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 (3.3 μm) while C. indicum can be characterized by long spine length (5.9 μm) 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 (7.9 μm) and (9.5 μm) 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 Dunganagi. Chrysanthemum segatum is distributed from Attock, Hasanabadal (Punjab) to Kashmir upto 3000 meters. Chrysanthemum leucanthemum is considered as an escape from gardens in Hilly station like Murree, Nathiagali and Dunganagli (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), Cilliars (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 grains 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.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Taxon</th>
<th>Locality</th>
<th>District</th>
<th>Voucher No.</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Chrysanthemum indicum</em> L.</td>
<td>Islamabad</td>
<td>Islamabad</td>
<td>225</td>
<td>M. Arif &amp; Sarfraz</td>
</tr>
<tr>
<td></td>
<td>Ashupa</td>
<td>Gilgit</td>
<td></td>
<td>65</td>
<td>A.B. Khan et al.,</td>
</tr>
<tr>
<td>2.</td>
<td><em>Chrysanthemum leucanthemum</em> L.</td>
<td>Tarakhal</td>
<td>Poonch</td>
<td>3471</td>
<td>Jan Mohammad</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upper Topa</td>
<td>Rawalpindi</td>
<td>317</td>
<td>Iqbal Dar et al.,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Murree</td>
<td>Rawalpindi</td>
<td>24</td>
<td>Iqbal Dar et al.,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kuldana</td>
<td>Rawalpindi</td>
<td>89</td>
<td>Shahzad et al.,</td>
</tr>
<tr>
<td>3.</td>
<td><em>Chrysanthemum marifolium</em> L.</td>
<td>Dilshan</td>
<td>Muzaffarabad</td>
<td>222</td>
<td>Jan Mohammad</td>
</tr>
<tr>
<td></td>
<td>Hattian</td>
<td></td>
<td></td>
<td>265</td>
<td>Jan Mohammad</td>
</tr>
<tr>
<td>4.</td>
<td><em>Chrysanthemum parthenium</em> (L.) Bernh</td>
<td>Gali Sheslamali</td>
<td>Muzaffarabad</td>
<td>3786</td>
<td>Jan Mohammad</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oga manu</td>
<td>Muzaffarabad</td>
<td>3792</td>
<td>Jan Mohammad</td>
</tr>
<tr>
<td>5.</td>
<td><em>Chrysanthemum segatum</em> L.</td>
<td>Hasanabadal</td>
<td>Cambelpur</td>
<td>624</td>
<td>Arif &amp; Hassan</td>
</tr>
<tr>
<td></td>
<td>Terhai</td>
<td>Rawalpindi</td>
<td></td>
<td>639</td>
<td>Manzoor &amp; Javid</td>
</tr>
<tr>
<td></td>
<td>Rawal Dam</td>
<td>Islamabad</td>
<td></td>
<td>839</td>
<td>Manzoor &amp; Javid</td>
</tr>
<tr>
<td></td>
<td>Shankoi</td>
<td>Chitral</td>
<td></td>
<td>1038</td>
<td>Muqarrab Shah &amp; Dilawar</td>
</tr>
<tr>
<td></td>
<td>Sargu</td>
<td>Chitral</td>
<td></td>
<td>1509</td>
<td>Muqarrab Shah &amp; Dilawar</td>
</tr>
<tr>
<td></td>
<td>Dabar Shai</td>
<td>Chitral</td>
<td></td>
<td>2330</td>
<td>Muqarrab Shah &amp; Dilawar</td>
</tr>
<tr>
<td></td>
<td>Lower Deosai</td>
<td>Skardu</td>
<td></td>
<td>02</td>
<td>Muqarrab Shah &amp; Dilawar</td>
</tr>
</tbody>
</table>

* = New report.
Table 2. Summary of pollen measurements, shape and sculpturing features in Chrysanthemum (Asteraceae-Compositae).

<table>
<thead>
<tr>
<th>Ser.</th>
<th>Taxon</th>
<th>Equatorial diameter (μm)</th>
<th>Polar diameter (μm)</th>
<th>P/E ratio</th>
<th>Exine thickness (μm)</th>
<th>Spine length (μm)</th>
<th>Number of spine rows between colpi</th>
<th>Shape in polar view</th>
<th>Shape in equatorial view</th>
<th>Aperture type</th>
<th>Pollen class</th>
<th>Sculpturing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Chrysanthemum indicum</em> L.</td>
<td>28.0±1.62 (24-30)</td>
<td>31.4±1.32 (30-34)</td>
<td>1.12</td>
<td>5.1±0.68 (3.5-7.5)</td>
<td>5.9±0.41 (5-7.5)</td>
<td>4-5</td>
<td>Circular to angular</td>
<td>Oblate-spheroidal</td>
<td>Non-lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>2</td>
<td><em>C. leucanthemum</em> L.</td>
<td>24.5±0.47 (22.5-23.5)</td>
<td>24.5±0.84 (22.5-25.5)</td>
<td>1.00</td>
<td>6.4±0.43 (6-7.5)</td>
<td>5.2±0.59 (4-6.5)</td>
<td>4-6</td>
<td>Angular</td>
<td>Prolate-spheroidal</td>
<td>Lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>3</td>
<td><em>C. marifolium</em> L.</td>
<td>23.5±0.80 (20-25)</td>
<td>32.0±0.82 (20-25)</td>
<td>1.36</td>
<td>5.5±0.57 (4-7)</td>
<td>4.8±0.67 (3-7)</td>
<td>4-5</td>
<td>Circular to angular</td>
<td>Subprolate</td>
<td>Lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>4</td>
<td><em>C. parthenium</em> (L.) Berth</td>
<td>25.7±0.52 (24-27.5)</td>
<td>28.6±0.43 (27.5-30)</td>
<td>1.11</td>
<td>9.5±0.53 (7.5-11)</td>
<td>4.6±0.22 (4-5)</td>
<td>4-5</td>
<td>Semi-angular</td>
<td>Spheroidal, oblate-spheroidal</td>
<td>Lacunate to non-lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>5</td>
<td><em>C. segetum</em> L.</td>
<td>27.0±0.84 (25-30)</td>
<td>30.1±1.61 (25-30)</td>
<td>1.07</td>
<td>5.8±0.64 (4-7.5)</td>
<td>3.5±0.25 (2.5-4)</td>
<td>4-6</td>
<td>Semi-circular to angular</td>
<td>Subprolate</td>
<td>Lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>6</td>
<td><em>C. sintekchae</em> Clarke</td>
<td>23.0±1.09 (20-25)</td>
<td>21.0±1.67 (20-25)</td>
<td>1.07</td>
<td>7.9±0.43 (6-5.5)</td>
<td>3.2±0.30 (2.5-3)</td>
<td>4-5</td>
<td>Circular to angular</td>
<td>Spheroidal</td>
<td>Non-lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
<tr>
<td>7</td>
<td><em>C. titubeum</em> H. &amp; T. In Clarke</td>
<td>24.9±0.57 (22.5-26)</td>
<td>25.7±0.44 (25-27.5)</td>
<td>1.03</td>
<td>4.1±0.41 (2.5-5)</td>
<td>5.8±0.3 (5-6.5)</td>
<td>3-4</td>
<td>Circular to angular</td>
<td>Prolate-spheroidal</td>
<td>Non-lacunate</td>
<td>Trizonocolporate</td>
<td>Echinate</td>
</tr>
</tbody>
</table>

*Mean values followed by min-max in parentheses. P= Polar, E= Equatorial, ± = Standard error.*
POLLEN MORPHOLOGY FOR IDENTIFICATION OF *CHRYSANTHEMUM*

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).
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. morifolium* (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.

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**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
Fig. 1. Light micrographs of pollen grains of *Chrysanthemum* species (X1000).

E. *Chrysanthemum tribeticum* 1. Polar view. 2. Equatorial view
F. *Chrysanthemum stoliczkaei* 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
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*
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. stoliczka*. 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. stoliczka* 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.

**Acknowledgement**

We would like to express our sincere thanks to the Department of Biological Sciences, Quaid-i-Azam University, Islamabad, for the use of Herbarium material of the *Chrysanthemum* species. We are grateful to Honourable Prof. Dr. Atta-ur-Rehman Chairman HEC in providing financial grant for scanning electron micrographs. Sincerest thanks to Prof Dr.Muhammad Riaz Khan, Department of Physics, University of Peshawar, for the skillful preparation of the scanning electron micrographs. We thank Malik Duri-Iman for preparing the typescript.

**References**


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(Received for publication 8 March 2003)