 $\pm$ 2.82)	1.34	2 3 3.5
2	<i>Peistrophe paniculata</i> (Forsk.) Brummitt	Acanthaceae	Monad/symmetrical/Prolate	35–40 (37.7 $\pm$ 3.53)	28–35 (31.5 $\pm$ 4.9)	1.2	6 – 5
3	<i>Carissa opaca</i> Stapf ex Haines	Apocynaceae	Monad/symmetrical/spheroidal	28.5–29 (28.5 $\pm$ 0.5)	28.5–28.5 (28.5 $\pm$ 0)	1	3 1 <2.5
4	<i>Chenopodium album</i> Linnaeus	Chenopodiaceae	Monad/symmetrical/spheroidal	20–22–23.5 (21.8 $\pm$ 1.75)	17–18–24.5 (19.8 $\pm$ 4.07)	1.1	∞ ∞ <2.5
5	<i>Amaranthus spinosus</i> Linn.	Amaranthaceae	Monad/symmetrical/spheroidal-prolate	15–18 (16.50 $\pm$ 1.75)	15–18 (16.50 $\pm$ 1.75)	1	∞ ∞ 2.45
6	<i>Artherna</i> sp.	Amaranthaceae	Monad/symmetrical/spheroidal	16.75–18 (17.37 $\pm$ 0.88)	17–17.5 (17.25 $\pm$ 0.35)	–	∞ ∞ 3
7	<i>Plantago ovata</i> Forsk.	Plantaginaceae	Monad/symmetrical/spheroidal	18–20 (19 $\pm$ 1.4)	18–25 (21.5 $\pm$ 4.9)	0.88	∞ ∞ 3
8	<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	Monad/symmetrical/spheroidal and dodecahedral	22–34 (28.4 $\pm$ 8.48)	22–34 (28.4 $\pm$ 8.48)	1	∞ ∞ 2.5–3
9	<i>Coriandrum sativum</i> Linn.	Apiaceae	Monad/symmetrical/dumbbell shaped	28.8–30–32.5 (30.5 $\pm$ 1.4)	12–15 (13.5 $\pm$ 2.12)	2.3	6 1 1.5–4
10	<i>Iris</i> sp.	Liliaceae	Monad/symmetrical/Prolate	90 $\pm$ 0	–	2	
11	<i>Artemisia dubia</i> Wall. ex Besser.	Asteraceae	Monad/symmetrical/prolate spheroidal	20–22–25 (22.3 $\pm$ 2.05)	18.7–22.5 (20.6 $\pm$ 2.68)	1.1	3 1 8.8
12	<i>Calendula arvensis</i> (Vail.) L.	Asteraceae	Monad/symmetrical/spheroidal	37.5 $\mu\text{m} \pm 3.53$	–	–	7.5
13	<i>Parthenium hysterophorus</i> L.	Asteraceae	Monad/symmetrical/angular-spheroidal	14.5–17–17.5 (16.3 $\pm$ 1.3)	14.8–17 (16.4 $\pm$ 1.37)	1	3 1 1.75
14	<i>Taraxicum arvensis</i> F.H. Wigg.	Asteraceae	Monad/symmetrical/spheroidal/fenestrated	27.5–30 (28.6 $\pm$ 0.99)	30–31–32 (31 $\pm$ 0.8)	1	6 1 4.45
15	<i>Sonchus asper</i> (L.) Hill	Asteraceae	Monad/symmetrical/fenestrated	32.5–32.5–32.5 (32.5 $\pm$ 0)	30–31–30 (30 $\pm$ 0)	1	6 3 3
16	<i>Conyzanthus conyzoides</i>	Asteraceae	Monad/symmetrical/fenestrated	30–30 (30 $\pm$ 0)	30–30 (30 $\pm$ 0)	1	6 0 5
17	<i>Eucalyptus globulus</i> Labill.	Myrtaceae	Monad/symmetrical/angular	22.5–22 (22.5 $\pm$ 0)	20.5–20.5 (20.5 $\pm$ 0)	1.05	3 2.1 1.9
18	<i>Callistemon citrinus</i> R. Br.	Myrtaceae	Monad/symmetrical/spheroidal to angular	14.4–15–14.4 (14.7 $\pm$ 0.2)	17–25.13–25 (22.37 $\pm$ 3.80)	0.91	3 1 <2.5
19	<i>Pinus roxburghii</i> Roxb.	Pinaceae	Monad/symmetrical/vesiculate	50–55–65 (56.6 $\pm$ 7.6)	41–43–43 (42 $\pm$ 1.4)	1.3	0 0 2.5
20	<i>Citrus sinensis</i> (Linn.) Osbeck	Rutaceae	Monad/symmetrical/spheroidal/tetracolporate	25–21.99 (23.49 $\pm$ 2.12)	27.25–21.20 (24.4 $\pm$ 4.12)	0.96	4 1 2.9
21	<i>Acacia nilotica</i> (Linn.) Delile	Fabaceae	Polyad/symmetrical/spheroidal	44–44–44 (44 $\pm$ 0)	40–42 (41 $\pm$ 1)	1.07	0 0 2.9
22	<i>Acacia modesta</i> Wall	Fabaceae	Polyad/symmetrical/spheroidal	44 μm	42 μm	–	– – –
23	<i>Trifolium alexandrinum</i> Linn	Fabaceae	Monad/symmetrical/tricolporate/prolate	22.5–30–32.5 (28.3 $\pm$ 5.2)	13.8–19.8 (16.9 $\pm$ 4.24)	1.6	3 0–3 2.9
24	<i>Rubinia pseudoacacia</i> L.	Fabaceae	Monad, symmetrical/tricolporate	22 $\pm$ 0	NA	–	NA 3 <2.5
25	<i>Alnus nitida</i> (Spach) Endl. Gen.	Betulaceae	Monad/symmetrical/Spheroidal	18.5–20 (19.25 $\pm$ 1.06)	22.4–22.9 (22.6 $\pm$ 0.32)	0.85	4.5 2.3 2.5
26	<i>Brassica campestris</i> Linn.	Brassicaceae	Monad/symmetrical/subprolate to spheroidal	14–15.25–16.75 (15.33 $\pm$ 1.37)	17–25.13–25 (22.37 $\pm$ 3.80)	1.08	3 2 2.7
27	<i>Melia azedarach</i> Linn.	Meliaceae	Monad/symmetrical/Spheroidal	32.7–34.1 (33.4 $\pm$ 0.98)	–	–	4 – 2.5
28	<i>Avena sativa</i> Linn.	Poaceae	Monad/symmetrical/spheroidal	27.9–23.9 (25.9 $\pm$ 2.82)	19.5–19.6 (19.55 $\pm$ 0.07)	1.3	1 0 <2.5
29	<i>Cymbopogon</i> (DC.) Stapf	Poaceae	Monad/symmetrical/rectangular	25–27 (26 $\pm$ 1.4)	25–27 (26 $\pm$ 1.4)	1	0 1 2.9
30	<i>Hordeum vulgare</i> L.	Poaceae	Monad/symmetrical/spherical	75–85 (80 $\pm$ 7.07)	80–92.5 (86 $\pm$ 8.8)	0.94	1 0 <5

TABLE 2 (Continued)

Plants found	Family	Type/shape/class	Size (µm)		P/E	Aperture (µm)		Exine (µm)
			P	E		P	E	
			X ± SD	X ± SD		P	E	
31 <i>Pennisetum glaucum</i> (L.) R. Br.	Poaceae	Monad/symmetrical/spheroidal	32–35 (33.5 ± 2.01)	35–35 (35 ± 0)	1.04	1	0	3
32 <i>Setaria</i> sp.	Poaceae	Monad/symmetrical/spheroidal	30–30 (30 ± 0)	30–30 (30 ± 0)	1	1	0	2.5
33 <i>Cyperus</i> sp.	Cyperaceae	Pseudomonad/symmetrical/prolate	25–30 (28.33 ± 3.5)	32.50–40 (35 ± 5.3)	1.2			2.41
34 <i>Eriobotrya japonica</i> (Thunb.) Lindl.	Rosaceae	Monad/symmetrical/spheroidal	28.5–28 (28.2 ± 0.2)	28.5–28 (28.2 ± 0.2)	1	1	1	4
35 <i>Prunus</i> sp.	Rosaceae	Monad/symmetrical/spheroidal	26 ± 0	25 ± 0	1	1	1	3
36 <i>Ziziphus jujuba</i> Mill.	Rhamnaceae	Monad/symmetrical/spheroidal	15–22.5–20 (19.1 ± 3.11)	17–25.13–25 (22.37 ± 3.80)	0.88	1	2	
37 <i>Cannabis sativa</i> Linn.	Cannabaceae	Monad/symmetrical/spheroidal	25 ± 0	17.5 ± 0	1.42	1	1.75	
38 <i>Dalbergia sissoo</i> Roxb.	Fabaceae	Monad/symmetrical/trilobed	52.7–52	47–47 (47 ± 0)	1.11	2.3	3(2–4)	
39 <i>Hyssopus officinalis</i> L.	Lamiaceae	Monad/symmetrical/spherical	22.1–24.1 (23.1 ± 1.4)	22–23 (22.5 ± 0.70)	1.01	2.3	5	
40 <i>Olea ferruginea</i> Linn	Oleaceae	Monad/symmetrical/trilobed	41–41	45–46 (45.5 ± 0)	0.9	1.2	<2.5	
41 <i>Abutilon indicum</i> (L.) Sweet	Malvaceae	Monad, symmetrical/echinate/periporate/spheroidal-globular	60 ± 0	60 ± 0	1	∞	6.45	

Abbreviations: E, equatorial view; P, polar view; SD, standard deviation; X, mean.

reported to be allergenic in Pakistan and throughout the world (Anjum Perveen & Sad-ul-Islam, 2007; D'amato et al., 1998; Galán et al., 1989; Ma, Hasnain, & Khan, 2010; Parveen et al., 2012; Perveen, Noori, Khan, & Qureshi, 2015). Among these three, Chenopodiaceae and Amaranthaceae are supposed to have originated from monophyletic lineage (Bittrich, 1993; Downie & Palmer, 1994). All three families possess pantoporate pollen along with plantaginaceae, which are difficult to differentiate. Pantoporate pollen with spinulose and punctate tectum are reported in both species of Amaranthaceae and Chenopodiaceae (Nowicke & Skvarla, 1979; Skvarla & Nowicke, 1976).

All families possess pori that are deeply recessed and mesoporia appeared as false reticulum. Punt et al. (1994) termed it as meta-reticulate condition. Borsch and Barthlott (1998) agree with this term. Generally, these families possess “Ektexinous elements” or isolated “ektexinous bodies,” these elements are small outgrowth that are called “flecks of ektexine” as reported by Skvarla and Nowicke (1976).

Chenopodiaceae is a stenopalynous family. The pollen of Chenopodiaceae appears to vary most in the number of apertures, size, and frequency of spinules in the ectexine. Pollen is usually radially symmetrical, apolar pantoporate, and spheroidal. Sexine is slightly thicker or thinner than nexine. Tectum is sparsely to densely punctate and rarely spinulose. However, on the basis of aperture numbers and exine ornamentation, this family was divided into four pollen types, viz., *Arthrocnemum indicum*-type, *Atriplex stocksii*-type, *C. album*-type, and *Haloxylon persicum*-type. Pollen morphology of this family is significantly helpful at specific and generic levels (Perveen & Qaiser, 2012).

Figure 4a has punctate tectum, meso poria flat with numerous, evenly spread micro spinules. Ektexinous bodies >15, more than 2.5 µm in diameter, distinctly separated from each other, deeper seated, and sharply set off against the tectum. The present features agree with Perveen and Qaiser (2012) and Borsch (1998), and they state that *Chenopodium* type pollen are generally spheroidal, pantoporate, and rounded aperture with scabrate ornamentation. Exine in the current study is around 2.5 µm with pore. Surface is also not uniform.

Borsch (1998) believes that light and scanning electron microscopy could explain many new characteristics of Amaranthaceae pollen's apertures, their ektexinous bodies (ektexinous elements), surrounding membrane, ultra-structure, and micro sculpture spread on exine and information on pollen kit. He proposed 17 different pollen types in his work. The taxonomic distribution of pollen characters in the Amaranthaceae indicates that pollen morphology is informative at different taxonomic levels. Pollen grains in the Amaranthaceae are tectate, tetrahedral, cubical, dodecahedric, or spheroidal monads and are consistently pantoporate. The pore number varies considerably, from 4 (*Nothos aerva brachiata*) to approx. 250 (*Froelichia floridana*) (Borsch, 1998). In some genera, metareticulate pollen occurs in several genera. The pollen of most genera is clearly tectate, apart from some metareticulate grains (thin mesopodium apparently looks in reticulate condition) in which the tectum is recessed in vertically oriented areas of the mesopodium adjacent to the apertures. The tectum is punctate or anulopunctate, with microspines (evenly distributed). In some

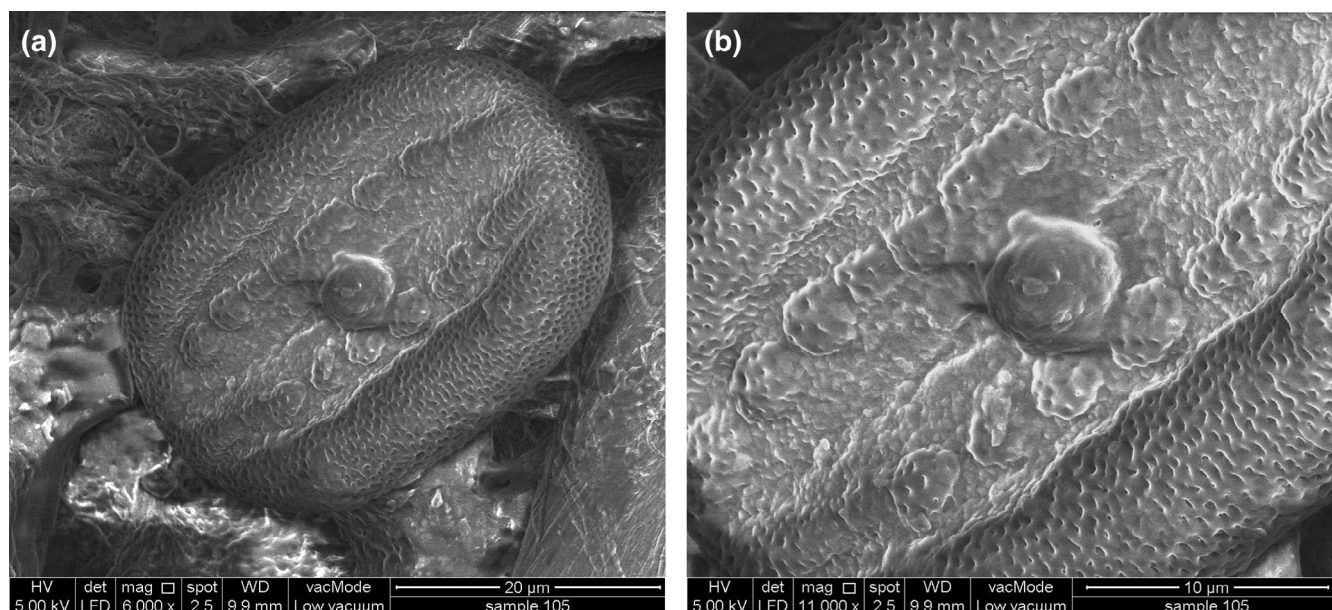


FIGURE 1 (a) *Justicia adhatoda* (polar view). (b) *Justicia adhatoda* (wall sculpturing)

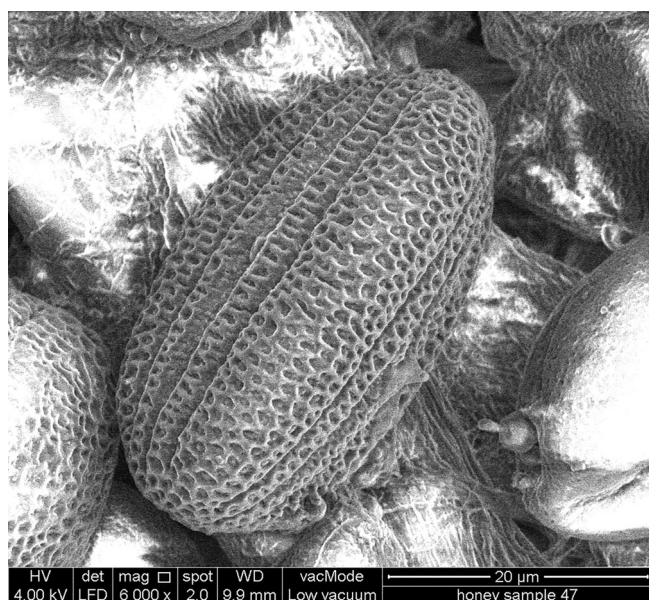


FIGURE 2 *Peristrophe paniculata* (polar view)

genera, metareticulate conditions exist with tooth shaped microspines or they are completely lacking (*Tidestromia* ssp., *Cyathula crispa*, *Psilotrichopsis* ssp., and *C. lanceolata*). Pores in these pollen are covered by bulging outgrowth named as ektexinous bodies varying in number with definite characteristics, shapes, and sizes covered with 1 to numerous microspines. Microspines are either separated or adjoined. Borsch (1998) believes that in related families (e.g., Chenopodiaceae) pollen with a morphology similar to that of taxa of the Amaranthaceae may occur, so that a joint classification as a single pollen type would seem to be reasonable. The pollen characters observed in *Silene* and *Dianthus* species (Family Caryophyllaceae)

were also tectate, polypantoporate, spheroidal, and spinulose micro perforate (Yildiz & Minareci, 2008).

In the present study, pollen in Figure 4b, *Amaranthaceae* pollen, has been categorized as Type II *Amaranthaceae* type following Borsch (1998). It is spheroidal, tectum punctate, mesoporia is flat but slightly moulded or undulate, ornamented with distinct but evenly spaced microspines, deeper seated ektexinous bodies often showing size difference, sharply set off against the tectum, and mesoporia with numerous microspines are present. Ektexinous bodies are evenly spread, while in Figure 5a,b pollen appears as *Arthraerua*-type (Family: *Amaranthaceae*). Pollen are spheroidal, mesoporia flat, or moderately and broadly vaulted, tectum punctate with numerous spines, evenly distributed over exine. Borsch (1998) names it Type III with ektexinous bodies, wide spheroidal-polyhedral, distinctly separated from each other, deeply seated, and sharply set off the tectum. Fifteen to forty are closely adjoined with arrangement in mosaic pattern, diameter equal to 2.0–4.0 µm, and marginal bodies forming an irregular borderline to the tectum. This description agrees with a current pollen sample, showing panto porate, spheroidal structure with tectate and spinulate tectum mesoporia flat with spinules ektexinous bodies (isolated small structures visible on exine) are more than 14. Pore membrane psilate, Ektexinous body deeply sunken, and echinae more than 2.5 µm in diameter. Distinctly separated from each other, evenly spread, and spheroidal, often conspicuously different in size, with microspines, deeper seated, and sharply set off against the tectum.

3.4 | Family: Caryophyllaceae

Pollen in Figure 6 is tectate spheroidal to dodecahedral and pantoporate. Mesoporia on pollen was spinulate and perforate.

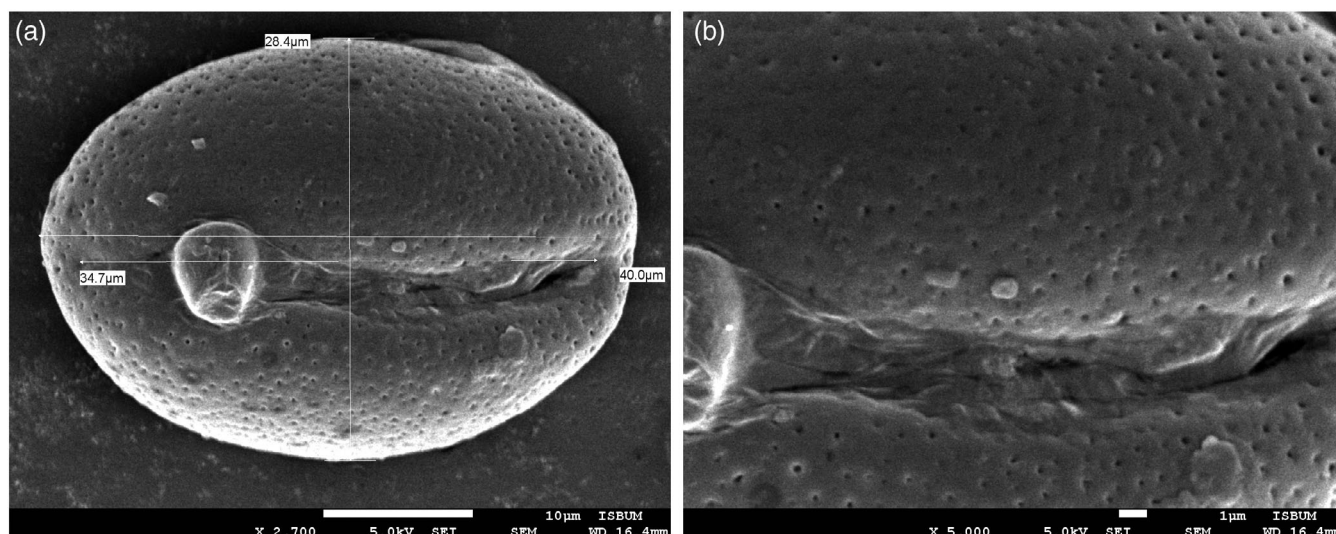


FIGURE 3 (a) *Carissa opaca* (equatorial view). (b) *Carissa opaca* (wall sculpturing)

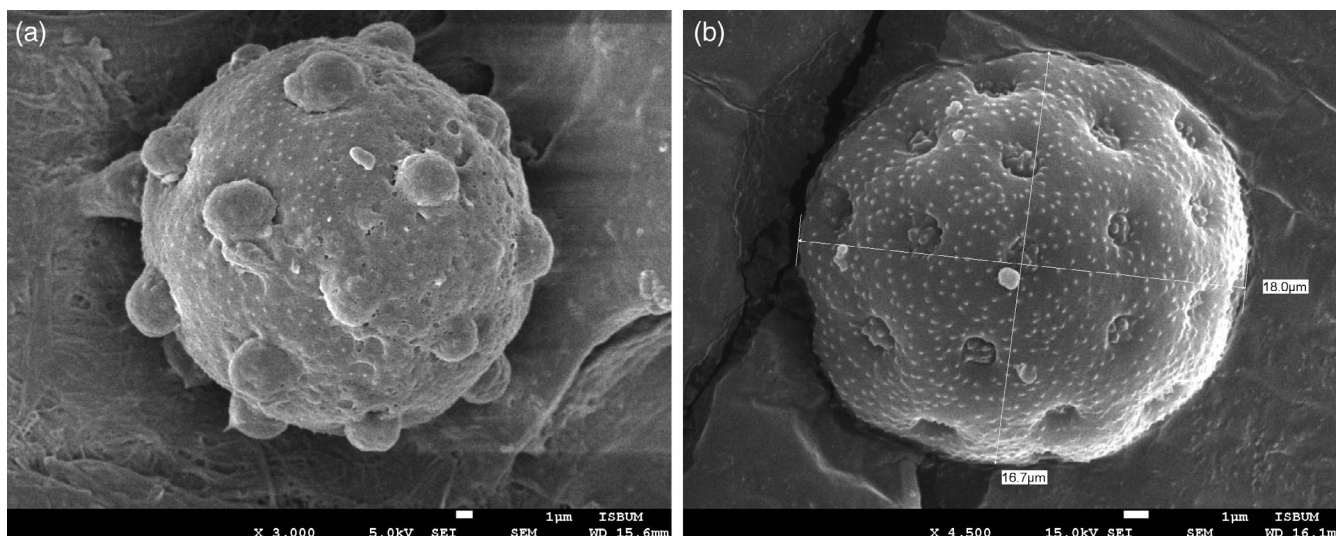


FIGURE 4 (a) *Chenopodium album*. (b) *Amaranthus viridis*

Ektexinous bodies are more than 14 and appeared star shaped, deeply seated in the exine, and demarcated from tectum. Diameter of the pori is in nanomicon or may be less. Pollen belonging to plants of Caryophyllaceae family are extensively studied by various scientists (Ataşlar, Erkara, & Tokur, 2009; Bittrich, 1993; Chanda, 1962; Erdtman, 1952; Eröz & Ataşlar, 2010; Ghazanfar, 1984; Külköylüoğlu et al., 2009; Mc Neill & Bassett, 1974; Moore & Webb, 1978; Perveen & Qaiser, 2006; Taia, 1994; Yildiz, 2001; Yildiz, Çirpici, & Dadandi, 2010; Yildiz, Dadandi, Minareci, & Çirpici, 2011). All agree with the general appearance of pollen in this family as tectate, pantoporate, spheroidal, spinulose-scabrate to punctate. Exine sometimes shows granulate nano-echinate ornamentations.

The current pollen appears closely to Type 4 pores (Borsch, 1998), which belong to Caryophyllaceae family possessing pollen of *Stelaria media* type as proposed by Parveen (2005) with

tectate, pantoporate, meso poria flat with spinules and pori. Ektexinous bodies >14, closely aligned to Type 4 pores (Borsch, 1998). "Stellate pore ornamentation" deeply seated in sexine sharply set off against the tectum. Distinctly separated from each other, evenly spread spheroidal, pores are nanomicon in diameter. Most of the genera in caryophyllaceae, including *Arenaria*, *Gypsophila*, *Lychnis*, *Petrorhagia*, *Cerastium*, *Silene*, *Vaccaria*, *Holosteum*, etc., possess pantoporate grains with either punctate or spinulose scabrate tectum. In the case of *Silene* genus, three species (*Silene brahuica*, *S. gonosperma*, and *S. indica*) possess reticulate tectum (Nowicke, 1976; Nowicke & Skvarla, 1979).

Pollen type *Stellaria media* is observed in subfamilies of Alsinoideae and Caryophylloideae. As they both possess porate pollen with spinulose punctate tectum. Palynologically, the family Caryophyllaceae is more or less similar to Illeceberaceae, both have

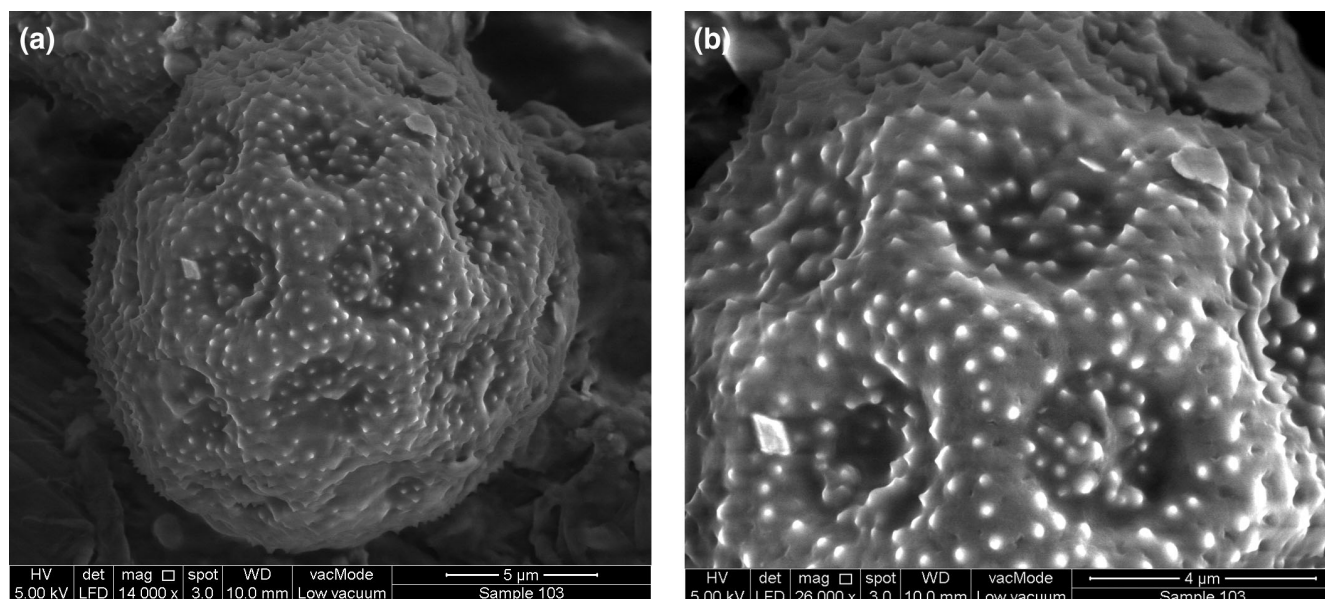


FIGURE 5 (a) *Arthernera* sp. indent. (b). *Arthernera* sp. indent (wall sculpturing)

porate and rarely colpate pollen with spinulose–punctate tectum (Perveen & Qaiser, 2003). There is diversity among different taxa with colpi ranging from tricolpate to panta colpate, shape ranges from sub oblate to sub prolate, including spheroidal and polyhedral conditions. With tectum punctate to annulopunctate; however, reticulate condition like in *Amaranthaceae* is rarely found. Most of the tectum was reported in microechinate. Two subfamilies are showing variation in pollen structure, viz., *Alsinoideae* and *Caryophylloideae*. Both have Pantoporate conditions with thick exine layer but pore numbers are variable. *Caryophylloideae* have 12–40 pores while *Alsinoideae* have 15–38 pores (Bittrich, 1993). Pore numbers are generally used to restrict taxa in these families. Pollen in the current sample appears to be belonging to caryophyllaceous family members.

Perveen and Qaiser (2003) believe *Caryophyllaceae* pollen grains are usually radially symmetrical, apolar rarely isopolar, pantoporate rarely 3–10 colpate, spheroidal to prolate-spheroidal to sub-prolate occasionally prolate. Sexine is thicker or thinner than nexine. Tectum spinulose–punctuate or scabrate-punctate often reticulate or reticulate-scabrate. On the basis of aperture type, three distinct pollen types, viz., *Silene indica*-type, *Spergula arvensis*-type, and *Stellaria media*-type are recognized. The current pollen appears more close to *Stellaria media* as it possess spinulose punctate exine, and size range fall close to it as compared to other two types.

3.5 | Family: Apiaceae

Paudyal and Gautam (2012) and Menemen and Stephen (2001) reported striato-rugulate sculpturing in majority of *Apiaceae* taxa. Distinctive characteristics include inner and outer outlines, slit-like ectocolpi, and broad band-like costae, and most important

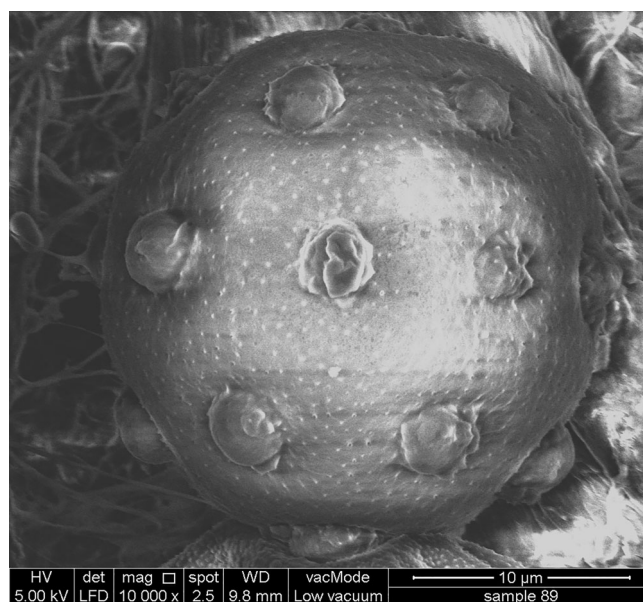


FIGURE 6 *Stellaria media*

reorganization character is bone shape pollen. *Apiaceae* pollen are reported to be present in honey (Andrada & Tellería, 2002; Dinkov & Ivanov, 2009; Salonen, Ollikka, Grönlund, Ruottinen, & Julkunen-Tiitto, 2009; Seijo, Aira, Iglesias, & Jato, 1992; Silici & Gökceoglu, 2007). In the current study, one type of pollen of *Coriandrum sativum* (Family *Apiaceae*) has been recovered, showing bone-shaped prolate pollen. Sculpturing over the exine is striato-reticulate or sometimes regulate. These striations run along 3–7 μm with crisscross pattern. These striations are visible over whole length except over the colpi. Colpi is protruding out and devoid of sculpturing (Figure 7a,b).

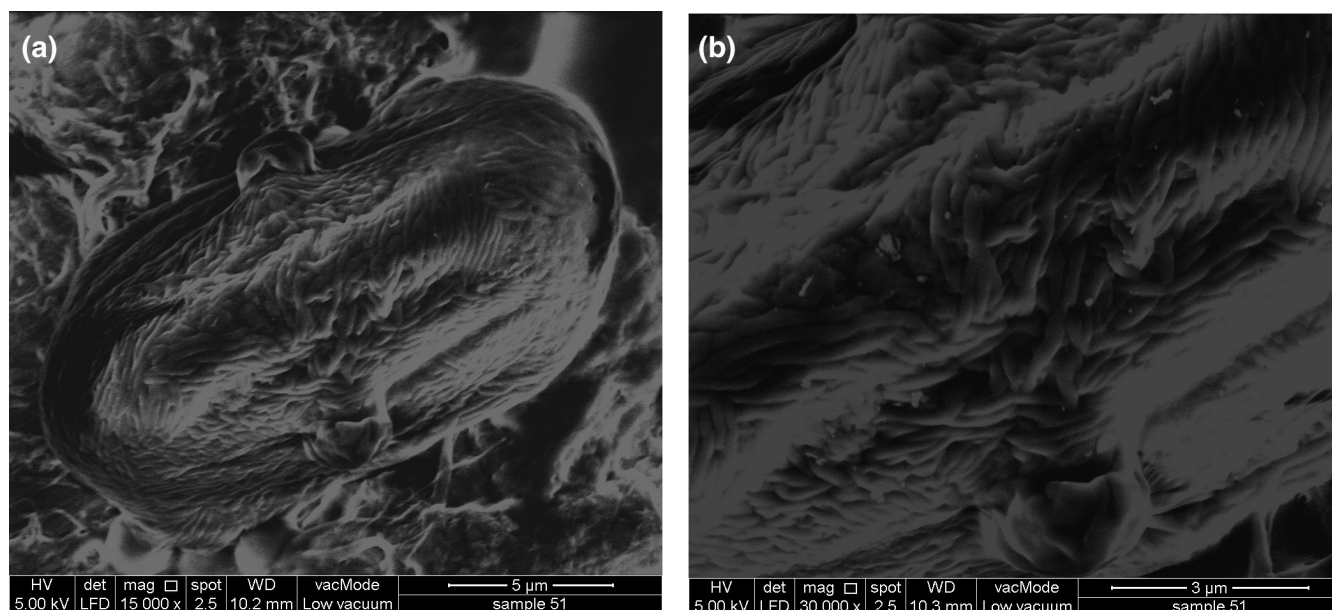


FIGURE 7 (a) *Coriandrum sativum*. (b) *Coriandrum sativum* (wall sculpturing)

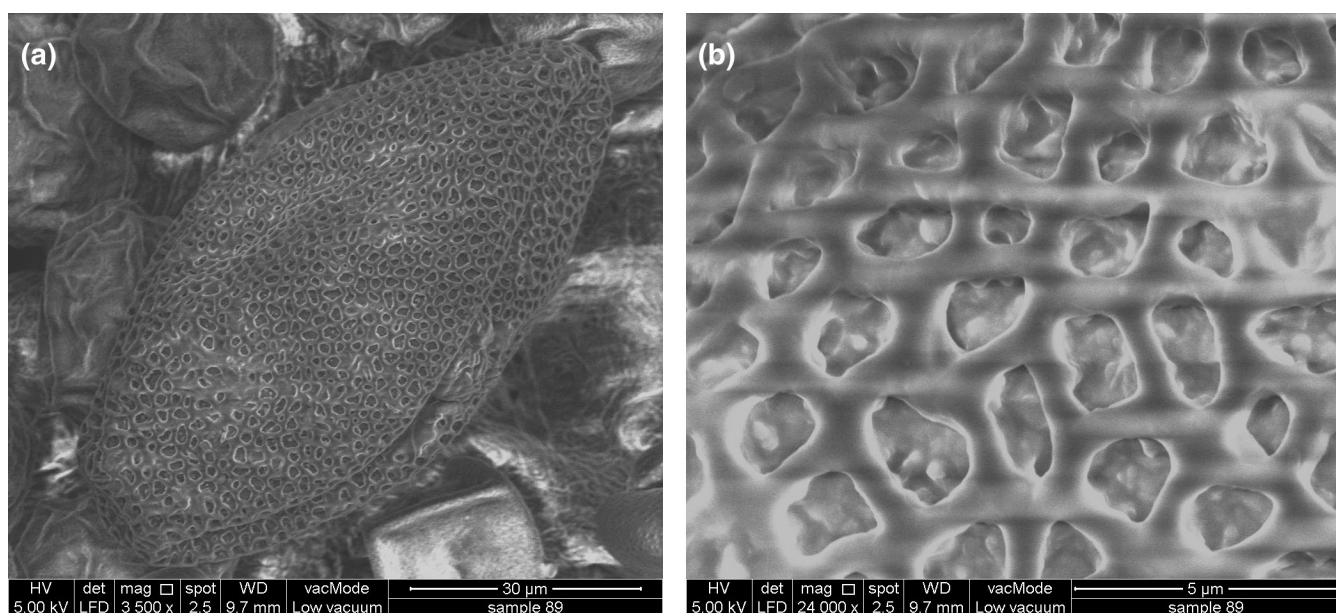


FIGURE 8 (a) *Iris sp. unindent*. (b) *Iris sp. unindent* (wall sculpturing)

3.6 | Family: Liliaceae

The present pollen (Figure 8a,b) belongs to family Liliaceae. Pollen was prolate with huge size range. Whole pollen is reticulate with prominent muri and lumen, and shapes are irregular. Papilla-like structures are visible in muri under SEM images. Colpi at the poles are narrow to broader at the equatorial region. Generally, perforation is believed to be from 0.2 to 2 μ m in diameter. Generally, pollen that belong to this family are monoletate, reticulate, and ellipsoidal (Ozler & Pehlivan, 2007). Pollen size is 90 μ m with one colpi. It extends along the whole length of pollen grain. In

Figure 8, the lumina is around 2 μ m. Reticulation is proposed to be of three types, that is, reticulate, micro reticulate, and perforate. Generally, pollen in liliaceae are monosulcate, which is in majority of monocot plants. Monosulcate condition is considered primitive.

3.7 | Family: Asteraceae

In family Compositae/Asteraceae, spines diversity and exine sculpturing are of taxonomic significance at generic and species level.

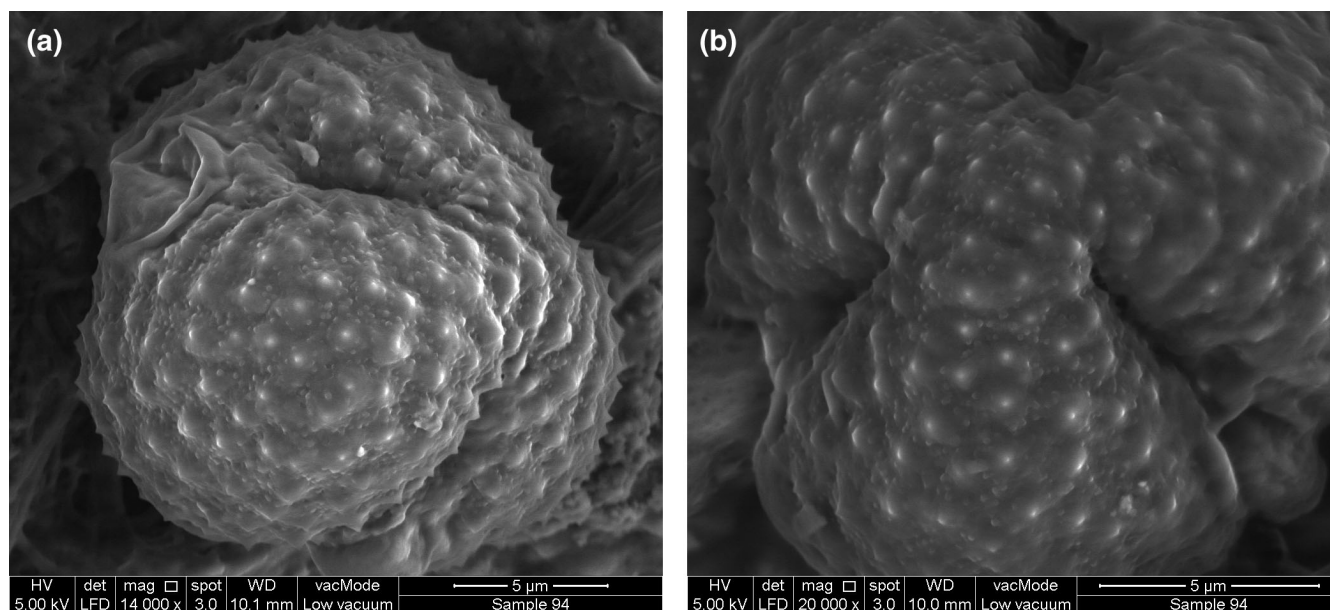


FIGURE 9 (a) *Artemisia dubia*. (b) *Artemisia dubia* (wall sculpturing)

3.7.1 | *Artemisia dubia*

The pollen in the current samples is tricolporate, with globular symmetry and reduced spines (spinules). Sexine is distinctly stratified as it is thicker in mesocolpium as compared to apocolpium, and this results in the formation of margo. Granules on exine are dispersed among the spinules. Spinules are micro-echines of less than 1 µm height. Broader base and slightly tapering toward the tip. Reduced spinules are diagnostic features of *Artemisia* (tribe Anthemideae of family Asteraceae) (Hayat et al., 2010; Martín, Torrell, Korobkov, & Vallès, 2003; Martin, Torrell, & Valles, 2001). These short spinules in the current samples are reported to be the result of evolution; primitive features were dense spinules with broad bases, granulated exine (Figure 9a,b), dispersed spinules, granulated, broad spinule bases, reduced exine, and thin colpi. These tiny spinules that are minor in size but numerous in numbers, such features are also reported by Bremer and Humpries (1993). Quantitative analysis by LM and SEM also supports the results.

A. dubia is an anemophilous plant; its pollen were observed in honey samples and are also reported allergenic (Tan, Zheng, & Tang, 1998). Present morphological features like small volume, dispersed spinules, thin colpi, and reduced spines show its shift to entomophilous mode of pollination. Its pollen were reported from honey samples collected from temperate, arid, and semiarid regions by Vallès and McArthur (2001). Martin et al. (2001) studied the pollen morphology of two members in tribe *Anthemideae* on the basis of spine size. One of the tribe with long spines is *Anthemis* type and other with short spines is *Artemisia* type. Spine length is the useful taxonomic marker for two genera segregation. Such characteristic pollen echines should be restricted to members from *Artemisia* of related aliens (Martín et al., 2003; Qasim Hayat, 2010).

Calendula arvensis pollen is observed in Figure 10, and appears close to *Calendula arvensis* on the bases of tricolporate shape, spine length,

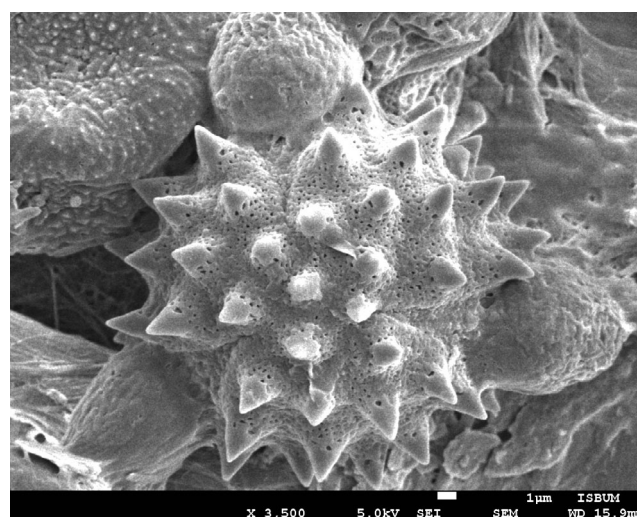


FIGURE 10 *Calendula arvensis*

perforate bases of spines, and number of spines between the colpi. Its colpi are bulging out with scabrate arrangement. Spines are four to five between colpi, which is its diagnostic feature. Pragłowski and Grafström (1980) viewed that structural exine characteristic in *Calendulae* and this tribe support consanguinity with *Heliantheae* and *Senecioneae*. However, there is a peculiar feature of spine, that is, tagellium type (perforated base), spines longer than broader, base dome shaped, tip acute, or curved apex, observed in the sample (Stanski, Luz, Nogueira, & Nogueira, 2013). Pollen in the honey sample from Sahiwal and Kashmir appear to have features as previously mentioned by people working on systematic aspects of pollen of *Helianthae*.

P. hysterothorus (Figure 11) pollen is tectate with echinate sexine. Echines have conical bases, each spine with 2 µm length and up to

3 μm width, showing much broader than longer spines and tapering toward the tip with two whorls of perforation at the bottom. At the base, spines are joined together and gradually taper toward the tips. *Parthenium* is reportedly allergenic throughout Pakistan.

3.7.2 | *Conyza bonerehensis*, *Sonchus asper*, and *Taraxicum officinalis*

All presented Lactuceae pollen types (Figure 12a–e) possess a characteristic trimerous pattern. Pollen sculpturing in both species was fenestrate/echinolophate (Fenestrate) according to Iversen and Troels-Smith (1950), whereas Wodehouse (1928) gave it a term Echinolophate. That exine is composed of elaborated system of ridges

enclosing lacunae possessing thin exine as compared to ridges. Those ridges are ornamented with sharp spines, therefore termed as echinolophate. The lacunae encompassing the pori are hexagonal in shape and termed as poral lacunae. Remaining lacunae are pentagonal and termed as abporal lacunae. Alternate to these lacunae are the paraporal lacunae. Overall, 15 lacunae are found in *Taraxicum*, especially with three poral, six abporal, and six paraporal. All three pollen are similar in texture, however, a huge size difference is observed among them: *Sonchus* size ranges up to 30 μm , *Taraxicum* is up to 25 μm , whereas *Conyza* shows more than 30 μm . Pollen class is hexazonocolporate. Pollen is lophate, having echines on the outer ridges. Lophae are perforated with numerous pores. Lacunae also appear slightly perforated. Spines are conical, sharp broad based with joined base connected to each other. No clear bases are visible. Pori protrudes out in the case of *Conyza* only.

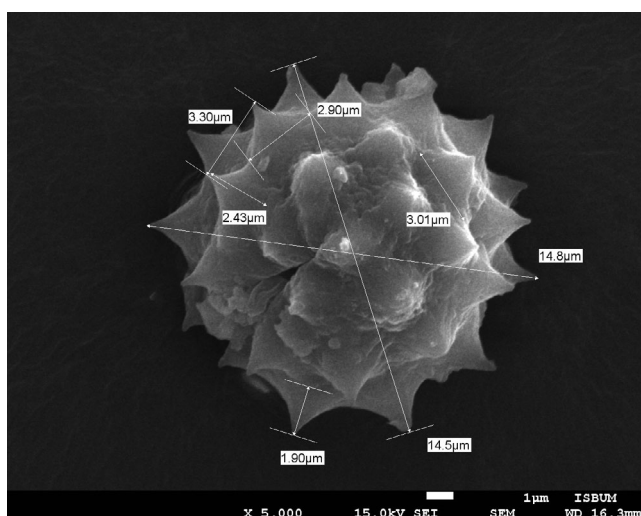


FIGURE 11 *Parthenium hysterophorus*

3.8 | Family: Myrtaceae

Common pollen features found in Myrtaceae pollen were monad, tricolporate, and angulo aperturate with angular at polar and oblate at equatorial axis. Apocolpial island is believed to be diagnostic feature (Thornhill & Crisp, 2012). Myrtaceae colpial morphology ranges from acolpial to parasyncolpate condition. Colpi develop a triangular apocolpial island if they do not meet at poles, other feature is brevicolpate in which colpi never join at pole. Another condition, Parasyncolpate appears in three forms, viz., Arcuate colpi (shape: convex, runs towards poles), Angular colpi (Apocolpial island in colpi), and Inverse arcuate (shape: concave, round away from poles). Syncolpate colpi meets at equatorial pole. Amb shapes are followed in P. view depending on endopore apex and classified as round, notched, and pointed. Apocolpial island: Ektexinous body present inside apocolpial field surrounded by margins of colpi. Apocolpial is either closely fitting

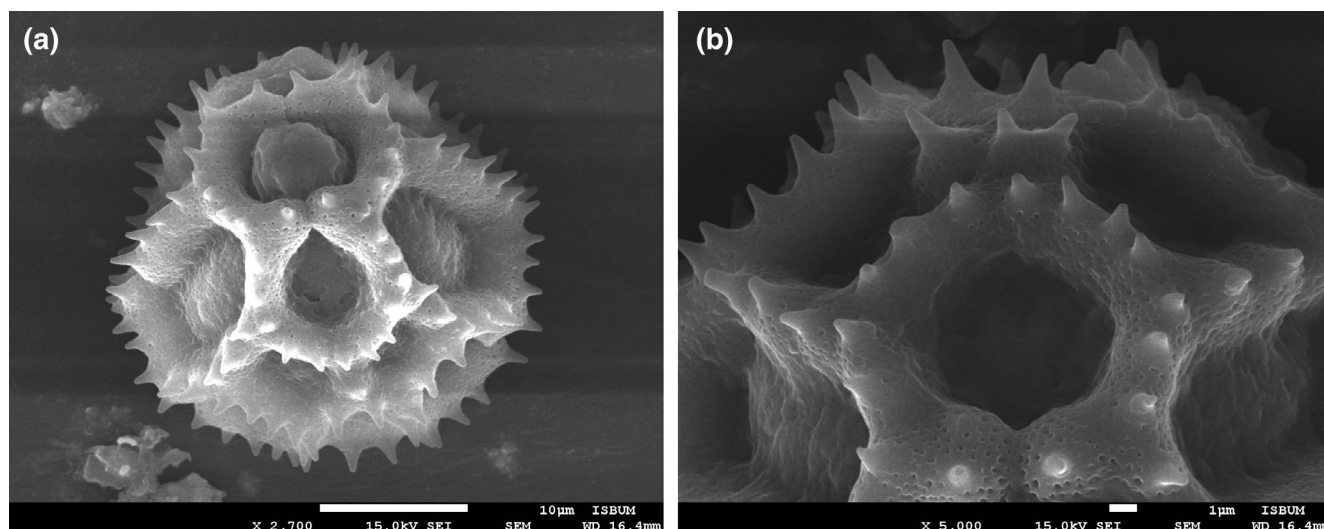


FIGURE 12 (a) *Taraxicum officinalis*. (b) *Taraxicum officinalis* (wall sculpturing). (c) *Sonchus asper*. (d) *Sonchus asper* (wall sculpturing). (e) *Conyza bonerehensis*

(ektexine piece that cover the entire apocolpial field), Small irregular (small ektexine piece that do not fill entire apocolpial field), and Globular (broken ektexine piece in apocolpial field).

E. globulus (Figure 13) pollen is tectate-rugulate. Margo is thick around the edges, however, in the center, colpi and margo are merged. Amb straight. Exine scabrate perforates syncolpate grain. Angular colpi are present. Apocolpial island is absent. Eucalyptus pollen size, exine, mesocolpial and apocolpial fields exine on mesocolpia, and margo are diagnostic features for separating *Eucalyptus* from other Myrtaceae pollen (Pickett & Newsome, 1997) while Eliseu and Dinis (2008) are of the view that typical *Eucalyptus* could be identified by characteristic triangular shape, parasyncolpate colpi, radial symmetry, and a distinct apocolpial fields.

C. citrinus (Figure 14) exine is tectate scabrate perforate. Colpi syncolpate. This ornamentation continues throughout meso and apocolpium, arcuate colpi, syncolpate, apocolpial island present, amb convex, apocolpial island globular. Apocolpia in *Callistemon* agrees with Pike (1956).

3.9 | Family: Pinaceae

Nakagawa, Yasuda, and Tabata (1996) believe that SEM is useful for identification of Himalayan *Pinus* pollen grains. Two types of pollen have been found in common in the Himalayas from where these honey samples were collected, namely *P. roxburghii* and *P. wallichiana*.

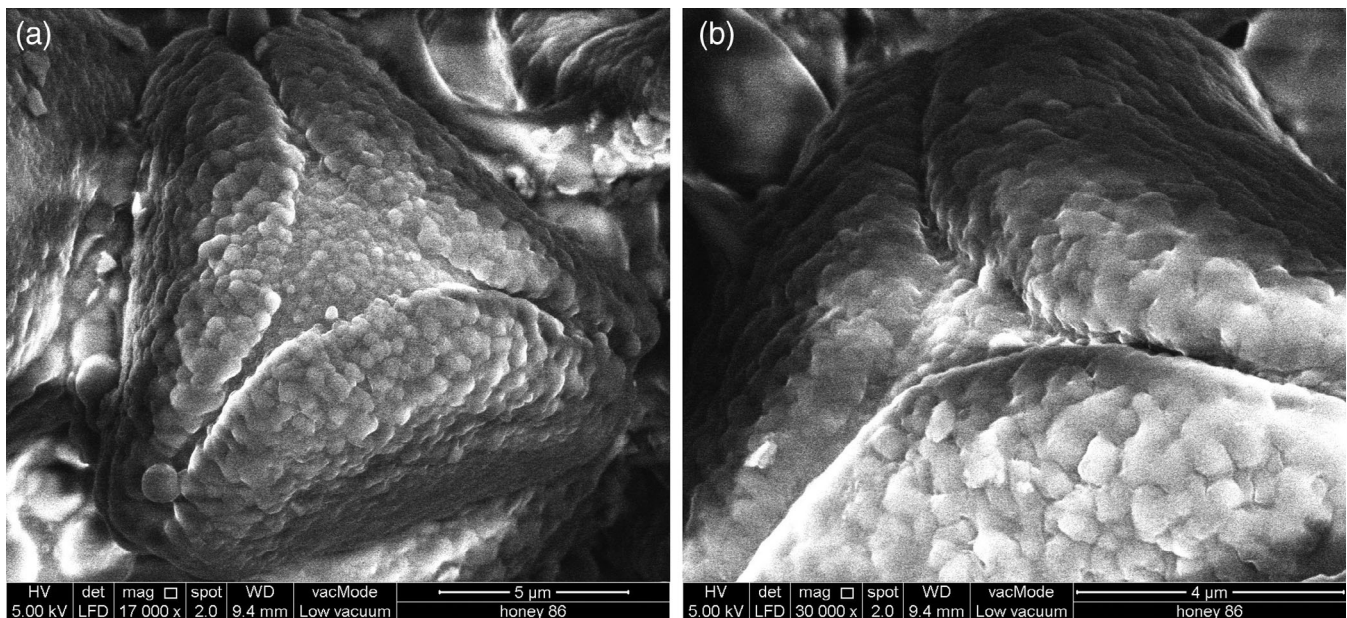


FIGURE 13 (a) *Eucalyptus cammoldulensis*. (b) *Eucalyptus cammoldulensis* (wall sculpturing)

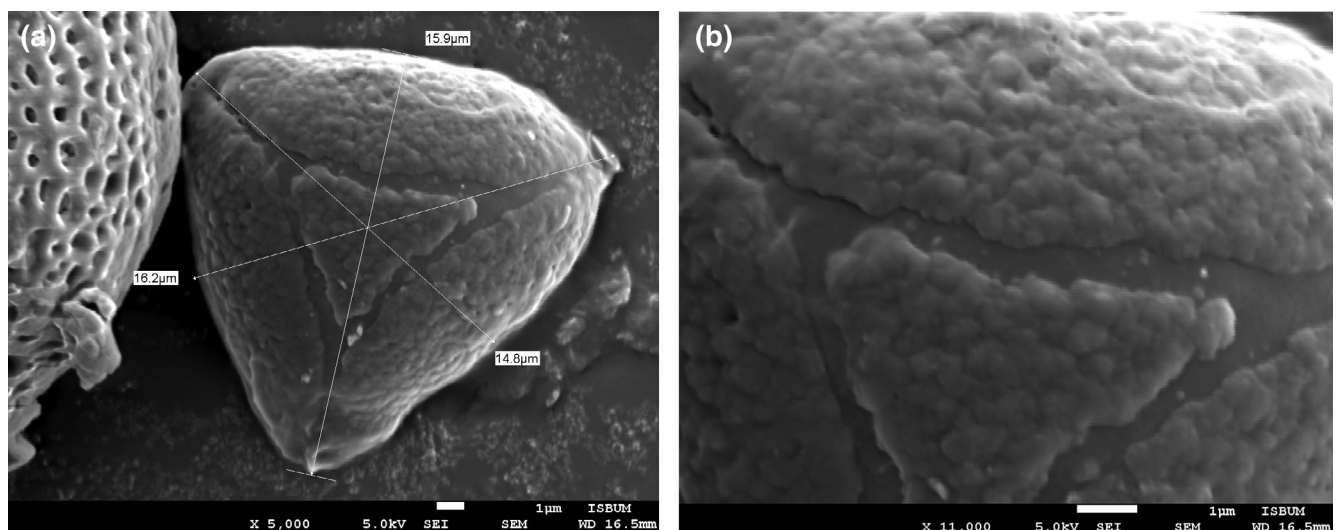


FIGURE 14 (a) *Callistemon citrinus*. (b) *Callistemon citrinus* (wall sculpturing)

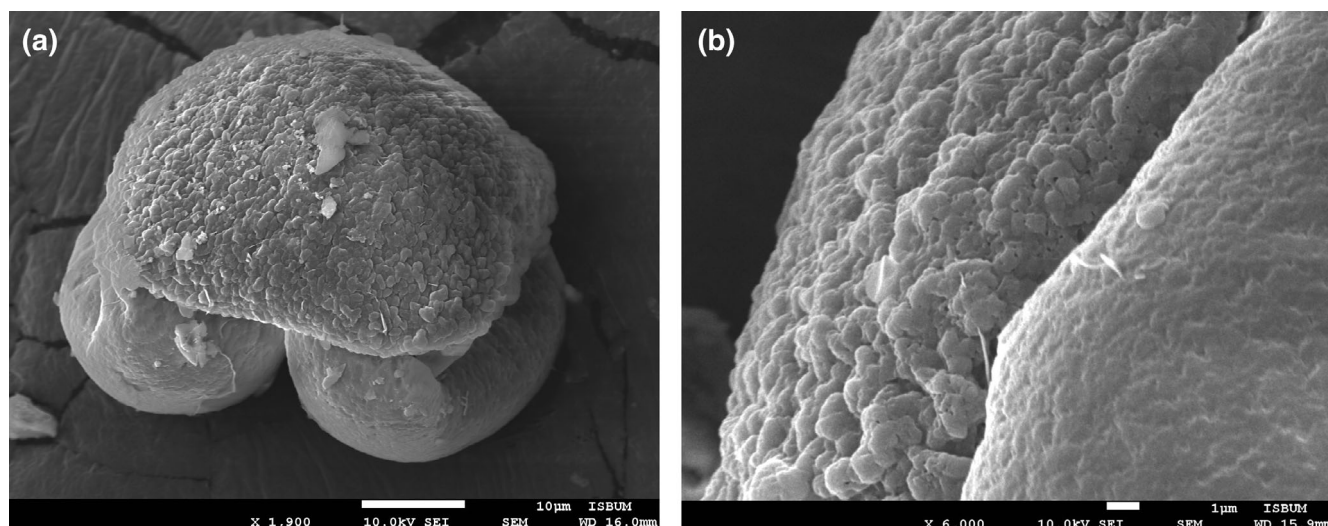


FIGURE 15 (a) *Pinus roxburghii*. (b) *Pinus roxburghii* (wall sculpturing)

Both of the species have bisaccate pollen (Nakagawa et al., 1996). With breadth of sacci of 46–53 μm and difference in exine of sacci and cappa exine patterns, also their point of attachment is sharp. The present pollen shows similar features to *P. roxburghii*. As leptoma and sacci have a smooth exine, very fine pinholes are scattered on the sacci while in case of *Pinus wallichiana*, exine on Leptoma and sacci is psilate. The exine sculpture of *P. roxburghii* (Figure 15a,b) is clearly rugulate and that of *P. wallichiana* is smooth or slightly rugulate.

3.10 | Family: Rutaceae

Pollen morphology in Rutaceae family has been extensively studied (Erdtman, 1952; Perveen & Qaiser, 2005), and observed their taxonomic significance at different levels of classification. Major diagnostic feature in citrus pollen is its shape and exine type, shape is generally round/radially symmetrical, isopolar, and colpate (4–5 in number). Exine width extends to 3 μm , and diameter falls between 22 and 45 μm . Four types of exine sculpturing are reported by Al-Anbari, Barusrux, Pornpongrungrueng, and Theerakulpisut (2015), who classified exine types into four types, viz., Type I: Macro reticulates at P & Eq. view. and perforate at P. axis. Type II: Macro reticulate at both P & Eq. sections. Type III: Micro reticulate at both P & Eq. axis. Type IV: Perforate at P & Eq. view. In *Citrus* also apertures have a vital role. In the present study, pollen exine appears as macroreticulate. Colpate is long and narrow, running through whole axis and ending at acute edges. Colpi is interrupted by pori in the middle. Pori protruding out. Exine thick around forming, and margocolpi thickened around edges. Exine section in colpi is scabrate. Lumina, more or less spheroidal, around 1 μm , with muri of psilate nature. There are some different views about sculpturing, that is, Yunus and Nair (188) observed rugulate-striate and rugulo-striate-reticulate ornamentation in Rutaceae and express its significance in distinguishing different genera (Figure 16a,b).

3.11 | Family: Fabaceae

3.11.1 | *Acacia nilotica*

Each pollen is 12–16 celled polyad (Figures 17 and 18). Each cell is spherical to rectangular with size ranging from 11.9 to 13.7 μm , perforation in cells is in nanometers and irregularly arranged. Pollen ornamentation is foveolate. Guinet (1981) and Perveen and Qaiser (1998) also agree with 12–16 cells in its pollen with foveolate sculpturing. Cells are compactly arranged with definite geometry. Ahmed (2015) reported 16 wedge-shaped cells in polyad pollen in *Acacia nilotica* with size range of 39.8–44.3 μm and mean diameter of 42 μm . The present results agree with her results. *Acacia* belongs to Mimosoideae family with eurypalynous taxon. Pollen, generally in this family, are monad to polyad with isopolar, oblate to spheroidal pollen with thick sexine. In the present study, pollen in Figure 17 appears similar to *Mimosa hamate* but the difference in cell number confirms it as *A. nilotica* (Guinet, 1969). Large-size cells are reported in *A. nilotica* type, this agrees with the present identification. Pollen in Figure 17 shows polyad with above 32 cells in a polyad. Cells in polyad are squarish with similar size and irregularly arranged in definite geometry. The surface of pollen appears undulate under LM. Foveolate-rugulate sculpturing with sculpturing consisting of more or less rounded depressions or lumina more than 1 nanometer in diameter and depressions are making irregular arrangements. Six different types of *Acacia nilotica* are reported in Flora of Pakistan, viz., *A. nilotica* ssp. *astringens*, *A. nilotica* ssp. *subalata*, *A. nilotica* ssp. *cupressiformis*, *A. nilotica* ssp. *indica*, *A. nilotica* ssp. *nilotica*, and *A. nilotica* ssp. *hemispherica*.

3.11.2 | *Trifolium repense*

Exine is reticulate and scabrate except on the membrane of colpi where exine is scabrate. Colpus is extended on both ends of pollen

with exine thickness below 2.5 μm . Koçyiğit, Daştan, Koçyiğit, Keskin, and Daştan (2013) agree on the same results while working on 16 taxa from *Trifolium* of beekeepers significance. They have fine to coarse reticulate exine, forming an area like a lip around colpi. The current pollen description also agrees with Moore, Webb, and Collison (1991) that muri and lumina are generally wider in *Trifolium* species and the shape of reticulum reduces or absent at the poles. In the current study, reticulum is absent at the poles. Apocolpium is large. *Trifolium* (Figure 19a,b) is believed to have elliptic endopori apocolpia convex and colpi without granules.

3.12 | Family: Betulaceae

Among the members of family Betulaceae, three closely related subgenera of *Alder* are *Alnobetula*, *Alnus*, and *Clethropsis* (Leopold et al., 2012). For pollen identification, the number of pori, polar archi, pollen diameter, and arci thickness are proposed as diagnostic features. Different palynologist prefer certain characteristics over the other Wodehouse (1959) stresses the thickening of surrounding of pori as a distinctive feature. This thickening extends from pori to pori. Erdtman (1963) proposed that polar arch and swollen arcus around

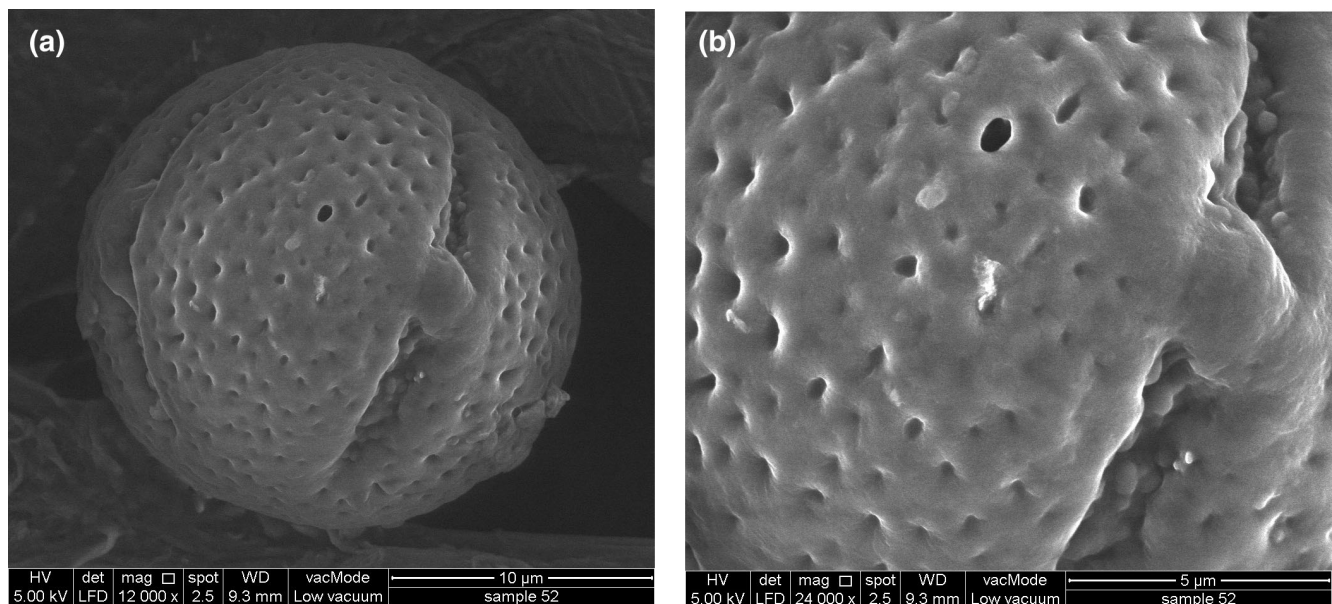


FIGURE 16 (a) *Citrus arvensis*. (b) *Citrus arvensis* (wall sculpturing)

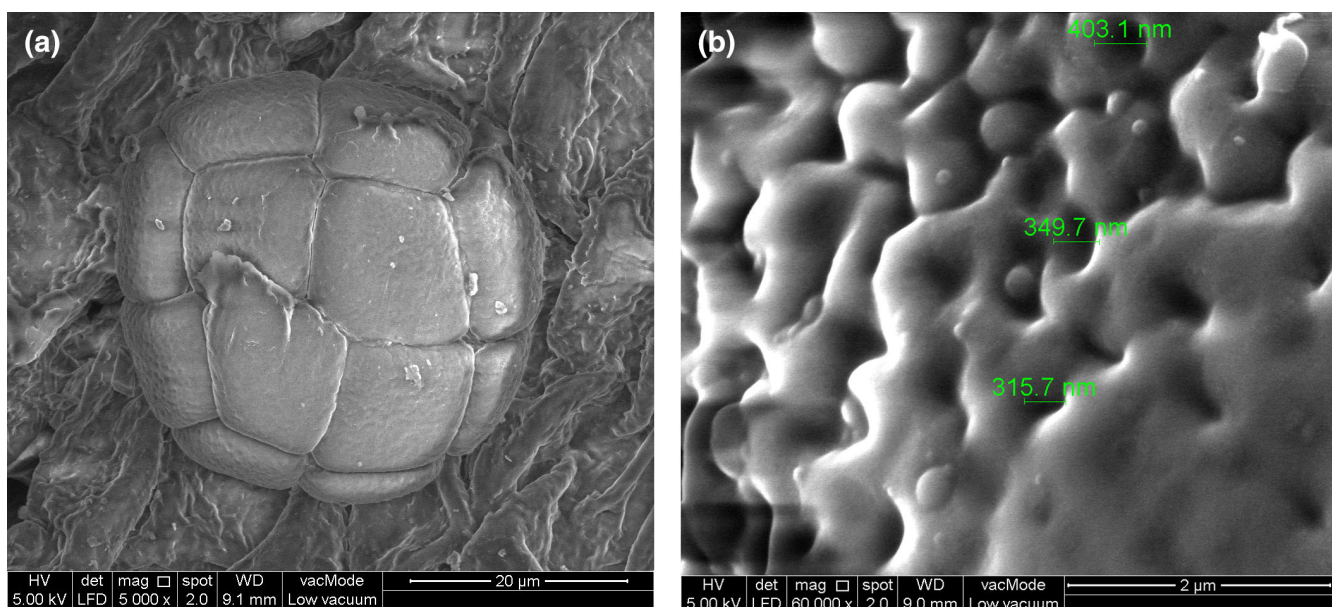


FIGURE 17 (a) *Acacia nilotica*. (b) *Acacia nilotica* (wall sculpturing) [Color figure can be viewed at wileyonlinelibrary.com]

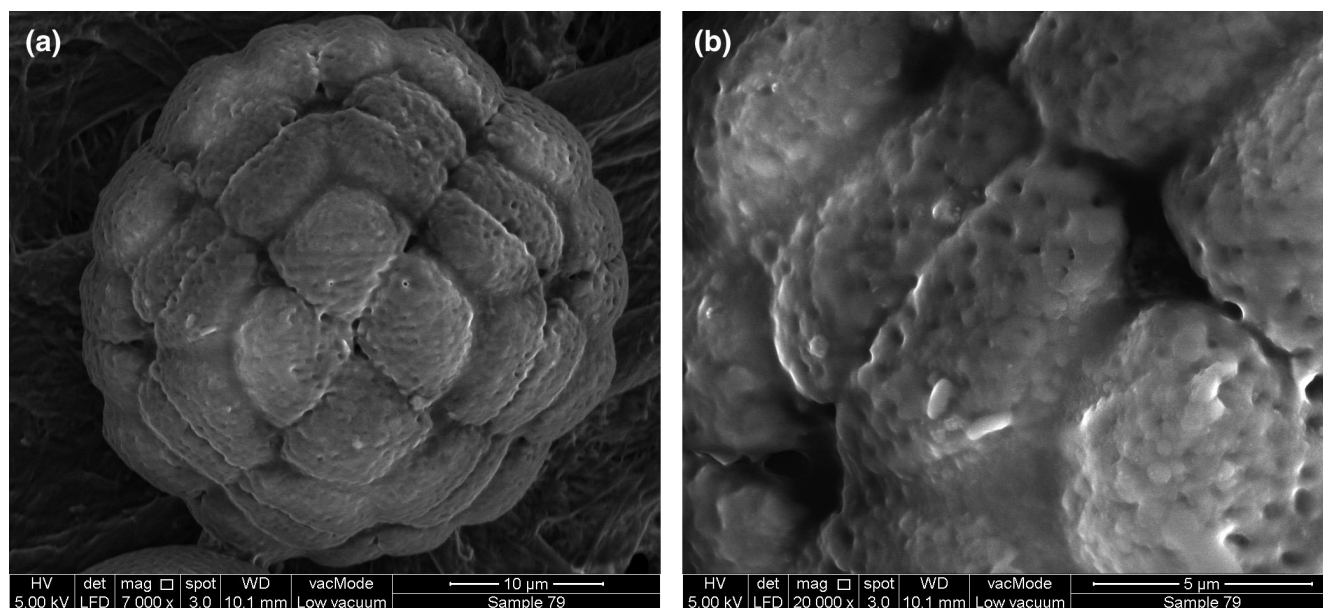


FIGURE 18 (a) *Acacia modesta*. (b) *Acacia modesta* (wall sculpturing)

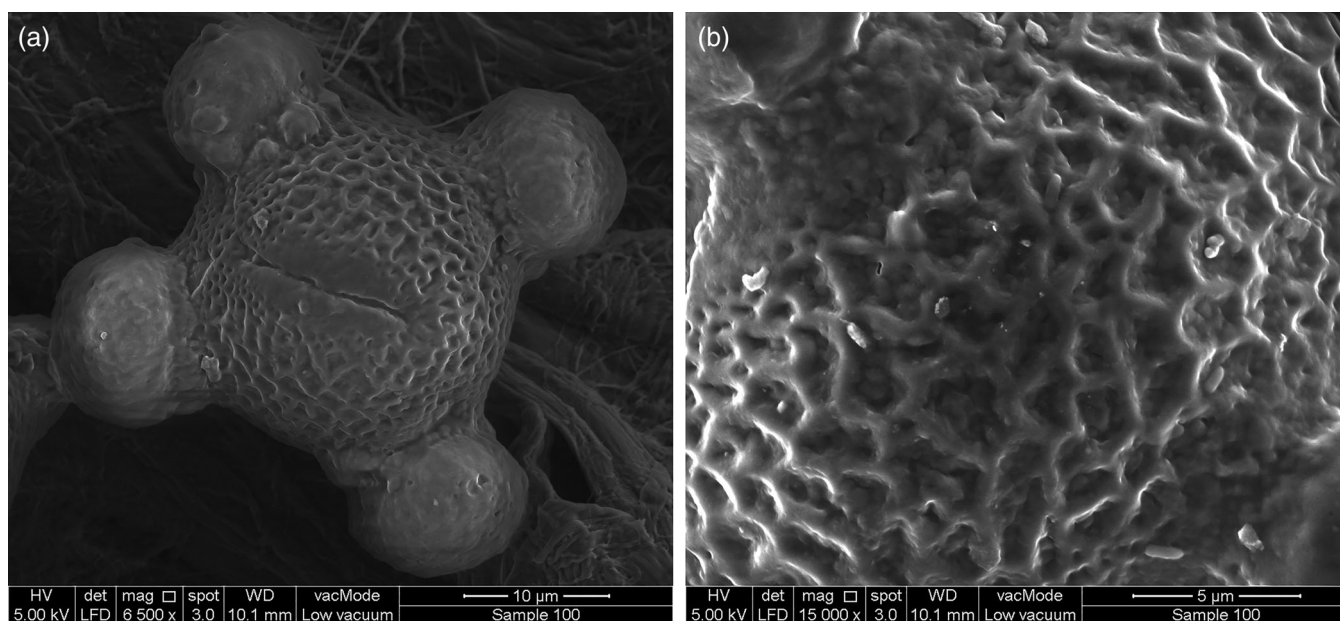


FIGURE 19 (a) *Trifolium repense*. (b) *Trifolium repense* (wall sculpturing)

the polar region are the diagnostic feature. After measuring pollen size and arcus width of 100 species of *Alnus* found in Kashmir Vishnu-Mittre and Sharma (1963) believes that size measurement and arcus width have no real significance. Dispersal pollen is impossible to identify through pollen size. Aspid was also found not significant.

They believe that the numbers of aperture are more reliable at species level. *Alnobetula* has 5, 6, or 7 pored pollen while *Alnus* have 4 or 5 pored pollen. *A. nitida* and *A. nepalensis* four pori have been reported in Pakistan and the Himalayas. Both of the species possess 3–5 apertures in *A. nitida* and 4–5 in *A. nepalensis* (Vishnu-Mittre &

Sharma, 1963). Blackmore, Steinmann, Hoen, and Punt (2003) identified that *Alnus* are distinguished by vestibulate and protruding pori and thickened pore, those thickness vary among different species (Mayle, Levesque, & Cwynar, 1993). The most distinctive feature of *Alnus* pollen is the presence of thickenings of the ectexine surrounding the pores (Blackmore et al., 2003). The current pollen samples possess 4–5 apertures showing its similarity to *A. nepalensis*. The morphology of the *Alnus* grain is presented by many authors (Blackmore et al., 2003; Erdtman, 1966a, 1966b; Erdtman, Berglund, & Praglowski, 1962) and recently by Reinink-Smith (2010).

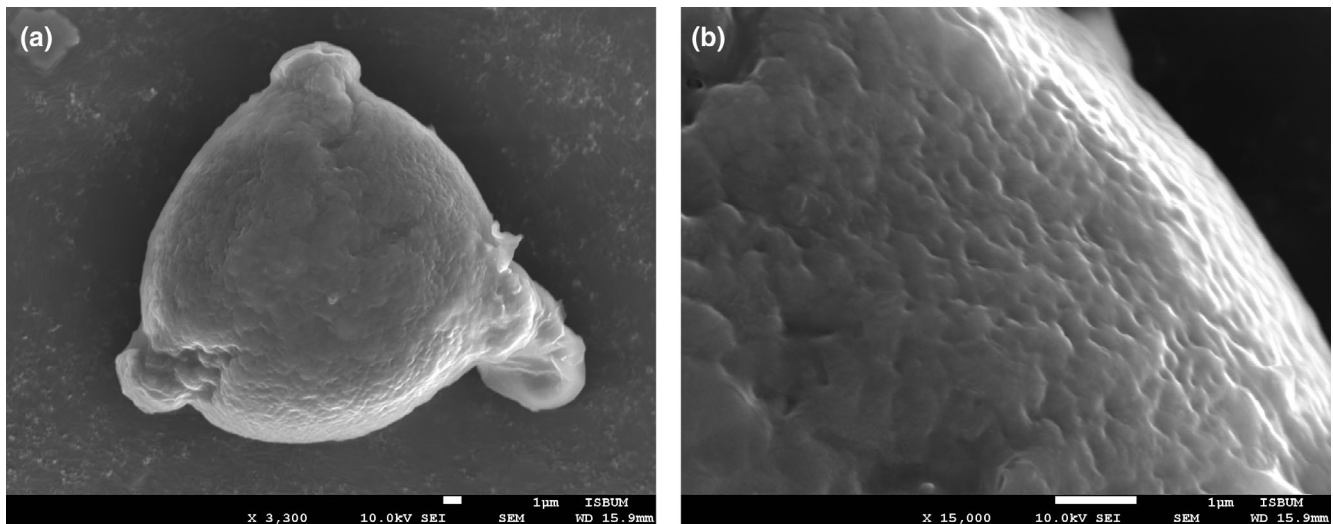


FIGURE 20 (a) *Rubinia pseudoacacia*. (b) *Rubinia pseudoacacia* (wall sculpturing)

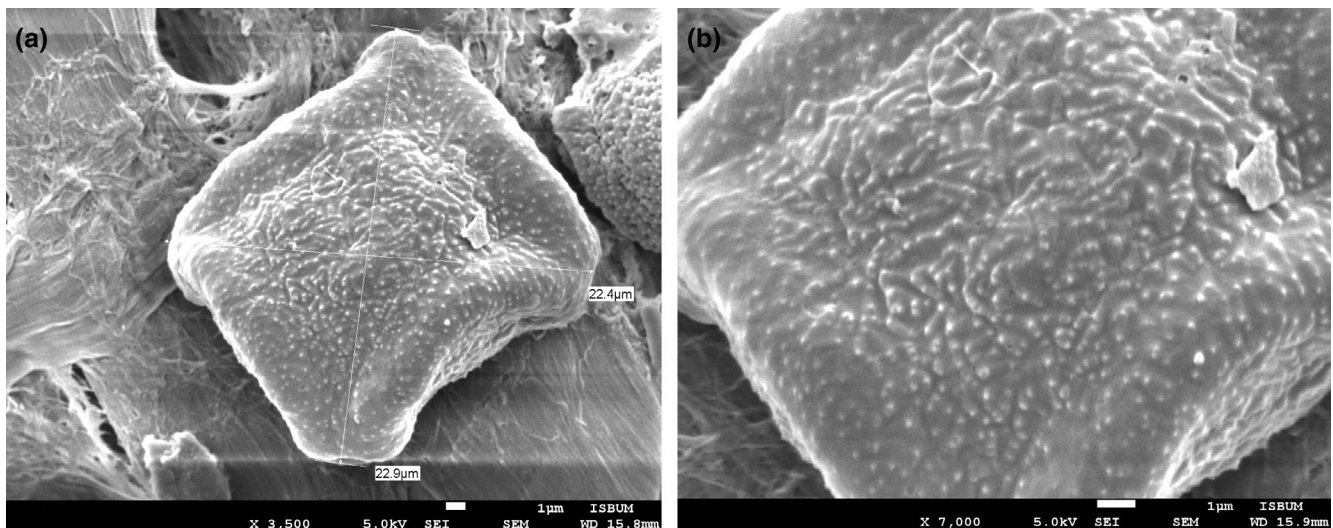


FIGURE 21 (a) *Alnus nitida*. (b) *Alnus nitida* (wall sculpturing)

Erdtman (1963) revealed polar archi in some species of *Alnus*, for example, *Alnus glutinosa*. Leopold et al. (2012) believe polar archi are rare in *Alnus*, and our pollen features do not agree with it. The present results agree that *Alnus* (Figure 21) pollen are psilate in light microscope, while in SEM, they appear scabrate. Wittborn, Rao, El-Ghazaly, and Rowley Jr. (1996) also express the same observation like short, irregular conical micro-rugulate ridges. Such micro-rugulate ridges are also present in the present sample. Exine thickness ranges between 1 and 1.5 μm in inter-arcus regions while in arcus it gets thickened more than 1.5 μm . Exine is split into ectexine and endexine, and they run parallel, but in polar region they are split to develop vestibular structure (Faegri, Kaland, & Krzywinski, 1989). The current samples also show such features. At the pore margin, the separation of the ectexine from the endexine creates a vestibule (Faegri et al., 1989), which is well developed in certain species (e.g., *Alnus lantana* and

Alnus incana). Vestibules are clearly observed in figure. Here, neighboring pori are connected to each other by arches, and those arches are nexine thickness with vestibulate pore (Moore et al., 1991). Pollen archi are also reported showing a donut-shape structure. Erdtman (1966a, 1966b) explains pollen wall as tegillate with thicker ectexine and thinner inner side (Figure 21a,b).

3.13 | Family: Brassicaceae

Ornamentation in the pollen is reticulate with muri having more or less regular pattern or reticulate-rugulate (Figure 22). Exine is semi tectate. Tricolpates with colpi are deeply sunken, having sharp acute ends, and colpi area is granulated and scabrate. No extension of reticulate sculpturing in colpi area, instead it appears scabrate. Lumina

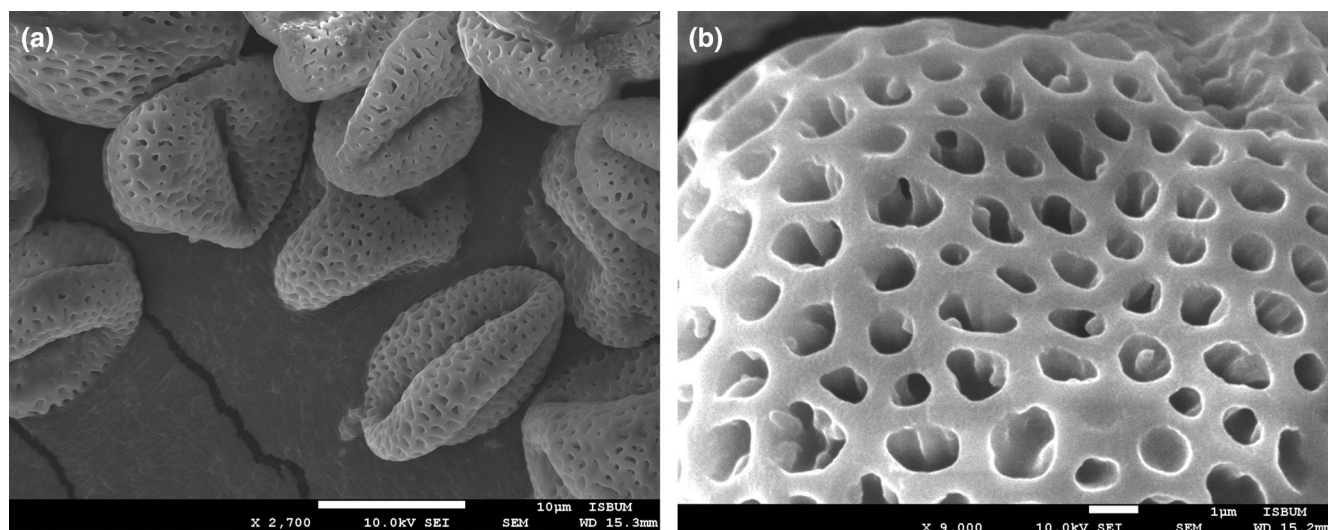


FIGURE 22 (a) *Brassica campestris* P & Eq. View. (b) *Brassica campestris* (wall sculpturing)

diameter ranges from 1 to 2.5 μm . Studies done on brassicaceae report it as stenopalynous family by Erdtman (1952). Parveen and Qaiser also confirm the homogenous nature of the family. Generally, its pollen shape ranges from subprolate to spheroidal. Majority of the genera are tricolporate. Moore and Webb, Appel and Alshehbaz reported the tricolporate and reticulate sculpturing in pollen. Its sculpturing is established as reticulate ranging from fine to coarse. The present results (Figure 22a,b) agree with Moore and Webb (1978), and they classify Brassicaceae pollen as having reticulate sculpturing with tricolporate grain. Khan (2003) is also agreed on reticulate sculpturing of Brassicaceae members.

3.14 | Family: Meliaceae

Meliaceae is stenopalynous family. In honey analysis of Adekanmbi and Ogundipe (2009), Paudyal and Gautam (2012), Freitas and Novais (2014), Nnamani and Uguru (2013) identified meliaceae in honey samples from Nepal, Southern Nigeria, Brazil, and Uganda. The identified tetracolporate and perforate pollen of meliaceae in the honey samples. It looks more close to *Melia azadirachta*, compared to *Azadirachta* species, *Melia* has numerous pores over surface also termed as foveolate (pore diameter less than 1 μm) with definite spheroidal structure as compared to *Azadirachta*, which has few perforations and indefinite structure. Colpi are narrow and long with bulging pori in the middle. Exine portion over colpi and pori is psilate. The end of colpi merges into surrounding sexine (Ora lalongate) (Figure 23a,b).

3.15 | Family: Oleaceae

Reticulum coarse, muri simpli-columellate is very thick, with the depth of muri is >length of thin short columella. Phyllyrea is similar grain but with more lumina across a mesocolpium than *Olea*. *Olea* shows fewer

lumina. *Fraxinus* has fine reticulum, muri with simpli-columellate but with duplicolumellate area at the intersection of muri. Muri depth is less than columella. *F. excelsa* and *F. ornus* have distinct thick sexine relative to nexine and longer colpi as compared to polar axis.

In the current honey sample, pollen extracted were ricolporate pollen with size range of 41 μm in P view and 45 μm in Eq. view. Its tectum is scabrate and very coarse, muri is always simpli-columellate and very thick and deep—depth of muri > length of the thin short columellae. Colpi exine reticulates. Colpi protruding out (Figure 24a,b).

3.16 | Family: Poaceae

A large number of studies are reported from around the world in the past for determining the significance of Poaceae pollen at different levels of hierarchy. Pollen size and sexine pattern were given preference in the past along with pore diameter, width of annuli, and orientation of pori were correlations at generic and major-group levels. Scanning electron microscopy seems unlikely to help significantly in identifying grass pollen in field or atmospheric samples, though it may be helpful in checking the identity and homogeneity of samples intended for medical use (Watson & Bell, 1975).

Poaceae pollen are considered to be differentiated on basis of size and sexine pattern. Size is the key feature to differentiate between cereal grains and wild grasses. Exine appeared in two forms, scabrate to areolate. Scabrate is composed of punctate structure or single granules, and areolate is composed of maculae or group of several granules (Page, 1978).

3.16.1 | *Avena sativa*

Exine brevi-ornate, undulate. Polygonal insulae studded with a number of spinules (small with diverse orientation) separated by narrow

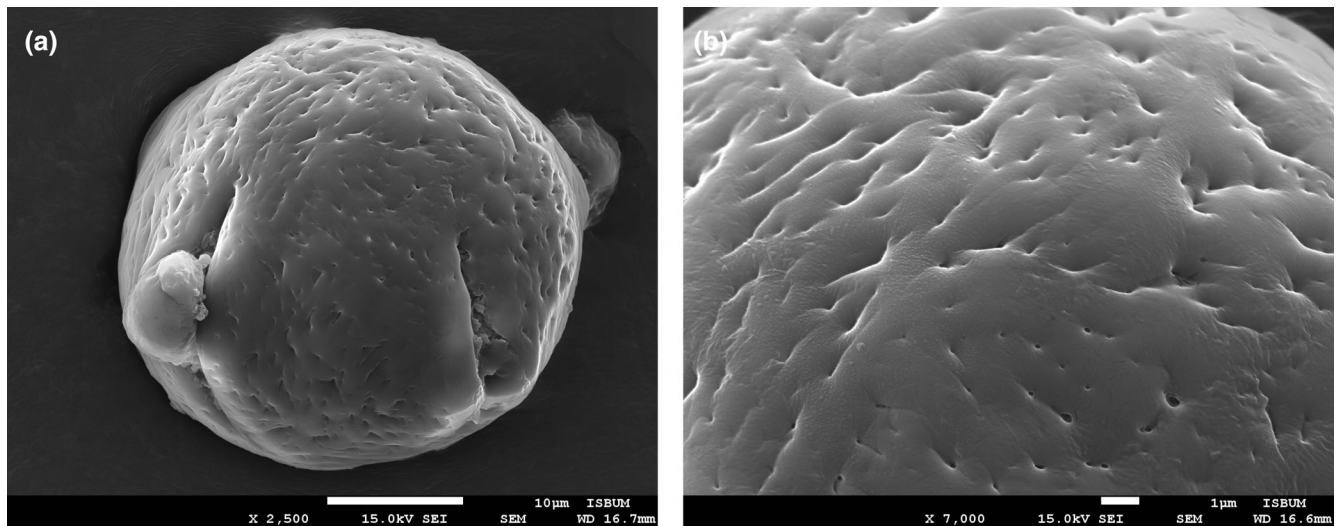


FIGURE 23 (a) *Melia azedarach*. (b) *Melia azedarach* (wall sculpturing)

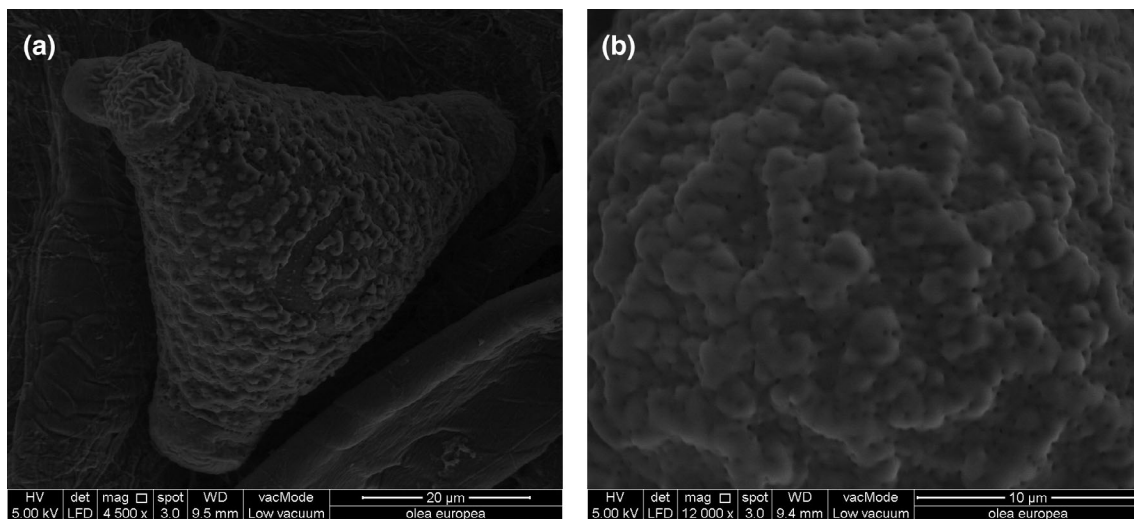


FIGURE 24 (a) *Olea europea*. (b) *Olea europea* (wall sculpturing)

clear, broad grooves, on annulus the exine insulae are aggregated (Figure 25). This type of sculpturing is also classified as areolate-verrucate type by Faegri and Iversen (1975). The shape of *Avena* is typical and not spheroidal (Figure 25a,b).

3.16.2 | Cymbopogan type

Irregular-spheroidal to polygonal. Insulae studded with few minute spinules. Exine undulate appears as reticulate under LM (Figure 26a,b).

3.16.3 | Hordeum vulgare

Echinulate, spinules are single, detached, and not grouped into insulae (Figure 27). Spinules are single, detached, and not grouped into insulae.

The surface of pollen appears as undulate or irregular or granulated under LM, such pollen is considered as *Hordeum* type by Watson and Bell (1975). The surface appears regulate or reticulate pattern because of absence of insulae and presence of scabrae. Köhler and Lange (1979) also agree with this identification of *Hordeum* having individual detached spinules not grouped in insulae, they appear as reticuloid under LM and such spinules are aggregated and more closely connected (Figure 27). Andersen and Bertelsen (1972) also pointed out smooth surface with crowded spinules, undulated exine in *Hordeum*.

3.16.4 | Pennisetum glaucum

Spinules are detached and not grouped, spinules are large closely placed near each other, and the size of spinules is not definite, ranging up to 0.5 μm (Figure 28). The shapes of pollen are not spheroidal,

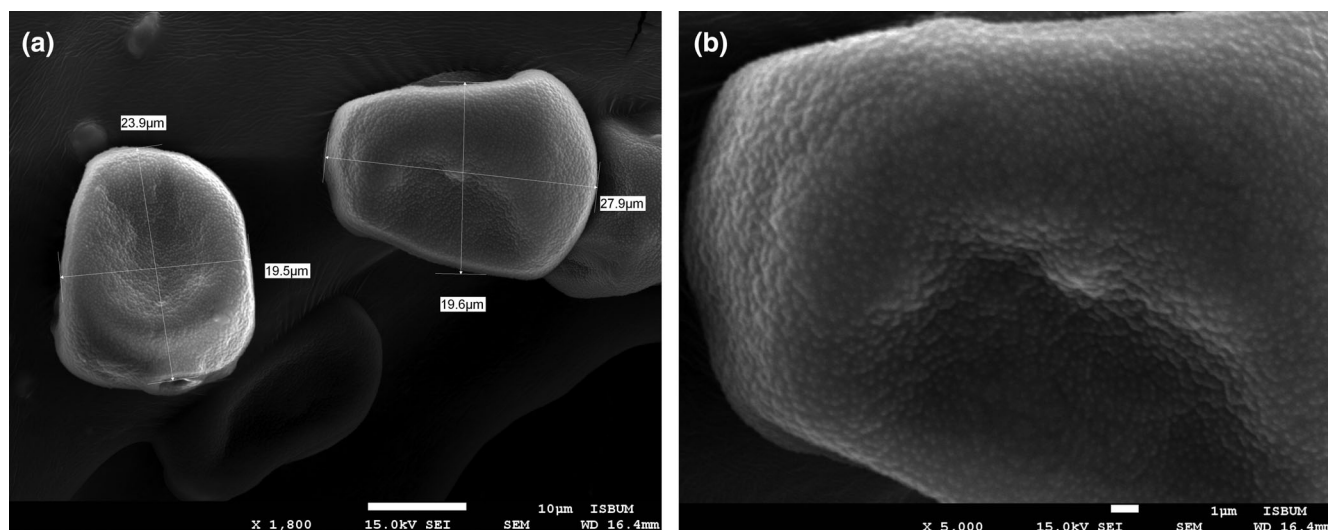


FIGURE 25 (a) *Avena sativa*. (b) *Avena sativa* (wall sculpturing)

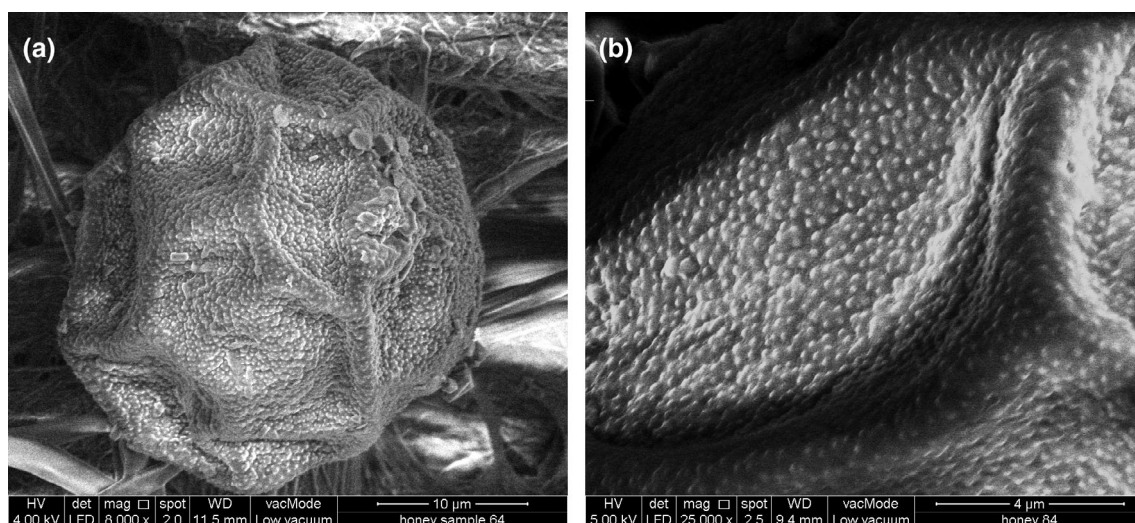


FIGURE 26 (a) *Cymbopogon* sp. indent. (b) *Cymbopogon* sp. indent (wall sculpturing)

instead it appears oblate. Among the pollen extracted from honey samples, major pollen allergy-causing plants reported from various areas of Pakistan are *Acacia* spp., *Amaranthaceae* spp., *Asteraceae* spp., *E. globulus*, *Melia azedarach*, *Brassica campestris*, *Cyperus* spp., *C. citrinus*, *Syzygium cumini* and members of family *Poaceae*, *Amaranthaceae*, *Chenopodiaceae*, and *Cyperaceae*. *P. roxburghii*, *Cannabis sativa*, *P. hysterophorus*, *Artemisia* species, and *C. album* are reported as allergenic airborne pollen (Anjum Perveen & Sad-ul-Islam, 2007; Ghufuran et al., 2013; Hasnain et al., 2012; Parveen et al., 2012; Perveen et al., 2015). Parveen et al. (2012) reported pollen types from *Amaranthaceae*, *Asteraceae*, and *Gramineae* families as allergenic in Tandogam Sindh. *Acacia* spp., *C. citrinus*, *Azadirachta indica*, *Cyperus* sp., *Brassica campestris*, and species from *Myrtaceae*. *Poaceae* shows the highest concentration followed by *Chenopodiaceae*/*Amaranthaceae*, *Cyperus*, *Eucalyptus*, *Typha*

angustifolia, *Brassica campestris*, and *Leucaena leucocephala* constitute the pollen spectrum of allergenic plants of Karachi (Shinwari, Tanveer, Yusuf, Perveen, & Khan, 2015), and their reports also include members from the family *Fabaceae*, *Amaranthaceae*, and *Chenopodiaceae*. *Poaceae* pollen constitute major contributor of airborne pollen followed by *Mimosaceae*, *Cyperaceae*, and *Amaranthaceae*/*Chenopodiaceae* in a study done in Karachi by Anjum Perveen and Sad-ul-Islam (2007). *Amaranthus viridis* (*Amaranthaceae*), *Atriplex stocksii* (*Chenopodiaceae*), *Salsola barysoma* (*Chenopodiaceae*), *Cynodon dactylon* (*Gramineae*), *Sorghum halepense* (*Gramineae*), *Secale cereal* (*Gramineae*), *Prosopis juliflora* (*Mimosaceae*), *Plantago ovata* (*Plantaginaceae*), *C. album* (*Chenopodiaceae*), *Eucalyptus* sp., and *Acacia nilotica* (*Mimosaceae*) are reported positive in skin prick test. Fungal spores were found to be another important source of respiratory allergies among children and adults.

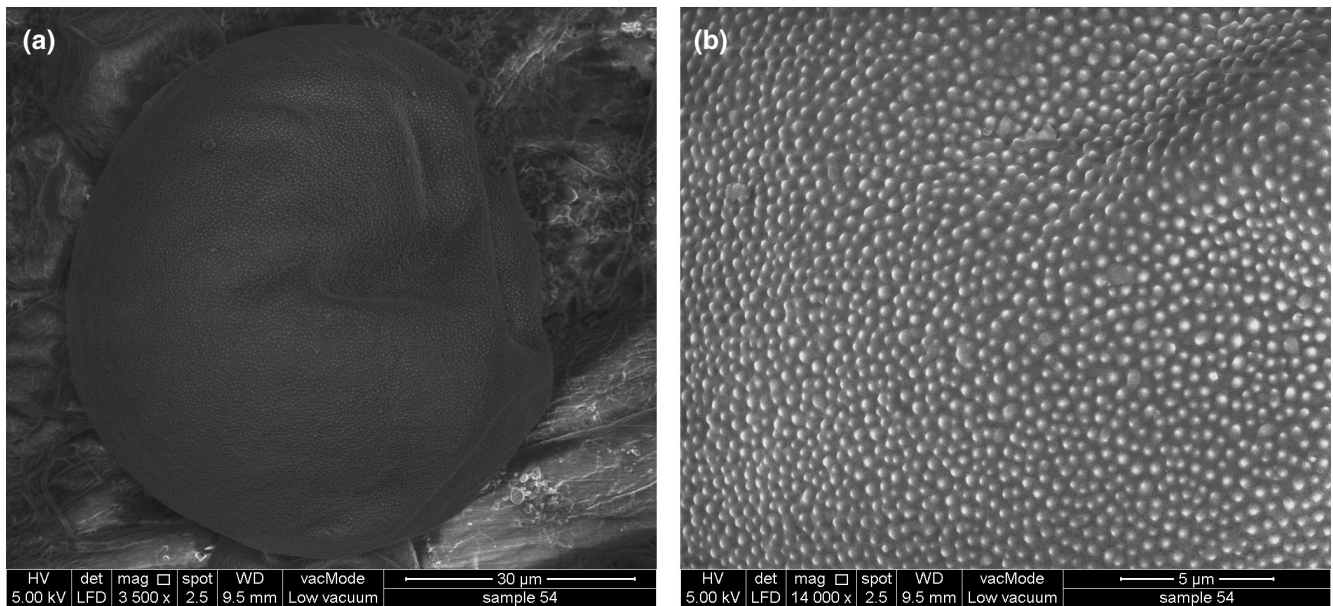


FIGURE 27 (a) *Hordeum* sp. indent. (b) *Hordeum* sp. indent (wall sculpturing)

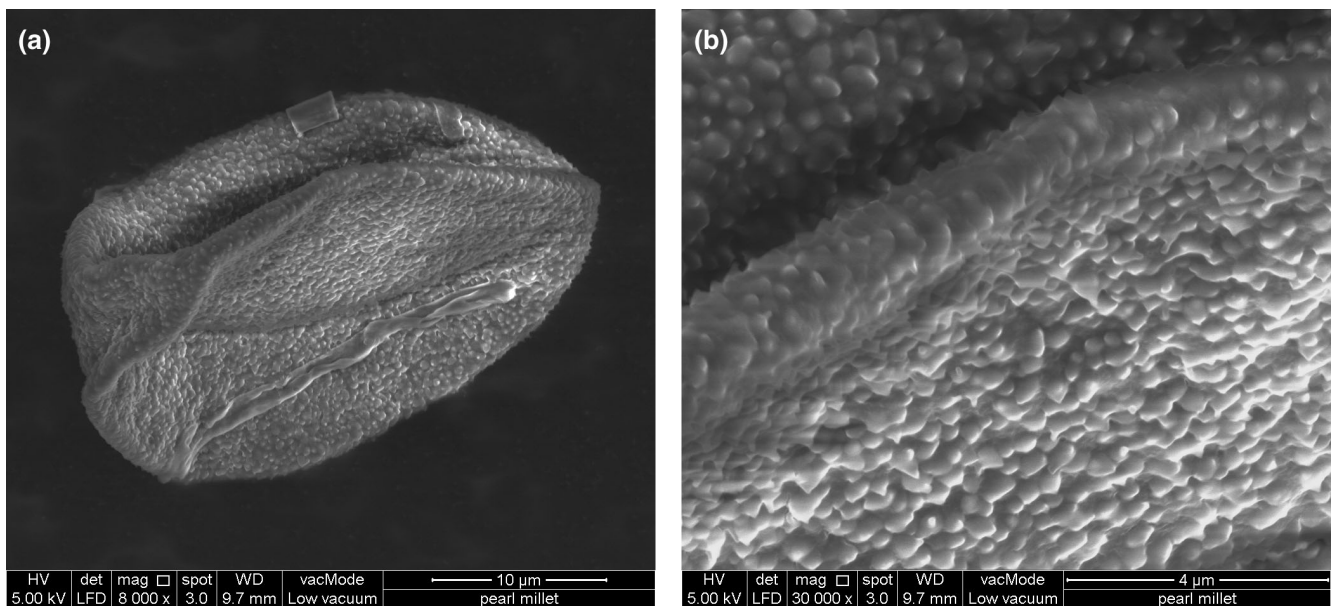


FIGURE 28 (a) *Pennisetum typhoides*. (b) *Pennisetum typhoides* (wall sculpturing)

3.16.5 | Setaria type

Exine brevi-ornate, undulate. Polygonal insulae studded with a number of spinules separated by narrow grooves, on annulus the exine insulae are aggregated (Figure 29). Insulae are over-aggregated and incisions are narrow and clearly defined. The pollen appears similar to *Avena* type, but the difference is sharp incision, insulae studded with 3–10 incisions. Also, annuli are not as much protrudes out as in *Setaria* type. Köhler and Lange (1979) categorize it as a *Setaria* type on the basis of insulae having irregular

polygonal outline. Those islands of insulae are studded with pointed spinules.

3.17 | Family: Cyperaceae

In family Cyperaceae, the pollen are not monad or polyad, instead they are pseudomonads, that is, they are degraded tetrads. According to Erdtmann (1969), three nuclei of the degraded mother cell and one functional one combine to form the pseudomonad during pollen

development. Cranwell (1952) worked on Cyperaceae and identified two pollen shapes, one is pear shape and the other is rounded. In the current study, *Cyperus* pollen (Figure 30a,b) are pear shaped. Pear shape is also confirmed by Moar and Wilmshurst (2003) with one convex and other rounded end. Cyperaceae pollen pori is not clearly demarcated. They possess slit-like demarcated areas on the proximal faces and sometimes considered as germ pore also. Here pori is represented by remnants of exine generally termed as areoli (Cranwell, 1952; Erdtmann, 1969). In Figure A, pores or furrow are not clearly segregated, and pollen appears to belong to *Cyperus* with

pear-shaped structure, slightly convex at one side and tapers toward the other end. They possess tectate exine.

Nagels et al. (2009) worked on Cyperaceae and identified oblate spheroidal to prolate pollen without apertures. Various diagnostic features of Cyperaceae pollen are known like size at polar axis, type and number of apertures, exine thickness, sculpturing, and shape (Erdtman, 1966a, 1966b; Wronska-Pilarek, Faegri, & Iverson, 1989). The size of Cyperaceae pollen in the current study ranges from 27.7 to 35 μm (P. axis), whereas in *Carex* it is 29.25–18.5 μm . Pollen possesses areolate exine studded with spinules. Depressions are minute and uniformly distributed over the tectum, lateral colpi is not prominent, whereas in *Carex* the three lateral colpi and one ulcerate pori are present. Surface in Cyperaceae is tectate, perforate with semi tectate condition having isolated island of sexine.

3.18 | Family: Rosaceae

In the current samples, the pollen from *Prunus* species (Figure 31) are tricolporate with striate perforate exine. Perforations are macro. Striations are separated by less than 1 μm distance. They appear like a rope, long, interwoven, branched, and often looped. Ropes are longer than 5 μm . Erdtman (1952) explained it as a diagnostic feature of Rosaceae Pollen. He categorizes pollen into three classes based on their length. Colpi of the pollen are syncolpate extending from poles and joining in the middle. Whereas *Eriobotrya japonica* (Figure 32) have striate exine that appears as psilate in LM but in SEM they are striate. More than 5 μm long, interwoven, few are parallel also connected to each other. We have found these characteristics close to the Rosaceae. Hebda and Chinnappa (1994) while working with LM and SEM analyses of pollen morphological feature explained pollen varieties of different tribes of Family Rosaceae, and found prolate to spheroidal pollen with

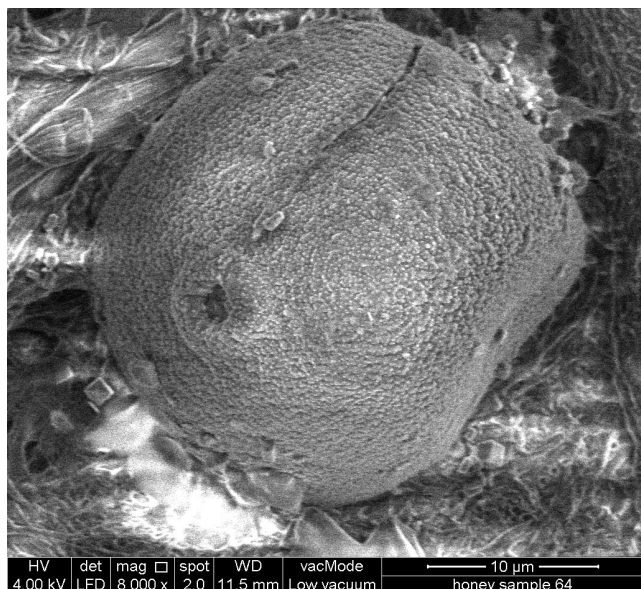


FIGURE 29 *Setaria* sp. indet

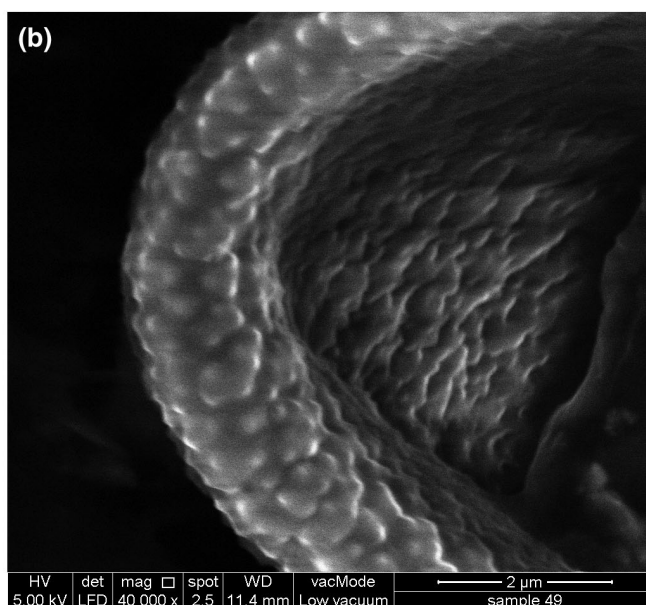
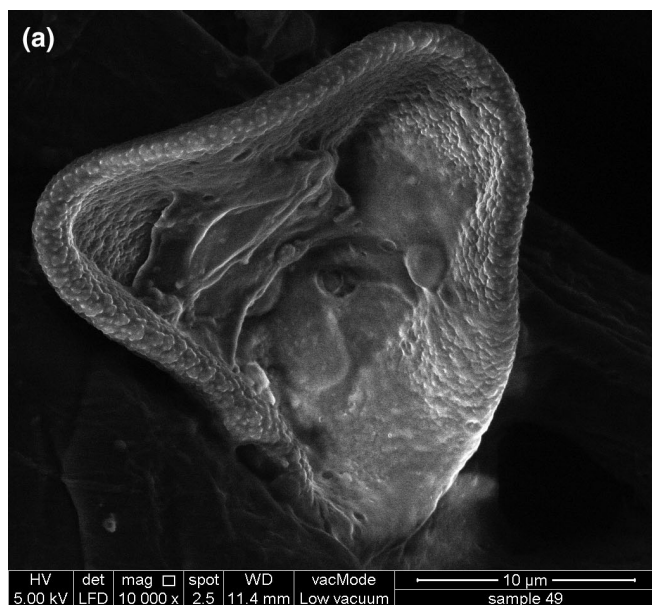


FIGURE 30 (a) *Cyperus rotundas*. (b) *Cyperus rotundas* (wall sculpturing)

tricolporate colpi, radially symmetrical pollen; they observed three different types of striate macro perforate pollen among 55 different genera of Rosaceae. Arzani, Nejatian, and Karimzadeh (2005) while working on Rosaceae pollen from Apricot cultivars described that ridges in exine are divided into two patterns, one is longitudinal and whirling and the other has interwoven ridges without whirling. The present study shows longitudinal and interwoven ridges, and it agrees with Fogle (1977); he also reported similar results for exine and pore characters for both peach and nectarine clone's pollen. The striations are in crisscross fashion extended over whole colpi and weaving ridges change their direction. However, they are neither parallel nor horizontal to colpi. Pollen are

tricolporate, that is, predominant character in Rosaceae pollen the colpi runs along whole length of polar region. Hebda and Chinnappa (1990) calculated 90% covering area at the polar side. Endoaperture at the center mimic the shape of dumbbell. Hebda and Chinnappa (1990) evidenced the presence of such endoaperture in *Prunus* sp. Evrenosoğlu and Misirli (2009) also witness striate perforate ornamentation among members of Rosaceae.

3.19 | Family: Rhamnaceae

Rhamnaceae has been reported as stenopalynous family (Erdtman, 1952). Its pollen are generally tricolporate and isopolar. Its ornamentation reportedly varies from reticulate to striate, striate reticulate, or psilate. It shows diversity in pollen size and exine type. Schirarend and Köhler (1993) divide the family into 12 types, considering *Rhamnus* type as suprareticulate rugulate while remaining 11 types are *Colletia* with tectum fossulate insulate, *Crumenaria* with tectum radiate-rugulate, *Gouania* with tectum perforate, *Helinustype* with tectum striate, *Hovenia* with striate-rugulate tectum, *Lasiodiscus* with tectum rugulate, *Maesopsis* with tectum baculate, *Phyllica* with reticulate tectum, *Pomaderris* with verrucate tectum, *Reissekia* with striate-reticulate tectum, and *Sageretia* with fossulate-perforate tectum. Perveen and Qaiser (2005) identified five diverse tectum types in this family and classify pollen into following types, viz. *Ziziphus oxyphylla*: Pollen striate, pollen length, and exine thickness are important features for delimitation; *Rhamnus prostrata*: Tectum psilate. *Berchemia pakistanica*: Tectum reticulate; *Sageretia thea*: Striate rugulate tectum but polar length is the important feature for delimitation; and *Ziziphus mauritiana*: Tectum rugulate.

Within *Ziziphus mauritiana*, three diverse features of tectum are present, that is, rugulate striate, rugulate, or striate. These taxa are



FIGURE 31 *Eriobotrya japonica*

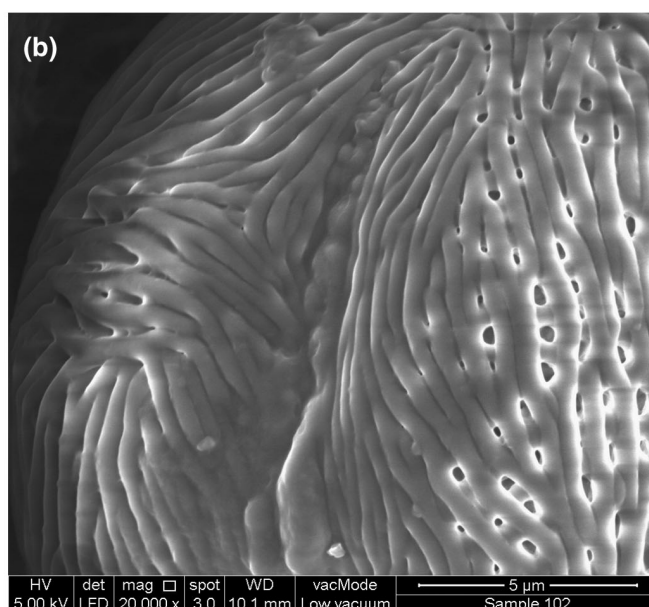
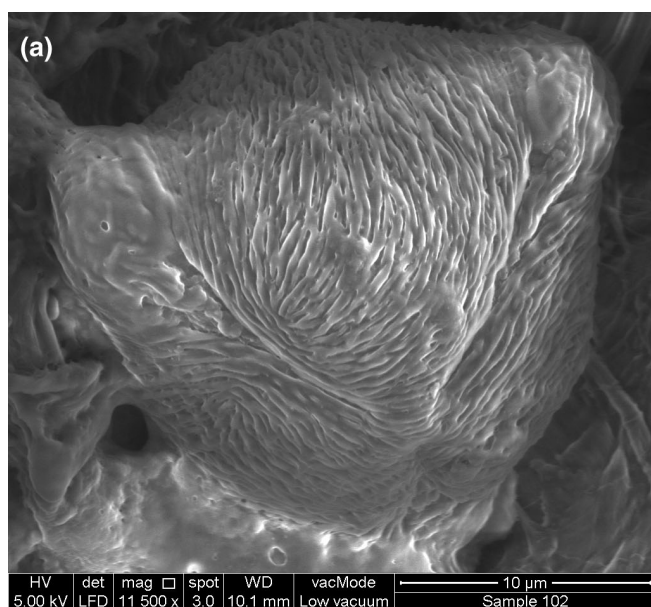


FIGURE 32 (a) *Prunus* sp. indent. (b) *Prunus* sp. indent (wall sculpturing)

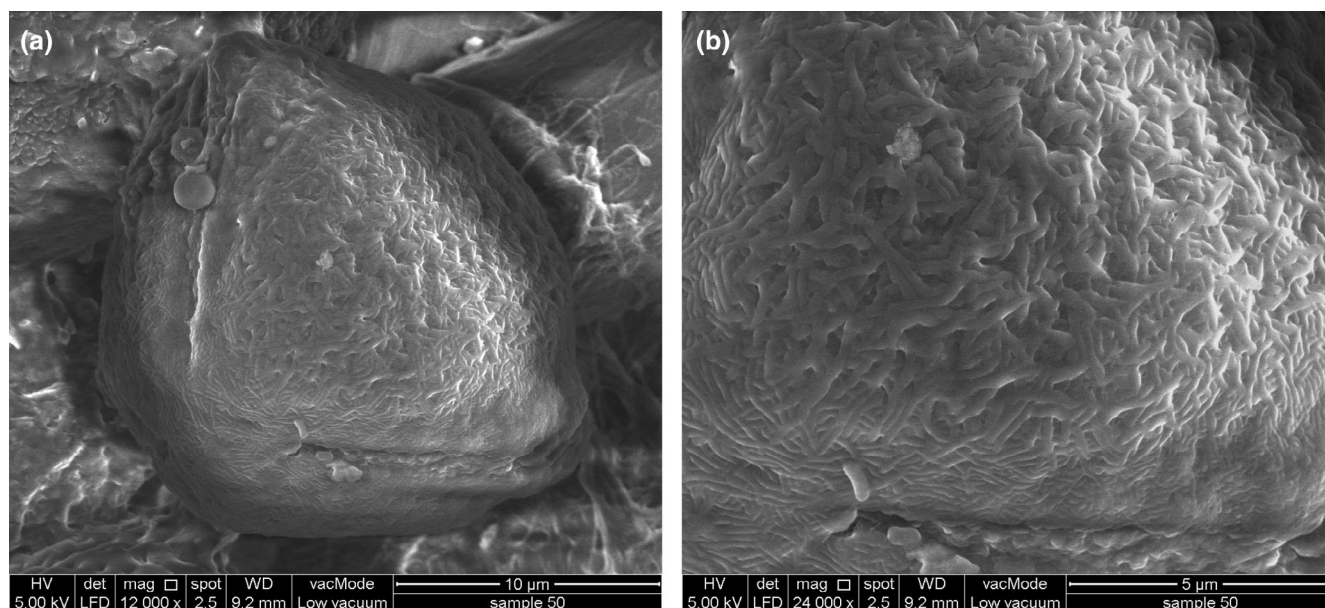


FIGURE 33 (a) *Ziziphus mauritiana*. (b) *Ziziphus mauritiana* (wall sculpturing)

easily delimited on the basis of polar length and exine thickness. Pollen morphology of the family Rhamnaceae is somewhat helpful at the generic level (Figure 33a,b). *Ziziphus mauritiana*—type with Exine tectate, tectum striate–rugulate. Decorated with elongated striated sexine elements, interwoven, length more than 1 µm long. Anglo-aperturate, tri zono colporate. Exine is psilate in margo region and does not show the extension of striato-rugulate tectum.

3.20 | Family: Cannabaceae

3.20.1 | *Cannabis sativa*

Pollen is spheroidal with three pori, surrounded with annulus. Size ranges from 18 to 21 µm. Shinwari et al. (2015) described *Cannabis* as a triporate, spheroidal annulate pollen with polar and equatorial diameter ranging between 8.41–and 23.67 µm and 21.04 µm, respectively. Palynological studies of allergenic pollen are indispensable as these studies are important in order to understand the underlying reasons of pollen being allergic and influence environmental factors on allergenicity of pollen. Thus, during the present study, salient morphological features of *Cannabis* pollen were studied (Pehlivan, 1987; Zavada & Dilcher, 1986). Cannabis pollen are triporate. Likewise, in the present study, the photo micrographs of cannabis pollen confirmed that pollen are triporate, confirming the present study's observation (Figure 34a,b).

3.21 | Family: Lamiaceae

Lamiaceae is considered as Eurypalynous by Erdtman (1952). He used aperture number to divide the family into two groups Lamioideae and

Nepetoideae with three and six colpi, respectively. Harley et al. (2004) further classified Nepetoideae into three tribes, Ocimeae, Mentheae, and Elsholzeae. Generally, they have radially symmetrical, isopolar (Bano et al., 2020) pollen ranging from oblate to spheroidal pollen having 3–6 colpi. In the present study, pollen are observed to belong to *Hissopus* genus (Figure 35a,b with six colpi, equidistantly arranged, size of the pollen range between 22 and 24 µm. The present pollen belongs to tribe Menthinae with bireticulate sculpturing. The reticulum is two layered primary and secondary reticula. Lumen in primary reticulum is regular, thicker, spheroidal, and little shallow than secondary reticulum. Secondary lumina narrow down toward poles and apertures. Moon, Vinckier, Smets, and Huysmans (2008) observed the bireticulate exine with prolate pollen in *Hissopus*. They believe this bireticulate ornamentation is its affinity toward old world taxa. Perveen and Qaiser (2004) on the base of pollen divided the family into seven types. They named the pollen with hexa colpate condition as *Nepeta laevigata* type. Moon et al. (2008) agree that Menthinae possess hexa colpate colpi expressing its synapomorphy with Nepetoideae.

3.22 | Family: Malvaceae

Generally, pollen grains within family Malvaceae are spheroidal-globular in P axis and oval-elliptic in Eq. view. Pollen are echinate and porate, may be polyporate, pantoporate, or zonoporate. Tectum is not properly psilate, instead it is punctated and granulated with scabrae spread in between spines. Spines are evenly spread over exine but their size, shape, and apex interspinal distance vary within species. Exine width ranges between 3 and 5 µm (Noreen Bibi, 2011). In the current sample, pollen (Figure 36) is spheroidal with P & Eq. mean diameter is ± 60 µm, Echinate with bulbous spines, spines are spread

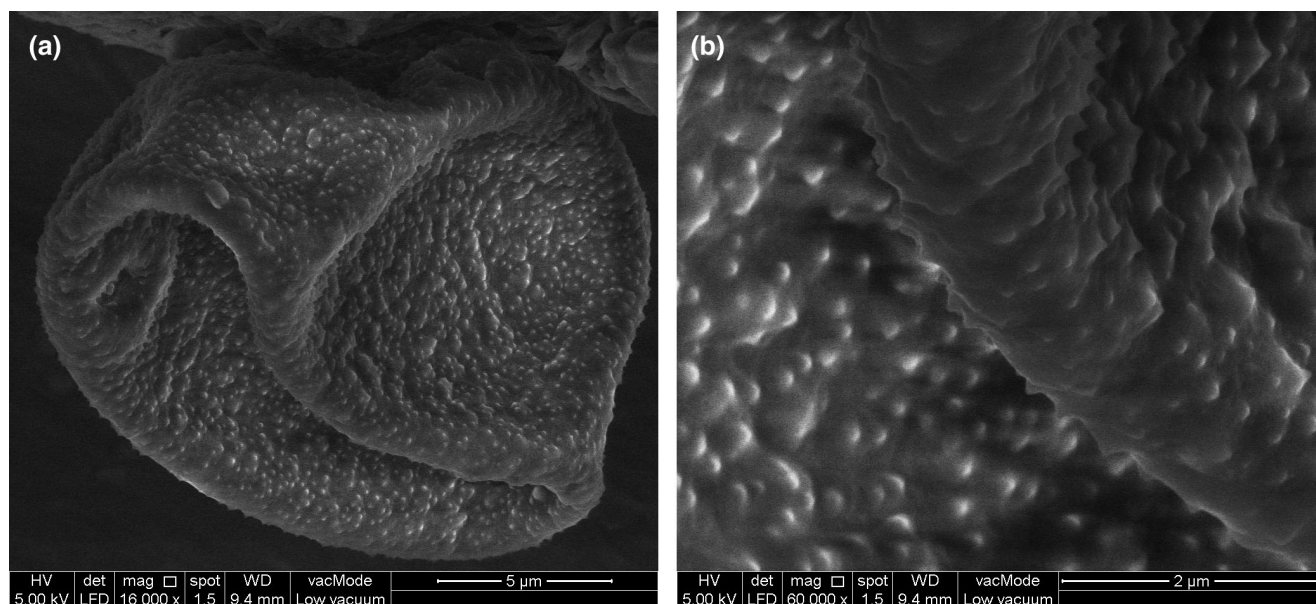


FIGURE 34 (a) *Cannabis sativa*. (b) *Cannabis sativa* (wall sculpturing)

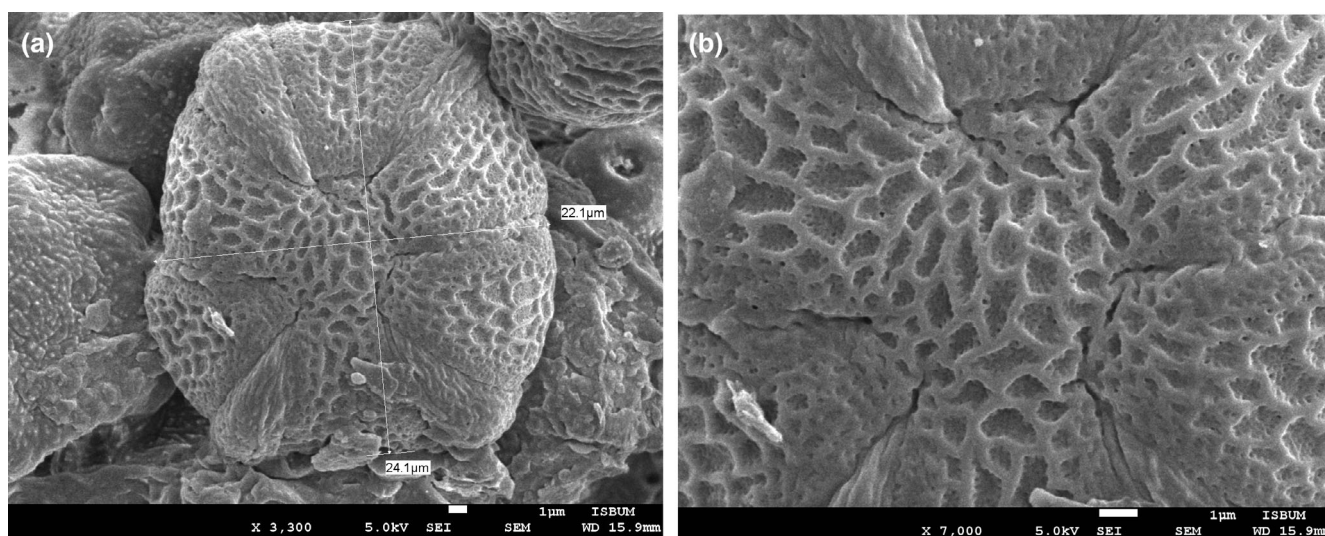


FIGURE 35 (a) *Hissopus sp. indent*. (b) *Hissopus sp. indent* (wall sculpturing)

over base, exine is semi psilate with punctate spread randomly between bulbous bases of spines.

Spines are zonoporate and monomorphic with bulbous base. Pore diameter between pore bases is not uniform. Diverse range of pollen shapes and sizes are reported in the literature. Culhane and Blackmore (1988) believe that six pollen types are present in honey, viz., *Abelmoschus crinitus*, *Abutilon indicum*, *Malva parviflora*, *Hibiscus arisi*, *Sinra incana*, and *Sida ovata* on the basis of diameter of grain, exine thickness, and spines. Many of the pollen in this family possess size above 100 µm or above. All are echinate but the echines are non-bulbous, and bases are without perforation; however, *A. indicum* shows such features, also pollen is isopolar, Parveen and

Noreen Bibi agree with that. Saad (1960) is of the view that because of greater number of distinct features, it is apparently possible to differentiate genera. Like bases of the spines are joined only in *A. indicum* and *Malvastrum coromandelianum*, but in the present case the size of pollen is smaller and *M. coromandelianum* is larger. Also, shape and number of spines are greater in *M. coromandelianum* compared to *A. indicum*. Spine base inter spinal distance, distribution, and apex show a diversity as they are intergeneric and interspecific. The spine shows considerable variations in base, apex, inter spinal distance, and their distribution on pollen surface. The variations are of value at different taxonomic levels, because they may occur not only between genera but also

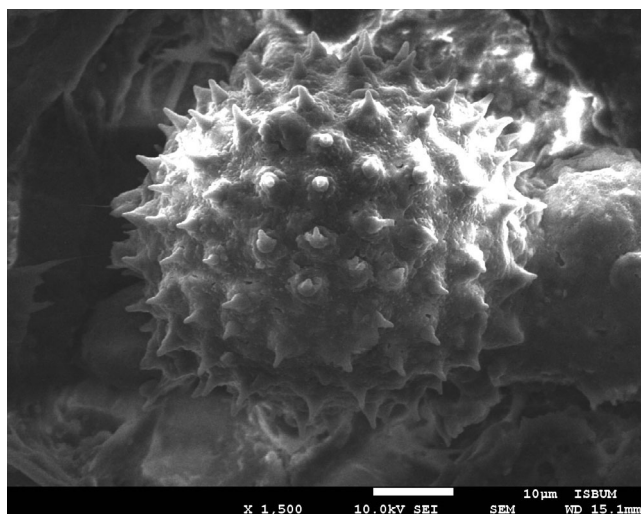


FIGURE 36 *Abutilon indicum*

between species of the same genus. Bulbous spines are only present in *A. indicum* (Figure 36).

It is generally considered that honey of specific areas can be easily recognized by its pollen content or pollen spectrum. But for such botanical source identification, well-defined pollen analytical characteristics are required. The present study is an attempt to achieve those objectives and to find out the presence of particular pollen within pollen spectrum that can present a geographical identity to most of the honey samples. Pollen features are proved to be identification marker or diagnostic feature for Mellitophilous plants and also served as excellent taxonomic marker, and these characterize site-specific taxa (Silici & Gökceoglu, 2007) like *A. nitida* pollen are well represented in honey samples from Kashmir. Only one species of *Alnus*, that is, *A. nitida*, is reported from temperate Himalaya ranges between Kunawar and Swat.

Our findings include pollen from many cultivated, indigenous, and exotic plant species, sometimes even representing the dominant pollen type in the honey samples. Pollen composition in the present study represents tropical–subtropical climatic zone. Many of the pollen assemblage belongs to entomophilous plants like *Citrus arvensis*, *Brassica campestris*, *Eriobotrya japonica*, etc., however, some anemophilous pollen such as *Alnus nepalensis*, *P. hysterothorus*, etc., are observed. Identification of various bee plants (nectar and pollen source) helps the beekeepers the proper placement and management of bee colonies for money-making honey production.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Mehwish Jamil Noor  <https://orcid.org/0000-0002-8849-7749>

Mushtaq Ahmad  <https://orcid.org/0000-0003-2971-2848>

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