

Palynological features of medicinal plants from Salt Ranges of Pakistan

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Abstract: Palynology is utilized for distinction and identification of various plants on the basis of their pollens that vary in quantitative as well as in qualitative structures. For this, medicinal plants were collected randomly from various sites of Salt areas. 33 plants belonging to 30 genera were selected and their locality, habitat, date of collection and inflorescence color were noted during collection visits and preserved in herbarium of Department of Botany, University of Agriculture, Faisalabad, Pakistan. Qualitative traits i.e., pollen shapes (both in equatorial and polar view), pollen types and presence of colpi, while quantitative features i.e., colpi width, length, number, exine thickness, polar and equatorial diameter, Polar/Equatorial ratio and pollen fertility were measured. Multivariate (cluster) analysis was used to assess relationship among diverse genera and species. In all species both qualitative and quantitative traits varied in one and in another respect that shows that these characters are vital in systematics and every species have its own pollen traits. Our study concluded that palynology can be useful in solving systematics problems. Each plant species has some unique morphological pollen characters, which can be further merged to extract generic and family level pollen traits. Exine ornamentation and sculpturing proved to be useful at generic and species level.

Keywords: Pollen, palynology, exine, colpi, systematics, taxonomy, medicinal plants, diversity

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1. Introduction

Pollen and spore study is called palynology and this technique is vital because pollen can be used as identifying marker and indicator in organization and used in evolutionary record (Chambers et al., 2011). Pakistan is a blessed country that has mixture of ecological surroundings, topographical areas and atmosphere that inherited with vital medicinal flora used in therapeutic medicine (Nisar et al., 2011). Therefore, taxonomist, ethnobotanist, phytochemist and ethnopharmacologists work hard to find more precious and medicinal plants from different sites of Pakistan (Nisar et al., 2011).

Plants species are vital in every respect on this planet (Ahmad et al., 2010). Medicinal properties of plants make them unique and their great varieties are present in saline areas of Pakistan (Mushtaq et al., 2012; Nadeem et al., 2013). Therefore, salt affected lands, enriched with plant diversity, always attracted

researchers (Ahmad et al., 2010). Choha Sadden Shah, Kalar Kahar, Soon Velly, roadsides of motorway and Khewra has excessive variety of plants that are all salt affected lands (Khan et al., 2011). Uncountable and distinctive natural resources are present at these sites. Their environmental conditions are suitable for these unique plant species (Ahmad et al., 2012). These plants have their individual commercial and curative values, many plants species of salted areas are about to loss so researchers focus on these areas recently so that they can conserve most important medicinal plants (Nawaz et al., 2012).

The great diversity of pollens makes them unique and crucial in systematic study (Devarkar, 2011). That helps in evolution record because of their distinctive characteristics of pollen structure, composition, exine, aperture of different flora when viewed through scanning and light microscope. That are very crucial is systematic studies (Chung et al., 2010).

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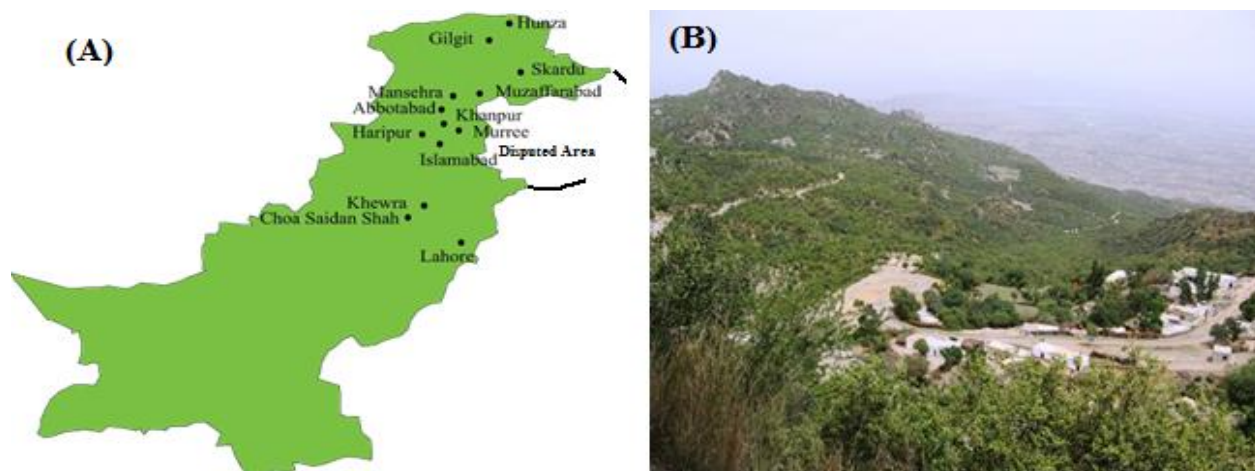


Fig. 1. Sample collection area (A) Map (B) panoramic view of Salt Range, Pakistan

Table 1: Source of Pollen material used in this study

Sr. No.	Family	Taxon	Locality	District	Collector
1	Acanthaceae	<i>Dicliptera bupleuroides</i>	Kalar Kahar	Chakwal	Authors
2	Apiaceae	<i>Unknown</i>	Kalar Kahar	Chakwal	Authors
3	Asclepiadaceae	<i>Calotropis procera</i>	Kalar Kahar	Chakwal	Authors
4	Asparagaceae	<i>Asparagus adscendens</i>	Sodhi	Khushab	Authors
5	Asteraceae	<i>Cotula hemisphaerica</i>	Kalar Kahar	Chakwal	Authors
6	Asteraceae	<i>Aster laevis</i>	Kalar Kahar	Chakwal	Authors
7	Boraginaceae	<i>Trichodesma indicum</i>	Kalar Kahar	Chakwal	Authors
8	Brassicaceae	<i>Nasturtium officinale</i>	Kalar Kahar	Chakwal	Authors
9	Brassicaceae	<i>Sisymbrium irio</i>	Kalar Kahar	Chakwal	Authors
10	Caryophyllaceae	<i>Stellaria media</i>	Kalar Kahar	Chakwal	Authors
11	Convolvulaceae	<i>Convolvulus arvensis</i>	Kalar Kahar	Chakwal	Authors
12	Euphorbiaceae	<i>Croton bonplandianus</i>	Kalar Kahar	Chakwal	Authors
13	Euphorbiaceae	<i>Euphorbia helioscopia</i>	Kalar Kahar	Chakwal	Authors
14	Fabaceae	<i>Prosopis juliflora</i>	Kanhati Garden	Khushab	Authors
15	Hypericaceae	<i>Hypericum calycinum</i>	Sakesar	Khushab	Authors
16	Lamiaceae	<i>Clerodendrum fragrans</i>	Kalar Kahar	Chakwal	Authors
17	Malvaceae	<i>Malvaviscus arborea</i>	Kalar Kahar	Chakwal	Authors
18	Myrsinaceae	<i>Anagallis arvensis</i>	Kalar Kahar	Chakwal	Authors
19	Oxalidaceae	<i>Oxalis corymbosa</i>	Kalar Kahar	Chakwal	Authors
20	Plantaginaceae	<i>Bacopa monnieri</i>	Kalar Kahar	Chakwal	Authors
21	Plantaginaceae	<i>Plantago lanceolata</i>	Kalar Kahar	Chakwal	Authors
22	Plantaginaceae	<i>Plantago major</i>	Kalar Kahar	Chakwal	Authors
23	Poaceae	<i>Cynodon dactylon</i>	Kalar Kahar	Chakwal	Authors
24	Poaceae	<i>Dichanthium annulatum</i>	Kalar Kahar	Chakwal	Authors
25	Poaceae	<i>Panicum antidotale</i>	Kalar Kahar	Chakwal	Authors
26	Polygonaceae	<i>Polygonum plebejum</i>	Sodhi	Khushab	Authors
27	Pontederiaceae	<i>Eichhornia crassipes</i>	Kalar Kahar	Chakwal	Authors
28	Ranunculaceae	<i>Ranunculus muricatus</i>	Kalar Kahar	Chakwal	Authors
29	Ranunculaceae	<i>Ranunculus sceleratus</i>	Kalar Kahar	Chakwal	Authors
30	Scrophulariaceae	<i>Mazus goodenifolius</i>	Naushahra	Khushab	Authors
31	Scrophulariaceae	<i>Verbascum thapsus</i>	Kanhati Garden	Khushab	Authors
32	Verbenaceae	<i>Verbena officinalis</i>	Kalar Kahar	Chakwal	Authors
33	Verbenaceae	<i>Verbena tenuisecta</i>	Kalar Kahar	Chakwal	Authors

It is hypothesized that palynology can be used as an aid in identification and differentiation of important medicinal plants of the area that can be used to solve systematics problems

2. Materials and Methods

The research was done during 2011-2013 in the Experimental Taxonomy Laboratory, Department of Botany, University of Agriculture, Faisalabad, Pakistan. The trial was limited to study the morphology and palynology of medicinal plants of Salt Range of Pakistan (Fig. 1).

2.1. Collection and Preservation of Medicinal Plant

During different season, many visits were made for collection of medicinal plants from salted areas of Pakistan. 33 plants species relevant to 30 genera were collected randomly from different sites (Table 1). Plants were dehydrated, preserved by using standard herbarium methods and deposited to the Herbarium of the Botany Department, University of Agriculture, Faisalabad.

2.2. Palynological Studies

Camera equipped with light microscope (Nikon 104, Japan) was used to study pollen morphology.

Table 2. Qualitative characteristics regarding pollen of different plant species from Salt Range of Pakistan

Sr. No.	Family	Taxon	Shape in polar view	Shape in Equatorial view	Pollen type	Pollen sculpturing
1	Acanthaceae	<i>Dicliptera bupleuroides</i>	Oblate spheroidal	Oblate	Tricolpate	Reticulate
2	Apiaceae	<i>Unknown</i>	Spheroidal	Curvilinear triangular	Tricolpate	Rugulate
3	Asclepiadaceae	<i>Calotropis procera</i>			Pollinium	
4	Asparagaceae	<i>Asparagus adscendens</i>	Spheroidal	Rhomboidal	Monolete	Reticulate
5	Asteraceae	<i>Cotula hemisphaerica</i>	Spheroidal	Rhomboidal	Tricolpate	Psilate
6	Asteraceae	<i>Aster laevis</i>	Oblate spheroidal	Rhomboidal	Monocolpate	Psilate
7	Boraginaceae	<i>Trichodesma indicum</i>	Spheroidal	Star shaped	Pentacolpate	Psilate
8	Brassicaceae	<i>Nasturtium officinale</i>	Oblate spheroidal	Ellipse	Tricolpate	Reticulate
9	Brassicaceae	<i>Sisymbrium irio</i>	Sub circular	Ellipse	Monocolpate	Reticulate
10	Caryophyllaceae	<i>Stellaria media</i>	Oblate spheroidal	Trapezoid	Monocolpate	Scabrate
11	Convolvulaceae	<i>Convolvulus arvensis</i>	Spheroidal	Ellipse	Zonocolpate	Reticulate
12	Euphorbiaceae	<i>Croton bonplandianus</i>	Oblate spheroidal	Ellipse	Tricolpate	Scabrate
13	Euphorbiaceae	<i>Euphorbia helioscopia</i>	Sub circular	Oval	Inaperturate	Scabrate
14	Fabaceae	<i>Prosopis juliflora</i>	Spheroidal	Oval	Dicolpate	Reticulate
15	Hypericaceae	<i>Hypericum calycinum</i>	Oblate spheroidal	Ellipse	Zonocolpate	Reticulate
16	Lamiaceae	<i>Clerodendrum fragrans</i>	Sub circular	Ellipse	Monocolpate	Reticulate
17	Malvaceae	<i>Malvaviscus arborea</i>	Marquise	Pentagone	Dicolpate	Rugulate
18	Myrsinaceae	<i>Anagallis arvensis</i>	Sub spheroidal	Ellipse	Monocolpate	Foveolate
19	Oxalidaceae	<i>Oxalis corymbosa</i>	Oblate spheroidal	Ellipse	Monocolpate	Foveolate
20	Plantaginaceae	<i>Bacopa monnieri</i>	Sub circular	Ellipse	Dicolpate	Foveolate
21	Plantaginaceae	<i>Plantago lanceolata</i>	Spheroidal	Heart shaped	Monocolpate	Foveolate
22	Plantaginaceae	<i>Plantago major</i>	Star shaped	Ellipse	Zonocolpate	Foveolate
23	Poaceae	<i>Cynodon dactylon</i>	Oblate	Triangular	Tricolpate	Verrucate
24	Poaceae	<i>Dichanthium annulatum</i>	Oval	Ellipse	Dicolpate	Rugulate
25	Poaceae	<i>Panicum antidotale</i>	Oblate spheroidal	Ellipse	Dicolpate	Verrucate
26	Polygonaceae	<i>Polygonum plebejum</i>	Heart shaped	Ellipse	Monocolpate	Reticulate
27	Pontederiaceae	<i>Eichhornia crassipes</i>	Sub circular	Oval	Monocolpate	Verrucate
28	Ranunculaceae	<i>Ranunculus muricatus</i>	Sub circular	Oval	Tricolpate	Scabrate
29	Ranunculaceae	<i>Ranunculus sceleratus</i>	Sub circular	Rectangular	Inaperturate	Scabrate
30	Scrophulariaceae	<i>Mazus goodenifolius</i>	Oval	Ellipse	Tricolpate	Scabrate
31	Scrophulariaceae	<i>Verbacum thapsus</i>	Sub circular	Curvilinear triangular	Tetrad	Scabrate
32	Verbenaceae	<i>Verbena officinalis</i>	Irregular	Ellipse	Tricolpate	Scabrate
33	Verbenaceae	<i>Verbena tenuisecta</i>	Spheroidal	Ellipse	Inaperturate	Scabrate

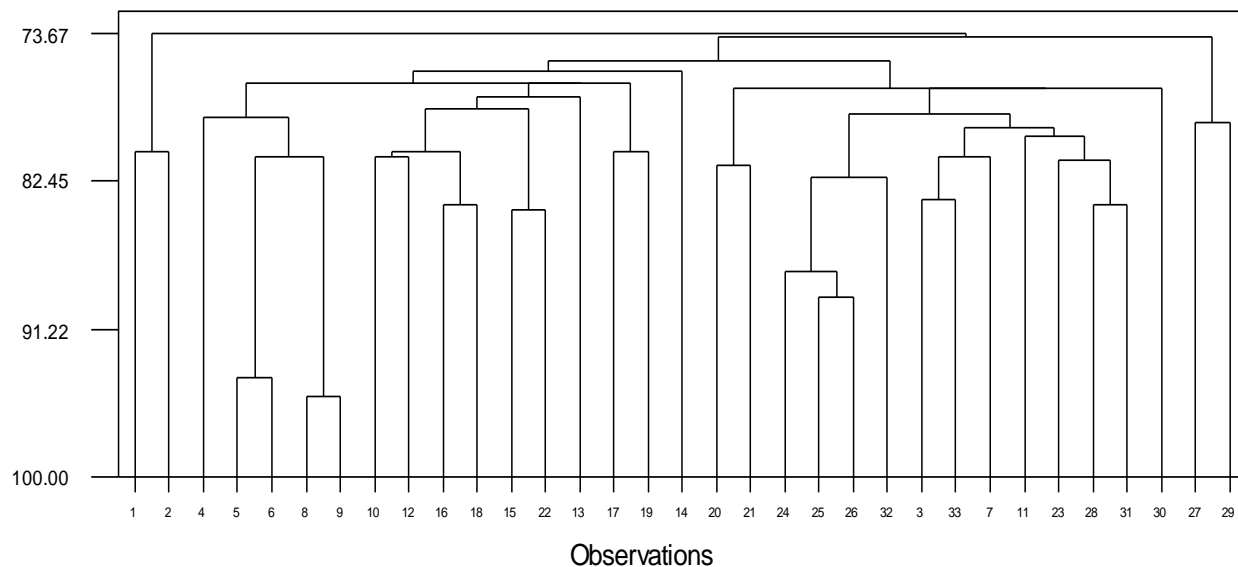


Fig. 2. Cluster analysis of qualitative morphological characteristics of different plant species from Salt Range of Pakistan. (1: *Dicliptera bupleuroides*, 2: Unknown, 3: *Calotropis procera*, 4: *Asparagus adscendens*, 5: *Cotula hemisphaerica*, 6: *Aster laevis*, 7: *Trichodesma indicum*, 8: *Nasturtium officinale*, 9: *Sisymbrium irio*, 10: *Stellaria media*, 11: *Convolvulus arvensis*, 12: *Croton bonplandianus*, 13: *Euphorbia helioscopia*, 14: *Prosopis juliflora*, 15: *Verbascum thapsus*, 16: *Hypericum calycinum*, 17: *Clerodendrum fragrans*, 18: *Malvaviscus arborea*, 19: *Anagallis arvensis*, 20: *Oxalis corymbosa*, 21: *Bacopa monnieri*, 22: *Plantago lanceolata*, 23: *Plantago major*, 24: *Cynodon dactylon*, 25: *Dichanthium annulatum*, 26: *Panicum antidotale*, 27: *Polygonum plebejum*, 28: *Eichhornia crassipes*, 29: *Ranunculus muricatus*, 30: *Ranunculus sceleratus*, 31: *Mazus goodenifolius*, 32: *Verbena officinalis*, 33: *Verbena tenuisecta*).

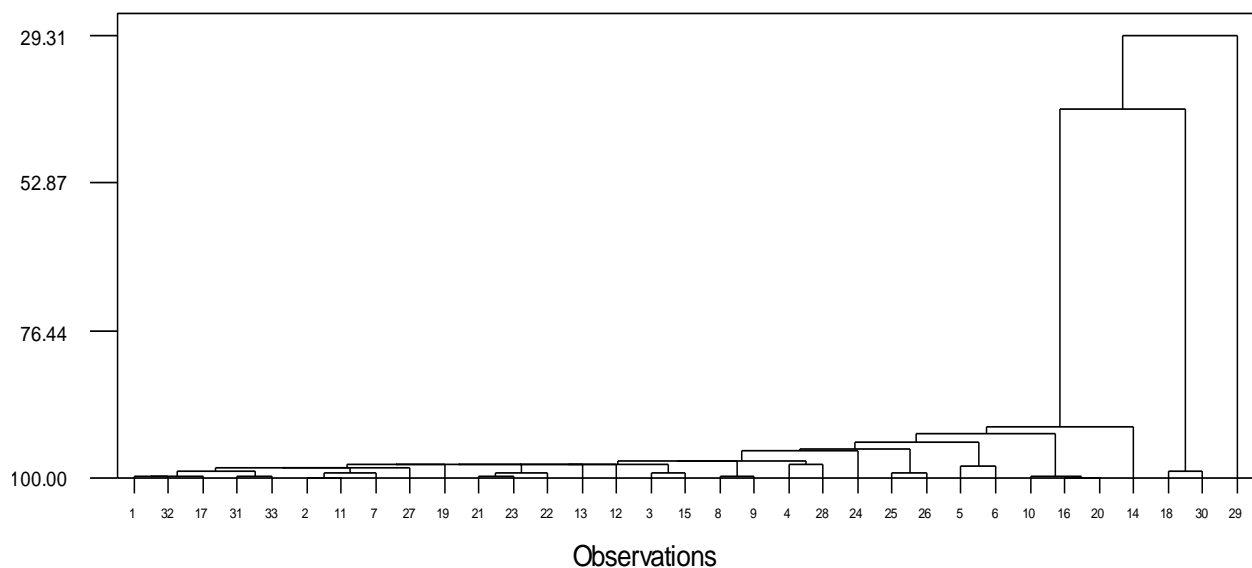


Fig. 3. Cluster analysis of quantitative morphological characteristics of different plant species from Salt Range of Pakistan. (1: *Dicliptera bupleuroides*, 2: Unknown, 3: *Calotropis procera*, 4: *Asparagus adscendens*, 5: *Cotula hemisphaerica*, 6: *Aster laevis*, 7: *Trichodesma indicum*, 8: *Nasturtium officinale*, 9: *Sisymbrium irio*, 10: *Stellaria media*, 11: *Convolvulus arvensis*, 12: *Croton bonplandianus*, 13: *Euphorbia helioscopia*, 14: *Prosopis juliflora*, 15: *Verbascum thapsus*, 16: *Hypericum calycinum*, 17: *Clerodendrum fragrans*, 18: *Malvaviscus arborea*, 19: *Anagallis arvensis*, 20: *Oxalis corymbosa*, 21: *Bacopa monnieri*, 22: *Plantago lanceolata*, 23: *Plantago major*, 24: *Cynodon dactylon*, 25: *Dichanthium annulatum*, 26: *Panicum antidotale*, 27: *Polygonum plebejum*, 28: *Eichhornia crassipes*, 29: *Ranunculus muricatus*, 30: *Ranunculus sceleratus*, 31: *Mazus goodenifolius*, 32: *Verbena officinalis*, 33: *Verbena tenuisecta*).

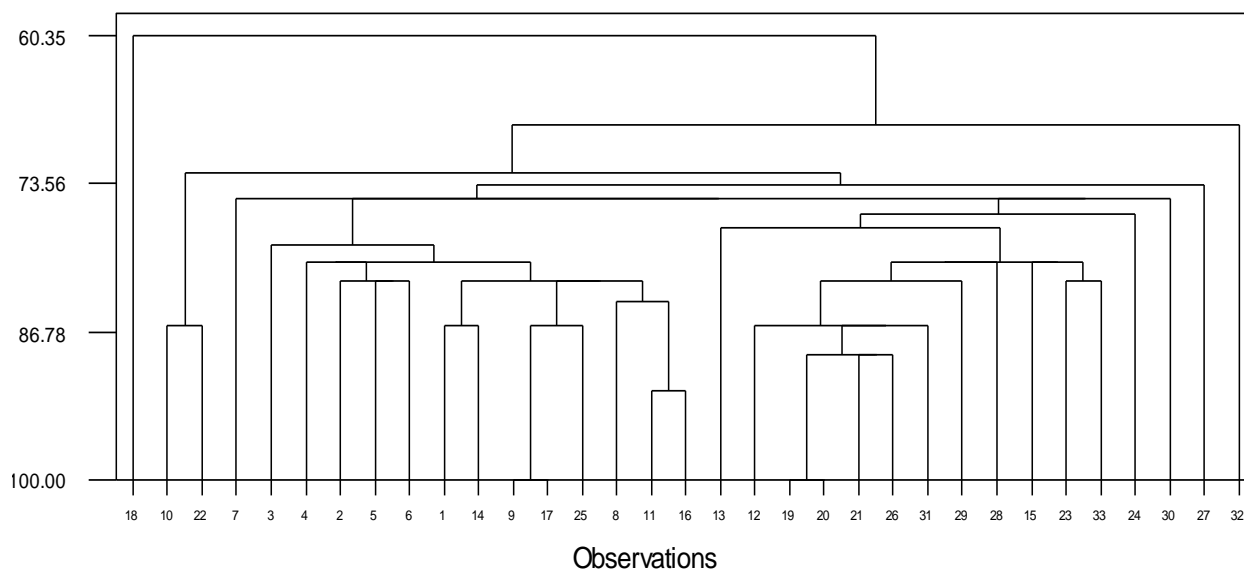


Fig. 4. Cluster analysis of qualitative palynological characteristics of different plant species from Salt Range of Pakistan (1: *Dicliptera bupleuroides*, 2: Unknown, 3: *Calotropis procera*, 4: *Asparagus adscendens*, 5: *Cotula hemisphaerica*, 6: *Aster laevis*, 7: *Trichodesma indicum*, 8: *Nasturtium officinale*, 9: *Sisymbrium irio*, 10: *Stellaria media*, 11: *Convolvulus arvensis*, 12: *Croton bonplandianus*, 13: *Euphorbia helioscopia*, 14: *Prosopis juliflora*, 15: *Verbascum thapsus*, 16: *Hypericum calycinum*, 17: *Clerodendrum fragrans*, 18: *Malvaviscus arborea*, 19: *Anagallis arvensis*, 20: *Oxalis corymbosa*, 21: *Bacopa monnieri*, 22: *Plantago lanceolata*, 23: *Plantago major*, 24: *Cynodon dactylon*, 25: *Dichanthium annulatum*, 26: *Panicum antidotale*, 27: *Polygonum plebejum*, 28: *Eichhornia crassipes*, 29: *Ranunculus muricatus*, 30: *Ranunculus sceleratus*, 31: *Mazus goodenifolius*, 32: *Verbena officinalis*, 33: *Verbena tenuisecta*).

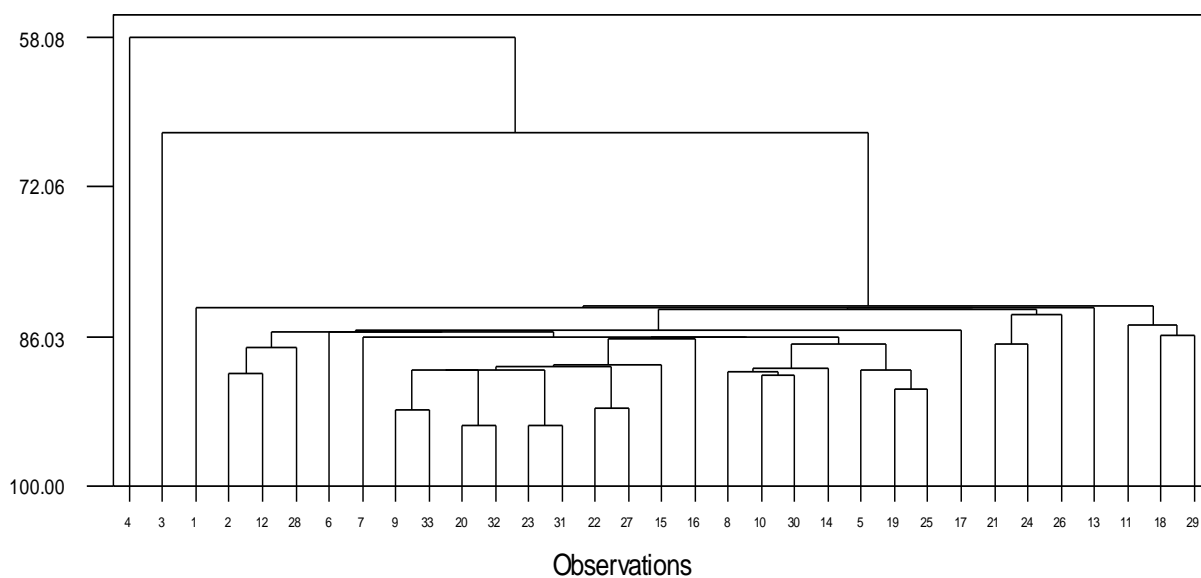


Fig. 5. Cluster analysis of quantitative palynological characteristics of different plant species from Salt Range of Pakistan (1: *Dicliptera bupleuroides*, 2: Unknown, 3: *Calotropis procera*, 4: *Asparagus adscendens*, 5: *Cotula hemisphaerica*, 6: *Aster laevis*, 7: *Trichodesma indicum*, 8: *Nasturtium officinale*, 9: *Sisymbrium irio*, 10: *Stellaria media*, 11: *Convolvulus arvensis*, 12: *Croton bonplandianus*, 13: *Euphorbia helioscopia*, 14: *Prosopis juliflora*, 15: *Verbascum thapsus*, 16: *Hypericum calycinum*, 17: *Clerodendrum fragrans*, 18: *Malvaviscus arborea*, 19: *Anagallis arvensis*, 20: *Oxalis corymbosa*, 21: *Bacopa monnieri*, 22: *Plantago lanceolata*, 23: *Plantago major*, 24: *Cynodon dactylon*, 25: *Dichanthium annulatum*, 26: *Panicum antidotale*, 27: *Polygonum plebejum*, 28: *Eichhornia crassipes*, 29: *Ranunculus muricatus*, 30: *Ranunculus sceleratus*, 31: *Mazus goodenifolius*, 32: *Verbena officinalis*, 33: *Verbena tenuisecta*).

2.3. Method of Pollen Study by Light Microscopy

Anthers from the specific flower separated with forceps, added 45% acetic acid (1-2 drops) and then crushed it with iron rod. Pollens were acetolysed by following procedure of Ahmad et al., (2008), who pursued Erdtman, (1952). Pollen stirred with needle on slide for equal spreading, then covers lip is put and transparent nail paint utilized for sealing slide corners. Labeled slides with the name of specific plant species with their location.

2.4. Pollen Parameters

Following parameters were studied under light microscope for pollen morphology.

2.5. Qualitative Characters

Shape in polar and equatorial view, type of pollen and sculpturing and presence of colpi

2.6. Quantitative Characters

Polar and equatorial diameter, P/E ratio, number, length and width of colpi and exine thickness

3. Results

Various qualitative features like pollen shape in equatorial and polar view, types of pollen and occurrence of colpi while quantitative features like equatorial and polar diameter, P/E ratio, number, length and width of colpi, exine thickness and pollen fertility was recorded. Then finally data was subjected to multivariate (cluster) analysis to found relationship between various genera and species (Fig. 2-Fig. 5).

In various species all quantitative and qualitative features were observed. It was recorded that qualitatively all species varied in their pollen shapes of equatorial and polar view, occurrence of colpi, exine sculpturing and pollen type are unique attributes of all plant species. And in the same way, quantitative attributes are also variate in equatorial and polar diameter, P/E ratio, number, length and width of colpi, exine thickness and pollen fertility. That features are vital in systematics studies.

Recent study revealed that pollen features of all species varied that helps to solve systematic problems. This study is important for identification of various species, and their division at family, generic and species level. It is recommended that molecular systematics can be reprocessed as an alternative methodology for identification of various plant species in future (Fig. 2-Fig. 9).

4. Discussion

Palynology is the study of spores and pollens and this study is also significant in this regard that pollen

study can be utilized as recognizing marker in evolutionary record (Chambers et al., 2011), that focus on pollen size, shape symmetry and sources (Annamaria et al., 2011). In all species both qualitative and quantitative traits significantly varied and showed that these characters are vital in systematics and every species have its own pollen traits. *Calotropis procera* member of family Asclepiadaceae exhibit distinct pollen features called pollinium that is unique from other pollen characters. It is indicative features of this family that makes them unique and isolate from other families and this is vital taxonomically (Sreenath et al., 2012).

In Plantaginaceae family three species *Plantago major*, *Bacopa monnieri* and *Plantago lanceolata* were studied. In polar view star shaped, sub circular and spheroidal shaped were found respectively while in equatorial view first two species showed ellipsoidal shaped and heart shaped was found in *P. lanceolata*. They were of zonocolpate, dicolpate and monocolpate type respectively. While all species have foveolate sculpturing that is common point of this family. So exine ornamentation feature is vital to understand correlation between species and polar and equatorial shapes are important to differentiate these species within the genus. Sosa et al., (2011) results are similar to present findings.

Verbena officinalis and *Verbena tenuisecta* have asymmetrical and spheroidal formed pollen in polar view, tricolpate and inaperturate pollen types were found respectively. While both species have common feature i.e. ellipse shaped in equatorial view and scabrate sculpturing and pattern that is prominent feature of this family are coordinated with previous studies of Sousa et al. (2013).

Highest polar diameter (34.67µm) and equatorial diameter (46.0µm) was perceived in *Dicliptera bupleuroides* of family Acanthaceae. Perveen and Qaiser, (2010) detected *Dicliptera bupleuroides* pollen size varied from 58.11µm and 35.89µm in polar and equatorial views respectively. Lowest pollen size (9.17µm) was observed in *Trichodesma indicum*, member of Boraginaceae family in polar view, while member of this family showed 13.08-20.02µm pollen size in polar view, which observed by Mehrabian et al. (2012) in *Onosma* L. that much close to present study, and in equatorial view, lowest pollen size (10.1µm) was found in *Verbascum thapsus* of family Scrophulariaceae. While all other species have pollen size in-between these range.

Table 3. Quantitative characteristics regarding pollen of different plant species from Salt Range of Pakistan

Sr. No.	Family	Taxon	Polar diameter (µm)	Equatorial diameter (µm)	P/E	Exine thickness (µm)	Number of colpi	Length of colpi (µm)	Width of colpi (µm)	Pollen fertility%
1	Acanthaceae	<i>Dicliptera bupleuroides</i>	34.68	45.9	0.75	1.02	3	7.14	10.2	84
2	Apiaceae	<i>Unknown</i>	22.44	30.6	0.73	2.04	3	4.08	5.1	69
3	Asclepiadaceae	<i>Calotropis procera</i>								77
4	Asparagaceae	<i>Asparagus adscendens</i>	18.36	19.38	0.94	3.06				28
5	Asteraceae	<i>Cotula hemisphaerica</i>	20.4	28.56	0.71	1.02	3	3.06	6.12	87
6	Asteraceae	<i>Aster laevis</i>	30.6	26.52	1.15	2.55	1	10.2	10.2	93
7	Boraginaceae	<i>Trichodesma indicum</i>	9.18	15.3	0.6	1.02	5	4.08	10.2	84
8	Brassicaceae	<i>Nasturtium officinale</i>	14.28	20.4	0.7	0.51	3	6.12	12.24	89
9	Brassicaceae	<i>Sisymbrium irio</i>	17.34	16.32	1.06	1.53	1	3.06	6.12	82
10	Caryophyllaceae	<i>Stellaria media</i>	23.46	23.46	1	1.53	1	5.1	7.14	93
11	Convolvulaceae	<i>Convolvulus arvensis</i>	12.24	24.48	0.5	1.02	1	4.08	5.1	62.5
12	Euphorbiaceae	<i>Croton bonplandianus</i>	18.36	33.66	0.54	2.55	3	4.08	5.1	64
13	Euphorbiaceae	<i>Euphorbia helioscopia</i>	22.44	23.46	0.95	1.02				64
14	Fabaceae	<i>Prosopis juliflora</i>	17.34	23.46	0.73	1.02	2	11.2	15.3	90
15	Hypericaceae	<i>Hypericum calycinum</i>	25.5	18.36	1.38	1.02	1	4.08	5.1	75
16	Lamiaceae	<i>Clerodendrum fragrans</i>	13.26	32.64	0.4	1.02	1	4.08	5.1	82
17	Malvaceae	<i>Malvaviscus arborea</i>	17.34	20.4	0.85	1.53	2	5.1	7.14	55
18	Myrsinaceae	<i>Anagallis arvensis</i>	24.48	27.54	0.88	2.5	1	5.1	7.14	82
19	Oxalidaceae	<i>Oxalis corymbosa</i>	12.24	22.44	0.54	1.02	1	5.1	7.14	82
20	Plantaginaceae	<i>Bacopa monnieri</i>	25.5	41.82	0.6	1.02	2	4.08	7.14	87
21	Plantaginaceae	<i>Plantago lanceolata</i>	13.26	15.3	0.86	0.51	1	5.1	8.16	76
22	Plantaginaceae	<i>Plantago major</i>	15.3	22.44	0.68	1.5	4	5.1	8.16	73
23	Poaceae	<i>Cynodon dactylon</i>	26.52	35.7	0.7	1.02	3	5.1	3.06	92
24	Poaceae	<i>Dichanthium annulatum</i>	29.58	24.48	1.2	2.5	2	5.1	7.14	81
25	Poaceae	<i>Panicum antidotale</i>	15.3	42.84	0.3	3.06	2	3.06	5.1	89
26	Polygonaceae	<i>Polygonum plebejum</i>	16.32	16.32	1	1.53	1	7.14	10.2	74
27	Pontederiaceae	<i>Eichhornia crassipes</i>	28.56	26.52	1.07	1.53	1	5.1	9.18	68
28	Ranunculaceae	<i>Ranunculus muricatus</i>	18.36	15.3	1.2	1.02	3	12.24	9.18	58
29	Ranunculaceae	<i>Ranunculus sceleratus</i>	17.34	22.44	0.77	2.04	2	7.14	9.18	94
30	Scrophulariaceae	<i>Mazus goodenifolius</i>	18.36	23.46	0.78	2.04	3	4.08	7.14	74
31	Scrophulariaceae	<i>Verbascum thapsus</i>	23.46	10.2	2.3	1.02	4	4.08	6.12	
32	Verbenaceae	<i>Verbena officinalis</i>	14.28	21.42	0.6	1.53	3	6.12	7.14	80
33	Verbenaceae	<i>Verbena tenuisecta</i>	17.34	13.26	1.3	1.53	1	5.1	7.14	85

The current research exposed the maximum P/E (2.31) was found in *Verbascum thapsus* of family Scrophulariaceae. Minimum P/E (0.29) ratio was observed in *Panicum antidotale* in Poaceae family, Ahmad (2009) stated P/E ratio 1.0 in *Panicum antidotale* and this feature is very vital.

Exine thickness seemed maximum (3.06 µm) in *Panicum antidotale* that do not relate with the Ahmad (2009), they found Exine thickness (0.8 µm) in *Panicum antidotale*. Minimum exine thickness (0.51 µm) was detected in two species *Nasturtium officinale* and *Plantago lanceolata* species of family Brassicaceae and Plantaginaceae respectively, Keshavarzi et al. (2012) reported exine thickness 1.63 µm in genus *Clypeola* of Brassicaceae family while in Plantaginaceae family exine thickness 1.0 µm was detected in *Stemodia* genus.

5. Conclusion

The present research showed all different species are unique in their pollen characters that makes them discriminated from other species. For species differentiation and correct identification only morphological studies are not enough then palynological studies exine features i.e., their ornamentation and sculpturing proved to be valuable at generic and species level are vital in systematics that states correct and accurate differentiation between relevant species. Every plant species has their own unique pollen features that can be further merged to extract generic and family level pollen traits.

List of Abbreviations: P: polar, E: equatorial

Competing Interest Statement: All the authors declare that they have no competing interest.

Author's Contribution: F. Ahmad designed the experiment. A. Zahoor conducted the experiment under the supervision of F. Ahmad. M. Naseer performed sectioning. N. Irum helps in collection of plant material. M. hameed. performed statistical analysis Finally, A. Zahoor approved the current version of manuscript. All the authors read and approved the final manuscript.

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