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POLLEN MORPHOLOGY AS AN AID TO THE IDENTIFICATION OF *CHRYSANTHEMUM* SPECIES (COMPOSITAE–ANTHEMIDEAE) FROM PAKISTAN

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Abstract

Pollen morphology of 7 species of *Chrysanthemum* has been examined from Pakistan by light and scanning electron microscopy. The pollen grains in all the species are trizonocolporate. Pollen shape in polar view is circular, inter-semi-angular and semi-circular to angular. Pollen is spheroidal, sub-spheroidal, oblate-spheroidal and prolate-spheroidal in equatorial view. Taxa of *Chrysanthemum* species can be distinguished by pollen size, exine thickness, spine length and number of spine rows between colpi. *Chrysanthemum indicum* has characteristic spines having a broad flattened base with grooves. *C.stoliczkai* has the small spine length (3.3µm) while *C. indicum* can be characterized by long spine length (5.9µm) and *C. tibeticum* can be distinguished due to lowest number of spine rows between colpi (3-4). *Chrysanthemum stoliczkai* and *C. parthenium* are determined by long exine thickness (7.9µm) and (9.5µm) within the genus. This study demonstrates the potential of pollen studies in distinguishing some taxonomic groups in Anthemideae. There is great range of variation in exine thickness which has proved useful at specific level. On the basis of exine thickness, 4 groups viz., Group I: *C. tibeticum*, Group II: *C. indicum*, *C. segatum*, Group III: *C. leucanthemum*, *C. murifolium* and Group IV: *C. stoliczkai*, *C. parthenium* are recognized.

Introduction

The genus *Chrysanthemum* L., of the family Compositae, tribe Anthemideae is annual or perennial herbs. Its taxonomy has been revised by Ghafoor (2002) in the Flora of Pakistan. It is represented by 11 species in Pakistan (Stewart, 1972). The species of *Chrysanthemum* grow on a variety of soils, in semi-dry turf, meadows and dry wood land. Wodehouse (1935) reported that the genus *Chrysanthemum* comprising of about 100 species is widely distributed in the Northern Hemisphere. All are insect pollinated. Stewart (1972) pointed out that the pretty daisy (*Chrysanthemum leucanthemum* L.) is distributed wild in grassy places in Murree and other hill stations. Hussain (2003) further reported that *C. leucanthemum* is distributed in Bansragali, Nathiagali, Thandiani, Kalabagh and Dungagali. *Chrysanthemum segatum* is distributed from Attock, Hasanabdal (Punjab) to Kashmir upto 3000 meters. *Chrysanthemum leucanthemum* is considered as an escape from gardens in Hilly station like Murree, Nathiagali and Dungagali (Stewart, 1972). It is also distributed in Poonch Valley of Kashmir. *Chrysanthemum murifolium* is mainly distributed in Muzaffarabad. *Chrysanthemum indicum* is commonly cultivated in gardens with vernacular name Guli Daudi. *Chrysanthemum tibeticum* is distributed in Rawalakot, Poonch Valley, Gilgit, Bultistan, Hazara and Ladak. *Chrysanthemum parthenium* is distributed in Kurrum and Kashmir Valley. *Chrysanthemum stoliczkai* is distributed in Chitral Valley. Huang (1972) reported four species viz., *Chrysanthemum arisanense, C. indicum, C. leucanthemum* and *C. segatum* in Taiwan. The family Compositae, which has been much exploited for palynological studies, is a eurypalynous (Erdtman 1952) and most of its genera possess

three zonocolporate pollen (Sachdeva & Malik, 1986). The pollen grains of Compositae are helianthoid, spherical or slightly flattened, mainly tricolporate, echinate with variation in size and colpus number (Wodehouse 1930, 1935; Skvarla *et al*., 1977). Throughout the span of the history of studies in pollen morphology, the Compositae invariably attracted botanical researchers, of which the studies made by Wodehouse (1935), formed the basis of contemporary growth of knowledge. The contribution of Stix (1960), and Skvarla *et al*., (1977) added uniqueness of exine architecture in the family, providing useful information toward the taxonomy and phylogeny of the group. The family is one of the largest among the angiosperms with a world-wide distribution and has attracted and fascinated botanists for over two centuries (Heywood *et al*., 1978). It comprises of ± 1535 genera and C. 2300 species distributed in 3 subfamilies and 17 tribes. It is also the largest plant family in Pakistan, represented by over 650 species distributed in 15 tribes (Ghafoor, 2002). Various workers viz., Keeley & Jones (1977), Robbin *et al*., (1979), Cilliers (1991), Nakajima & Monteiro (1995), Kaya *et al*., (1996), Isawumi *et al*., (1996), Diez *et al*., (1999), Pinar & Dönmez (2000), Dawar *et al*., (2002), Meo & Khan (2003 ab), Meo & Khan (2004 ab) published pollen morphological studies of certain representatives of Compositae. Pollen size, spine length and number of spine rows between colpi can help in distinguishing genera. These characters are utilized in systematic studies in Asteraeae (Clark *et al*., 1980). Palynology can play an important role in the formation of natural groups and help in the assessment of taxonomical relationship between species. A little research work has been carried out on palynology in Pakistan. Khan & Memon (1970), Zahur *et al*., (1978), Meo *et al*., (1988ab, 1989), Meo (1999), Nasreen & Khan (1998), Perveen & Qaiser (2003), Dawar *et al.*, (2002) have critically reported pollen morphology of different families in Pakistan. However, pollen morphology of Chrysanthemum species has not yet been studied in Pakistan. Although Stewart (1972) reported 11 species of the genus in Pakistan yet *Chrysanthemum segatum*, *C. murifolium* and *C. stoliczkai* are new reports from Pakistan in the present investigation. Since there are no reports on the pollen morphology of these taxa from our area, the present report gives an account of the micromorphological characters in order to strengthen the recognition of 7 species of Anthemideae tribe from Pakistan.

Materials and Methods

Pollen samples were obtained from Quaid-i-Azam University Herbarium (ISL), Islamabad. Mature, unopened buds were removed from the herbarium specimen. The pollen grains were processed by the standard methods described by Erdtman (1966). The pollen grain were prepared for light microscopy by mounting in glycerine jelly and observations were made with a Nikon-Labophot microscope under oil immersion. For SEM, acetolyzed pollen grains were mounted on stubs with double adhesive tape. The prepared stubs were air dried and then coated with gold for 3 minutes using JEE-420, Jeol Vacuum Evaporator. The specimens were examined and photographed with a Jeol JSM-5910 scanning electron microscope using Monocrom films. The measurements are based on 20 readings from each specimens. Polar axis (P), equatorial diameter (E), P/E ratio, Exine thickness, shape in polar view, shape in equatorial view, aperture type, spine length, number of spine rows between colpi were measured. The data was statistically analyzed (Table 2). The terminology used is in accordance with Erdtman (1952), Kremp (1965), Faegri & Iverson (1964), Huang (1972), Punt *et al*., (1994), Mesfin *et al*., (1995). Table 1 represents species names and voucher specimens from which pollen was investigated.

Table 1. Specimens investigated.

S. No.	Taxon	Locality	District	Voucher No.	Collector
1.	*Chrysanthemum indicum* L.	Islamabad	Islamabad	225	M. Arif & Sarfraz
		Ashupa	Gilgit	65	A.B. Khan *et al*.,
2.	*Chrysantheum leucanthemum* L.	Tarakhal	Poonch	3471	Jan Mohammad
		Upper Topa	Rawalpindi	317	Iqbal Dar *et al*.,
		Murree	Rawalpindi	24	Iqbal Dar *et al*.,
		Kuldana	Rawalpindi	89	Shahzad *et al*.,
3.	**Chrysantheum murifolium* L.	Dilshan	Muzaffarabad	222	Jan Mohammad
		Hattian	Muzaffarabad	265	Jan Mohammad
4.	*Chrysantheum parthenium* (L.) Bernh	Gali Sheslamali	Muzaffarabad	3786	Jan Mohammad
		Oga manu	Muzaffarabad	3792	Jan Mohammad
5.	**Chrysantheum segatum* L.	Hasanabadal	Cambelpur	624	Arif & Hassan
		Terlai	Rawalpindi	639	Manzoor & Javaid
		Rawal Dam	Islamabad	839	Manzoor & Javaid
6.	**Chrysantheum stoliczkai* Clarke	Gorogol	Chitral	1126	Muqarrab Shah & Dilawar
		Shankoi	Chitral	1038	Muqarrab Shah & Dilawar
		Sagru	Chitral	1509	Muqarrab Shah & Dilawar
7.	*Chrysantheum tibeticum* H.&T. in Clarke	Rawalakot	Poonch	550	Bashir Ahmad & Javaid
		Dabar Shai	Chitral	2330	Muqarrab Shah & Dilawar
		Lower Deosai	Skardu	02	Muqarrab Shah & Dilawar

* = New report.

Table 2. Summary of pollen measurements, shape and sculpturing features in Chrysanthemum (Anthemideae-Compositae).

Ser.	Taxon	Equatorial diameter (µm)	Polar diameter (µm)	P/E ratio	Exine thickness (µm)	Spine length (µm)	Number of spine rows between colpi	Shape in polar view	Shape in equatorial view	Aperture type	Pollen class	Sculpturing
1.	*Chrysanthemum indicum* L.	*28.0±0.62 (24-30)	31.4±1.32 (30-34)	1.12	5.1±0.68 (3.5-7.5)	5.9±0.41 (5-7.5)	4-5	Circular to angular	Oblate-spheroidal	Non-lacunate	Trizono-colporate	Echinate
2.	*C. leucanthemum* L.	24.5±0.47 (22.5-32)	24.5±0.84 (22.5-27.5)	1.00	6.4±0.43 (6-7.5)	5.2±0.50 (4-6.5)	4-6	Angular	Prolate-spheroidal	Lacunate	Trizono-colporate	Echinate
3.	*C. murifolium* L.	23.5±0.89 (20-25)	32.0±0.82 (20-25)	1.36	5.5±0.57 (4-7)	4.8±0.67 (3-7)	4-5	Circular to angular	Subprolate	Lacunate	Trizono-colporate	Echinate
4.	*C. parthenium* (L.) Bernh	25.7±0.52 (24-27.5)	28.6±0.43 (27.5-30)	1.11	9.5±0.53 (7.5-11)	4.6±0.22 (4-5)	4-5	Semi-angular	Spheroidal, oblate-spheroidal, prolate-spheroidal	Lacunate to non-lacunate	Trizono-colporate	Echinate
5.	*C. segatum* L.	27.0±0.84 (25-30)	30.1±1.61 (25-30)	1.07	5.8±0.64 (4-7.5)	3.5±0.25 (2.5-4)	4-6	Semi-circular to angular	Subprolate	Lacunate	Trizono-colporate	Echinate
6.	*C. stoliczkai* Clarke	23.0±1.09 (20-25)	31.0±1.67 (25-35)	1.07	7.9±0.43 (6.5-9)	3.3±0.30 (2.5-4)	4-5	Circular to angular	Spheroidal	Non-lacunate	Trizono-colporate	Echinate
7.	*C. tibeticum* H. & T. In Clarke	24.9±0.57 (22.5-26)	25.7±0.44 ((25-27.5)	1.03	4.1±0.41 (2.5-5)	5.8±0.3 (5-6.5)	3-4	Circular to angular	Prolate-spheroidal	Non-lacunate	Trizono-colporate	Echinate

*Mean values followed by min-max in parentheses. P= Polar, E= Equatorial, ± = Standard error.

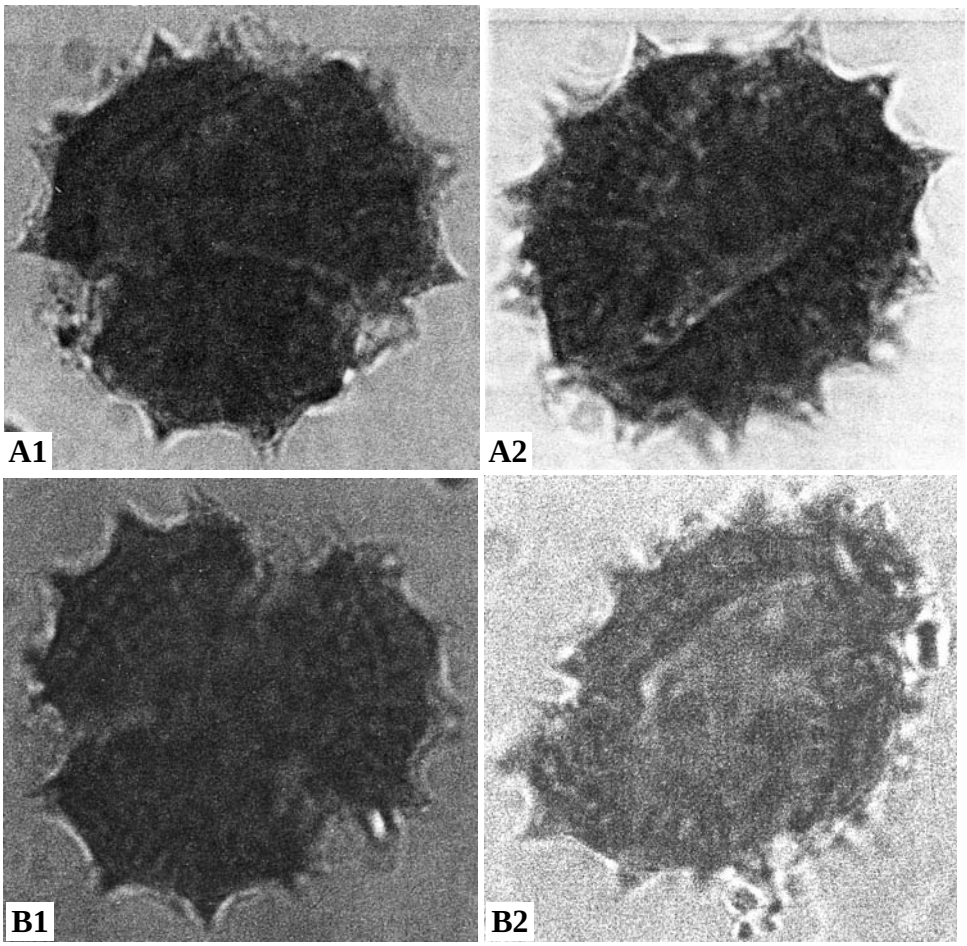

Fig. 1. Light micrographs of pollen grains of *Chrysanthemum* species (X1000).
A. *Chrysanthemum segatum* 1. Polar view. 2. Equatorial view
B. *Chrysanthemum leucanthemum* 1. Polar view. 2. Equatorial view

Results

Table 2 summarizes the measurements of pollen grains from the taxa examined. Light microscopic micrographs of *Chrysanthemum* species are presented in Fig. 1 and scanning electron micrographs are presented in Figs. 2-3.

Size: The size of the pollen grains (polar–equatorial diameter, excluding spines) of the species of Chrysanthemum ranges from 24.6-23μm to 32-23.5μm in *C. stoliczkai* and *C. murifolium*. There is little variation in the size of pollen grains. *C. stoliczkai*, *C. tibeticum* have pollen grains similar in size (Table 2).

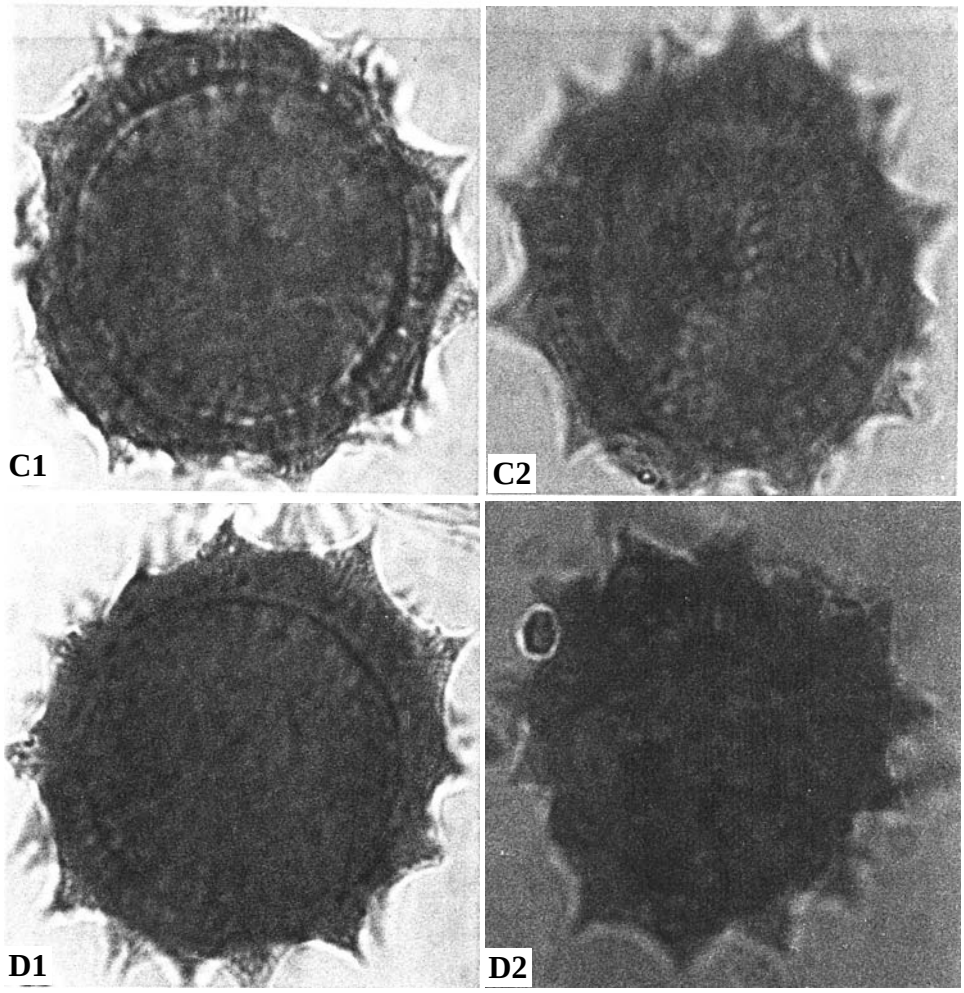

Fig. 1. Light micrographs of pollen grains of *Chrysanthemum* species (X1000).
C. *Chrysanthemum morifolium* 1. Polar view. 2. Equatorial view
D. *Chrysanthemum indicum* 1. Polar view. 2. Equatorial view

Symmetry and shape: The pollen grains are radially symmetrical, isopolar, spheroidal to oblate spheroidal. Pollen shape in equatorial view is spheroidal, sub-spheroidal, oblate-spheroidal and prolate-spheroidal while circular, inter-semi-angular to angular in polar view. The P/E (polar/equatorial diameter) ratio varied from 1.00 to 1.36. The ratio appear to be the lowest in *Chrysanthemum leucuanthemum* (1.00) while the highest in *C. murifolium* (1.36) which represents that pollen shape varied among the species (Table 2).

Aperture: The pollen grain is trizonocolporate with lalongate ora. The ora are elliptic or quite circular except *C. indicum* where aperture is non-lacunate. Apertural membrane is echinate. Colpi and pores are minute in *Chrysanthemum* species.

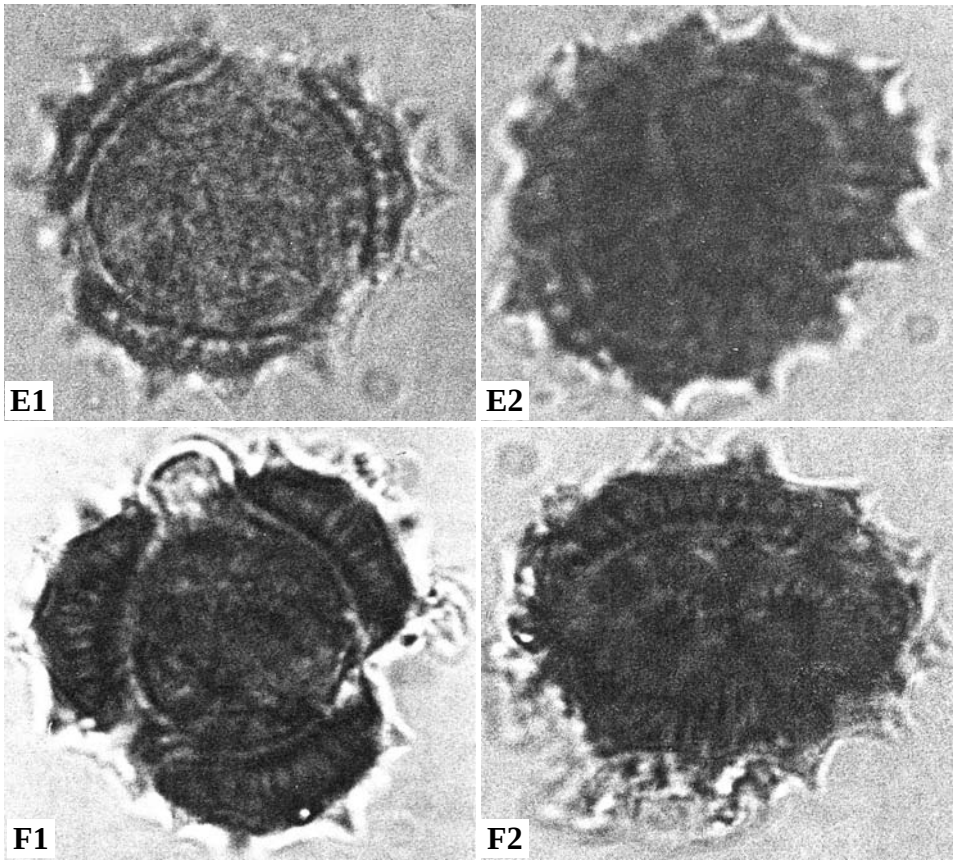

Fig. 1. Light micrographs of pollen grains of *Chrysanthemum* species (X1000).
E. *Chrysanthemum tribeticum* 1. Polar view. 2. Equatorial view
F. *Chrysanthemum stoliczkai* 1. Polar view. 2. Equatorial view

Spines: The spines are commonly conical with a broad base and a blunt apical portion. The spine length varies between 3.3μm to 5.9μm among the species. The number of spine rows between colpi varies from 3 to 6 among the species. This feature can be determined easily in polar view by light microscope. Spines are characteristic in *C. indicum*. They have a broad flattened base with grooves.

Exines: Exine thickness varies between 4.1–7.9μm among the taxon. Exine surface is granulated in *Chrysanthemum* species. Exine is much thicker than intine. Columella is somewhat aggregated and granulated in *Chrysanthemum* species.

Ornamentation: Scanning electron microscopic studies was carried out to observe the pattern of variation of exine sculpturing in the genus *Chrysanthemum*. Pollen grains are caveate, echinute and spine length in *Chrysanthemum indicum* is higher as compared to *C. parthenium* (Fig. 2-B). Light microscopic studies also confirm the larger length of pollen spine in *C. indicum* (5.9μm) and in *C. parthenium* (4.6μm) (Table 2). Ora circular in both the species, but much larger in *C. indicum* as compared to *C. parthenium*.

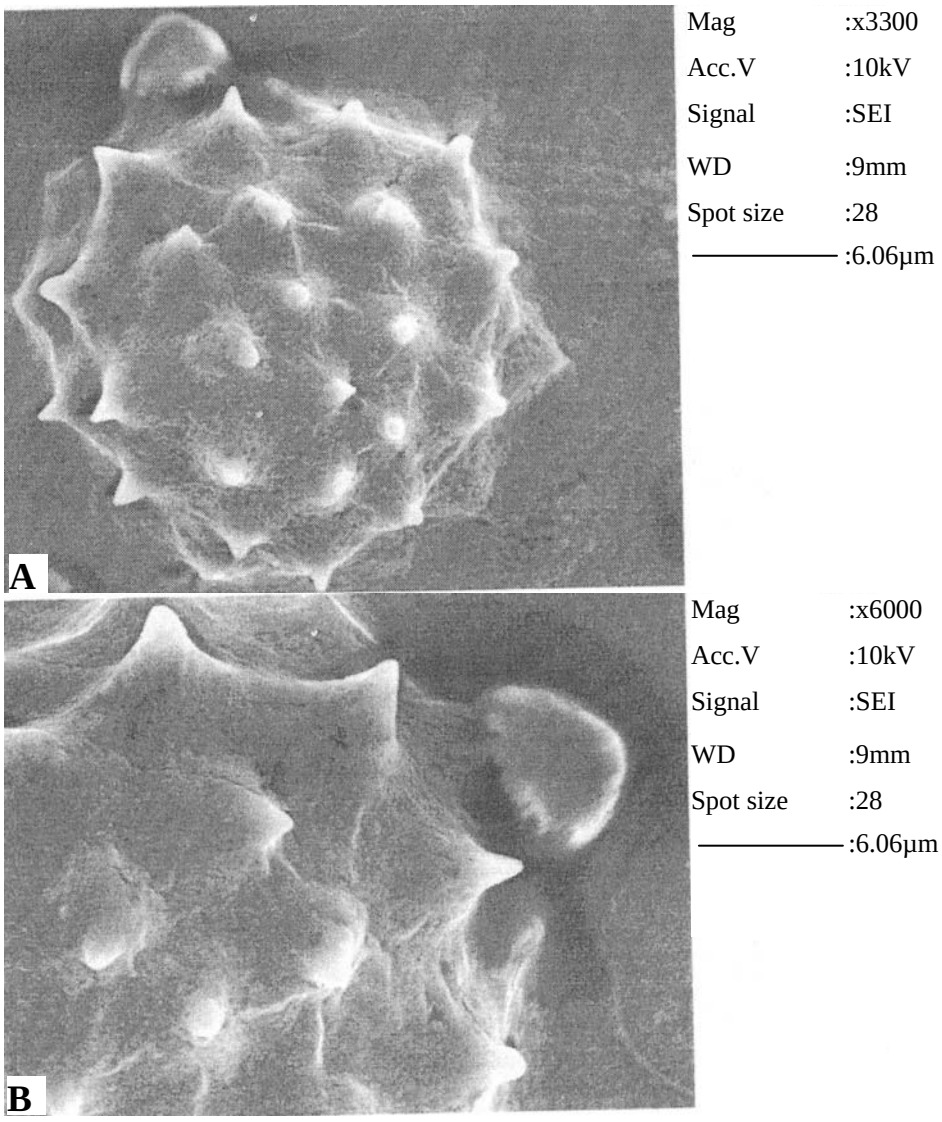

Fig. 2. Scanning electron micrographs of pollen grains of *Chrysanthemum indicum*. A. Equatorial view, B. Exine pattern.

Key to the species of *Chrysanthemum* is presented for identification.

Key to the species of *Chrysanthemum*

1. P/E ratio is 1.36 —— *C. murifolium*
1. P/E ratio is 1.0 —— 2
2. No. of spine rows between colpi 3 – 4 —— *C. tibeticum*
2. No. of spine rows between colpi 4 – 6 —— 3

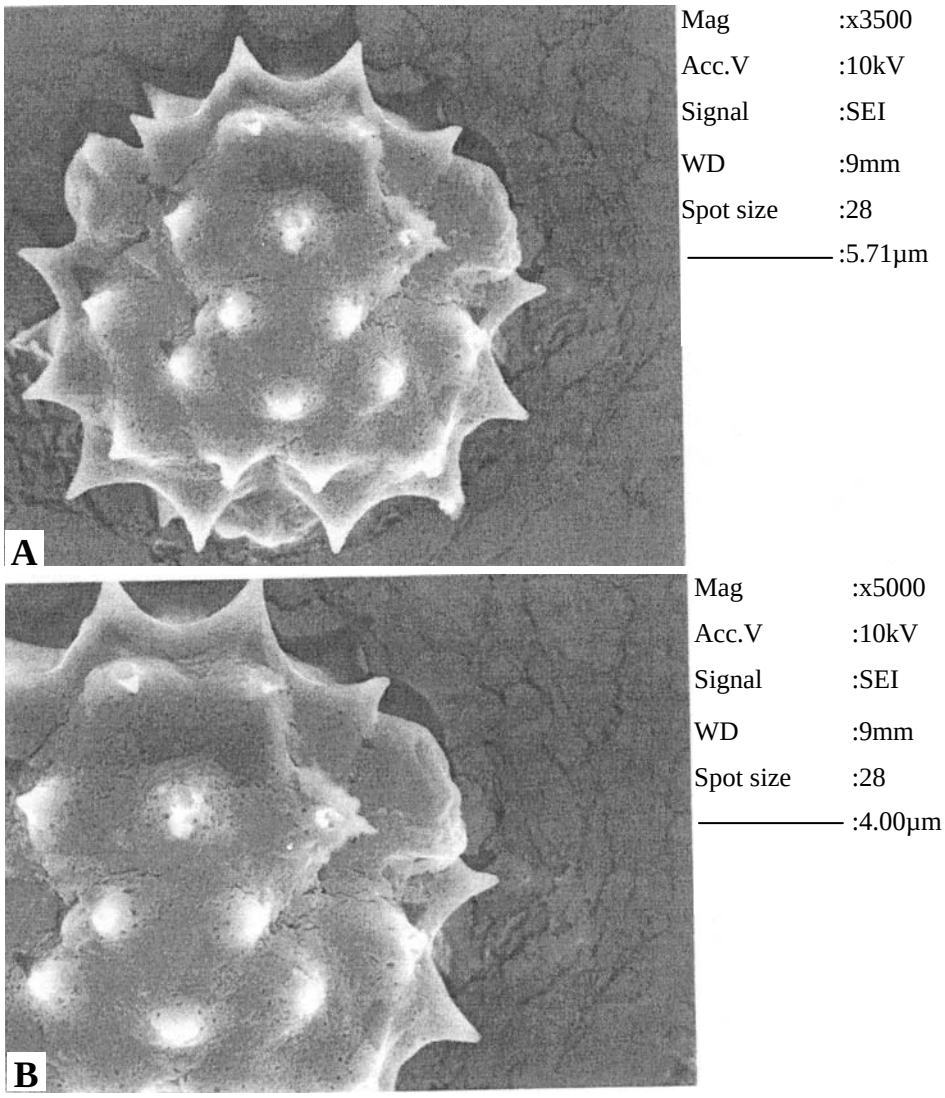

Fig. 3. Scanning electron micrographs of pollen grains of *Chrysanthemum parthenium*.
A. Equatorial view, B. Exine pattern.

3. Exine thickness 9.5µm in *C. parthenium*
3. Exine thickness 5.8 – 7.9µm —— 4
4. Spine length 5.9µm – *C. indicum*
4. Spine length 3.5 – 5.2µm —— 5
5. Sub-prolate – *C. segatum*
5. Prolate-spheroidal – *C. leucanthemum*

Discussion

The taxa examined in seven species of Chrysanthemum revealed that there is a great range of variation of pollen size of potential taxonomic value for identification and delimitation of species within the genus. The maximum pollen size 32.0 µm was in *Chrysanthemum murifolium* in polar view while the minimum size 24.5µm in *C. leucanthemum*. Similarly, the maximum pollen size 28.0µm was recorded in *C. indicum* in equatorial view and minimum size 23.0µm in *C. stoliczkai*. The rest of the species possessed intermediate sized pollen. Wodehouse (1935) reported pollen diameter range from 24.2 to 34.2µm in *Chrysanthemum* species which corroborate with the present findings. Wodehouse (1965) further described the size range 31-34µm in *C. murifolium*. He believed that *C. murifolium* is a hybrid with an admixture of *C. indicum*. Malik *et al*., (1964) recorded pollen size 32 x 28µm in *Chrysanthemum cinerariefolium* with similar findings as of present research. Huang (1972) noted the size ranged from 20–31 x 18-31µm to 29–31 x 30-36µm in *Chrysanthemum arisanensis, C. indicum, C. leucanthemum* and *C. segatum* which also correspond with our findings. According to Zahur *et al*., (1978) the size as 27(30) 33µm in *Chrysanthemum indicum* indicates the similar trend of pollen size in the genus with some variation within the species which might be due to variation of pollen size due to ploidy variation (Meo, *et al*., 1988b). Hussain (2003) observed pollen grain dimension as 23.99 (29.79) 41.32µm in *Chrysanthemum leucanthemum* which differ with our results in *C. leueanthemum* while in *C. indicum* (1.12) and *C. murifolium* (1.36) is observed to be the highest in the genus. Meo *et al*., (1988b) stated that pollen size increases corresponding with ploidy level. Hence the pollen size is the variable and useful feature in the *Chrysanthemum*. The exine thickness generally proved to be a very useful character in Compositae and similar trend has also been observed in this genus. Exine thickness emerged to be highest in *Chrysanthemum parthenium* (9.5µm) and lowest in *C. tibeticum* (4.1µm). Such a diversity is of taxonomic significance and varies at the specific level. Huang (1972) reported exine 2.0 – 2.5µm thick in four species of *Chrysanthemum* from Taiwan which does not correspond with our findings as exine thickness is much higher in the taxa of *Chrysanthemum* from our area. Aggregated and granulated columella is characteristic of all species of *Chrysanthemum*. Apart from exine, spine feature proved to be the most useful character of systematic value in present investigation. The number of spine rows between colpi varies from 3 to 6 in *Chrysanthemum* species. The number of spine rows between colpi and exine width is useful character to distinguish *Chrysanthemum* species. In *C. tibeticum* the number of spine rows between colpi varies from 3-4 with exine thickness 4.1µm while in *C. leucanthemum* the number of spine rows between colpi varies from 4-6 with exine thickness 6.4µm demonstrates correlation of characters and strengthen the taxonomic potential for segregations of species within the genus. Spine length also varies in *Chrysanthenium* species. *C. stoliczkai* spine length 3.3µm, *C. segatum* 3.5µm and in *C. leucanthemum, C. murifolium, C. indicum, C. parthenium* and *C. tibeticum* spine length is 4.6–5.9µm indicating the variation in spine length which can be used as an additional character for the identification and classification of the species of *Chrysanthemum*. Wodehouse (1935) reported 2.3 to 4.6µm long spines in *Chrysanthemum* species. Hall (1928), Clark *et al*., (1980) studied the Astereae and distinguished some genera on the basis of pollen characters. Huang (1972), Tomsovic (1997) utilized pollen characters as

an additional information for systematic purposes. Tomsovic (1997) noted that species of Echinops are similar in their main characters. Nevertheless one of them is an exception: *E. strigosus* L. distributed in the Mediterranean. The pollen grains are almost rounded, La. 40μm in diameter and the ectoexine is simple with unbranch baculae. On the basis of all available characters he considered this species as a separate genus *Psectra* containing one species only. Similarly, Pinar & Dönmez (2000) reported that spine cavities of pollen exine can be utilized as diagnostic characters in the genera of Compositae. Dawar *et al*., (2002) grouped 22 taxa in *Inula* on the basis of pollen characters. Meo & Khan (2004) recognized 3 groups in Scorzonera (Cichorieae–Compositae) on the basis of exine thickness. Variation in exine thick is also prominent in this genus. The character of exine thickness in this genus can be useful at specific level since almost all the species have different exine thickness (Table 1). *C. tibeticum* can be separately placed in the first group and *C. indicum, C. segatum* can be placed in the second group. *C. leucanthemum, C. murifolium* can be placed in the third group. However, *C. stoliczkai, C. parthenium* can be placed in the fourth group due to highest value of exine thickness. Exine sculpturing pattern through scanning electron microscopy showed a distinct difference of larger pollen spines in *Chrysanthemum indicum* as compared to *C. parthenium*. Such a different sculpturing pattern might proved to be of much taxonomic value in the rest of the species of the genus.

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