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Full Length Research Paper

Palynological diversity in selected medicinal plant species of *Asteraceae (Compositae)* from flora of Kaghan Valley

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Pollen morphology of seven species, belonging to seven Asteraceae genera from the flora of Kaghan Valley was investigated by light and scanning electron microscopy. The species named *Achillea millefolium* Linn., *Chrysanthemum leucanthemum* Linn., *Gerbera gossypina* (Royle) Beauv., *Senecio chrysanthemoides* DC. Prodr., *Sonchus asper* Linn., *Tagetes erecta* Linn. and *Xanthium strumarium* Linn. were studied which showed considerable variation in pollen shape, size, length and number of spines, colpi and sculpturing in light as well as electron microscopy. The maximum pollen size was found in *G. gossypina* which was 38.3 µm in polar view and 57.75 in µm equatorial view whereas minimum pollen size in polar view that is, 23.65 µm was found in *X. strumarium* and 19 µm in equatorial view was found in *A. millefolium*. Psilate pollen sculpturing was observed in *G. gossypina* however the other species had spines on their pollen. The psilate sculpturing was considered as an advanced feature when compared to echinate sculpturing of pollen which was regarded as the primitive form. It was concluded that this research work has provided new dimensions towards further investigations in the field of palynology.

Key words: Palynology, psilate, echinate.

INTRODUCTION

The flora of the Kaghan is very interesting. Variety of bryophytes, pteridophytes, angiosperms and gymnosperms are present in the area. The general vegetation is of the scrub type which includes the shrubs or medium size trees (Mustafa, 2003). There is a great variety of medicinal plants growing throughout the area which are being used by the local people. Wodehouse (1935) outlined the principles of morphological evolution of spine form in Compositae and suggested the reduction series from long to minute spines. The spinate pollen character is considered as a primitive feature as compared to spineless pollen. Asteraceae is an extremely natural taxon, with unique floral theme and micro-morphological features including those of pollen grains. It is a eurypalynous family (Erdtmann, 1952) and most of its genera are zonoporate pollen (Sachdeva and Malik, 1986).

Most of the taxonomists identify plant species on the basis of phenotypic characters of plants like root, stem and leaf structures, but now the scientists believed that

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palynological studies can provide more accurate basis for the identification of plant species (Daiz and Lifante, 1991). There are many disciplines associated with plant taxonomy, which are used by taxonomists as an aid, or to improve the identification, classification and systematic position of plant taxa. Among these disciplines palynology is one of the most significant tools used by modern taxonomist to identify and differentiate closely related taxa.

The study of pollen biology has direct relevance in agriculture, horticulture, forestry, plant breeding and biotechnology. Pollen grain have potential use in gene transfer, monitoring cytotoxic effect of bioactive chemicals such as herbicides, pesticides and pollutants understanding the organization and function cytoskeleton and association proteins, studies on expression and cloning of gene and research on intracellular differentiation and polarity (Zafar et al., 2007).

The purpose of the present study is to evaluate palynological characters of seven medicinal plants species of Asteraceae from Kaghan Valley for taxonomic purposes and to solve problems of identification, classification and evolution of spine in the family Asteraceae. The present study can also be helpful to compare the pollen characters of studied species with the species present in other areas to observe affect of climate on pollen characters of these species.

METHODOLOGY

Palynological studies were conducted in the Plant Taxonomy Laboratory, Herbarium of Quaid-i-Azam University, Islamabad and Centralized Science Laboratory University of Karachi. The study was confined to palynological description of the medicinal plants of Kaghan Valley of District Mansehra. Pollen material of fresh specimens was used which was collected from Kaghan Valley during field trips. For every species 4 to 5 samples were collected from different individuals. Floral parts of plants were kept in zip transparent envelops labeled with the name of genus and species.

Light microscopy

The Glycerin jelly was prepared according to modified method of Meo and Khan (2005). A beaker contained 50 ml of distilled water was taken and heated on a hot plate, when temperature reaches to 70 to 80°C, 35 g of gelatin was added. After increase in temperature it became a viscous liquid. The whole solution was kept on heating for one hour then 35 g of glycerin were mixed in it with few crystals of phenol. After that 0.1 safranine was added by 1/8 volume with glycerin jelly. It was stirred till uniform pink color appeared. Jelly was stabilized at room temperature.

First acetolysed anthers were removed from filaments of stamen with the help of dissecting needles and then crushed to release pollen grains on a clean glass slide. Anther wall material was discarded. Then pollen grains were mounted in glycerin jelly stained with 1% safranine. The slide was placed on hot plate to melt glycerin jelly and to remove bubbles from the slide. Cover slip was placed on the prepared pollen-glycerin jelly mixture. When cooled, the glass slide was labeled and edges of the cover slip were sealed with transparent nail vanish. The prepared slides were studied under the light microscope.

Detailed qualitative and quantitative characters were studied under light microscope for pollen morphology. The micro-graphs of these mounted materials were taken using a digital camera (Meiji CCD Model 00179048, Canada) fitted on the Lieca light microscope (DM 1000, Germany). All photographs were taken on different object lens by using oil emulsion.

Scanning electron microscopy (SEM)

The pollen grains were prepared for scanning electron microscopy (SEM) by the standard methods described by Erdtmann (1952). For SEM studies, pollen grains were taken from plants and transferred to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber (Ion-sputter JFC-1100). Coating was restricted to 150 A. The S.E.M examination was carried out on a Jeol microscope JSM-2. Desired views of pollen like polar view, equatorial view and sculpturing elements were focused in photography. The measurements are based on 15-20 readings from each specimen. Polar axis (P) and equatorial diameter (E), aperture size, apocolpium, mesocolpium and exine thickness. The terminology used is in accordance with Erdtmann (1952), Kremp (1965), Faegri and Iversen (1964) and Walker and Doyle (1975).

RESULTS AND DISCUSSION

This research was carried out to examine the value of pollen morphology in taxonomy of seven species of Asteraceae, investigated by light and scanning electron microscopy (Table 1). The findings are represented in alphabetical order of species with botanical name, local name, English name, distribution and palynological description (Tables 1 and 2).

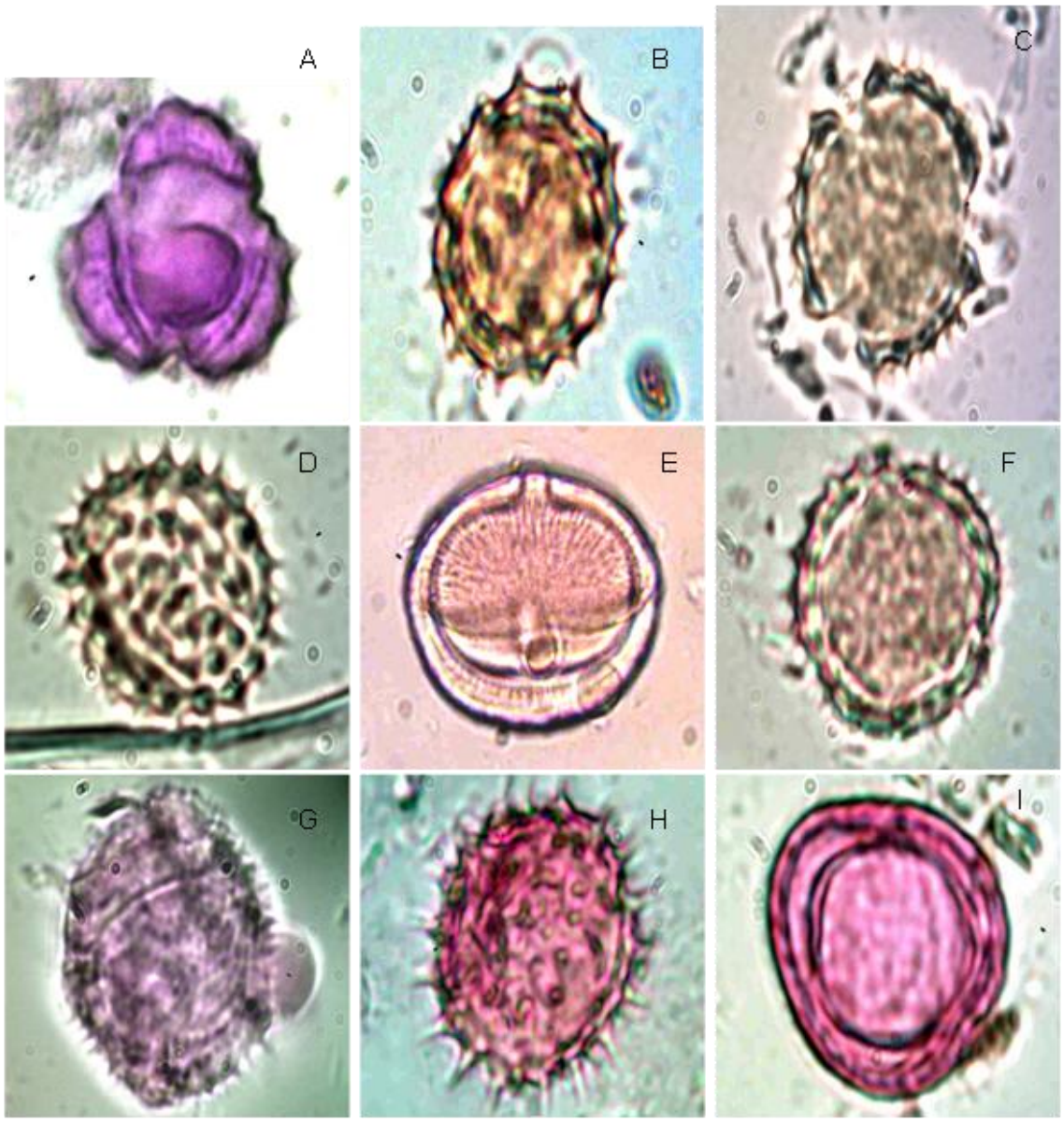
There was a great diversity in the pollen morphology of all species studied. Variation mostly found in size, shape, spine length and number and colpi morphology. All these variations were observed by light microscopy whereas variation in the sculpturing was observed by scanning electron microscopy of pollen grains (Table 2). The pollen types in all seven species were monad and tricolporate but tri-tetracolporate pollen grains were seen in *Senecio chrysanthemoides* and *Sonchus asper* (Figures 1F and G). In polar view circular shape was seen in *Chrysanthemum leucanthemum, S. chrysanthemoides* and *Sonchus asper* (Figures 1C, D, F and G). Circular to circular-lobate in *Achellia millefolium* and *Gerbera gossypina* (Figures 1A, B and E) while circular to sub-angular in *Xanthium strumarium* (Figure 1I). Maximum pollen size that is, 38.3 µm in polar view and 57.75 in µm equatorial view was found in *G. gossypina* whereas

Table 1. List of Asteraceae species investigated, with local name, English name, distribution and voucher numbers.

S/N	Species	Local name	English name	Distribution in Pakistan	Distribution worldwide	Voucher number
1	*Achillea millefolium* Linn.	Biranjasaif	Yarrow	Punjab, Hazara, Gilgit, Astor, Swat, Muree, Poonch, Kaghan, Naran and Shogran.	Europe including Britain and East to western Asia	P-1
2	*Chrysanthemum leucanthemum* Linn.	Daudi	Oxeye Daisy	Muree, Kalabagh, Kaghan, Naran, Shogran, Nathiagali and such similar areas	Native to Europe including Britain and temperate regions of Asia and Siberia	P-2
3	*Gerbera gossypina* (Royle) Beauv.	Chita Patra	Traugott gerber	Punjab, Hazara, Balakot, Kaghan, Naran, Muree and AJK	West Nepal and India	P-3
4	*Senecio chrysanthemoides* DC. Prodr.	Ghopga	-	Gilgit, Baltistan, Zanskar, Hazara, Muree, Poonch and Kashmir	China, India, Nepal, Pakistan, Spain	P-4
5	*Sonchus asper* Linn.	Hind	Spiny-leaved Sow thistle	Sindh, Baluchistan, N.W.F.P (Swat, Gilgit, Ladak, Kurram), Punjab and Kashmir	Europe, including Britain, south and east from Scandanavia to N. Africa, N. and W. Asia.	P-5
6	*Tagetes erecta* Linn.	Genda	Marigold	Kaghan, Naran, Gilgit, Muree, Abbottabad, Gilgit.	USA, Mexico, Georgia, Columbia, UK, Spain and Sweden	P-6
7	*Xanthium strumarium* Linn.	Chota Datura	Cocklebur	N.W.F.P, Balochistan, Sindh and AJK	North America and widespread across Southern Canada, most of the contiguous United States, and Mexico.	P-7

Table 2. Quantitative and qualitative characters of palynological investigations of studied plant species.

S/N	Species	Type of pollen	Shape in polar view	Shape in equatiorial view	Polar diameter (µm)	Equatorial diameter (µm)	P/E ratio (µm)	Exine thickness (µm)	Sculpturing (Under SEM)
1	*Achillea millefolium* Linn.	Monad, tricolporate	Circular to circular-lobate	Spheroidal and prolate	26	19	1.36	2.5	Echinate with spine broad smaller base and short muronate tip
2	*Chrysanthemum leucanthemum* Linn.	Monad, tricolporate	Circular	Spheroidal, prolate-spheroidal and prolate	27	26.5	1.0	1.58	Echinate with spine broad at the base, tip mucronate
3	*Gerbera gossypina* (Royle) Beauv.	Monad and tricolporate	Circular to circular-lobate	Prolate, polate-spheroidal and suboblate	38.3	57.73	0.66	2.5	Psilate with smooth surface
4	*Senecio chrysanthemoides* DC. Prodr.	Monad, tricolporate to tetra-coloporate, fenestrate and periporate	Circular	Spheroidal, prolate and subprolate	25.75	24.37	1.05	3.03	Echinate with small rudimentary spines
5	*Sonchus asper* Linn.	Monad, tri to tetracoloporate and fenestrate	Circular	Spheroidal, prolate and rectangular	37.75	45.75	0.82	6.08	Echinate, spine slightly thick at the base with gradually tapering tip
6	*Tagetes erecta* Linn.	Monad and tri-colporate	Circular	Prolate to spheroidal	39	45.5	0.85	2.1	Echinate with slightly thick base and gradually tepering tip
7	*Xanthium strumarium* Linn.	Monad and tricolporate	Circular and sub-angular	Spheriodal, spheroidal-oblate, suboblate, and peroblate	23.75	23.65	1.00	1.53	Ecihnate with spines less than 1 µm

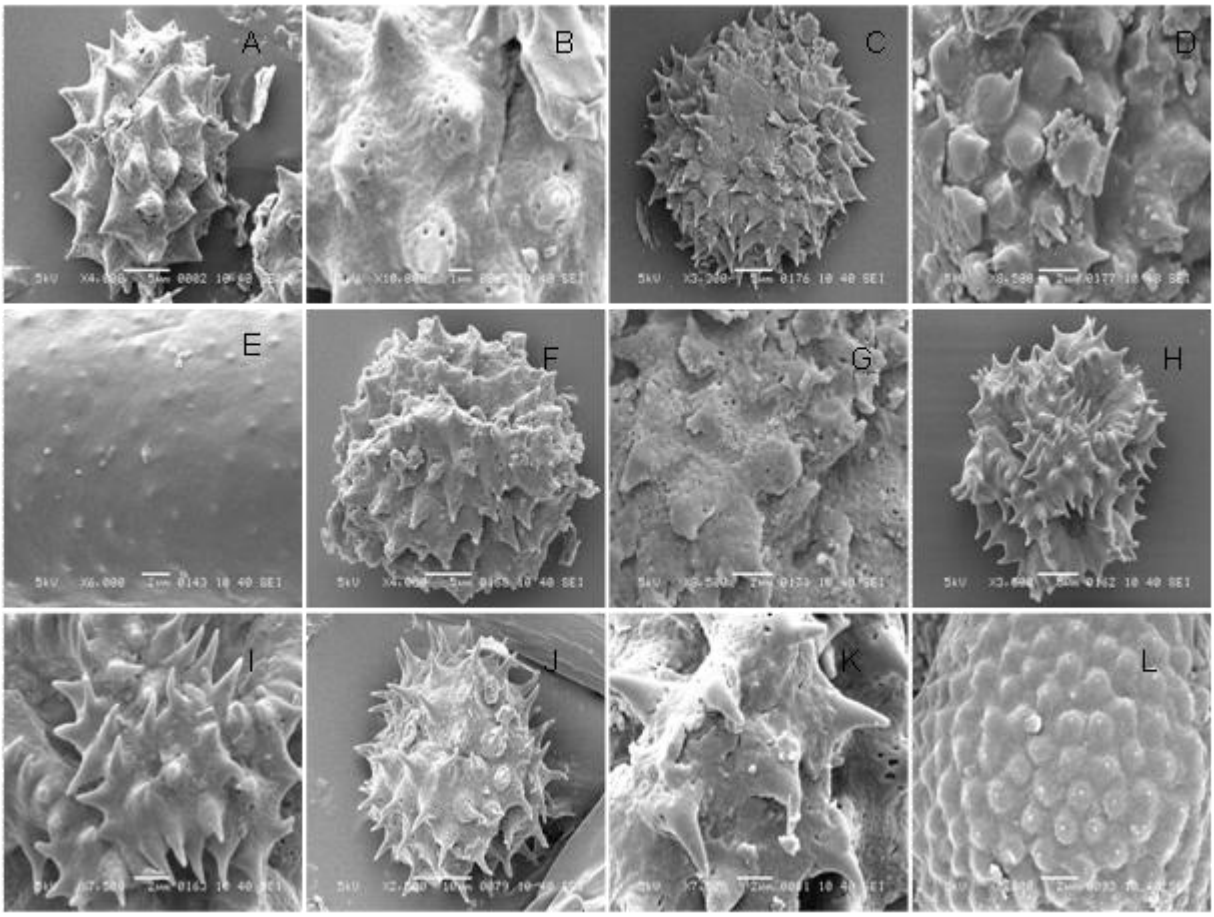


Figures 1. LM microphotographs of pollen grains. *Achillea millefolium:* A and B. Polar and Equatorial view. C and D. *Chrysanthemum leucanthemum:* Polar and equatorial view. E. *Gerbera gossypina:* Equatorial view. F. *Senecio chrysanthemoides:* Polar view. G. *Sonchus asper:* Polar view. H. *Tagetes erecta:* Polar view. I. *Xanthium strumarium:* Polar view.

minimum pollen size in polar view that is, 23.65 µm was found in *X. strumarium* and 19 µm in equatorial view was the minimum size found in *A. millefolium* (Table 2).

Maximum P/E ratio was 1.36 µm found in *A. millefolium* and minimum in *G. gossypina* that is, 0.66 µm. Similarly, exine thickness also varied; maximum exine thickness was 3.03 µm found in *S. chrysanthemoides* and minimum 1.53 µm in *X. strumarium* (Table 2).

The character of pollen spine is significant in evolution and at specific and generic level in classification of this family. Spineless pollen was observed in *G. gossypina* (Figures 1E and 2D) while the other six species present



Figures 2. SEM of pollen sculpturing showing all echinate pollen except the *Gerbera gossypina. Achillea millefolium*: A and B. *Chrysanthemum leucanthemum:* C and D. *Gerbera gossypina:* E. *Senecio chrysanthemoides:* F and G. *Sonchus asper:* H and I. *Tagetes erecta:* J and K. *Xanthium strumarium:* L.

spines on their pollen. These findings are in agreement with the findings of Keeley and Jones (1977) who reported pinate and spineless pollen in some *Veronica* species and other observed that both pollen and vegetative characters indicates a divergence due to independent line of evolution of spine isolation. Wodehouse (1935) outlined the principles of morphological evolution of spine form in Asteraceae and suggested the reduction series from long to minute spines.

The spineless or psilate pollen character is considered as an advanced feature as compared to echinate pollen which is regarded as the primitive feature. In all seven species the number of spines also varies from species to species. The number of spines between colpi also varies from species to species. In *S. asper*, pollen grains were densely covered with numerous small spines (Figures 1G, 2H and I). In other species numbers of spines were different as other than *S. asper*, large numbers of spines were found in *C. leucanthemum* (Figures 1C, D, 2C and D) that is, 15 to 24 per pollen and in *S. chrysanthemoides* (Figures 1F, 2F and G) it was 14 to 19, in *A. millefolium* (Figures 1A, B, 2A and B) 13 to 15 and 11 to 20 in *Tagetes erecta* (Figures 1H, 2J and K). Overall number of spines between colpi ranges from 4 to 7 in all these 4 species.

Similarly, Meo and Khan (2003) has investigated and compared two species of *Achellia* that is, *A. millefolium* and *A. santolina*. He has compared both species very well by light microscopy. According to him numbers of spines rows between colpi in *A. millefolium* were 5 to 6 and 4 to 5 in *A. santilina.* Another study was carried out

by Hussain (2003) on palynological and ethanobotanical studies of *Chrysanthemum leucanthemum* L. from Gallies (Abbottabad) N.W.F.P., Pakistan. He has described well the palynomorph features of pollen of *C. leucanthemum*. According to his studies, sculpturing of pollen was sub-echinte and reticulate but according to studies pollen sculpturing of *chrysanthemum leucanthemum* under scanning electron microscope is echinate with spine broad at the base, tip mucronate. The sculpturing varies from echinate to psilate. Psilate pollen grains with smooth surface were observed in *G. gossypina*, whereas pollen grains of rest of the six species that is, *A. millefolium*, *C. leucanthemum, S. chrysanthemoides, S. asper, T. erecta* and *X. strumarium* were echinate.

Conclusion

Palynological characters particularly exine sculpturing serve as an aid in delimitation of taxa for identification and classification at specific and generic level in family Asteraceae. However, for any taxonomic conclusion it is suggested that other aspects like morphology, anatomy, cytology and finger printing should be used.

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