PALYNOLOGICAL OBSERVATIONS ON THE GENUS **CALENDULA** (CALENDULEAE-COMPOSITAE) FROM PAKISTAN

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**Abstract**

The pollen morphology of *Calendula* species was investigated by light and scanning electron microscopy. Pollen grains are prolate-spheroidal in equatorial view and semi-angular in polar view, tri. to tetrazoncolporate, caveate and echinate. Size of polar axis and equatorial diameter, exine thickness, spine length are presented. Variation exists at species level i.e. exine thickness is higher in *C.arvensis* L. (6.3 μm) as compared to *C.officinalis* L. (3.3 μm). Existence of simple and closely placed pair of spines are highly diagnostic in *Calendula*. In *C. officinalis* spines are mostly paired and in *C. arvensis* mostly simple but closely placed pairs of spines also exist.

**Introduction**

Compositae (Astesaceaee) is one of the largest plant families consisting of herbaceous plants with only few exceptions. It is cosmopolitan in distribution, occurring in all continents except Antarctica (Hicking & King, 1997). The family is most abundant in mountain sub-tropical latitude. It is highly evolved family among angiosperms and is generally regarded as occupying the highest position in plant kingdom due to its great preponderance and cosmopolitan range (Chopra, 1970). The family comprises ± 1535 genera and over 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).


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There are only two species of Calendula viz., *C. officinalis* and *C. arvensis* in Pakistan (Stewart, 1972). *Calendula officinalis* is native of S.Europe and cultivated in every flowery garden. *C. arvensis* is distributed in South Europe, Caucasus, Iran, Afghanistan and Pakistan (Nasir & Rafiq, 1995). *Calendula arvensis* is abundant, spring, annual, weed and is doubtfully native. It is found as gregarious in open places, wastelands, road side, graveyard and field margins. *Calendula* spp., is a repeated medical plant in homeopathic system of medicine. Seeds, flowers and leafs of *Calendula arvensis* are medicinally important. Calendulin, salicylic acid, oleanic acid, faradial and arvidiol chemicals are obtained from *Calendula arvensis* and its flowers are locally used in making an ointment for skin (Khalid, 1995).

Palynologically, the tribe is rather stenopalynous. However, certain pollen morphological features, recorded in the genera and species of the tribe, are undoubtedly interesting for taxonomic purposes. The most important pollen morphological studies of the taxa included in the Calenduleae were previously made by Stix (1960), Dimon (1971), Norlindh (1978) and Skvarla et al., (1978). Pragowski & Grafstrom (1980) viewed that a brief palynological investigation of the tribe would be useful. The tribe Calenduleae consists of 113 species and large number of subspecies (Norlindh, 1978). The present report give an account of the palynological observations on the genus *Calendula*.

**Materials and Methods**

Pollens were removed from dry herbarium material from Quaid-i-Azam University Herbarium (ISL), Islamabad. A list of the specimens used in this study and the Herbarium data are presented in the Appendix 1. Pollen grains were prepared according to the acetolysis method as described by Erdtman (1966). Light microscopical work was carried out using a Nikon Labophot microscope (1000 X) under oil immersion. Florets were treated in acetic acid for 5 minutes. Pollen grains were mounted on slides in glycerine jelly. For scanning electron microscopy (SEM), pollen was air dried on specimen stubs and then coated with gold for 3 minutes for using JEE-420, Jeol Vacuum evaporator. The specimens were examined and photographed with a Jeol JSM- 5910 scanning electron microscope using Mono-Crom film.

Polar axis (P), Equatorial diameters (E), P/E ratio, Exine thickness, shape in polar view, shape in equatorial view, spine length, number of spine rows between colpi and aperture types were recorded. The data was statistically analyzed (Table 1). Terminology as used by Erdtman (1952), Huang (1972), Walker & Doyle (1975), and Punt. et al., (1994) has been followed. The permanent slides of *Calendula* species have been placed in the pollen reference collection of the Department of Biological Sciences, Quaid-i-Azam, University, Islamabad.

**Results**

The pollen morphology of genus *Calendula* is generally radially symmetrical, usually prolate-spheroidal, polar axis 38.9-39.7 μm, equatorial diameter 32.1-36.4 μm, trizonocolporate to tetrizonocolporate, caveate and echinate.

Table 1 summarizes the measurements of pollen grains from the taxa examined. Light microscopic micrographs of *Calendula* species are presented in Fig. 1 and scanning electron micrographs are presented in Figs. 2-3.
Appendix 1. Source of pollen material used in this study.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality</th>
<th>District</th>
<th>Collected by</th>
<th>Voucher No.</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calendula officinalis</em> L.</td>
<td>Kolpur</td>
<td>Quetta</td>
<td>Manzoor Hussain &amp; M. Arif.</td>
<td>352</td>
<td>11/4/1978</td>
</tr>
<tr>
<td></td>
<td>Attock</td>
<td>Attock</td>
<td>Muqarrab shah &amp; Ayaz Abassi</td>
<td>414</td>
<td>8/4/1977</td>
</tr>
<tr>
<td></td>
<td>Samangli</td>
<td>Quetta</td>
<td>Manzoor &amp; Arif</td>
<td>944</td>
<td>26/4/1978</td>
</tr>
<tr>
<td></td>
<td>Odegram</td>
<td>Swat</td>
<td>Muqarrab Shah &amp; Dilavar Khan</td>
<td>70</td>
<td>20/4/1976</td>
</tr>
<tr>
<td></td>
<td>Mathakis (Tribal area)</td>
<td>Dir</td>
<td>Ghulam Farooq</td>
<td>15</td>
<td>22/3/1976</td>
</tr>
</tbody>
</table>

Table 1. Summary of pollen measurements, shape and sculpturing features in *Calendula* (Calenduleae) species.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Equatorial view (μm)</th>
<th>Polar view (μm)</th>
<th>P/E ratio</th>
<th>Exine thickness (μm)</th>
<th>Number of spine rows between colpi</th>
<th>Spine length (μm)</th>
<th>Shape in equatorial view</th>
<th>Shape in polar view</th>
<th>Class</th>
<th>Aperture type</th>
<th>Sculpturing</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calendula officinalis</em> L.</td>
<td>36.4 ± 1.46 (31-40.5)</td>
<td>38.9 ± 1.19 (34-41.5)</td>
<td>1.07</td>
<td>3.3 ± 0.30 (2.5-4)</td>
<td>5-9</td>
<td>6.5 ± 0.42 (5-7.5)</td>
<td>Prolate-spheroidal</td>
<td>Semi-angular</td>
<td>Tri &amp; Tetrazono colporate</td>
<td>Non-lacunate</td>
<td>Echinate</td>
</tr>
<tr>
<td><em>Calendula arvensis</em> L.</td>
<td>32.1 ± 0.30 (31-33)</td>
<td>39.7 ± 1.12 (36-42.5)</td>
<td>1.24</td>
<td>6.3 ± 0.85 (4-8.5)</td>
<td>4-8</td>
<td>7.1 ± 0.48 (5-7.5)</td>
<td>Prolate-spheroidal</td>
<td>Semi-angular</td>
<td>Tri &amp; Tetrazono colporate</td>
<td>Lacunate</td>
<td>Echinate</td>
</tr>
</tbody>
</table>

*Mean values followed by min-max in parentheses. P= Polar, E = Equatorial, ± = Standard error*
Fig. 1. Light micrographs of pollen grains of *Calendula* species (X1000) *Calendula officinalis* L. A. Polar view; B. Equatorial view. *Calendula arvensis* L. C. Polar view; D. Equatorial view

**Size:** The size of the pollen grain (Polar axis-equatorial diameter excluding spines) of *Calendula* species ranges from 38.9/36.4μm to 39.7/32.1μm. There is a little variation in the size of the pollen grains. *Calendula officinalis* and *Calendula arvensis* have almost similar sized pollen and P/E ratio is 1.09 in *C. officinalis* and 1.24 in *C. arvensis*.

**Symmetry and shape:** The pollen grains are radially symmetrical, isopolar and isodiametric Pollen shape is prolate-spheroidal in equatorial view and semi-angular in polar view.

**Aperture:** The pollen grains are trizonocolporate and tetrazonocolporate in both of the species. The Aperture type is non-lacunate in *Calendula officinalis* and lacunate in *C. arvensis*. Apertural membrane is spinate. Colpi is long in *C. arvensis* and short in *C. officinalis*. 
PALYNOLOGICAL OBSERVATIONS ON *CALENDULA* FROM PAKISTAN

515

Fig. 2. Scanning electron micrographs of pollen grains *Calendula officinalis* L. A. Polar view, B. Exine pattern.

**Spine:** The spine length varies from 6.5μm in *C. officinalis* to 7.1 μm in *C. arvensis*. The number of spine rows between colpi varies from 5-9 in *C. officinalis* and 4-8 in *C. arvensis*. Existence of thin paired spines is taxonomically a highly distinguishing character in the genus *Calendula*. The pairs of closely placed thin spines appear as bifurcated in both the species.

**Exine:** Exine thickness varies from 3.3μm in *C. officinalis* to 6.3μm in *C. arvensis*. Surface view of pollen sculpturing is echinate in both the species.
Ornamentation: Pollen grains were examined through scanning electron microscope. Pollen grains in both the species of *Calendula arvensis* and *C. officinalis* are caveate, Columella weakly developed and the foot layer is vestigeal or lacking. Sculpturing is echinate with pointed end, sometimes curved. The feature of spines appear to be the most diagnostic to distinguish *Calendula arvensis* from *C. officinalis* (Figs. 2 & 3). The spines are short and thin in *Calendula officinalis* as compared to *C. arvensis*. 
Discussion

The family Asteraceae has not yet been published in Flora of Pakistan, neither flora of pollen is completed. Palynological studies not only provide additional information but also provide taxonomic characters, which proved to be helpful to improve the systematic position of taxa within their respective classification. The most significant pollen morphological studies of the taxa included in *Calendula* were previously made by Stix (1960), Dimon (1971), Praglowski (in Norlindh 1978), Skvarla *et al.*, (1977) and Praglowski & Grafstron (1980). Pollen morphology of *Calendula* is helpful at the specific level. Praglowski & Grafstron (1980) felt that a brief palynological investigation of tribe Calenduleae is helpful for taxonomic purposes. Existence of paired spines i.e., two closely placed spines and simple spines of pollen grain is diagnostic feature of the genus *Calendula* within the tribe Calenduleae has been discovered in the present investigation. However, the spines mostly appear paired in *Calendula officinalis* and mostly simple and some paired spines are found in *C. arvensis*. Such paired spines might be helpful in the delimitation of species and the classification of the tribe Calenduleae in its respective position in Asteraceae. Among the other characters exine thickness is a diagnostic character in *Calendula officinalis* (3.3μm) and *C. arvensis* (6.3μm) in the present study (Table 1). Praglowski & Grafstron (1980) viewed that structural exine characteristic in this tribe support a consanguinity with Heliantheae and Senecioneae.

Nair (1961) reported 3-zonocolporate pollen in *Calendula*. However in the present investigation both the species have 4-zonocolporate (tetrazonocolporate) pollen as mentioned by Malik, *et al.*, (1964) only in *Calendula arvensis*. The presence of trizonocolporate and tetrazonocolporate pollen in *C. officinalis* and *C. arvensis* is an evolutionary trend which would be helpful to establish a phylogenetic relationship of species within the genus *Calendula*. Praglowski & Grafstron (1980) studied pollen morphology of 8 genera of the tribe Calenduleae and all the taxa were trizonocolporate. The emergence of tetrazonocolporate condition which exists in *Calendula arvensis* and *Calendula officinalis* has been reported here for the first time. Praglowski & Grafstron (1980) consider the pollen of Calenduleae to show affinity with Astereae, Senecioneae and Heliantheae. They consider the consanguinity of Calenduleae with Senecioneae as expressed by Small (1919), Norlindh (1946) and Cronquist (1955b).

Praglowski & Grafstron (1980) concluded that Calenduleae should be considered as offshoot of the Senecioneae rather than a direct derivative of Heliantheae. *Calendula arvensis* can be distinguished due to higher spine length (7.1μm) from *Calendula officinalis* (6.5 μm). Number of spine rows between colpi is usually a useful taxonomic character in the family Compositae. In *C. arvensis* the number of spine rows between colpi is 4-8 and 5-9 in *C. officinalis*. Praglowski & Grafstron (1980) reported numerous slender spines (100-130) on the pollen of *Calendula*-type. Tomsovic (1997) studied the pollen characters of Echinops and investigated that in the genus Echinops, the species *E. strigosus* is very different and should be classified as a separate genus *Psectra* (Endllicher) including one species *Psectra strigosa* (L.). Tomsovic (1997), Hall (1928) and Clark *et al.*, (1980) studied the Asteraceae and distinguished some genera on the basis of pollen features. There is little variation in size of the pollen grains of *Calendula* species in the present studies as both species have almost similar sized pollen ranged from 38.9-36.4μm to 39.7-32.1μm. Praglowski & Grafstron (1980) pointed out with reference to taxonomy that pollen grains of *Calendula* (the type-genus for the tribe), possess some
morphological characteristics of this genus. These features include size of the pollen grains, which is the largest within the tribe (equatorial diameter up to 50 μm) with long solid spines and presence of comparatively thick exine. *Calendula* represents a characteristic pollen, morphologically well delimited and easily recognizable group, which can hardly be confused with any other representative of the tribe (Meo, 2005). Malik et al., (1964) studied pollen morphology of some Pakistani medicinal plants, which include *Calendula arvensis*. However, *C. officinalis* and *C. arvensis* can be distinguished on the basis of exine thickness, spine length, pair of closely placed spines, numbers of spine rows between colpi and the aperture type. Similarly, Pinar & Dönmez (2000) observed that spine cavities of pollen exine could be used as a diagnostic character in the genera of Compositae. On the basis of palynological studies it can be concluded that the pollen characters in the genus *Calendula* are helpful at the specific level. As revealed from scanning electron micrographs that spines appears to be the most diagnostic to distinguish *C. arvensis* from *C. officinalis*. The spines are short and thin in *C. officinalis* as compared with *C. arvensis* (Fig. 2). The light microscopic results also confirm the fact that spine length is higher in *C. arvensis* as compared to *C. officinalis* (Table 1). Key to the species of *Calendula* is presented for identification.

**Key to the species of *Calendula***

1. Exine thickness 3.3 μm, number of spine rows between colpi 5-9 ......... *C. officinalis*
1. Exine thickness 6.3 μm, number of spine rows between colpi 4-8 ........... *C. arvensis*

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**References**


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