

Kanwal K *et al.* (2024) Notulae Botanicae Horti Agrobotanici Cluj-Napoca Volume 52, Issue 2, Article number 13462 DOI:10.15835/nbha52213462 Research Article



# Exploring the micromorphological diversity of palynomorphic flora from lesser Himalaya biodiversity hotspot

Kainat KANWAL<sup>1</sup>, Muhammad ZAFAR<sup>1\*</sup>, Mushtaq AHMAD<sup>2</sup>, Mona S. ALWAHIBI<sup>3</sup>, Mohamed S. ELSHIKH<sup>3</sup>, Trobjon MAKHKAMOV<sup>4</sup>, Akramjon YULDASHEV<sup>5</sup>, Sokhib ISLAMOV<sup>6</sup>, Dilnoza SOTIBOLDIYEVA<sup>7</sup>, Nasibakhon NARALIYEVA<sup>8</sup>, Heba F. EID<sup>9</sup>, Imomova MUKAMMAL<sup>10</sup>, Mozaniel Santana de OLIVEIRA<sup>11</sup>, Salman MAJEED<sup>12</sup>, Vokhid FAYZIEV<sup>13</sup>

<sup>1</sup>Quaid-i-Azam University, Department of Plant Sciences, Islamabad, Pakistan; Kainatkanwal90@gmail.com; zafar@qau.edu.pk (\*corresponding author) <sup>2</sup>Normal University, College of Life Science, Neijiang 641000, China; mushtaqflora@hotmail.com

<sup>3</sup>King Saud University, College of Science, Department of Botany and Microbiology, Riyadh 11451, Saudi Arabia; malwhibi@ksu.edu.sa;

melshikh@ksu.edu.sa

<sup>4</sup>Tashkent State Agrarian University, Department of Forestry and Landscape Design, 2 A., Universitet Str., Kibray district, 100700, Tashkent region, Uzbekistan; mturobzhon@mail.ru

<sup>5</sup>Andijan State University, Department of Biology and Ecology, 129, Universitet Str., 170100, Andijan, Uzbekistan; akramyuldashev@mail.ru

<sup>6</sup>Tashkent State Agrarian University, Department of Technology of Storage and Processing of Agricultural Products, 2 A., Universitet Str., Kibray district, 100700, Tashkent region, Uzbekistan; islamov.s80@mail.ru

<sup>7</sup>National University of Uzbekistan, Department of Botany and Plant Physiology, 4 University Street, Tashkent 100174, Uzbekistan; dilnozabiolog@mail.ru

<sup>8</sup>Andijan State University, Department of Botany, Andijan, Uzbekistan; n\_naralieva@mail.ru
<sup>9</sup>Al-Azhar University, Faculty of Science, Cairo, Egypt; Heba.fathy@azhar.edu.eg

<sup>10</sup>Fergana State University, Department of Botany and Biotechnology, 712000, Uzbekistan; mukammalxon75@mail.ru

<sup>11</sup>Campus de Pesquisa do Museu Paraense Emílio Goeldi, Av. Perimetral, 1901 - Terra Firme, 66077-830, Belém,

Brazil; mozaniel.oliveira@yahoo.com.br

<sup>12</sup>University of Mianwali, Department of Botany, Mianwali, 42200 Pakistan; salmansunny61@gmail.com <sup>13</sup>Chirchik State Pedagogical University, Department of Biology, 104., Amir Temur Str., 100700, Uzbekistan; fvaxid@mail.ru

# Abstract

Palynology, a prominent field in plant systematics and biodiversity studies, plays a vital role in identifying and determining the plant species present in a specific region. The current study was performed to evaluate the micromorphological traits of pollen from flora of Lesser Himalaya. Pollen microstructural variations aid in the identification of species belonging to specific botanical families and various geographic habitats. Flowers of 24 selected species categorized into 16 families were collected, preserved and then acetolysis protocol followed. Pollen was examined under a light and scanning microscopy (LM and SEM) for palynomorph description. The palynomorphs characteristics such as size, shape, exine surface, and aperture

*Received: 16 Oct 2023. Received in revised form: 18 Mar 2024. Accepted: 23 May 2024. Published online: 03 Jun 2024.* From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers. orientation, were examined. Status of these plants show that herbs are being dominant (11 species), while shrubs (7 species), climbers (3 species), bulbous plants (2 species), small tree, sedge, weed (1 species each). Pollen shape determined in equatorial view were; spheroidal, sub-prolate, oblate-spheroidal, prolate, spherical and sub-oblate. The variations were seen among pollen types; tricolpate, tricolporate and polyporate in most of the species. Exine stratification was observed mostly scabrate while echinate, cristate-reticulate, granulate, punctate, rugulate-perforate, striate-rugulate, verrucate, cristate-foveolate was visualized in each different species. Palynomorph apertural patterns were observed sunken, furrowed, slightly bulged, scabrate, granulate, slit like, and perforate. The largest polar diameter was measured in *Hymenocallis littoralis* (138.6  $\mu$ m) whereas smallest in *Parthenium hysterophorus* (14.70  $\mu$ m). Equatorial distance was calculated maximum for *Cascabela thevetia* (110.1  $\mu$ m) and minimum for *Hibiscus rosa-sinensis* (1.7  $\mu$ m). P/E ratio was calculated largest in *Hymenocallis littoralis* (1.8) and lowest in *Duranta erecta* (0.89). The palynomorphs taxonomic characters investigated can be helpful in species level identification and provide a baseline to conduct more systematic research with respect to specific plant families and genera.

Keywords: mesocolpium distance; palynomorphs; pollen furrows; SEM; sculpturing; taxonomy

#### Introduction

The diversity of flora in a given region is important to note, since it is sometimes defined to intense competition among closely related plant species for survival in their natural environments. Palynology is the scientific study of living or fossil pollen grains and spores, produced by male part of angiosperm plants. The term palynology was first introduced by Hyde and William in 1945. It is an extensive field that includes the study of microfossils taken from ancient rocks and sediments and pollen morphology (Majeed *et al.*, 2020).

Pollen characters are genetically sustained and persistent exhibiting significant variations in wall structure, exine sculpturing, size, shape, aperture ornamentation, polarity, and symmetry etc. These traits are useful to determine the systematic categorization of angiosperm species (Chatterjee *et al.*, 2014). The pollen is microscopic but exceptionally durable due to the presence of sporopollenin, a complex polymer found in the outer layer known as exine, while the inner layer (intine) is formed from pecto-cellulosic substances. Exine sculpturing is particularly important in taxonomy and systematic classification. The arrangement and orientation of pollen apertures are interesting aspects of pollen identification. Both pollen apertures and shapes are key criteria for identifying flora.

Palynology also deals with the seasonal allergenic diseases that affect almost 400 million people worldwide, reported by World Health Organization (Kailas *et al.*, 2017). The analysis of the different types of pollen in the honey can reveal the sources of nectar and the foraging habits of honeybees (Al-Kahtani *et al.*, 2020). The observation of pollen in forensic palynology has made easier to investigate the various crimes and legal problems. Pollen study can also be used to determine the season and time of the year that aids in forensic investigations of suspectable crime scene (Pasarelli and Cortes, 2017).

Palynological data has been used in various disciplines as applications to resolve ambiguity. In Pakistan, applied and basic palynological research has prevailed, although there is still a lot of work to be done in this area (Majeed *et al.*, 2020). Generally, the potential study of micromorphological features, in the field of plant systematics has increased globally (Lu *et al.*, 2015). Nowadays, it's critical to study pollen features for new plant species, especially to understand their phylogenetic and evolutionary interactions (Xu *et al.*, 2008).

The examination of micromorphological traits are valuable for identification and classification plant species within various angiosperm including ferns, bryophytes, and monocots (Katata-Seru *et al.*, 2018; Khan *et al.*, 2019a). Taxonomists rely on the examination of morpho-palynological characters to define accurate

taxonomic hierarchical of diverse floral species (Amina *et al.*, 2020; Majeed *et al.*, 2022; Noor *et al.*, 2023; Nazish *et al.*, 2019).

Light microscopy is commonly used to determine basic features of pollen; diameter, shape, size, mesocolpium, and aperture ornamentation and exine patterns. However, in depth features of pollen grains and exine sculpturing can be studied using SEM more effectively as compared to light microscopy (Ashfaq *et al.*, 2020; Attique *et al.*, 2022). SEM enhanced high resolution to visualize ultrastructural micron level features. This powerful technique allows for the qualitative analysis of samples, providing insights into their unique characteristics and properties (Gul *et al.*, 2021; Rashid *et al.*, 2022). SEM imaging has significantly advanced the field of plant taxonomy by enabling better visualization of pollen structural features (Muniraja *et al.*, 2020; Ullah *et al.*, 2022). Naz *et al.* (2020) explored pollen flora of lactiferous plants of Apocynaceae from Rawalpindi, while (Ullah *et al.*, 2022) examined the honeybee flora from the Margalla Hills surrounding to elaborate palyno-morphological features.

This study represents the first attempt to use SEM tool to identify the pollen micromorphological characters of 24 angiosperm species belong to Lesser Himalayan region. This morpho pollinic illustration was essential for conservation efforts of flora as its help to identify endangered species. However, the palynological description of green angiosperm flora allows for a comprehensive dynamic of the regional plant biodiversity, providing valuable insights into the ecological and evolutionary relationships.

## Materials and Methods

## Sampling area and climatic conditions

Pakistan is occupied by tallest mountain peaks known as Himalayas, reflected in the attractive beauty and unique cultures of the people who live in their shadow. Lesser Himalayas have a total area of 23295 km<sup>2</sup> (Khan *et al.*, 2019b) and the whole population of this region is around 10 million (Yang *et al.*, 2012). The area possesses sub-tropical and semi-humid zones with temperature lower in October to February warm and moist summers from month of May to June and rainy season during July to August as summarized in Table 1 (Usma *et al.*, 2022a). Its geographical aspects, altitudes, heights, vegetation cover and climatic conditions vary dramatically (Waheed *et al.*, 2013; Umber *et al.*, 2022; Yousaf *et al.*, 2022).

Sr. No	Plant taxa	Family	English	Habit	Flowering	Global distribution	Distribution in Pakistan	Collection	Coordina	Vouch er no.
1.	Bougainvillea spectabilis Willd.	Nyctaginaceae	Great bougainvil lea	Climbin g shrub	Throughout year	Africa North & Central America	Islamabad, Rawalpindi, Ayubia, Lahore	Shamsabad	33°44'44" N 73 ° 08'18" E	BS-01
2.	Cannabis sativa L.	Cannabaceae	Marijuana or Hemp	Herb	Late summer- Early fall	Worldwide, Native of Brazil, widely found In Tropics	Throughout Pakistan	QAU Islamabad	33°44'44" N 72 ° 08'14" E	CS-02
3.	<i>Cascabela thevetia</i> (L.) Lippold	Apocynaceae	Yellow oleander	Small tree/shru b	Late summer- Early fall	North America Africa	Rawalpindi, Gujranwala, Jhelum, Lahore,	Murree road	33°44'56" N 73 ° 08'21" E	CT-14
4.	Celosia argentea L.	Amaranthaceae	Silver cock's comb	Annual herb	Summer-Fall	East Africa and Several of The Indian Ocean Islands	W. Pakistan, Swat and Hazara Eastwards	QAU Islamabad	33°42'44" N 73 ° 08'11" E	CA-15
5.	Convolvulus arvensis L.	Convolvulaceae	Bindweed	Climbin g/trailing herb	Summer- Early autumn	America, Tropical Africa	Lahore, Gujranwala, Chakwal, Faisalabad	Chakwal	33°43'44" N 73 ° 08'16" E	CP-16
6.	<i>Cucumis melo</i> var. agrestis Naudin	Cucurbitaceae	Wild melon	climbing vine	Summer- Early autumn	Tropical And Subtropical Asia and Africa	Islamabad, Murree, Kashmir, Himalayas	Rawalpindi	33°44'51" N 73 ° 08'17" E	CM-13

Table 1. Sampling data and GPS coordinates and distribution of selected flora from Lesser Himalaya

7.	Cyperus niveus Retz.	Cyperaceae	White galingale	Perennial sedge	Summer- Early autumn	Ethiopia to Africa, Syria to Arabian Peninsula	Throughout the Pakistan	Chakwal	33°44'54" N 73 ° 08'14" E	CN-07
8.	Datura stramonium L.	Solanaceae	Jimson weed	Annual/ biennial herb	Summer- Autumn	Tropical & S. Africa to S. Central China	Mianwale, Bhakar, Chakwal, Rawalpindi	Pindora	33°44'44" N 73 ° 08'18" E	DS-06
9.	Duranta erecta L.	Verbenaceae	Golden dewdrop	Evergree n shrub, small tree	Summer-Fall	America, West Indies, Western USA.	Punjab, Sindh, Bhakar, Mianwale	Rawat	33°45'44" N 73 ° 08'16" E	DE-17
10.	<i>Dodonaea viscosa</i> (L.) Jacq.	Sapindaceae	Hopbush	Shrub/tr cc	Summer-Fall	Throughout Tropical America from S. USA Southwards	Tropical And Subtropical Areas of Pakistan	QAU Islamabad	32°44'44" N 73 ° 86'18" E	DV-18
11.	Hibiscus rosa-sinensis L.	Malvaceae	Chinese hibiscus	Shrub/s mall tree	Throughout ycar	India: Assam, Bihar, Gujarat, Maharastra, Manipur	Rawalpindi, Islamabad, Balochistan, Sindh	Margalla hills	33°44'50" N 73 ° 08'19" E	HR-24
12.	<i>Hymenocallis littoralis</i> (Jacq.) Salisb.	Amaryllidaceae	Beach spider lily	Bulbous perennial	Summer-Fall	India, Native to South America, Pakistan	Rawalpindi, Islamabad, Peshawar, Lahore	Shahdara	32°44'44" N 73 ° 09'18" E	HL-19
13.	<i>Ipomoea cairica</i> (L.) Sweet	Convolvulaceae	Mile-a- minute vine	Climbin g vine	Throughout ycar	Minnesota, Oregon, Texas, The Dakotas, California,	Attock, Faisalabad, Vehari, Sialkot, Thatta Badin, Swat,	Gujhar Khan	33°43'44" N 72 ° 08'18" E	IB-21
14.	<i>Ipomoea carnea</i> Jacq.	Convolvulaceae	Pink morning glory	Shrub	Summer-Fall	Tanzania To South Africa, Pakistan to India	Mainly In the Tropical and Subtropical Regions of Pakistan	Margalla hills	33°44'44" N 73 ° 08'18" E	IA-20
15.	Lantana camara L.	Verbenaceae	Common lantana	Perennial shrub	Throughout year	South Africa Mexico, Colombia	Tropical And Subtropical Regions of Pakistan	Chakwal	33°44'44" N 73 ° 08'03" E	LC-04
16.	Malvastrum coromandeli anum (L.) Garcke	Malvaceae	Indian mallow	Perennial herb or sub- shrub	May- November	Tropical & S. Africa, Arabian Peninsula to Temp. E. Asia.	Chakwal, Sindh, KPK, Islamabad, Rawalpindi	Ramli	33°44'54" N 73 ° 08'13" E	MC-08
17.	Parthenium hysterophorus L.	Asteraceae	Congress grass	Annual/ biennial herb	Throughout year	Asia, Africa, and North America	Chakwal, Sindh, Peshawar, Islamabad, Rawalpindi	Mandra	33°44'44" N 73 ° 08'18" E	PH-09
18.	Physalis angulata L.	Solanaceae	Cut leaf ground cherry	Annual herb	Summer-Fall	Tropical Africa and Madagascar India to North Australia	Through Pakistan,	Margalla hills	31°43'44" N 73 ° 08'16" E	PA-03
19.	Ricinus communis L.	Euphorbiaceae	Castor bean plant	Perennial shrub/tre c	Summer-Fall	Southeastern Asia, India, Australia, Africa Climates	Sindh, Chakwal, Multan, Rawapindi	QAU Islamabad	33°44'44" N 73 ° 06'18" E	RC-22
20.	<i>Taraxacum officinale</i> (L.) Weber ex F.H.Wigg.	Asteraceae	Dandelio n	Herbace ous perennial	Spring-Early summer	West Indies and Some Southern States of the USA	Lahore, Gujranwala, Sindh, Chakwal	Margalla hills	33°44'54" N 73 ° 08'16" E	TO-05
21.	Tribulus terrestris L.	Zygophyllaceae	Puncturev ine	Perennial herb	Summer-Fall	China, India, Japan, Myanmar, <i>Paki</i> <i>stan</i> , Sri Lanka, Vietnam;	Sindh, Balochistan, Bhakar, Mianwali	Rawalpindi	33°45'44" N 73 ° 09'18" E	TT-11
22.	Trichodesma indicum (L.) Lehm.	Boraginaceae	Indian borage	Perennial herb	Late winter- Early summer	Mexico And extending to Ecuador and Venezuela.	Northern Areas of Pakistan	QAU Islamabad	33°44'45" N 73 ° 05'18" E	TI-12
23.	Verbena officinalis L.	Verbenaceae	Common vervain	Perennial herb	Summer-Fall	Tropical And Sub-Tropical America, Mexico	West Pakistan, Swat	NARC Islamabad	32°44'44" N 74 ° 08'18" E	VO-10
24.	Zephyranthes carinata H erb.	Amaryllidaceae	Pink rain lily	Perennial herb	Summer-Fall	Tropical & Subtropical Asia to N.	Tropical And Subtropical Areas of Pakistan	Faizabad	33°44'44" N 72 ° 08'13" F	ZC-23

Kanwal K et al. (2024). Not Bot Horti Agrobo 52(2):13462

## Collection and preservation of plant specimens

The Margalla Hills has an area of about 12,606 hectares. The research area, where plant species from various families were collected, included Margalla Hills and the surrounding areas of Islamabad. Masks, surgical gloves, cutters, scissors, zipper bags and newspapers have been used for the plant's assemblage. The flowered plant species present abundantly were collected. These plant species belonging to the sixteen different families were brought in the Herbarium of Pakistan (ISL) and compared with preserved specimens and expert taxonomist.

## LM analysis of pollen grains

Slides of pollen grains were made according to the acetolysis method of Erdtman (1969). Mature flowers were collected from different field trips, anthers have been cast out from flowers and put on the glass slides by the help of forceps. 1-2 droplets of acetic acid were added on the anthers and mashed them by using needles. Debris was removed from the crushed anthers using the camel brush. Then glycerin jelly was added on the slides for staining of pollen. Finally slide was covered with cover slip and tagged. After visualizing the presence of pollen by light microscope, slides were made long lasting by the help of melted paraffin (Umber *et al.*, 2022). Slides of pollen grains were observed in the Meiji Techno Japan Microscope (Model: MT4200H) at 100X and 400× magnification. Pollen photomicrographs were captured on  $40\times$  resolutions of Leica Dialux 20 Light microscope. Almost 10-15 pollen were observed, and 10-20 readings were noted for each character of pollen. Morphological characters calculated involve polar and equatorial diameter, P/E index, number of colpi and spores, colpi length, colpi width, mesocolpium, exine size, no of pores, pores length, pore width, pollen type, pollen shape and size (Bano *et al.*, 2012; Candeias *et al.*, 2021).

# Utilization of SEM for pollen imaging and sculpturing applications

Scanning electron microscopy is used an advanced tool to differentiate closely related species by visualizing the ultra-sculpture of pollen wall. Anthers were treated with 45% acetic acid and then crushed, before being placed on metallic stubs coated with gold palladium and observed under (Model: JEOL JSM5910) SEM at the Central Resources Library (CRL), Department of Physics at the University of Peshawar.

#### Quantitative statistical analysis

Five consecutive measurements were recorded and analyzed using SPSS 16 statistical programming of quantitative parameters in randomly 15 grains per species. The mean value and standard error (SE) for each element were computed and presented in a table format as mean (minimum-maximum)  $\pm$ SE. The existing terminology reported by Punt *et al.* (2007) and Halbritter *et al.* (2018) was followed to describe the qualitative morpho pollinic aspects.

### P/E index

Polar to equatorial diameter was calculated to determine the pollen shape. The given formula (Majeed *et al.*, 2022) was followed.

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

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

## Pollen fertility and sterility estimation

The percentage of pollen fertility and sterility was calculated based on the color of the pollen. Pollen not tainted or damaged were considered infertile, whereas stained pollen was determined fertile. According to below formula, fertility and sterility was calculated (Raza *et al.*, 2020).

Fertility % = Fertility of pollen /  $F + S \times 100$ Sterility % = Sterility of pollen /  $S + F \times 100$ 

S indicates sterile and F indicates the fertile pollen.

# Results

In this study, 24 plant species belonging to the 16 different families from Lesser Himalayas were collected and observed for their palynomorphs characteristics (Figures 1-3). Qualitative and quantitative micromorphology of pollen flora were examined using the LM and SEM (Figures 4 to 11; Tables 2, 3 and 4).



**Figure 1.** Lesser Himalaya plants field pictorial view: (a) *Bougainvillea spectabilis*, (b) *Cannabis sativa*, (c) *Cascabela thevetia*, (d) *Celosia argentea*, (e) *Convolvulus arvensis*, (f) *Cucumis melo*, (g) *Cyperus niveus*, (h) *Datura stramonium*, (i) *Duranta erecta* 



**Figure 1**. Lesser Himalaya plants field pictorial view: (a) *Dodonaea viscosa*, (b) *Hibiscus rosa-sinensis*, (c) *Hymenocallis littoralis*, (d) *Ipomoea cairica*, (e) *Ipomoea carnea*, (f) *Lantana camara*, (g) *Malvastrum coromandelianum*, (h) *Parthenium hysterophorus*, (i) *Physalis angulata* 



**Figure 2**. Lesser Himalaya plants field pictorial view: (a) *Ricinus communis*, (b) *Taraxacum officinale*, (c) *Tribulus terrestris*, (d) *Trichodesma indicum*, (e) *Verbena officinalis*, (f) *Zephyranthes carinata* 



**Figure 3.** Light microscope pollen photomicrographs. Polar view and equatorial view: (a) *Bougainvillea* spectabilis, (b,c) Cannabis sativa, (d,e) Cascabela thevetia, (f) Celosia argentea, (g,h) Convolvulus arvensis, (i,j) Cucumis melo, (k) Cyperus niveus, (l,m) Datura stramonium, (n,o) Duranta erecta, (p) Dodonaea viscosa, (q) Hibiscus rosa-sinensis, (r) Hymenocallis littoralis, (s) Ipomoea cairica, (t) Ipomoea carnea



**Figure 4**. Light microscope pollen photomicrographs. Polar view and equatorial view: (a) *Lantana camara*, (b) *Malvastrum coromandelianum*, (c,d) *Parthenium hysterophorus*, (e,f) *Physalis angulata*, (g,h) *Ricinus communis*, (i,j) *Taraxacum officinale*, (k) *Tribulus terrestris*, (l) *Trichodesma indicum*, (m,n) *Verbena officinalis*, (o) *Zephyranthes carinata* 



**Figure 5.** Pollen SEM micrographs showing polar view (PV), equatorial view (EV) and exine sculpturing (ES). *Bougainvillea spectabilis* (A) PV-5µm (B) EV-10 µm (C) ES-5µm; *Cannabis sativa*, (D) PV-5µm (E) EV-5µm (F) ES-2 µm; *Cascabela thevetia*, (G) PV-10µm (H) EV-10µm (I) ES-5µm; *Celosia argentea* (J) PV-5µm (K) EV-10µm (L) ES-2 µm



**Figure 6.** Pollen SEM micrographs showing polar view (PV), equatorial view (EV) and exine sculpturing (ES). *Convolvulus arvensis* (A) PV-10µm (B) EV-10µm (C) ES-5µm; *Cucumis melo* (D) PV- 10µm (E) EV-10µm (F) ES-5µm; *Cyperus niveus* (G) PV-2µm (H) EV-10µm (I) ES-5µm; *Datura stramonium* (J) PV-10µm (K) EV-10µm (L) ES-5µm



**Figure 7.** Pollen SEM micrographs showing polar view (PV), equatorial view (EV) and exine sculpturing (ES). *Duranta erecta* (A) PV-2µm (B) EV-10µm (C) ES-5µm; *Dodonaea viscosa* (D) PV-10µm (E) EV-10µm (F) ES-5µm *Hibiscus rosa-sinensis*, (G) PV-20µm (H) EV-20µm (I) ES-10µm *Hymenocallis littoralis*. (J) PV-10µm (K) EV-10µm (L) ES-5µm



**Figure 8.** Pollen SEM micrographs showing polar view (PV), equatorial view (EV) and exine sculpturing (ES). *Ipomoea cairica* (A) PV-20µm (B) EV-20µm (C) ES-10µm; *Lantana camara* (D) PV- 10µm (E) EV-10µm (F) ES-5µm *Malvastrum coromandelianum* (G) PV-10µm (H) EV-10µm (I) ES-10µm *Parthenium hysterophorus* (J) PV-5µm (K) EV-5µm (L) ES-2µm.



**Figure 9**. Pollen SEM micrographs showing polar view (PV), equatorial view (EV) and exine sculpturing (ES). *Physalis angulata* (A) PV-5µm (B) EV-5µm (C) ES-2µm; *Ricinus communis* (D) PV- 5µm (E) EV-5µm (F) ES-2µm; *Taraxacum officinale* (G) PV-5µm (H) EV-5µm (I) ES-5µm *Tribulus terrestris* (J) PV-10µm (K) EV-10µm (L) ES-5µm.



**Figure 10**. Pollen SEM micrographs showing polar view (PV), equatorial view (EV) and exine sculpturing (ES). *Trichodesma indicum* (A) PV-5µm (B) EV-5µm (C) ES-2µm; *Verbena officinalis* (D) PV-10µm (E) EV-10µm (F) ES-2µm Zephyranthes carinata (G) PV-10µm (H) EV-10µm (I) ES-5µm

Sr.No	Plant taxa	Polar diameter Mean (Min-Max) S.E µm	Equatorial diameter Mean (Min-Max) S.Ε μm	P/E ratio	Exine thickness Mean (Min-Max) S.Ε μm	Mcsocolpium Mean (Min-Max) S.E μm	Fertilit y %	Sterili ty %
1.	Bougainvillea spectabilis Willd.	35.05(29.75-38.75) ±1.66	34.80(27.75-38.50) ±1.96	1.0	2.55(2.25-2.75) ±0.12		85%	3%
2.	Cannabis sativa L.	20.4 (20.25-20.75) ± 0.09	19.05 (18.25-19.75) ± 0.26	1.07	0.35 (0.25-0.50) ± 0.06		83%	10%
3.	Cascabela thevetia (L.) Lippold	113.1(110.5-115.2) ±0.83	110.1(108.7-111.2) ±0.45	1.02	3.20(2.75-3.75) ±0.16	87.0(80.25-109.5) ±5.64	72%	8%
4.	Celosia argentea L.	30.3(27.75-32.0) ±0.73	29.3(28.0-30.25) ±0.40	1.03	2.95(2.75-3.25) ±0.09		80%	16%
5.	Convolvulus arvensis L.	71.2(70.50-72.0) ±0.31	68.2(67.25-69.50) ± 0.40	1.04	4.50(4.25-4.75) ±0.11	45.0(43.75-45.75) ±0.35	84%	12%
6.	Cucumis melo var. agrestis Naudin	54.75(53.0-55.50) ±0.45	52.0(49.75-53.0) ±0.58	1.05	2.15(2.0-2.25) ±0.61	48.75(48.50-49.0) ± 0.07	90%	5%
7.	Cyperus niveus Retz.	28.55(27.25-29.75) ±0.47	24.90(24.50-25.25) ±0.15	1.16	1.80(1.25-2.25) ±0.16	÷	74%	5%
8.	Datura stramonium L.	56.8(56.5-57.0) ±0.10	55.0(53.7-56.0) ±0.37	1.03	0.40(0.25-0.50) ±0.06	-	89%	11%
9.	Duranta erecta L.	26.4(6.25-32.2) ±5.04	29.4(28.75-29.7) ±0.18	0.89	0.7(0.50-1.0) ±0.09	18.74(16.65-21.24) ±0.40	74%	26%
10.	Dodonaea viscosa (L.) Jacq.	23.9(23.7-24.2) ±0.09	23.4(23.2-23.7) ±0.09	1.02	3.05(2.75-3.50) ±0.14	10.34(10.25-10.50) ±0.06	82%	18%
11.	Hibiscus rosa-sinensis L.	1.7(145.2-190.2) ±8.34	1.7(147.2-198.1) ±8.22	1.0	2.8(2.25-3.25) ±0.16	-	78%	22%
12.	Hymenocallis littoralis (Jacq.) Salisb.	138.6(134.75-149.5) ±2.75	76.60(40.50-91.0) ±9.47	1.80	3.25(3.0-3.50) ±0.11	-	83%	6%
13.	Ipomoea cairica (L.) Sweet	97.10(95.50-98.0) ±0.43	89.80(89.0-90.50) ±0.25	1.08	2.60(2.25-3.0) ±0.15	-	85%	4%
14.	Ipomoea carnea Jacq.	102.85(92.0-111.25) ±3.26	102.80(94.75- 114.5) ±3.43	1.0	3.55(3.0-4.50) ±0.26	-	80%	4%
15.	Lantana camara L.	27.65(25.50-32.25) ±1.22	29.85(27.75-33.0) ±1.23	0.92	2.30(2.0-2.75) ±0.12	-	74%	7%
16.	Malvastrum coromandelianum (L.) Garcke	85.0(80.25-92.25) ±2.14	84.10(78.75-93.75) ±2.68	1.01	2.60(2.25-3.0) ±0.15	-	81%	2%
17.	Parthenium hysterophorus L.	14.70(14.25-15.25) ±0.16	14.50(14.25-14.75) ±0.11	1.01	1.30(1.0-1.50) ±0.09	10.30(9.75-10.75) ±0.16	88%	5%
18.	Physalis angulata L.	33.60(33.0-34.0) ±0.16	31.80(31.25-32.25) ±0.18	1.05	2.50(2.0-2.75) ±0.15	25.05(20.50-28.25) ±1.80	88%	5%
19.	Ricinus communis L.	25.3(25.2-25.5) ±0.06	28.5(28.2-28.7) ±0.09	0.81	2.41(2.2-2.7) ±0.11	24.3(24.0-24.7) ±0.12	79%	21%
20.	Taraxacum officinale (L.) Weber ex F.H.Wigg.	26.2(25.2-27.0) ±0.36	24.9(24.5-25.2) ±0.15	1.05	2.45(2.25-2.75) ±0.12	17.4(15.2-18.7) ±0.58	81%	19%
21.	Tribulus terrestris L.	53.40(50.25-58.0) +1.53	55.30(47.75-58.25) +1.94	0.96	2.40(2.0-2.75) ±0.15		84%	8%
22.	Trichodesma indicum (L.) Lehm.	25.25(24.75-25.50) ±0.13	24.50(24.0-24.75) ±0.13	1.03	1.60(0.50-5.0) ±0.85	14.9(14.50-15.25) ±0.15	58%	4%
23.	Verbena officinalis L.	46.25(43.0-49.75) ±1.10	48.75(47.0-50.25) ±0.67	0.84	2.40(2.0-2.75) ±0.15	34.85(32.75-37.75) ±0.95	82%	8%
24.	Zephyranthes carinata Herb.	78.55(74.75-82.75) ±1.31	58.45(56.60-59.75) ±0.70	1.34	2.45(2.25-2.75) ±0.12	-	82%	10%

Table 2. Quantitative analysis of pollen morphology and size in the studied taxa

Abbreviation: -, Absent; %, Percentage; S.E, Standard Error; Min, minimum; Max, Maximum

S.r		Number of				м	Length ean (Min-Max) S.E	μm	Width Mean (Min-Max) S.E µm			
No	Plant name	Colpi	Spines	Pores	Spines between colpi	Colpi	Spine	Pores	Colpi	Spine	Pores	
1.	Cascabela thevetia (L.) Lippold	03	-	-	-	5.25(4.75-5.50) ±0.13	-	-	15.80(15.25- 16.25) ±5.64	-	-	
2.	Celosia argentea L.	-	-	05	-	-	-	4.6(4.25-4.75) ±0.10	-	-	3.0(2.75-3.25) ±0.07	
3.	Convolvulus arvensis L.	03	-	•	-	20.4(20.25- 20.75) ± 0.09	-	-	20.6(20.25-21.0) ±0.16	-	-	
4.	Cucumis melo var. agrestis Naudin	03	-	-	-	2.30(2.0-2.75) ±0.32	-	-	6.60(5.50-7.25) ±0.32	-	-	
5.	Duranta erecta L.	03	-	-	-	10.43(8.74- 11.23) ±0.44	-	-	7.23(5.50-9.24) ± 0.70	-	-	
6.	<i>Dodonaea viscosa</i> (L.) Jacq.	03	-		-	2.70(2.0-3.0) ±0.18		-	6.90(6.75-7.25) ±0.10	-	-	
7.	Hibiscus rosa-sinensis L.		17	•	-	•	15.4(15.25- 15.75) ±0.09	-	-	10.5(10.2- 11.2) ±0.18	•	
8.	Ipomoea cairica (L.) Sweet	-	27	28	-	-	5.0(4.50-5.75) ±0.22	5.5(5.25-5.75) ±0.09	-	2.75(2.25-3.0) ±0.13	6.05(5.75- 6.25) ±0.09	
9.	<i>Ipomoea carnea</i> Jacq.	-	38	48	-	-	9.45(8.75-9.75) ±0.18	5.4(5.25-5.75) ±0.09	-	2.40(2.0-2.75) ±0.15	6.70(6.25-7.0) ±0.14	
10.	<i>Malvastrum coromandelianum</i> (L.) Garcke	-	38	-	-	-	4.55(4.25-4.75) ±0.09	-	-	3.25(3.0-3.50) ±0.11	-	
11.	Parthenium hysterophorus L.	03	16	-	05	0.50(0.25-0.75) ±0.07	0.95(0.75-1.25) ±0.09	-	4.30(3.75-4.75) ±0.18	0.04(0.25- 0.50) ±0.06	-	
12.	Physalis angulata L.	04	-	-	-	4.20(2.75-5.50) ±0.60	-	-	5.95(5.50-6.50) ±0.18	-	-	
13.	Ricinus communis L.	03	-	-	-	0.4(0.25-0.50) ±0.06	-	-	0.3(0.25-0.50) ±0.06	-	-	
14.	<u>T</u> araxacum officinale (L.) Weber ex F.H.Wigg.	06	29	-	11	2.45(2.25-2.75) ±0.12	0.06(0.50-0.7) ±0.06	-	5.35(5.25-5.50) ±0.06	0.35(0.25- 0.50) ±0.06	-	
15.	Trichodesma indicum (L.) Lehm.	03	-	-	-	3.25(3.0-3.50) ±0.07	-	-	8.10(7.75-8.50) ±0.12	-	-	
16.	Verbena officinalis L.	04	-	-	-	2.60(2.0-3.0) ±0.20	-	-	6.05(4.75-7.0) ±0.36	-	-	

**Table 1.** Variations in quantitative readings of colpi, spines and pores among pollen grains of examined species

Abbreviation: -, Absent; %, Percentage; S.E, Standard Error; Min, minimum;

Table 4. Qualitative features of pollen flora from Lesser

Sr. No	Plant taxa	Pollen Shape	Shape in polar view	Pollen size	Polarity	Dispersal unit	Pollen type	Aperture orientation	Spine shape	Fastigium cavity	Exinc ornamentation
1.	Bougainvillea spectabilis Willd.	Spheroidal	Spherical	Medium	Apolar	Monad	Tricolpate	Sunken	i.	-	Cristate- reticulate
2.	Cannabis sativa L.	Prolate- spheroidal	Spherical	Small	Isopolar	Monad	Tricolpate	Sunken and furrowed		+	Scabrate and rough
3.	Cascabela thevetia (L.) Lippold	Prolate- spheroidal	Triangular	Very large	Isopolar	Monad	Tricolpate	Slightly bulged, scabrate		-	Scabrate
4.	Celosia argentea L.	Prolate- spheroidal	Round	Medium	Apolar	Monad	Polyporate	Circular, bulged and granulate		-	Microgranulate and punctate
5.	Convolvulus arvensis L.	Prolate- spheroidal	Round	Large	Isopolar	Monad	Tricolpate	Slit like, bulged, and regulate, granulate	-	+	Rough and microscabrate- punctate
6.	<i>Cucumis melo var. agrestis</i> Naudin	Prolate- spheroidal	Triangular	Large	Isopolar	Diad	Tricolporate	Slightly bulged, circular, perforate	-	-	Rough and rugulate perforate
7.	Cyperus niveus Retz.	Sub-prolate	Spherical	Medium	Apolar	Monad	-	-	-	-	Scabrate- granulate
8.	Datura stramonium L.	Prolate- spheroidal	Spherical	Large	Apolar	Diad	-			-	Striate-rugulate
9.	Duranta erecta L.	Oblate- spheroidal	Triangular	Medium	Heterop olar	Monad	Tricolpate	Bulged and scabrate	-	-	Scabrate with slight bumps and ridges
10.	<i>Dodonaea viscosa</i> (L.) Jacq.	Prolate- spheroidal	Round	Small	Apolar	Monad	-	-	-	-	Verrucate with rough and warty texture
11.	Hibiscus rosa-sinensis L.	Spheroidal	Spherical	Very large	Apolar	Monad	-	ł	Regular spines with blunt tips and bulged base	-	Echinate- scabrate
12.	Hymenocallis littoralis (Jacq.) Salisb.	Prolate	Oval	Large - Very large	Heterop olar	Diad	Diocolpate	Bulged and scabrate	Regular, small with blunt tips	-	Rough and Microechinate

13.	<i>Ipomoea cairica</i> (L.) Sweet	Prolate- spheroidal	Spherical	Large	Apolar	Monad	-	÷	Regular, pointed spines with bulged bases.		Echinate
14.	Ipomoea carnea Jacq.	Spherical	Spherical	Large - Very large	Isopolar	Monad	Polyporate	-	Regular, broad, and bulbous at base, blunt tip apex	-	Echinate with conical spines
15.	Lantana camara L.	Oblate- spheroidal	Round	Medium	Isopolar	Monad	Tricolpate	Sunken and perforated	-	-	Scabrate
16.	<i>Malvastrum coromandelianum</i> (L.) Garcke	Prolate- spheroidal	Round	Large	Isopolar	Monad	-	-	Regular, broad, and bulbous at base, blunt tip apex	÷	Echinate
17.	Parthenium hysterophorus L.	Prolate- spheroidal	Round	Small	Isopolar	Monad	Tricolpate	Sunken	Regular, perforated, and bulbous base, pointed apex	-	Echinate and perforated
18.	Physalis angulata L.	Prolate- spheroidal	Spherical	Medium	Isopolar	Diad	Tri or tetracolpate	Slit like psilate but bulged, and granulate at tips	÷	+	Microscabrate- punctate
19.	Ricinus communis L.	Sub-oblate	Triangular	Medium	Isopolar	Polyad	Tricolpate	Sunken and slit like	-	+	Scabrate to perforate
20.	<i>Tarax.acum officinale</i> (L.) Weber ex F.H.Wigg.	Prolate- spheroidal	Triangular	Medium	Isopolar	Monad	Tricolporate	Bulged and circular	Psilate pointed spines with broader base and acute tips	÷	Echinate
21.	Tribulus terrestris L.	Oblate- spheroidal	Round	Medium -Large	Apolar	Monad	÷	-	-	-	Cristate- reticulate with slightly wavy margins
22.	Trichodesma indicum (L.) Lehm.	Prolate- spheroidal	Triangular	Small	Isopolar	Tetrad	Tricolpate	Bulged and microgranulate	-	+	Microgranulate
23.	Verbena officinalis L.	Sub-oblate	Triangular	Medium -Large	Heterop olar	Monad	Tri or tetracolpate	Sunken and slit like	-	+	Microscabrate
24.	Zephyranthes carinata Herb.	Prolate	Oval	Large	Isopolar	Monad	-	-	-	-	Cristate- foveolate

Kanwal K et al. (2024). Not Bot Horti Agrobo 52(2):13462

# Qualitative analysis

Prolate-spheroidal shape was dominantly observed (13 species) followed by spheroidal, sub-prolate, oblate-spheroidal, prolate, spherical and sub-oblate. Slight variations were seen in pollen types; tricolpate, tricolporate, polyporate in most of the species whereas dicolpate in *Hymenocallis littoralis* and tetracolpate in *Physalis angulata*. Exine sculptural patterns was examined mostly scabrate. However, echinate stratification was visualized in Asteraceous, Malvaceous and Convolvulaceous species. The exine muri elements in other species were cristate-reticulate, granulate, micro-granulate, punctate, micro-scabrate, rugulate-perforate, striate-regulate, verrucate and cristate-foveolate. Various aperture patterns were observed; sunken, furrowed, slightly bulged, scabrate, granulate, slit like, perforate, circular as shown in Table 4. The *Cucumis melo* has the highest fertility (90%), with the sterility and fertility percentages of each species were mentioned in Table 2.

#### Quantitative parameters

## Pollen diameter and P/E ratio

The largest polar diameter was observed in *Hymenocallis littoralis* (138.6  $\mu$ m) while the smallest was observed in *Parthenium hysterophorus* (14.70  $\mu$ m). Equatorial diameter is measured maximum in *Cascabela thevetia* (110.1  $\mu$ m) and minimum in *Hibiscus rosa-sinensis* (1.7  $\mu$ m) as shown in Figure 12. P/E ratio was calculated to be largest in *Hymenocallis littoralis* (1.8), while minimum in *Duranta erecta* (0.89).

Kanwal K et al. (2024). Not Bot Horti Agrobo 52(2):13462



Figure 12. Polar and equatorial diameter comparison among palynomorphs

# Aperture size

The largest colpi length and width was noted maximum in *Convolvulus arvensis* (20.4  $\mu$ m and 20.6  $\mu$ m) whereas the smallest was in the *Ricinus communis* (0.4  $\mu$ m and 0.3  $\mu$ m) as shown in Figure 13. Length of spine was observed largest in *Hibiscus rosa-sinensis* (15.4  $\mu$ m) and smallest in *Taraxacum officinale* (0.06  $\mu$ m). Width of spine was observed greater in *Hibiscus rosa-sinensis* (10.5  $\mu$ m) while minimum in *Parthenium hysterophorus* (0.04  $\mu$ m). The largest pore length was noted in *Ipomoea cairica* (5.5  $\mu$ m) whereas smallest was observed in the *Celosia argentea* (4.6  $\mu$ m). *Ipomea carnea* has the widest pores 6.70  $\mu$ m, while *Celosia argentea* has the narrowest 3.00  $\mu$ m.



Figure 13. Mean variations in colpi size variations among palynomorphs

Exine thickness and mesocolpium distance

Exine thickness was observed maximum in *Ipomoea carnea* (3.55  $\mu$ m) whereas minimum in the *Cannabis sativa* (0.35  $\mu$ m). Mesocolpium is the distance between two colpi. It was observed maximum in the

*Cascabela thevetia* (87.05 µm) while minimum in *Parthenium hysterophorus* (10.30 µm) illustrated in Figure 14.



Figure 14. Mean variations in mesocolpium distance among palynomorphs

### Discussion

The relationship between various plant families and groups can be more easily determined by the taxonomic study of pollen (Joujeh *et al.*, 2019). Researchers classify plants of various areas and regions on basis of pollen morphology (Najar *et al.*, 2022). The Lesser Himalayan region boasts a breath-taking array of plant life, with many species still waiting to be uncovered.

*Bougainvillea spectabilis* pollen was observed radially symmetrical, oblate, tricolpate, having long colpate colpi without endo apertures, reticulate exine sculpturing and hetero brochate lumina in past research (Maw *et al.*, 2020). While we have observed pollen of *Bougainvillea spectabilis*. as spheroidal shape, medium sized, a polar, monad, tricolpate, sunken colpi with cristate-reticulate exine sculpturing. In the past study pollen of *Cannabis sativa* was observed as triporate, when viewed from the polar and equatorial angles the shape of the pollen appeared either oblate or spheroidal. (Kayani *et al.*, 2015). While in our findings pollen of *Cannabis sativa L.* was recorded prolate- spheroidal, spherical, small, iso-polar, monad, having sunken and furrowed 3-colpate, with scabrate and rough exine surface.

*Cascabella thevetia* pollen were noted as monad, sub-spheroidal shape with three colpi, the outline appears lobate, colpus have obtuse ends, which are classified as brevicolporus. The exine sculpturing was observed micro-reticulate to perforate in the previous research (Naz *et al.*, 2019). However, our findings on *Cascabella thevetia* revealed pollen were prolate- spheroidal, triangular, isopolar, very large monad, three slightly bulged scabrate colpi and scabrate exine surface. *Celosia argentea* grains were found to be monad with radial symmetry, spheroidal shape, and small size. They have a total of 18 apertures and poly-pantoporate, collapsed circular-like shapes and sparse granules. The exine sculpturing was rough and punctuate with numerous sparsely distributed granules (Usma *et al.*, 2022b). In our research we have observed pollen of *C. argentea* as

prolate- spheroidal, round, medium, a polar and monad. Polyporate, circular, bulged and granulate colpi with exine surface micro-granulate and punctate.

Pollen of *Convolvulus arvensis* were medium sized, prolate shape, tricolpate, perforate-scabrate exine sculpturing. The colpi are long and narrow, and the colpus membrane is sparsely perforated and does not have any spines (Amina *et al.*, 2020; Ashfaq *et al.*, 2020). Our findings indicate the pollen of *C. arvensis* as prolate-spheroidal, round, large, iso-polar, monad. Three colpi that are slit like, bulged, and rugulate, granulate while with exine surface is rough and micro scabrate-punctate. Pollen of *Cucumis melo* var. *agrestis* were recorded prolate, sub-oblate to oblate, reticulate ornamentation previously (Perveen and Qaiser, 2008). While in present research pollen of *C. melo* var. *agrestis* were recorded prolate-spheroidal, triangular, iso-polar, dyad and large size. Colpi are slightly bulged, circular and rugulate perforate while exine sculpturing is rough and perforate.

Pollen of *Cyperus niveus* was monoporate, spherical in polar view, oblate-spheroidal in the equatorial view with P/E ratio 0.94 and exine thickness 0.89  $\mu$ m (Ahmad *et al.*, 2020). While we observed pollen of *C. niveus* was sub-prolate, spherical, medium, a polar, monad and scabrate-granulate exine. The pollen grains of *Datura stramonium* were observed either roughly spherical or oblate in shape, with a circular impressions, three furrowed, brevi-colpate (Kayani *et al.*, 2019). While we have observed pollen of *D. stramonium* prolate-spheroidal, spherical, large, a polar, and exine surface striate-regulate.

Pollen of *Duranta erecta* were previously characterized as medium-sized, triangular, prolate-spheroidal and is either di-colporate or tricolporate with psilate exine. Pollen were oblate-spheroidal, tricolporate, and have a semi-reticulate exine (Sousa *et al.*, 2011). However, our research indicated the pollen of *D. erecta* oblate-spheroidal, triangular, medium sized, heteropolar and monad. Tricolpate, bulged apertures with scabrate exine having slight bumps and ridges are present. *Dodonaea viscosa* grains were observed monad, medium size with a prolate spheroidal shape that can appear either circular or triangular. It was also discovered iso-polar, tricolpate, and has a psilate exine (Alnagger *et al.*, 2022). In our research, pollen of *D. viscosa* were observed prolate-spheroidal, round, small, a polar, monad, exine surface was verrucaate with rough and warty texture.

In previous findings *Hibiscus rosa-sinesis* pollen were seen spheroidal shape, a polar, displays radial symmetry in polar view and bilateral symmetry in equatorial view. The spines on the surface were mostly curved and directed away from the center with blunt or rounded tips (NAGGAR, 2004). While in our study pollen of *H. rosa-sinesis* were spheroidal, spherical, very large, a polar, monad and having regular spines with blunt tips bulged base, exine ornamentation echinate-scabrate. Previous studies on *Hymenocallis littoralis* grains were described as large, circular, scabrate with a prolate shape and the absence of colpi (Zafar *et al.*, 2022). In another research it was showed scabrate, verrucate colpi, psilate and scabrate exine at the apocolpium, while rest of the area reticulate with well oriented muri (Bahadur *et al.*, 2018). We observed pollen of *H. littoralis* as prolate, oval shape, large to very large, heteropolar, two colpi that are bulged, regular, small spines with blunt tips and exine sculpturing rough and micro-echinate present.

Pollen of *Ipomoea cairica* were described medium sized, sub-spheroidal, scabrate and echinate (Ashfaq *et al.*, 2019). While this research observed pollen of *I. cairica* as prolate- spheroidal, spherical, large, monad, regular, pointed spines with bulged bases with echinate exine. In previous literature the pollen of *Ipomoea carnea* were spherical in shape, has multiple pores, and is covered with spines that create a tetragonal pattern. At the base of these spines, there are bulbous projections, and the pollen also features circular apertures (Basarkar and Technology, 2017; Farid, 2022). In current study pollen of *I. carnea* were spherical, large to very large, a polar, monad and polyporate. Exine sculpturing echinate with conical spines. Spines are regularly arranged on the surface with broad and bulbous at base, gradually tapering toward apex with blunt tips.

Previous studies have described the pollen of *Lantana camara* having different characteristics. (*Zafar et al.*, 2022) identified it as tricolporate, medium-sized, round, and scabrate. (Basarkar and Technology, 2017) explained *L. camara* pollen were tricolporate, elliptical, triangular, and psilate. Whereas current findings described *L. camara* grains were oblate-spheroidal, round, medium, iso-polar, monad, tricolpate, sunken and

perforated colpi with scabrate exine. Past studies on pollen data of *Malvastrum coromandelianum* shown large, oblate-spheroidal in shape, with multiple pores, and a surface covered in spines and small spinules. (Cheema, 2018; Zafar *et al.*, 2022). We observed *M. coromandelianum* grains were prolate- spheroidal, round, large, isopolar and monad. Exine is echinate with regular spines that are broad and bulbous at base and blunt tip apex.

Previous research identified pollinic features of *Parthenium hysterophorus* as small, circular, and oblate in shape. It was perforate and echinate, with three colpi visible on its surface (Attique *et al.*, 2022; Basarkar and Technology, 2017; Nabila *et al.*, 2022). However, this study described *P. hysterophorus* pollen were observed prolate-spheroidal, round, small, iso-polar, monad and tricolpate. Echinate and perforated exine having regular, spines that consist perforated bulbous base and pointed apex. Earlier study was identified the pollen of *Physalis angulata* were psilate, oblate-spheroidal or prolate in shape, with four or three colpi (Bhat *et al.*, 2018). The present work represent pollen of *P. angulata* were as prolate-spheroidal, spherical, medium and iso-polar.

According to Bahadur *et al.* (2022) the pollen of *Ricinus communis* were oblate-spheroidal in shape, with two colpi visible on its surface, echinate exine, covered in small spines. We described in this study pollen of *R. communis* were observed sub-oblate, triangular, medium, iso-polar. Tricolpate, sunken and slit like apertures with fastigium cavity visible while exine surface is scabrate to perforate.

Nabila *et al.* (2022) elaborate *Taraxacum officinale* grains were monad, with six colpi visible on its surface exine is echinate, covered in small spines. In polar view, the shape of the pollen is circular to semi-angular, while in equatorial view, it is also circular to semi-angular. In our finding grains of *T. officinale* were prolate-spheroidal, triangular, medium size, iso-polar, monad and having tricolporate, bulged and circular apertures. Echinate exine consisting of pointed spines with broader base and acute tips. Past studies have reported that the pollen grains of *Tribulus terrestris* were oblate-spheroidal in shape have multiple pores, including both polyporate and pantoporate types and feature a reticulated exine (Thakur *et al.*, 2020). Whereas current observation explained pollen of *T. terrestris* were oblate-spheroidal, round, medium-large, a polar and monad. Cristate-reticulate exine with slightly wavy margins.

Kumar *et al.* (2020) described the pollen of *Trichodesma indicum* was tricolporate, with a sub-prolate shape and a small size. The colpi are longicolpate that are elongated in shape. The pollen surface features obscurely reticulate sculpturing. While in our results pollen of is *T. indicum* were examined prolate- spheroidal, triangular, small, iso-polar, and tetrad. Colpi are bulged, micro-granulate, three in number with fastigium cavity visible

(Perveen and Qaiser, 2007) reported that the grains of *Verbena officinalis* were oblate-spheroidal in shape, striate-regulate exine sculpturing and has three colpi. However, grains pollen of *V. officinalis* were observed sub-oblate, triangular, medium-large, heteropolar, monad, tri or tetracolpate, sunken and slit like colpi with microscabrate exine in present work. The pollen of *Zephyranthes carinata* were described previously round and has a textured surface with ridges and furrows that form hexagonal or pentagonal shapes. This texture is due to the muri on the exine, which gives it an ornamental appearance. (Bahadur *et al.*, 2018). Present study interprets the pollen of *Z. carinata* were oval, large, iso-polar, monad, prolate and oval. Colpi are nonvisible while exine sculpturing is cristate-foveolate.

Thus, the study of pollen morphology can offer valuable tools for the identification of different plant species. Further, these palynological method can be applied in comparative and taxonomic studies to provide important insights into evolutionary relationships and accurate classification of different angiosperms grouping.

# Conclusions

Pollen morphological traits from selected flora of the Lesser Himalayas, such as pollen shape, size, type, aperture orientation, and exine sculpturing were significant for accurate species identification. Exine stratification were visualized as echinate, cristate-reticulate, granulate, punctate, scabrate, rugulate-perforate, striate-rugulate, verrucate, cristate-foveolate patterns. Pollen types observed were tricolporate, tricolpate, polyporate. Major shapes observed in polar view were spherical, round, triangular, tetragonal while prolate-spheroidal, spherical, oblate-spheroidal, prolate and sub-oblate in equatorial view. Palynomorphs with the highest mesocolpium distance were measured in *Cascabela thevetia* (87.05  $\mu$ m). Whereas exine thickness was noted maximum for *Ipomoea carnea* (3.55  $\mu$ m). Colpi size was calculated largest for *Convolvulus arvensis* (20.4  $\mu$ m). The variations in morpho pollinic features highlights the diversity of species found in Lesser Himalaya and this knowledge of pollen flora description was helpful to resolve systematics and correct identification of problematic taxa.

#### Authors' Contributions

KK: Contributed to the conceptualization of the study, data collection, and analysis. MZ: Provided guidance throughout the research process, contributed to the writing and revision of the manuscript. MA: Participated in data analysis and interpretation. MSA: Contributed to literature review and manuscript writing. MSE: Funding, Provided expertise in a specific aspect of the research methodology. TM: Contributed to data interpretation and statistical analysis. AY: Involved in data collection and organization. SI: Contributed to the discussion section of the manuscript. DS: Provided critical feedback and revisions to the manuscript. NN: Participated in the design of the study and data interpretation. HFE: Contributed to the conceptualization and design of the study. IM: Participated in data collection and analysis. MSdeO: Involved in manuscript preparation and formatting. SM: Provided expertise in a specific area of the research topic. VF: Contributed to the overall supervision and coordination of the research project. All authors read and approved the final manuscript.

#### Ethical approval (for researches involving animals or humans)

Not applicable.

#### Acknowledgements

The authors extend their appreciation to the Researchers supporting project number (RSP2024R173), King Saud University, Riyadh, Saudi Arabia.

# **Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

# References

- Ahmad S, Zafar M, Ahmad M, Ozdemir F, Yaseen G, Sultana S, Kutlu MJ (2020). Palynological studies of winter weeds melliferous flora of district Bannu, Khyber Pakhtunkhwa, Pakistan. Annali di Botanica 10:77-86. https://doi.org/10.13133/2239-3129/14863
- Al-Kahtani SN, Taha E-K, Khan KA, Ansari MJ, Farag SA, Shawer DM, Elnabawy ESM (2020). Effect of harvest season on the nutritional value of bee pollen protein. PloS One 15:e0241393. https://doi.org/10.1371/journal.pone.0241393
- Elnaga AA, Alnagger AA, Rabei, S (2022). Pollen morphology of some woody plants in new Damietta City. Journal for Damietta Faculty of Science 12:163-173. *https://doi.org/10.21608/sjdfs.2022.137961.1045*
- Amina H, Ahmad M, Bhatti GR, Zafar M, Sultana S, Butt MA, Bahadur S, Haq IU, Ghufran MA (2020). Microscopic investigation of pollen morphology of Brassicaceae from Central Punjab-Pakistan. Microscopy Research and Techniques 83:446-454. https://doi.org/10.1002/jemt.23432
- Ashfaq S, Ahmad M, Zafar M, Sultana S, Bahadur S, Ahmed SN, Gul S, Nazish M (2020). Pollen morphology of family Solanaceae and its taxonomic significance Anais da Academia Brasileira de Ciências 92:1-16. https://doi.org/10.1590/0001-3765202020181221
- Ashfaq S, Ahmad M, Zafar M, Sultana S, Bahadur S, Ullah F, Zaman W, Ahmed SN, Nazish MJF (2019). Foliar micromorphology of Convolvulaceous species with special emphasis on trichome diversity from the arid zone of Pakistan. Flora 255:110-124. https://doi.org/10.1016/j.flora.2019.04.007
- Attique R, Zafar M, Ahmad M, Zafar S, Ghufran MA, Mustafa MRU, Yaseen G, Ahmad L, Sultana S, Nabila, Majeed, S (2022). Pollen morphology of selected melliferous plants and its taxonomic implications using microscopy. Microscopy Research and Techniques 85:2361-2380. https://doi.org/10.1002/jemt.24091
- Bahadur S, Ahmad M, Mir S, Zafar M, Sultana S, Ashfaq S, Arfan M (2018). Identification of monocot flora using pollen features through scanning electron microscopy. Microscopy Research and Techniques 81:599-613. https://doi.org/10.1002/jemt.23015
- Bahadur S, Taj S, Long W, Ahmad M (2022). Pollen morphology and its implication in the taxonomy of some selected taxa of the bi and tri-ovulate Euphorbiaceae of the Hainan Island by using multiple microscopic techniques. Microscopy Research and Techniques 85:2045-2060. https://doi.org/10.1002/jemt.24062
- Bano A, Ahmad M, Khan MA, Zafar M, Sultana S, Ullah Z (2012). Pollen morphology of four endemic species of *Pedicularis* L. from alpine zone of the Deosai Plateau, Himalayan range. Bangladesh Journal of Plant Taxonomy 19:1-5. http://dx.doi.org/10.3329/bjpt.v19i1.10935
- Basarkar U (2017). Light microscopic studies of pollen grains by acetolysis method. International Journal of Researches in Biosciences, Agriculture and Technology 3:1-10.
- Bhat NA, Jeri L, Mipun P, Kumar Y (2018). Systematic Studies (micro-morphological, leaf architectural, anatomical and palynological) of genus *Physalis* (Solanaceae) in Northeast India. Plant Archive 18:2229-2238.
- Candeias J, Schmidt-Weber CB, Buters JJ (2021). Dosing intact birch pollen grains at the air-liquid interface (ALI) to the immortalized human bronchial epithelial cell line BEAS-2B. PloS One 16:e0259914. https://doi.org/10.1371/journal.pone.0259914
- Chatterjee R, Sarkar S, Rao G (2014). Pollen morphology and palynotaxonomical studies of common garden plants of Apocynaceae, in Kolkata, West Bengal, India. International Journal of Environment 3:109-116.
- Cheema P (2018). Palynological studies on some medicinal mallows from Punjab, India. Annals of Plant Sciences 7:2166-2169. http://dx.doi.org/10.21746/aps.2018.7.3.13
- Farid MH (2022). Comparative botanical studies on four genera of family Convolvulaceae. Azhar Journal of Agricultural Research 47:10-25. *https://doi.org/10.21608/ajar.2022.277827*
- Gul S, Ahmad M, Zafar M, Bahadur S, Zaman W, Ayaz A, Shuaib M, Butt MA, Ullah F, Saqib S (2021). Palynological characteristics of selected Lamioideae taxa and its taxonomic significance. Microscopy Research and Techniques 84:471-479. https://doi.org/10.1002/jemt.23603
- Halbritter H, Ulrich S, Grímsson F, Weber M, Zetter R, Hesse M, Buchner R, Svojtka M, Frosch-Radivo A (2018). Pollen morphology and ultrastructure. Illustrated Pollen Terminology 37-65. https://doi.org/10.1007/978-3-319-71365-6 3

- Joujeh R, Zaid S, Mona S (2019). Pollen morphology of some selected species of the genus *Centaurea* L. (Asteraceae) from Syria. South African Journal of Botany 125:196-201. *https://doi.org/10.1016/j.sajb.2019.07.040*
- Kailas JG, Naik MC, Bheemalingappa M, Ramakrishna H, Rao B (2017). Arboreal diversity of the Andaman Islands, India, based on pollen analysis. Palynology 41:370-388. *https://doi.org/10.1080/01916122.2016.1209592*
- Katata-Seru L, Moremedi T, Aremu OS, Bahadur I (2018). Green synthesis of iron nanoparticles using *Moringa oleifera* extracts and their applications: Removal of nitrate from water and antibacterial activity against *Escherichia coli*. Journal of Molecular Liquids 256:296-304. https://doi.org/10.1016/j.molliq.2017.11.093
- Kayani S, Ahmad M, Sultana S, Shinwari ZK, Zafar M, Yaseen G, Hussain M, Bibi, T (2015). Ethnobotany of medicinal plants among the communities of Alpine and Sub-alpine regions of Pakistan. Journal of Ethnopharmacology 164:186-202. https://doi.org/10.1016/j.jep.2015.02.004
- Kayani S, Hussain M, Ahmad M, Zafar M, Sultana S, Butt MA, Ali S, Shah GM, Mir S (2019). Scanning electron microscopy (SEM) and light microscopy (LM)-based Palyno-morphological views of Solanaceae in Western Himalaya. Microscopy Research and Techniques 82:63-74. https://doi.org/10.1002/jemt.23097
- Khan SAR, Sharif A, Golpîra H, Kumar A (2019a). A green ideology in Asian emerging economies: From environmental policy and sustainable development. Sustainable Development 27:1063-1075. *https://doi.org/10.1002/sd.1958*
- Khan SU, Zafar M, Ahmad M, Anjum F, Sultana S, Kilic O, Ozdemir FA, Nazir A, Yaseen G, Aabidin S.Z (2019b). Pollen micromorphological analysis of tribe Acacieae (Mimosaceae) with LM and SEM techniques. Microscopy Research and Techniques 82:1610-1620. https://doi.org/10.1002/jemt.23327
- Khan MR, Zafar M, Ahmad M, Al-Ghamdi AA, Elshikh MS, Makhkamov T, Majeed S, Kumar, P (2023). Exploring intraspecific pollen morphology variation in Apocynaceae: A roadmap for horticultural innovation. Folia Horticulturae 35:479-493. https://doi.org/10.2478/fhort-2023-0034
- Kumar A, Sharma SS, Kumar B (2020). Palynological studies on some plants of Boraginaceae. Journal of Biotechnology and Biochemistry 6:4-12. *https://doi.org/10.9790/264X-0605020412*
- Lu L, Wortley AH, Li D-Z, Wang H, Blackmore S (2015). Evolution of angiosperm pollen. 2. The basal angiosperms. Annals of the Missouri Botanical Garden 100:227-269.
- Majeed, S., Ahmad, M., Ozdemir, F. A., Demirpolat, A., Şahan, Z., Makhkamov, T. Nabila (2023). Micromorphological characterization of seeds of dicot angiosperms from the Thal desert (Pakistan). Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology 157(2):392-418. https://doi.org/10.1080/11263504.2023.2165553
- Majeed S, Zafar M, Ahmad M, Kilic O, Sultana S, Raza J, Yaseen G, Gul H, Mir S, Jabeen, M (2020). Pollen morphological investigations of family Cactaceae and its taxonomic implication by light microscopy and scanning electron microscopy. Microscopy Research and Techniques 83:767-777. https://doi.org/10.1002/jemt.23467
- Majeed S, Zafar M, Ahmad M, Zafar S, Ghufran A, Ayoub M, Sultana S, Yaseen G, Raza J (2022). Morpho-palynological and anatomical studies in desert cacti (*Opuntia dillenii* and *Opuntia monacantha*) using light and scanning electron microscopy. Microscopy Research and Techniques 85:2801-2812. https://doi.org/10.1002/jemt.24129
- Majeed S, Zafar M, Althobaiti AT, Ramadan MF, Ahmad M, Makhkamov T, Yaseen G (2022). Comparative petiole histology using microscopic imaging visualization among Amaranthaceous taxa. Flora 297:152178. https://doi.org/10.1016/j.flora.2022.152178
- Maw KH, Than KN, Yee NN (2020). Pollen morphology of some ornamental plants in Yadanabon university campus, Mandalay, Third Myanmar Korea Conference Research Journal 3:203-210.
- Muniraja M, Vijayalakshmi G, Lakshmipathi Naik M, Terry R, Sha Valli Khan PJP (2020). Ultrastructural observations of anthers, staminodes, and pollen grains of mango (*Mangifera indica* L. var. Beneshan; Anacardiaceae). Palynology 44:565-574. https://doi.org/10.1080/01916122.2019.1633437
- Nabila Ahmad M, Zafar M, Bahadur S, Sultana S, Taj S, Celep F, Majeed S, Research RJM, Technique (2022). Palynomorphological diversity among the Asteraceous honeybee flora: An aid to the correct taxonomic identification using multiple microscopic techniques. Microscopy Research and Techniques 85:570-590. https://doi.org/10.1002/jemt.23932
- Naggar, S.M (2004). Pollen morphology of Egyptian Malvaceae: An assessment of taxonomic value. Turkish Journal of Botany 28:227-240.

- Najar ZH, Kashtwari M, Zargar SA, Wani AAJV (2022). Assessment of morphological diversity of apple (*Malus*× *domestica* Borkh.) Germplasm in North Kashmir, India. Vegetos 1-10. *https://doi.org/10.1007/s42535-022-00435-x*
- Naz S, Zafar M, Ahmad M, Memon RA, Sultana S, Bahadur S, Ozdemir FA, Siddiq Z, Shah M.A(2019). Palynological investigation of lactiferous flora (Apocynaceae) of District Rawalpindi, Pakistan, using light and scanning electron microscopy. Microscopy Research and Techniques 82:1410-1418. https://doi.org/10.1002/jemt.23293
- Nazish M, Zafar M, Ahmad M, Sultana S, Ullah R, Alqahtani AS, Ullah F, Ahmad S, Ashfaq S, Ullah F (2019). Palynomorphological investigations of halophytic taxa of Amaranthaceae through SEM from Salt range of Northern Punjab, Pakistan. Microscopy Research and Techniques 82:304-316. https://doi.org/10.1002/jemt.23173
- Noor W, Zafar M, Ahmad M, Althobaiti AT, Ramadan MF, Makhkamov T, Khan A (2023). Petiole micromorphology in Brassicaceous taxa and its potential for accurate taxonomic identification. Flora 303:152280. https://doi.org/10.1016/j.flora.2023.152280
- Pasarelli M, Cortes M (2017). Pollen as a Forensic tool in a zone of wetlands from Buenos Aires, Argentina. Journal of Forensic Research 8:1-3. https://doi.org/10.4172/2157-7145.1000366
- Perveen A, Qaiser M (2007). Pollen flora of Pakistan-LIII. Verbenaceae. Pakistan Journal of Botany 39:663-669.
- Perveen A, Qaiser M. (2008). Pollen flora of Pakistan-LVI. Cucurbitaceae. Pakistan Journal of Botany 40:9-16.
- Punt W, Hoen P, Blackmore S, Nilsson S, Le Thomas, A (2007). Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology 143:1-81. https://doi.org/10.1016/j.revpalbo.2006.06.008
- Rashid N, Zafar M, Ahmad M, Malik K, Shah SN, Sultana S, Zahid N, Noshad Q, Siddiq Z (2022). Use of scanning electron microscopy to analyze sculpturing pattern and internal features of pollen grain wall in some members of Astragaleae (subfamily: Papilionoidae). Microscopy Research and Techniques 85:1631-1642. https://doi.org/10.1002/jemt.24023
- Raza J, Ahmad M, Zafar M, Athar M, Sultana S, Majeed S, Yaseen G, Imran M, Nazish M, Hussain, A (2020). Comparative foliar anatomical and pollen morphological studies of Acanthaceae using light microscope and scanning electron microscope for effective microteaching in community. Microscopy Research and Techniques 83:1103-1117. https://doi.org/10.1002/jemt.23502
- Sousa SM, Reis AC, Silva PS, Viccini LFJC (2011). An increment in the *Duranta repens* L. (Verbenaceae) knowledge: DNA content, karyology, meiosis and palynology. Caryologia 64:110-116. https://doi.org/10.1080/00087114.2011.10589770
- Thakur M, Nanda, V (2020). Composition and functionality of bee pollen: A review. Trends in Food Science & Technology 98:82-106. https://doi.org/10.1016/j.tifs.2020.02.001
- Ullah F, Ahmad M, Zafar M, Parveen B, Ashfaq S, Bahadur S, Safdar Q, Safdar LB, Alam F, Luqman M (2022). Pollen morphology and its taxonomic potential in some selected taxa of Caesalpiniaceae observed under light microscopy and scanning electron microscopy. Microscopy Research and Techniques 85:1410-1420. https://doi.org/10.1002/jemt.24004
- Ullah, T., Zafar, M., Ahmad, M., Alkahtani, J., Elshikh, MS., Makhkamov, T., Majeed, S, Nizomova, M (2024). Integrating morpho pollinic traits for systematic classification of rubiaceous species and conservation implications. Genetic Resources and Crop Evolution 1-18. *https://doi.org/10.1007/s10722-024-01917-4*
- Umber F, Zafar M, Ullah R, Bari A, Khan MY, Ahmad M, Sultana S (2022). Implication of light and scanning electron microscopy for pollen morphology of selected taxa of family Asteraceae and Brassicaceae. Microscopy Research and Techniques 85:373-384. https://doi.org/10.1002/jemt.23912
- Usma A, Ahmad M, Ramadan MF, Khan AM, Zafar M, Hamza M, Sultana S, Yaseen G (2022a). Micro-morphological diversity of pollen among Asteraceous taxa from Potohar Plateau-Pakistan. Microscopy Research and Techniques 85:2467-2485. https://doi.org/10.1002/jemt.24102
- Usma A, Ahmad M, Zafar M, Sultana S, Ullah F, Saqib S, Ayaz A, Zaman WJA (2022b). Palynological study of weed flora from Potohar Plateau. Agronomy 12:2500. https://doi.org/10.3390/agronomy12102500
- Waheed A, Ahmad H, Abbasi FM, Hamid F, Shah A, Safi F, Ali, H. (2013). Pollen sterility in wide crosses derivatives of rice (*Oryza sativa* L.). Journal of Materials and Environmental Science 4:404-409.
- Xu F-X, Kirchoff BK (2008). Pollen morphology and ultrastructure of selected species of Magnoliaceae. Review of Palaeobotany and Palynology 150:140-153. https://doi.org/10.1016/j.revpalbo.2008.01.007

- Yang S, Zheng Z, Huang K, Zong Y, Wang J, Xu Q, Rolett BV, Li JJTH (2012). Modern pollen assemblages from cultivated rice fields and rice pollen morphology: Application to a study of ancient land use and agriculture in the Pearl River Delta, China. The Holocene 22:1393-1404. https://doi.org/10.1177/0959683612449761
- Yousaf Z, Zafar M, Ahmad M, Sultana S, Rozina Ozdemir FA, Abidin SZU, (2022). Palyno-anatomical microscopic characterization of selected species of Boraginaceae and Fabaceae. Microscopy Research and Techniques 85:1332-1354. https://doi.org/10.1002/jemt.23999
- Zafar A, Zafar M, Ahmad M, Khan AM, Mahmood T, Kilic O, Fatima A, Habib D, Sultana S, Majeed S (2022). Microscopic (LM and SEM) visualization of pollen ultrastructure among honeybee flora from lower *Margalla* Hills and allied areas. Microscopy Research and Techniques 85:3325-3338. https://doi.org/10.1002/jemt.24187



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



**License** - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

#### Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- <u>Responsibilities</u>: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.