agronomy

Article

Palynological Study of Weed Flora from Potohar Plateau

Anwer Usma [1], Mushtaq Ahmad [1,*], Muhammad Zafar [1], Shazia Sultana [1], Fazal Ullah [2], Saddam Saqib [3,4], Asma Ayaz [5] and Wajid Zaman [6,*]

[1] Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan
[2] State Key Laboratory of Grassland Agro-Ecosystems, School of Life Sciences, Lanzhou University, Lanzhou 730000, China
[3] State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
[4] University of Chinese Academy of Sciences, Beijing 100049, China
[5] State Key Laboratory of Biocatalysis and Enzyme Engineering, School of Life Sciences, Hubei University, Wuhan 434200, China
[6] Department of Life Sciences, Yeungnam University, Gyeongsan 38541, Korea
* Correspondence: mushtaqflora@hotmail.com (M.A.); shangla123@gmail.com (W.Z.)

Abstract: The pollen morphology of weeds was investigated by scanning electron microscopy (SEM). A morpho-palynological investigation of 18 species of weeds that belongs to 16 angiosperms families was performed using SEM to document distinguishable microscopic features. The main objective of the present study was to provide basic knowledge about morpho-palynological features of weed species that helps delimit the weed flora of the Potohar Plateau. The results show diversity among the qualitative and quantitative characteristics of pollen shape, equatorial and polar axis diameter, the exine's thickness, and the exine's surface ornamentation. The pollen grains were spherical, prolate-spheroidal, oblate-spheroidal, and sub-oblate. The exine ornamentation in most species was reticulate, scarbate, aerolate, faveolate, reticulate-perforate, and reticulate-scabrate. All the species described possessed tricolpate pollen. The variations found in the thickness of the exine and other characters were helpful at the genus and species-specific levels. In accordance with these variations, a taxonomic key was prepared using these characteristics to identify and differentiate weed plant species. SEM images of pollen grains can help delimit the taxa to the species level. This study provides baseline information to distinguish the species of weeds.

Keywords: exine sculpturing; palynomorph; pollen grains; scanning electron microscopy; Pakistan

check for updates

Citation: Usma, A.; Ahmad, M.; Zafar, M.; Sultana, S.; Ullah, F.; Saqib, S.; Ayaz, A.; Zaman, W. Palynological Study of Weed Flora from Potohar Plateau. *Agronomy* **2022**, *12*, 2500. https://doi.org/10.3390/agronomy12102500

Academic Editor: Rodolfo Gentili

Received: 5 September 2022
Accepted: 11 October 2022
Published: 13 October 2022

1. Introduction

Weeds are considered undesirable plants competing with crops' growth and development. The growth of weeds in agricultural fields can limit the production of staple crops and causes severe damage to their production [1]. According to Khan et al. [2] weeds grow in cultivated and wild habitats and damage the growing crop plants [3]. Weedy plants threaten human health, welfare, biodiversity, ecosystem services, and food security. Here, weeds are defined as any plants that have detrimental socioeconomic and environmental effects, endanger human health, and jeopardize global food security, biodiversity, and ecosystem services [4]. Weeds play an important role in achieving an ecological balance in a cropping system by supporting different life forms. Throughout the world, major vegetation of weeds belongs to families such as Asteraceae, Poaceae, Amaranthaceae, and Fabaceae. The growth of weeds depends mostly on climatic conditions [5]. In some previously reported studies, the most commonly grown weeds of cotton crops are *Cynodon dactylon*, *Amaranthus viridis*, *Phyla nodiflora*, and *Phragmites australis* [6]. Common weeds of wheat crops are *Phragmites* sp., *Cynodon* sp., *Ranunculus* spp., and *Polypogon* sp. [6]. Similarly, *Achyranthes aspera* is a common weed in sugarcane crops and *Plantago lanceolata* is an important weed in chickpea fields [7]. Weeds belonging to the family Fabaceae can obtain essential nutrients from the soil and compete with crops because they gain nutrition faster than other plants [8]. These plants obtain nitrogen from their surroundings and convert it to nitrogen-rich compounds to enhance the growth of essential microorganisms, thereby increasing soil fertility [9]. Many researchers work on the world's weed flora and documented 50 parasitic weeds from Pakistan [10].

In Pakistan, the region of Potohar is located on the northeast side, with an area of approximately 25,000 km^2. Topographically, hilly and plain areas are found in this region [11]. This area also comprises of river Indus and the Jhelum, stretching from the salt range to the foothills of the Himalayas. The Potohar region is between latitude 32°5′34″ N and longitude 71°30′73″ E. The Jhelum plains lie at the lowest altitude, i.e., 825 ft (250 m). Non-native plant species have been introduced into the study area for greenery, which competes with native plant species. The natural vegetation of the Potohar region includes dry deciduous scrubby forests and few species of trees. This area's agriculture purely depends on the monsoon rainfall [12]. The construction of roads, houses, and industries has affected the natural vegetation, resulting in a change in soil composition.

The study of pollen grains plays a vital role in studying plant taxonomy and biodiversity because it helps identify plant species in a particular area [13]. Many taxonomists use different disciplines of plant systematics to identify plant species, such as morphology, anatomical studies, and palynological studies, to find the precise position of plants within taxa [14–19]. On the other hand, the studies of pollen characters are considered the most important tools for classifying and identifying morphologically similar plants. Considerable research has been done on pollen grains in Pakistan and worldwide. Meo and Khan [19] conducted Morpho-palynological studies on weeds for the first time in Rawalpindi, Pakistan. Meo et al. [20] reported the diverse features of weeds and pollen grains belonging to the family Asteraceae. On the other hand, there is not a single document on the morpho-palynology of weeds from the Potohar region of Pakistan. Current research aims to provide detailed information about weeds in the Potohar region of Pakistan, the diversity of their pollen grains, and their exine structure based on qualitative and quantitative characters using SEM as a basis for future studies. A taxonomic key is made based on microscopic characteristics, which helps to distinguish the micro-morphological characteristics to strengthen the identification of complicated weed species. The purpose of the present study was twofold: (1) to determine the pollen fertility of economically important weed species, which will help identify the freely reproducing species for conservation purposes; (2) to the taxonomic identification of the weed species through pollen morphological characteristics.

2. Materials and Methods

2.1. Specimen Collection and Plant Identification

From different sites in the Potohar region of Pakistan (Figure 1), 18 weeds were collected in a single season between August 2021 and November 2021. Approximately three to five samples were collected from each collection site randomly. Table 1 lists the collection site and voucher numbers. After collection, plants were identified either by comparing their morphological characteristics with the flora of Pakistan or by matching their morphology with specimens placed in the Herbarium of Pakistan (ISL). After mounting, the botanical names and author citations were validated using the International Plant Name Index (IPNI) (www.ipni.org (accessed on 7 July 2022)) and accessioned into the Herbarium of Pakistan (ISL) at Quaid-i-Azam University Islamabad, Pakistan.

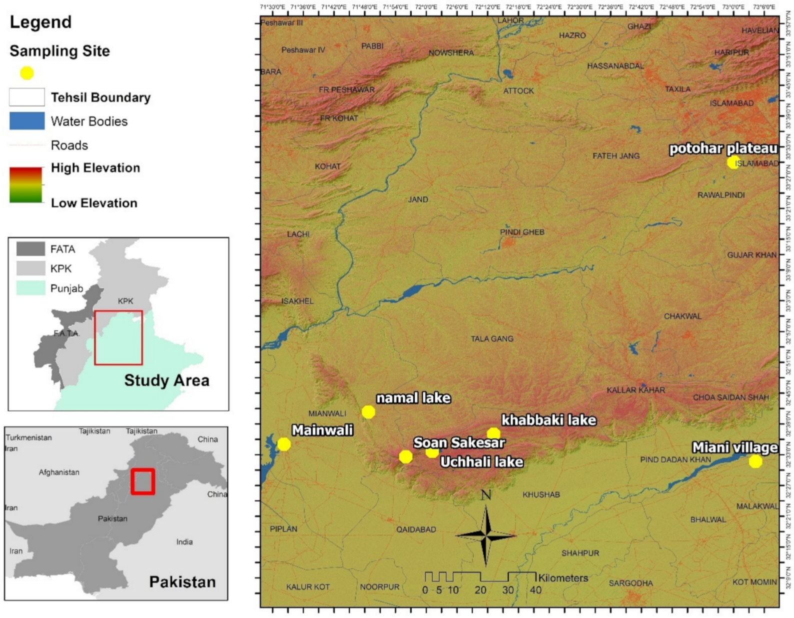

Figure 1. Map of the collection sites of the study area. (Source: Google).

Table 1. Weed samples collected from the Potohar region.

Sr. No	Plant Taxa	Family	Locality	Voucher Specimen Number
1.	*Achyranthus aspera L.*	Amaranthaceae	Salt Range Chakwal	AU-107
2.	*Aerva javanica* (Burm.f.) Juss. ex Schult.	Amaranthaceae	Miani Village Talagang	AU-113
3.	*Amaranthus graecizans* L.	Amaranthaceae	Khewra mines	AU-115
4.	*Asphodelus tenuifolius* Cav.	Asphodelaceae	Uchali Lake	AU-99
5.	*Atriplex stocksii* Boiss.	Amaranthaceae	Uchali Lake	AU-77
6.	*Boerhavia procumbens* Banks ex Roxb.	Nyctaginaceae	Rawalpindi	AU-65
7.	*Brassica furatii* Mouterde	Brassicaceae	Musa khel Mianwali	AU-39
8.	*Capparis decidua* (Forssk.) Edgew.	Capparaceae	Miani Village	AU-55
9.	*Celosia argentea* L.	Amaranthaceae	Kabkbi Lake	AU-110
10.	*Cenchrus ciliaris* L.	Poaceae	Islamabad	AU-205
11.	*Chenopodium murale* L.	Chenopodiaceae	Salt Range	AU-209
12.	*Corchorus depressus* (L.) Stocks	Tiliaceae	Attock	AU-211
13.	*Cissampelos pareira* L.	Menispermaceae	Salt Range	AU-215
14.	*Citharexylum spinosum* L.	Verbenaceae	Islamabad	AU-217
15.	*Cleome brachycarpa* (Forssk.) Vahl ex DC	Capparaceae	Miani village Talagang	AU-31
16.	*Cleome viscosa* L.	Capparaceae	Miani village Talagang	AU-45
17.	*Cucumis melo* subsp. *agrestis* (Naudin) Pangalo.	Cucurbitaceae	Miani village Talagang	AU-53
18.	*Euphorbia granulata* Forssk.	Euphorbiaceae	Thoha Bahadur Chakwal	AU-69

2.2. Study of Pollen under an Optical Microscope

Either dry or fresh plant material extracts pollen grains from anthers. With the help of sharp needles, the anthers were separated from the flowers and placed on glass slides with a single drop of acetic acid, which helps appropriately crush anthers. A slight modification of the method reported by Butt et al. [21] was used to prepare the pollen grains slides. One drop of glycerin jelly was added to the pollen grains, and the extra debris was removed from the glass slide with the help of needles. Glycerin jelly will help stain the pollen grains [21,22]. A cover slip was placed on the pollen grains, and the pollen characteristics were observed by optical microscopy (OM, Leica Dialux 20).

2.3. Study of Pollen under a Scanning Electron Microscope

The pollen grains were prepared for SEM using a slight modification of the methodology reported by Erdtmann [23]. In this method, pollen grains were crushed with 45% acetic acid and were placed on double-sided tape fixed to aluminum stubs using a fine pipette and sputter coated with gold to 150Å. Subsequently, these slides were examined by scanning electron microscopy (Model JEOL JSM- 5910) installed in the Central Resource Library (CRL), Department of Physics, University of Peshawar, Pakistan.

2.4. Data Analysis

The minimum represented the quantitative features (mean ± standard error) and maximum (e.g., 27.7 (85.2 ± 10.8) 105). Five readings of each character were noted for each pollen grain. The quantitative data were noted and processed using SPSS software (version 16) to determine the minimum, maximum, mean, and standard error. These data are very helpful in identifying species and the nature of the different pollen grains. These indices provide information on pollen diameter, colpi and pore size, exine thickness, and polar-to-equatorial ratio.

2.5. Percentages of Sterility and Fertility among Pollen

The pollen fertility was determined using the techniques reported by Khan and Stace [24]. The sterility and fertility percentages were determined using the formula [5,25].

The formula for fertility is given as:

$$\text{Fertility} = (\text{F}/\text{F} + \text{S}) \times 100$$

The formula of sterility is expressed as:

$$\text{Sterility} = (\text{S}/\text{F} + \text{S}) \times 100$$

In the formula, F denotes the number of fertile pollen on a single ocular, while S denotes the number of sterile pollen on one ocular.

2.6. Taxonomic Key

After examining the pollen grains under OM and SEM, different microscopic characteristics were observed, such as pollen, exine ornamentations, and colpi. The taxonomic keys have been developed based on these distinguishing characteristics.

3. Results

A palynological study of 18 weed species from the Potohar Plateau of Pakistan belonging to 12 families was analyzed by OM and SEM. The examined species were explained quantitatively and qualitatively in Tables 2–4. The palynological study showed that weedy species of the study area were quite diverse, and their pollen structure helped identify them. Using these qualitative characters, a taxonomic key was prepared to identify and differentiate the weed plant species (Table 5).

Table 2. Qualitative data of the pollen of weeds members from the Potohar region of Pakistan.

Sr. No	Taxa	Shape	Aperture Condition	Mesocolpium	Exine Ornamentation	Colpi Orientation
1.	*Achyranthus aspera*	Oblate-spheroidal	Tricolporate	Scabrate	Scabrate	Sunken, angular
2.	*Aerva javanica*	Prolate-spheroidal	Tricolporate	Aerolate	Reticulate	Sunken, angular, margins distinct, and ends tapering
3.	*Amaranthus graecizans*	Prolate-spheroidal	Trizonocolporate	Reticulate	Scabrate-reticulate	Prominent and rounded at ends
4.	*Asphodelus tenuifolius*	Oblate-spheroidal	Tricolporate	Scabrate-reticulate	Aerolate	Sunken, slit-like margins, and end tapering
5.	*Atriplex stocksii*	Oblate	Tricolporate	Scabrate	Reticulate-Perforate	Prominent and rounded at ends
6.	*Boerhavia procumbens*	Oblate-spheroidal	Tricolporate	Reticulate	Scabrate	Sunken, angular, margins distinct
7.	*Brassica furatii*	Spherical	Tricolporate	Scabrate	Echinate	Sunken, further divided into three slit-like portions, ends are tapering
8.	*Capparis decidua*	Oblate-spheroidal	-	Aerolate	Faveolate	Sunken, slit-like, margins wavy and slightly pointed at ends
9.	*Celosia argentea*	Oblate-spheroidal	Tricolporate	Perforate	Scabrate	Sunken, slit-like, margins distinct, and pointed at ends
10.	*Cenchrus ciliaris*	Prolate-spheroidal	-	Scabrate	Reticulate	Prominent margins
11.	*Chenopodium murale*	Oblate-spheroidal	Tricolporate	Reticulate	Aerolate	Sunken, long and slit-like
12.	*Corchorus depressus*	Oblate-spheroidal	Not visible	Scabrate	Reticulate-scabrate	Prominent and tapering ends
13.	*Cissampelos pareira*	Sub-oblate	Tricolporate	Reticulate	Perforate	Sunken, angular, margins distinct
14.	*Citharexylum spinosum*	Oblate-spheroidal	Tricolporate	Aerolate-Perforate	Scabrate	Long and slit-like, tapering ends
15.	*Cleome brachycarpa*	Oblate-spheroidal	Tricolporate	Reticulate	Reticulate	Sunken and tapering ends
16.	*Cleome viscosa*	Prolate-spheroidal	Tricolporate	Scabrate	Scabrate-reticulate	Long and slit-like margins
17.	*Cucumis melo* subsp. *agrestis*	Oblate-spheroidal	Not Visible	Perforate	Perforate	Sunken, slit-like, wavy margins
18.	*Euphorbia granulata*	Prolate-spheroidal	Tricolporate	Scabrate-reticulate	Aerolate	Sunken, wavy, and margins distinct

Table 3. Quantitative data of pollen of weeds members from Potohar region of Pakistan.

Plant Name	Exine Thickness Mean (Min-Max) S.E µm	Polar Diameter Mean (Min-Max) S.E µm	Equatorial Diameter Mean (Min-Max) S.E µm	P/E Ratio	No of Pores	Colpi Length Mean (Min-Max) S.E µm	Colpi Width Mean (Min-Max) S.E µm	Pores Length Mean (Min-Max) S.E µm	Pores Width Mean (Min-Max) S.E µm
Achyranthus aspera	1.56 (1.35–1.95) ± 0.11	14.5 (12.1–15.6) ± 0.63	15.6 (12.3–18.0) ± 0.96	0.93	–	3.81 (1.65–7.95) ± 3.81	2.55 (1.50–4.50) ± 0.53	–	–
Aerva javanica	1.80 (1.35–2.55) ± 0.23	18.03 (16.8–18.6) ± 0.34	17.8 (15.3–19.6) ± 0.84	1.01	–	6.12 (1.65–10.20) ± 1.40	3.45 (1.95–7.20) ± 0.97	–	–
Amaranthus graecizans	1.50 (1.20–1.80) ± 0.11	28.8 (25.5–33.15) ± 1.65	27.0 (25.6–30.3) ± 1.2	1.07	–	10.05 (4.05–17.55) ± 2.17	6.24 (2.85–13.20) ± 1.92	–	–
Asphodelus tenuifolius	6.12 (1.65–10.20) ± 1.40	3.45 (1.95–7.20) ± 0.97	6.12 (1.6–10.20) ± 1.40	0.97	–	12.2 (10.0–15.1) ± 2.44	5.79 (3.00–8.55) ± 2.31	–	–
Atriplex stocksii	2.01 (1.80–2.10) ± 0.10	11.7 (11.4–11.9) ± 0.15	17.3 (17.2–17.5) ± 0.09	0.67	–	12.30 (9.30–15.10) ± 1.80	7.20 (5.70–9.20) ± 1.03	–	–
Boerhavia procumbens	0.6 (0.5–0.75) ± 0.06	13.3 (12.2–15.25) ± 0.5	13.5 (12.2–15.5) ± 0.54	0.98	–	4.2 (3.2–5.00) ± 0.52	5.4 (4.9–6.3) ± 0.45	–	–
Brassica furatii	1.55 (1.0–2.0) ± 0.16	23.9 (22.75–25.5) ± 0.52	23.9 (22.2–25.5) ± 0.72	1.00	–	5.5 (4.6–6.7) ± 0.62	2.9 (2.3–3.5) ± 0.34	–	–
Capparis decidua	1.05 (0.75–1.25) ± 0.09	13.9 (12.0–15.50) ± 0.63	13.4 (12.0–15.5) ± 0.70	0.99	–	5.2 (4.2–6.1) ± 0.15	6.4 (5.7–7.3) ± 0.15	–	–
Celosia argentea	2.5 (2.25–2.75) ± 0.11	21.0 (15.5–24.75) ± 1.63	22.6 (18.0–25.2) ± 1.3	0.92	–	7.8 (6.8–9.1) ± 0.9	4.1 (3.4–5.1) ± 0.4	–	–
Cenchrus ciliaris	2.65 (2.25–3.0) ± 0.12	15.3 (14.7–15.75) ± 10.16	14.8 (14.0–15.7) ± 0.3	1.03	1	4.8 (4.4–5.4) ± 0.29	2.7 (2.4–3.1) ± 0.2	7.00 (5.25–8.50) ± 0.54	4.05 (3.00–5.00) ± 0.40
Chenopodium murale	1.9 (1.0–2.75) ± 0.34	14.0 (13.0–14.7) ± 0.32	14.5 (13.5–15.2) ± 0.3	0.96	–	6.7 (5.8–8.1) ± 0.6	3.1 (2.4–4.00) ± 0.4	–	–
Corchorus depressus	2.95 (2.75–3.25) ± 0.09	36.9 (33.7–43.00) ± 1.74	38.7 (37.2–40.5) ± 0.65	0.95	–	7.8 (5.8–9.5) ± 1.07	4.3 (3.7–5.00) ± 0.3	–	–
Cissampelos pareira	2.80 (2.25–3.25) ± 0.16	40.4 (35.7–45.50) ± 1.68	47.2 (45.2–48.5) ± 0.65	0.85	–	1.86 (1.35–2.40) ± 0.17	1.23 (.75–1.95) ± 0.21	–	–
Citharexylum spinosum	0.78 (0.6–1.1) ± 0.09	27.3 (22.7–32.7) ± 1.65	30.0 (24.5–33.5) ± 1.77	0.91	–	1.65 (1.05–2.40) ± 0.22	1.68 (1.05–2.10) ± 0.20	–	–
Cleome brachycarpa	2.5 (2.25–2.75) ± 0.11	34.05 (29.0–39.0) ± 1.69	35.1 (32.2–39.2) ± 1.32	0.96	–	2.25 (0.75–3.45) ± 0.47	2.64 (2.10–3.30) ± 0.20	–	–
Cleome viscosa	2.15 (1.5–3.0) ± 0.3	16.2 (13.5–18.0) ± 0.85	15.6 (13.2–18.5) ± 1.0	1.03	–	2.70 (1.95–3.30) ± 0.22	3.03 (2.40–3.60) ± 0.20	–	–
Cucumis melo **subsp.** *agrestis*	0.95 (0.75–1.25) ± 0.09	13.9 (12.0–15.5) ± 0.63	13.4 (12.0–15.5) ± 0.70	0.99	–	1.14 (0.75–1.50) ± 0.13	1.77 (1.50–2.10) ± 0.12	–	–
Euphorbia granulata	2.0 (1.50–2.25) ± 0.13	19.8 (17.2–21.25) ± 0.69	18.2 (16.2–20.5) ± 0.69	1.08	1	–	–		

Table 4. Pollen Fertility and Sterility percentages of examined taxa.

Sr. No	Taxa	No. of Fertile Pollen	No. of Sterile Pollen	Fertility %	Sterility %
1.	*Achyranthus aspera*	70	15	82	17
2.	*Aerva javanica*	90	40	69	30
3.	*Amaranthus graecizans*	77	10	88	11
4.	*Asphodelus tenuifolius*	99	12	89	10
5.	*Atriplex stocksii*	88	22	80	20
6.	*Boerhavia procumbens*	50	9	84	15
7.	*Brassica furatii*	80	2	97	2
8.	*Capparis decidua*	80	7	91	8
9.	***Celosia argentea***	75	10	88	11
10.	*Cenchrus ciliaris*	90	11	89	10
11.	*Chenopodium murale*	118	7	94	5
12.	*Corchorus depressus*	102	10	91	8
13.	*Cissampelos pareira*	90	5	95	5
14.	*Citharexylum spinosum*	110	14	89	11
15.	*Cleome brachycarpa*	86	11	89	11
16.	*Cleome viscosa*	99	12	89	10
17.	***Cucumis melo*subsp. *agrestis***	143	9	94	5
18.	*Euphorbia granulata*	81	14	85	14

Table 5. Dichotomous key for identifying the Pothor (Pakistan) weed species based on the pollen morphological characteristics.

Link Characters	Present (+) /Absent (−)	Diagnostic Characters	Species Name
1	+	pollen shape oblate-spheroidal, mesocolpium scabrate, colpi orientation sunken, angular	*Achyranthus aspera*
	−	pollen shape prolate-spheroidal, mesocolpium aerolate, colpi orientation Sunken, angular, margins distinct, and ends tapering	2
2	+	aperture tricolporate, exine reticulate	*Aerva javanica*
	−	aperture trizonocolporate, exine scabrate-reticulate	3
3	+	The shape of pollen prolate-spheroidal, mescolopium reticulate	*Amaranthus graecizans*
	−	pollen shape oblate-spheroidal, mescolopium scabrate-reticulate	4
4	+	aperture tricolporate, colpi sunken, slit like margins, and end tapering	*Asphodelus tenuifolius*
	−	aperture tricolporate, colpi prominent, and rounded at ends	5
5	+	shape oblate, mescolopium scabrate	*Atriplex stocksii*
	−	Shape oblate-spheroidal, mescolopium reticulate	6
6	+	exine scabrate, colpi Sunken, angular, margins distinct	*Boerhavia procumbens*
	−	exine echinate, colpi Sunken, further divided into three slit-like portions, ends are tapering	7
7	+	pollen shape spherical, mescolopium scabrate	*Brassica furatii*
	−	pollen shape oblate spheroidal, mescolpium aerolate	8
8	+	exine faveolate, colpi sunken slit-like, margins wavy, and slightly pointed at the end	*Capparis decidua*
	−	pollen shape oblate spheroidal, exine scabrate	9
9	+	aperture tricolporate, mesocolpium perforate	*Celosia argentea*
	−	aperture not clear, mescolpium scabrate	10
10	+	pollen shape prolate-spheroidal, exine reticulate	*Cenchrus ciliaris*
	−	pollen shape oblate-spheroidal, exine aerolate	11
11	+	aperture tricolporate, colpi sunken, long and slit like	*Chenopodium murale*
	−	aperture not visible, colpi prominent, and tapering	12

Table 5. *Cont.*

Link Characters	Present (+) /Absent (−)	Diagnostic Characters	Species Name
12	+	pollen shape oblate-spheroidal, mesocolpium scabrate	*Corchorus depressus*
	−	pollen shape sub-oblate, mescolpium perforate	13
13	+	aperture tricolporate, exine sunken, angular, margins distinct	*Cissampelos pareira*
	−	aperture tricolporate, exine long and slit like, tapering ends	14
14	+	pollen shape oblate-spheroidal, mesocolpium aerolate-perforrate	*Citharexylum spinosum*
	−	pollen shape oblate-spheroidal, mescolpium reticulate	15
15	+	exine reticulate, colpi sunken, and tapering ends	*Cleome brachycarpa*
	−	exine scabrate-reticulate, colpi long and slit-like margins	16
16	+	pollen shape prolate-spheroidal, aperture tricolporate	*Cleome viscosa*
	−	pollen shape oblate-spheroidal, aperture not visible	17
17	+	mesocolpium perforate, exine perforate	*Cucumis melo* subsp. *agrestis*
	−	mescolpium scabrate-reticulate, exine aerolate	*Euphorbia granulata*

3.1. Pollen Size, Shape, and P/E Ratio

Variations in grain shape were observed in equatorial and polar views, size, and pollen shapes. The P/E ratio helps determine the shapes of pollen. Pollen grains of the analyzed species have spherical, prolate-spheroidal, oblate-spheroidal, oblate, and sub-oblate shapes (Figure 2). The maximum polar diameter was 40.40 µm for *Cissampelos parira* and the minimum was 13.95 µm for *Atriplex stoeksii*. *Cissampelos parira* have a maximum equatorial diameter of 47.20 µm, whereas *Cucumis melo* subsp. *Agrestis* have a minimum of 13.45 µm (Figure 3). Similarly, *Euphorbia grandulata* have a maximum P/E ratio of 1.08, and *Cissampelos parira* have a minimum of 0.85 (Figure 4).

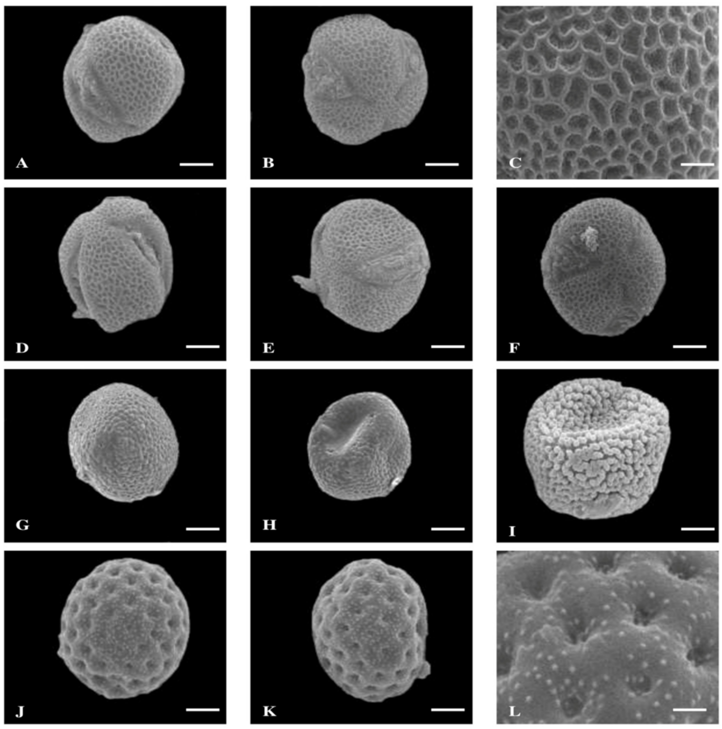

Figure 2. SEM images of pollen grains (Equatorial and Polar axis) and pollen surface ornamentation (**A**–**C**), The scale bar represents 5 µm for *Achyranthus aspera* (**D**–**F**), 5 µm for *Aerva javanica* (**G**–**I**), 10 µm for *Amaranthus graecizans* (**J**–**L**), and 5 µm for *Asphodelus tenuifolius*.

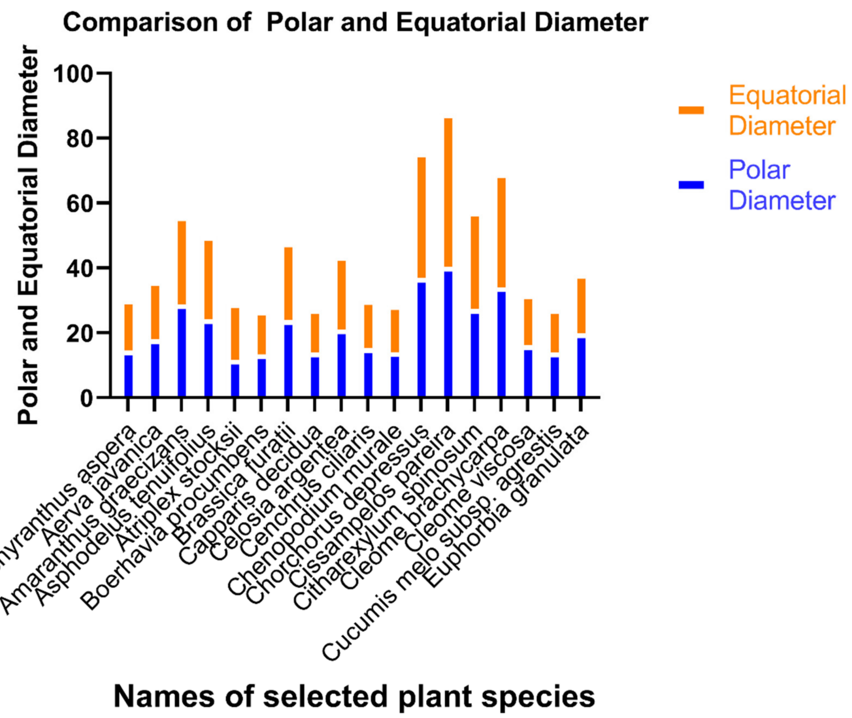

Figure 3. Comparison of the polar and equatorial diameter (µm) of the pollen of selected plant species.

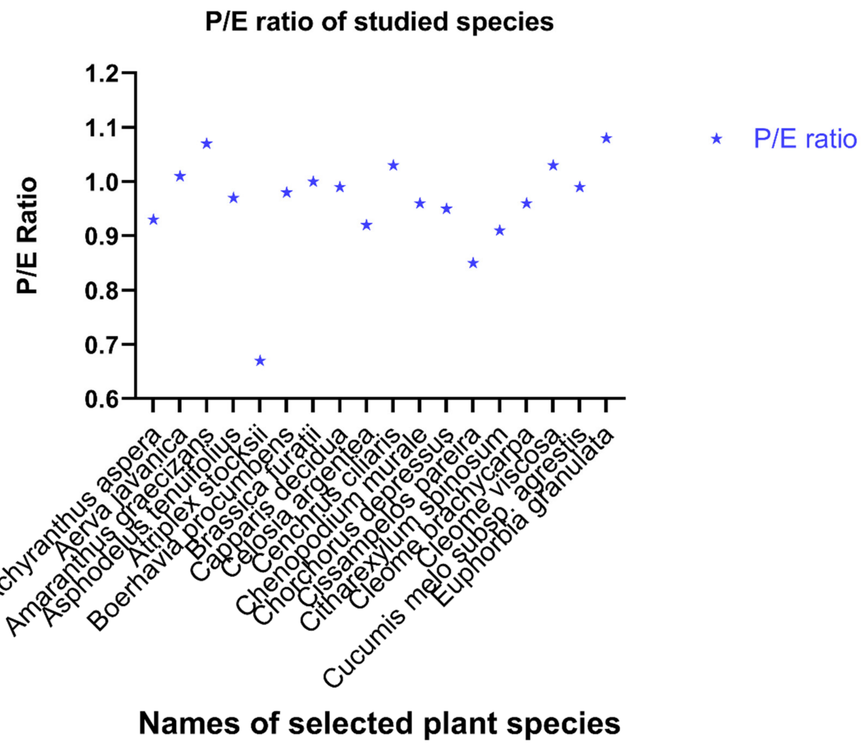

Figure 4. P/E ratio of different species of weeds collected from the Potohar region of Pakistan.

3.2. Quantitative and Qualitative Characters of Colpi

All the documented species contain colpi (Figure 5) in their pollen except for *Euphorbia grandulata*. *Atriplex stoeksii* had a maximum colpi length of 12.30 µm, and *Citharexylum spinosum* had a minimum of 2.25 µm. *Atriplex stoeksii* had a maximum colpi width of 7.20 µm, and *Cissampelos parira* had a minimum of 1.23 µm. All species examined had tricolporate pollen (Figure 6).

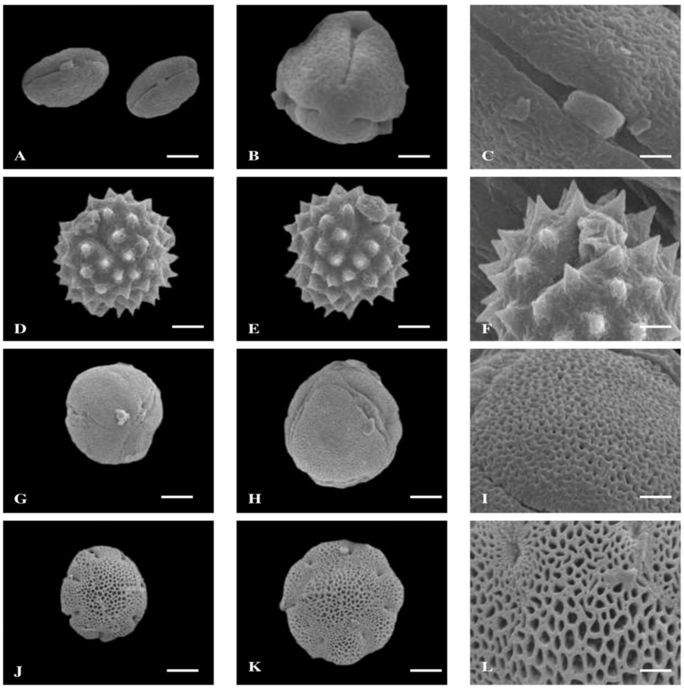

Figure 5. SEM images of pollen grains (Equatorial and Polar axis) and pollen surface ornamentation (**A**–**C**). The scale bar represents 5 µm for *Atriplex stoeksii* (**D**–**F**), 5 µm for *Boerhivia procumbense* (**G**–**I**), 10 µm for *Brassica furatii* (**J**–**L**), and 10 µm for *Capparis decidua*.

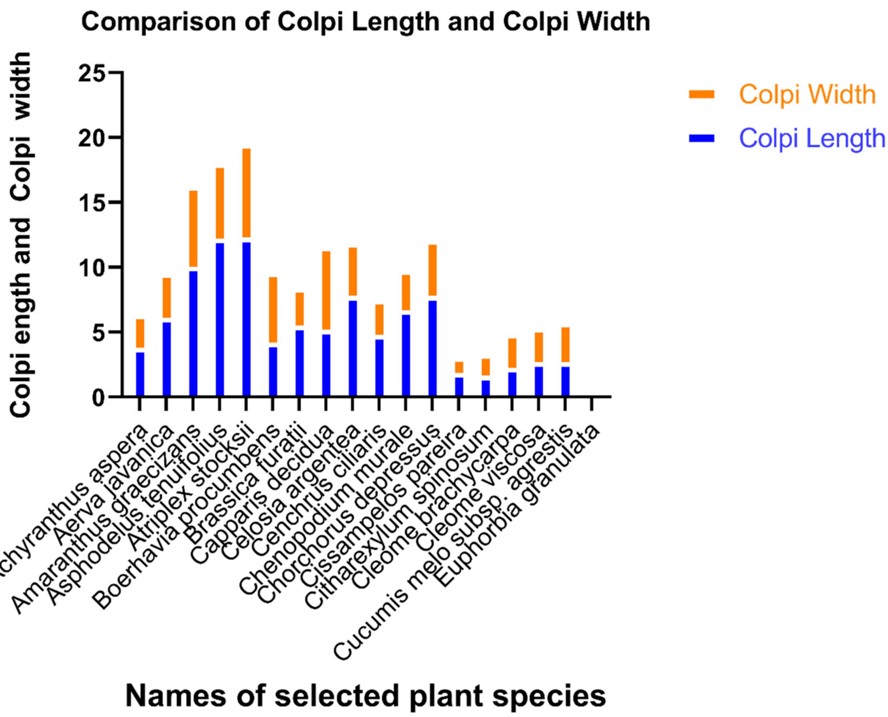

Figure 6. Comparison of the Colpi length and width (µm) of selected plant species.

3.3. Exine Thickness

The quantitative pollen characters were observed by SEM and OM (Figure 7). The maximum exine thickness was 2.95 µm for *Chorchorus depressus*, and the minimum was 0.60 µm for *Boerhivia procumbense* (Figure 8).

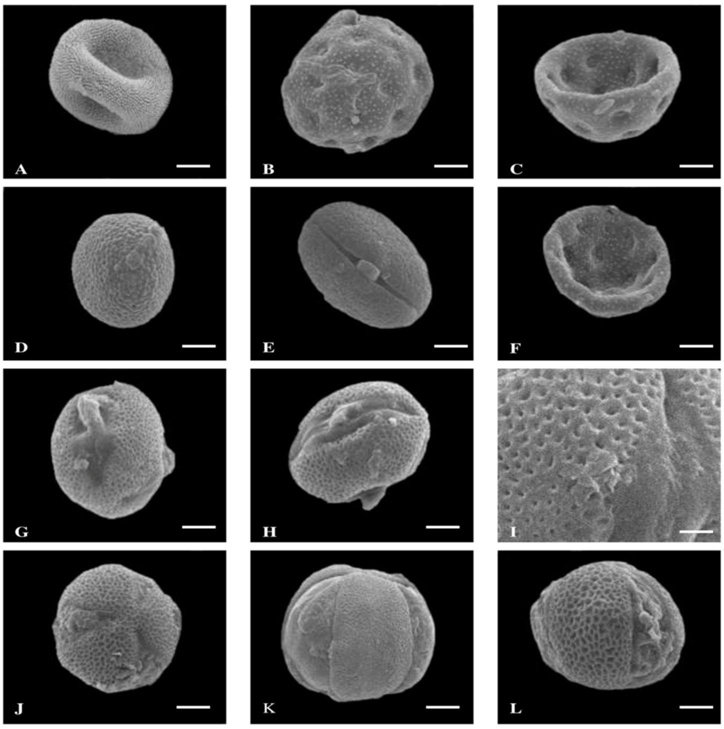

Figure 7. SEM images of pollen grains (Equatorial and Polar axis) and pollen surface ornamentation (**A**–**C**). The scale bar represents 5 µm for *Celosia aegentae* (**D**–**F**), 10 µm for *Cenchrus ciliaris* (**G**–**I**), 5 µm for *Chenopodium murale* (**J**–**L**), and 5 µm for *Chorchorus depressus*.

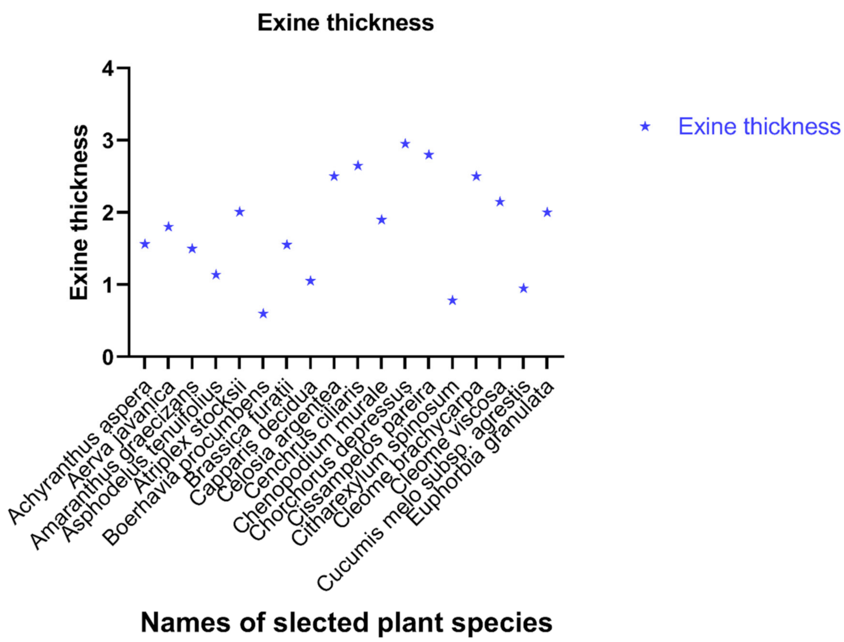

Figure 8. Graph representing the exine thickness of the selected plant species.

3.4. Exine Sculpturing

The exine is the most distinguishing characteristic of the species (Figures 9 and 10). All the pollen grains included in this study had different exine sculpturing. Exine ornamentations are essential features for identifying closely related species and genera with each other. The present study recorded the following ornamentations: reticulate, carbonate, aerolate, faveolate, reticulate-perforate, and reticulate-scabrate. *Achyranthus aspera*, *Celosia aegentae*, and *Citharexylum spinosum* have scarbate exine ornamentations. *Aerva javanica*, *Cleome* *brachycarpa*, and *Cenchrus ciliaris* have reticulate *ornamentations. Amaranthus gracigiense* and *Cleome viscosa* have scarbate-reticulate. *Asphodelus tenuifolius*, *Euphorbia grandulata*, and *Chenopodium murale* have aerolate, while *Cissampelos parira* and *Cucumis melo* subsp. *agrestis* have perforated exine sculpturing.

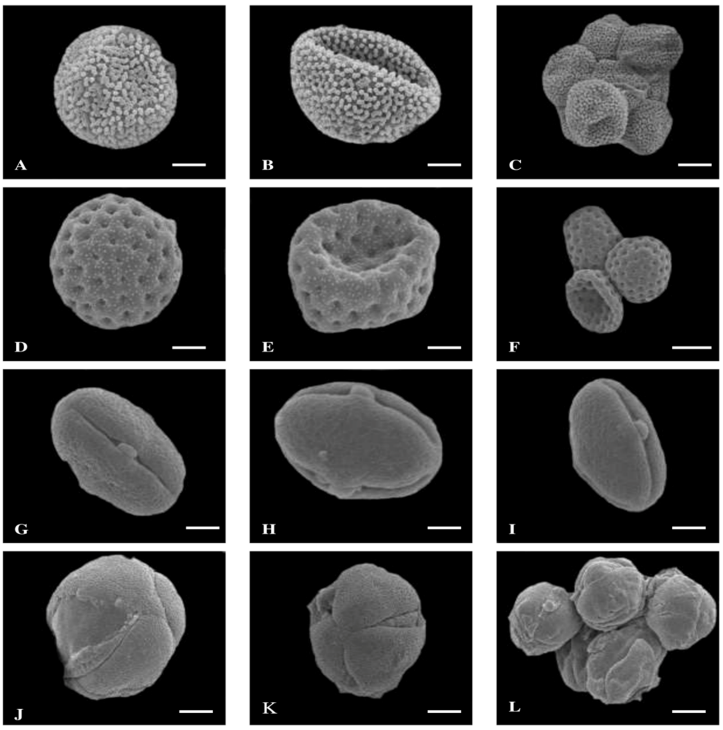

Figure 9. SEM images of pollen grains (Equatorial and Polar axis) and pollen surface ornamentation (**A**–**C**). The scale bar represents 10 µm for *Cissampelos parira* (**D**–**F**), 5 µm for *Citharexylum spinosum* (**G**–**I**), 5 µm for *Cleome brachycarpa* (**J**–**L**), and 5 µm for *Cleome viscosa*.

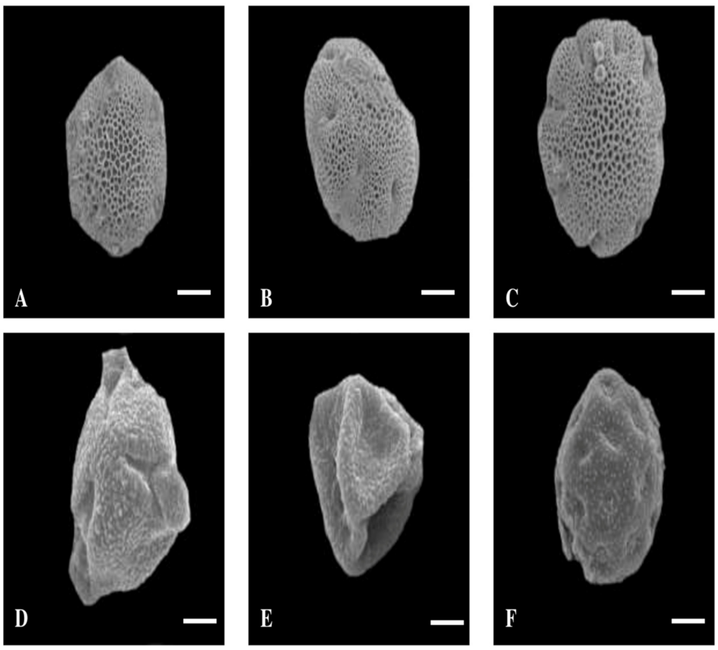

Figure 10. SEM images of pollen grains (Equatorial and Polar axis) and pollen surface ornamentation (**A**–**C**). The scale bar represents 10 µm for *Cucumis melo* subsp. *agrestis* (**D**–**F**) and 5 µm for *Euphorbia grandulata*.

3.5. Fertility and Sterility Percentages

Table 4 lists the pollen fertility and sterility results. The highest fertility percentage was observed in *Brassica furatii* (97%) and *Cissampelos pareira* (95%), whereas the lowest percentage of fertility was observed in *Aerva javanica* (69%). In the case of sterility, the highest sterile pollen was observed in *Aerva javanica* (30%), while the lowest sterile pollen grains were found in *Brassica furatii* (2%).

4. Discussion

This study examined the role of palynological studies for species identification from the Potohar regions of Pakistan using microscopic techniques. The pollen grains of the weeds varied in shape, aperture, mesocolpium, colpi orientation, and exine ornamentations. Pollen shape, exine sculpturing, spines, number of colpi, and exine thickness of weeds from district Bannu were described by Ahmad et al. [26]. A wide variation in pollen parameters helps differentiate closely related species and genera. The morphological characteristics of pollen provide additional knowledge in plant taxonomy and systematics. The pollen investigated through LM showed great variability in equatorial diameter, polar axis diameter, width and length of colpi, and exine thickness. An extensive range of variations was observed in the pollen morphology of selected species of weeds from the study area showing that the field of palynology plays a vital role in the evolutionary studies of plant species. Variations in exine sculpturing are the diagnostic characteristic in plant systematic studies.

The current study showed that pollen morphological features, particularly the shape of pollen grains, are the major distinguishing feature for identifying weeds species. The present study varies from the previous studies conducted on weeds in terms of the qualitative and quantitative microscopic characteristics. Amaranthaceae were considered the dominant family of weeds in the present research family, followed by Capparaceae. In contrast, the remainder of each family, i.e., Euphorbiaceae, Cucurbitaceae, Verbenaceae, Menispermaceae, Teliaceae, Chenopodiaceae, Poaceae, Asphodelaceae, Brassicaceae, and Nyctaginaceae, contain single species. Pollen grains were in the form of monad, with most species having scabrate exine ornamentation. Similar findings have been reported [27]. Nazish et al. [28] examined the apolar, spheroidal, and scabrate. Micro Echinate pollen ornamentations and sunken pore ornamentations have several pores, ranging from 11 to 23.

Various researchers working on the palynological study of weeds believed that exine ornamentation is the key feature for differentiating taxonomic characteristics [25–30]. Significant differences were observed in the following: the exine thickness, the diameter of the equatorial and polar axis, the size of the colpus, and the number of pores. Palynological studies are used for species identification in plant taxonomy and systematics. The study is linked directly to environmental sciences, molecular biology, plant ecology, pharmaceutical studies, genetics, and aerobiology [13]. In the present study, some pollen has the same aperture conditions, i.e., tricolporate. Khan et al. [31] worked on the pollen morphological features of 16 plants using scanning electron microscopy, which is somewhat similar to our results [32]. They used OM and SEM and described the pollen micro-morphology of 24 wetland weeds. Bolick et al. [33] recorded the exine sculpturing patterns of the pollen of Asteraceae with particular emphasis on evolution. The morphological pollen characteristics can be valuable in solving complications linked with the systematic study of grass. The study of the pollen provides the base for more structures to properly identify the plant species [34]. A palynological study of Asteraceous taxa from the Potohar region helps differentiate species taxonomically [35]. Research work has been done on many aspects of flora. The study area has diverse species, but no work has been performed on the pollen morphological study of weedy species.

Oblate-spheroidal observations were observed abundantly in this study. Most of the colpi orientations noted were sunken and angular. In addition, many prominent, waxy, long, slit-like, and tapering ends of colpi were observed in the pollen. The pollen examined in this study was reticulate in sculpturing, while some contained faveoelate. *Cenhrus* *ciliaris* contained a single pore in the center of the pollen. Fertility and sterility percentages were determined for the stability of pollen in the study area. The fertility and sterility percentages in pollen grains indicated the relationship among the species' ancestors. The fertility of pollen grains helps identify the genetic variations in flora [36]. Meo et al. [37] reported different shapes of pollen grains of the genus *Cenchrus*. They observed spherical to perbolate shape in *C. pennisetiformis* and spheroidal to subulate in *C. setigerus*. The pollen of this genus is monoporate with psilate exine ornamentation. The position of pores was ectoporous, but endoporus pores were also observed in some cases. Three species, i.e., *C. ciliaris*, *C. biflorus*, and *C. pennisetiformis* are endoporus, whereas *C. setigerus* is ectoporus. The size and shape of pollen grains of the genus *Cenchrus* are used to distinguish the species. It is a valuable tool for calculating the strength of species in a specific region. The pollen examined in this study were oblate, suboblate, oblate-spheroidal, and prolate-spheroidal in shape with the aperture conditions of tricolporate, mesocolpium scarbate, aerolate, reticulate, and perforated exine ornamentations. By contrast, the colpi orientations were long, slit-like, sunken, angular, margins distinct, waxy, rounded, and tapering ends.

Perveen et al. [38] reported the morphological characteristics of pollen of the family Brassicaceae, which have reticulate exine ornamentation compared to the current study results *Brassica furatii* exhibit echinate type of exine surface. Appel and Al-Shehbaz [39] reported that all pollen grains observed in their research exhibit tricolpate with the reticulate type of exine, similar to the present study results in that *Brassica furatii* has tricolpate pollen grains. Moore et al. [40] reported tricolpate pollen with reticulate sculpturing of the exine. Considerable work has been done on weeds of the family Brassicaceae by [41,42], and it was concluded that the shape of the lumen is the most significant characteristic in the delimitation of different species in the family Brassicaceae.

Weed flora has been documented previously by many researchers. Reference [43] reported 73 weed species that are medicinally important and utilized for treating different diseases of both human beings and animals. These weed species help manufacture different plant yields and the indigenous sellers of crude drugs of plants. The pattern of distribution, richness, and abundance of weed species (e.g., *A. viridis*, *P. lanceolata*, *D. annulatum*, *V. thapsus*, and *C. dactylon*) depend on the different environmental conditions, such as $CaCO_3$, pH, organic matter, soil texture, phosphorous, electrical conductivity, and concentration of nitrogen. Rafay et al. (2014) reported that weeds play a significant role in the agroecosystem by increasing the cropping system's environmental heterogeneity and floral diversity. Williams and Kremen [44] reported that non-crop plants support different types of invertebrates because many insects depend upon specific weeds for survival.

On the other hand, weeds play a serious role in crop production and threaten cultivated lands because they use soil nutrients. Weeds adversely affect the yield and growth of crop plants because of the nutrient demand. Weed vegetation is eliminated using different control measures, e.g., chemical approaches, cultural procedures, and biological mechanisms. In cultural operations, weeds are thrown away from the rice field [43]. Identification of different weed species and farming practices plan with revised management has been suggested [45].

The results of the present study of this family were compared with previously published data showing that these species are quite similar to the previously reported species, with few variations. Butt et al. [32] described the palynological study of weeds for pollen features, i.e., pore number, the diameter of the equatorial and polar axis, length, width of colpi, and its outline, which are very helpful for classifying species in plant taxonomy. According to the results, these characteristics are insufficient for species identifications; sometimes, many species hold similar features. The most vital characteristics are exine thickness, mesocolpium, and aperture conditions. The study also provides information on the delimitation of species from the genus to the family level. Nevertheless, further research will be needed to achieve more conclusive results using more cosmopolitan species.

5. Conclusions

A microscopic study of weeds and pollen grains collected from the Potohar region of Pakistan has been documented. The studied pollen helps identify the weed plant species, genus, and family. Eighteen plant species belonging to 16 families were investigated in the present research; the morpho-palynological characters were investigated quantitatively and qualitatively. In the case of palynomorph, major variations were observed in size, appearance, and diameter of the equatorial and polar axis, the wideness of the exine, and its ornamentation. The pollen traits were proven to have taxonomic potential to support and strengthen the systematics of this subfamily. The dominant family of weeds in the present study is Capparaceae followed by Amaranthaceae, and the oblate shape of pollen is commonly observed in weeds. All the pollen grains were tricolpate with scabrate exine sculpturing. The current study was compared with previous studies, confirming that the pollen morphology under OM and SEM provides important information for identifying weeds species. The microscopic features provide considerable knowledge and have important information for correctly identifying flora. This study also contributed to collecting the data on weeds plants, i.e., preventive, therapeutic, and diagnostic potential. Further phylogenetic, pharmacognosy, and molecular studies are recommended for weed plants in the future to support and strengthen the systematics of weed flora.

Author Contributions: A.U., M.A., M.Z. and S.S. (Shazia Sultana) designed the research, performed the experiments, and analyzed the data., W.Z., F.U., A.A. and S.S. (Saddam Saqib) visualization, methodology, writing—review and editing, investigation, re-sources, data curation. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: All the authors are thankful to (CRL) Central resource library, Department of the Physics University of Peshawar, for providing the facility of Scanning Electron microscopy.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Oerke, E.C.; Dehne, H.W. Safeguarding production—Losses in major crops and the role of crop protection. *Crop Prot.* **2004**, *23*, 275–285. [CrossRef]
2. Khan, F.; Muhammad, Z.; Khan, K.; Ahmad, S.; Khan, M.J.; Bakht, T.; Kamal, A. Palynological investigation of allergenic and invasive weeds plants for biodiversity in district Lakki Marwat using scanning electron microscopy. *Pak. J. Weed Sci. Res.* **2020**, *26*, 349–365. [CrossRef]
3. Qureshi, R.; Waheed, A.; Arshad, M. Weed communities of wheat crop in district Toba Tek Singh, Pakistan. *Pak. J. Bot* **2009**, *41*, 239–245.
4. Neve, P.; Barney, J.N.; Buckley, Y.; Cousens, R.D.; Graham, S.; Jordan, N.R.; Lawton-Rauh, A.; Liebman, M.; Mesgaran, M.B.; Schut, M. Reviewing research priorities in weed ecology, evolution and management: A horizon scan. *Weed Res.* **2018**, *58*, 250–258. [CrossRef]
5. Ullah, F.; Zafar, M.; Ahmad, M.; Dilbar, S.; Shah, S.N.; Sohail, A.; Zaman, W.; Iqbal, M.; Bahadur, S.; Tariq, A. Pollen morphology of subfamily Caryophylloideae (Caryophyllaceae) and its taxonomic significance. *Microsc. Res. Tech.* **2018**, *81*, 704–715. [CrossRef] [PubMed]
6. Mamoona, M.; Mir, A.K.; Mushtaq, A.; Nighat, S.; Sidra, N.A.; Kanwal, T.; Saira, T.; Tehmeena, M.; Madhia, A.; Shazia, B. Foliar epidermal anatomy of some ethnobotanically important species of wild edible fruits of northern Pakistan. *J. Med. Plants Res.* **2011**, *5*, 5873–5880.
7. Jogi, Q.; Hajano, G.A.; Kandharo, M.N.; Shah, A.N.; Soomro, A.A.; Abbasi, Z.A.; Burioro, M.; Baloch, S.K.; Kalwar, Z.A.; Abbasi, N.A. Examine different weed management techniques in sugarcane (*Saccharum officinarum* L.). *Pure Appl. Biol. (PAB)* **2019**, *8*, 151–159.
8. Mehsud, A.; Mehmood, S.; Muhammad, A.; Khan, R.U.; Khan, S.U.; Khan, H.U.; Wazir, R.; Hussain, Z. Morphology and anatomy of some weeds from flora of district Bannu, Pakistan. *Pak. J. Weed Sci. Res.* **2013**, *19*, 437–445.
9. Amarger, N. Rhizobia in the field. *Adv. Agron.* **2001**, *23*, 741–748.
10. Marwat, K. A checklist and key to parasitic weeds of Pakistan. *Pak. J. Weed Sci. Res.* **1933**, *6*, 1–15.
11. Naz, J.; Ahmad, M.; Sultana, S.; Zafar, M.; Naz, S.; Zaman, W.; Ullah, F. Invasive alien species of Potohar Plateau, Northern Pakistan. *Ann. Bot.* **2021**, *11*, 171–179.

12. Rashid, K.; Rasul, G. Rainfall variability and maize production over the Potohar Plateau of Pakistan. *Pak. J. Meteorol.* **2011**, *8*, 63–74.
13. Muhammad, Z.; Khan, M.; Mushtaq, A.; Shazia, S. Palynological and taxonomic studies of some weeds from flora of Rawalpindi. *Pak. J. Weed Sci. Res.* **2006**, *12*, 99–109.
14. Hameed, A.; Zafar, M.; Ahmad, M.; Sultana, S.; Akhter, M.S.; Zaman, W.; Saqib, S.; Ullah, F. Micromorphology, phytochemical and pharmacological evaluation of *Isodon rugosus* (wall. Ex benth.) Codd. *J. Anim. Plant Sci.* **2022**, *32*, 736–745.
15. Jehanzeb, S.; Zafar, M.; Ahmad, M.; Sultana, S.; Zaman, W.; Ullah, F. Comparative petioler anatomy of tribe Mentheae subfamily Nepetoideae, Lamiaceae from Pakistan. *Feddes Repert.* **2020**, *131*, 163–174. [CrossRef]
16. Shah, S.N.; Ahmad, M.; Zafar, M.; Ullah, F.; Zaman, W.; Malik, K.; Rashid, N.; Gul, S. Taxonomic importance of spore morphology in Thelypteridaceae from Northern Pakistan. *Microsc. Res. Tech.* **2019**, *82*, 1326–1333. [CrossRef]
17. Ullah, F.; Ayaz, A.; Saqib, S.; Parmar, G.; Bahadur, S.; Zaman, W. Taxonomic implication of leaf epidermal anatomy of selected taxa of Scrophulariaceae from Pakistan. *Microsc. Res. Tech.* **2020**, *84*, 521–530. [CrossRef]
18. Usma, A.; Ahmad, M.; Zafar, M.; Sultana, S.; Lubna; Kalsoom, N.; Zaman, W.; Ullah, F. Micromorphological variations and taxonomic implications of caryopses of some grasses from Pakistan. *Wulfenia* **2020**, *27*, 86–96.
19. Zaman, W.; Ullah, F.; Parmar, G.; Saqib, S.; Ayaz, A.; Park, S. Foliar micromorphology of selected medicinal Lamiaceae taxa and their taxonomic implication using scanning electron microscopy. *Microsc. Res. Tech.* **2022**, *2022*, 1–20. [CrossRef]
20. Meo, A.; Khan, M. Palynological studies of some weeds of asteraceae from Pakistan. *Pak. J. Weed Sci. Res. (Pak.)* **2004**, *10*, 87–92.
21. Ronald, O.K. *Pollen and Spores*, 2nd ed.; American Association of Stratographic Palynologists Foundation: College Station, TX, USA, 2000; pp. 13–21.
22. Ashfaq, S.; Ahmad, M.; Zafar, M.; Sultana, S.; Nazish, M.; Khan, A.N. Systematics of medicinally important weeds of genus *Convolvulus*: Convolvulaceae. *Planta Daninha* **2020**, *38*. [CrossRef]
23. Erdtmann, G. Pollen morphology and plant taxonomy III, Morina L. with an addition on pollen morphological terminology. *Sven. Bot. Tidskr.* **1952**, *39*, 187–191.
24. Khan, M.A.; Stace, C.A. Breeding relationships in the genus *Brachypodium* (Poaceae: Pooideae). *Nord. J. Bot.* **1999**, *19*, 257–269. [CrossRef]
25. Ahmad, S.; Zafar, M.; Ahmad, M.; Yaseen, G.; Sultana, S. Microscopic investigation of palyno-morphological features of melliferous flora of Lakki Marwat district, Khyber Pakhtunkhwa, Pakistan. *Microsc. Res. Tech.* **2019**, *82*, 720–730. [CrossRef]
26. Ahmad, S.; Zafar, M.; Ahmad, M.; Ozdemir, F.; Yaseen, G.; Sultana, S.; Kutlu, M. Palynological studies of winter weeds melliferous flora of district Bannu, Khyber Pakhtunkhwa, Pakistan. *Ann. Bot.* **2020**, *10*, 77–86.
27. Raees, K.; Abidin, S.Z.U.; Mumtaz, A.S.; Jamsheed, S.; Ullah, H. Comparative leaf and pollen micromorphology on some Grasses taxa (Poaceae) distributed in Pakistan. *Int. J. Nat. Life Sci.* **2017**, *1*, 72–82.
28. Nazish, M.; Zafar, M.; Ahmad, M.; Sultana, S.; Ullah, R.; Alqahtani, A.S.; Ullah, F.; Ahmad, S.; Ashfaq, S.; Ullah, F. Palyno-morphological investigations of halophytic taxa of Amaranthaceae through SEM from Salt range of Northern Punjab, Pakistan. *Microsc. Res. Tech.* **2019**, *82*, 304–316. [CrossRef]
29. Amina, H.; Ahmad, M.; Bhatti, G.R.; Zafar, M.; Sultana, S.; Butt, M.A.; Bahadur, S.; Haq, I.U.; Ghufran, M.A.; Ahmad, S. Microscopic investigation of pollen morphology of Brassicaceae from Central Punjab-Pakistan. *Microsc. Res. Tech.* **2020**, *83*, 446–454. [CrossRef]
30. Perveen, A.; Qaiser, M. Pollen flora of Pakistan-LXIX. Poaceae. *Pak. J. Bot.* **2012**, *44*, 747–756.
31. Khan, K.; Ahmad, M.; Zafar, M.; Malik, K.; Sultana, S.; Ahmad, S.; Khan, F.; Ullah, K. Palyno-Morphological Study of Weedy Melliferous (Bee Visited) Plants Using Light Microscopic Techniques From Southern Khyber Pakhtunkhwa, Pakistan. *Pak. J. Weed Sci. Res.* **2021**, *27*, 163–172. [CrossRef]
32. Butt, M.A.; Zafar, M.; Ahmad, M.; Kayani, S.; Bahadur, S.; Ullah, F.; Khatoon, S. The use of taxonomic studies to the identification of wetlands weeds. *Adv. Weed Sci.* **2021**, *39*, 1–13. [CrossRef] [PubMed]
33. Bolick, M.R. Taxonomic, evolutionary, and functional considerations of Compositae pollen ultrastructure and sculpture. *Plant Syst. Evol.* **1978**, *130*, 209–218. [CrossRef]
34. Aftab, R.; Perveen, A. A palynological study of some cultivated trees from Karachi. *Pak. J. Bot.* **2006**, *38*, 15–28.
35. Usma, A.; Ahmad, M.; Ramadan, M.F.; Khan, A.M.; Zafar, M.; Hamza, M.; Sultana, S.; Yaseen, G. Micro-morphological diversity of pollen among Asteraceous taxa from Potohar Plateau-Pakistan. *Microsc. Res. Tech.* **2022**, *85*, 2467–2485. [CrossRef] [PubMed]
36. Ullah, F.; Ullah, A.; Sohail, A.; Khan, R. Diversity, distribution and ecological importance of weeds in the maize crop at Maidan Valley, Dir (l), Khyber Pakhtunkhwa, Pakistan. *Pak. J. Weed Sci. Res.* **2015**, *21*, 543–553.
37. Meo, A.; Hafiz, H.; Baig, I. Pollen grain description of 13 non allergic and four allergic Graminaceous species. *J. Pure Appl. Sci.* **1989**, *8*, 19–26.
38. Perveen, A.; Qaiser, M.; Khan, R. Pollen Flora of Pakistan–XLII. Brassicaceae. *Pak. J. Bot.* **2004**, *36*, 683–700.
39. Appel, O.; Al-Shehbaz, I. Cruciferae. In *Flowering Plants Dicotyledons*; Springer: Berlin/Heidelberg, Germany, 2003; pp. 75–174.
40. Moore, P.D.; Webb, J.A.; Collison, M.E. *Pollen Analysis*; Blackwell Scientific Publications: Hoboken, NJ, USA, 1991.
41. Brochmann, C. Polyploid evolution in arctic-alpine (Brassicaceae). *Sommerfeltia* **1992**, *4*, 1–44. [CrossRef]
42. Al-Shehbaz, I.; Beilstein, M.; Kellogg, E. Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. *Plant Syst. Evol.* **2006**, *259*, 89–120. [CrossRef]

43. Khan, M.; Kakar, S.; Marwat, K.; Khan, I. Differential response of Zea mays L. in relation to weed control and different macronutrient combinations. *Sains Malays.* **2013**, *42*, 1405–1411.
44. Williams, N.M.; Kremen, C. Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecol. Appl.* **2007**, *17*, 910–921. [CrossRef] [PubMed]
45. Iqbal, M.; Khan, S.M.; Khan, M.A.; Ahmad, Z.; Abbas, Z.; Khan, S.M.; Khan, M.S. Distribution pattern and species richness of natural weeds of wheat in varying habitat conditions of district Malakand, Pakistan. *Pak. J. Bot.* **2017**, *49*, 2371–2382.