# PALYNOLOGICAL STUDIES OF *ARTEMISIA* LINN. FROM MURREE AND HAZARA

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## Abstract

Palynological studies of twelve species of the genus *Artemisia* Linn. (family Asteraceae) were analyzed. Pollen grains in each species differ, especially of those, which resemble in their plant forms (gross morphology). Characters like pollen shape, polar diameter 'P', equatorial diameter 'E', 'P/E' ratio, exine thickness and pore diameter are found considerably important. *Artemisia japonica* and *A.desertorum* are distinguished due to pollen grain size and the absence of operculum on the pore of *A.desertorum*. *A.dubia* is distinguished from its closely resembling species due to its double columella layer in its exine. Therefore, palynological studies in this genus are potentially significant in species' delimitation.

## Introduction

Diversity in pollen morphology has made such studies a valuable taxonomic tool. Palynological research, while studying plant taxonomy, has proved useful in dealing critical and disputed taxonomic problems. The family *Asteraceae*, which has been much exploited for palynological studies, is a typical example of eurypalynous group and most of its genera possess zonocolpate pollen. (Suchdeva and Malik, 1986).

The present palynological study is a part of classical plant taxonomy, carried out to understand the taxonomic status of *Artemisia* spp. found in Murree-Hazara region of Pakistan. Since the work on palynology of *Artemisia* has not yet been done in Pakistan, it is worth knowing the potential of pollen features of this group. Normally, tricolpate pollens are found in the genus. The general expanded shape is spheroidal or oblately flattened. The size varies from 17.6 to 28.5 $\mu$m in diameter. The furrows are long and tapering while pollens are usually fertile. Broadly, the membranes are smooth and provided with germinal aperture. Exine is thicker and coarsely granular. The outer layer of exine is thick and slightly overlapping the furrow membranes along their margins. (Woodhouse, 1935).

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## Materials and Methods

The florets from mature capitula were taken from the duplicates of herbarium specimens of Quaid-i-Azam University, Islamabad. The fresh material collected from Murree, Nathia Gali and Kaghan valley was also used. The grains were acetolysed using the conventional procedure of Erdtman (1966) and slides were prepared by using 1% safranin mixed in glycerin jelly (usually called 'Gel Safranin'). Glycerin jelly was prepared by dissolving 70 g of Gelatin in 42 ml distilled water in a beaker. The beaker was placed in another metalled pot, containing boiling water. The gelatin was stirred for 1-2 hours, after which 35 ml of glycerin was added in it followed by an additional of 1 g Phenol crystals. This was then poured into 1% safranin solution in 1:1 ratio to make 'Gel Safranin'. The homogenized mixture was preserved in a vile and used for staining the pollen grains. Five slides of each species were prepared. The slides thus prepared were observed under light microscope. Characters like pollen class, pollen shape (in equatorial and polar view), polar and equatorial diameter, 'P/E' ratio, number of colpi, exine thickness, pore diameter, and wall sculpturing have been considered important in *Artemisia*.

## Results

The palynological data using light microscopic techniques are recorded for each species. Both quantitative and qualitative characters are tabulated in Table 1. Multiple observations helped us to calculate the range, average and standard error for polar diameter, equatorial diameter and P/E ratio for each species, thus giving an idea of their exact value (Table 2). Pollen morphology of each *Artemisia* sp. is given as follows:

***A.amygdalina* Decne.**

The exine layers are quite distinct, the edges do not taper sharply. The cytoplasm is found in the center away from the wall (Plate 1, Table 1&2).

***A.biennis* Willd.**

The exine layers are hardly distinct and appear as thin outer layer. The mesocolpium is neither much deep nor wide (Plate 1, Table 1 & 2).

***A.brevifolia* Wall. ex DC.**

The exine is quite distinct and tapering towards the edges (Plate 1, Table 1 & 2).

***A.desertorum* Spreng.**

It has the minimum equatorial diameter 'E' i.e. 13 $\mu$m. The exine layers are distinct, the edges do not taper sharply. The pores are operculate (Plate 1, Table 1 & 2).

***A.dubia* Wall. ex Bess.**

The colpi are much wider and the intine offers no gap to the cell cytoplasm (Plate 1, Table 1 & 2).

***A.gmelinii* Web. ex Stech.**

The colpi deepens sharply and running all along the pollen length. The cytoplasmic inclusions are much uniform (Plate 1, Table 1 & 2).

***A.japonica* Thunb. var. Parriflora (Roxb) Pamp.**

The exine is extraordinary thick and tapering towards the colpi but does not appear pointed. The colpi are much deeper (Plate 1, Table 1 & 2).

***A.laciniata* Willd.**

The exine is much thicker tapering much sharply towards the edges. The colpi do not sink deep into the cell inclusions rather an outward bulging of the cytoplasm is observed (Plate 1, Table 1 & 2).

***A.moorcroftiana* Wall. ex DC.**

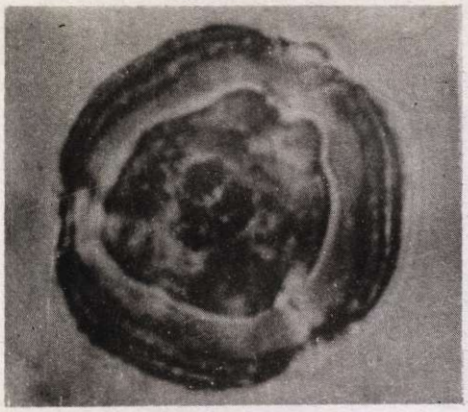
The mesocolpium is widely projected across the exine giving an appearance of double columella layer. The edges taper near the colpi (Plate 1, Table 1 & 2).

***A.roxburghiana* Wall. ex Besser.**

The exine layer is thin with a distinct columella layer. The edges taper towards deeply sunken colpi. The cytoplasmic inclusions are distinctly apart from the indistinct exine intine layer (Plate 1, Table 1 & 2).

***A.scoparia* Waldst. & Kit.**

The exine is even thinner to *A.roxburghiana*. The columella is not distinctly seen. The colpi are much wider and run along the pollen length (Plate 11, Table 1&2).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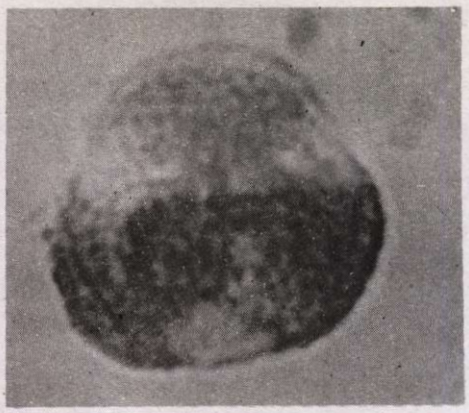
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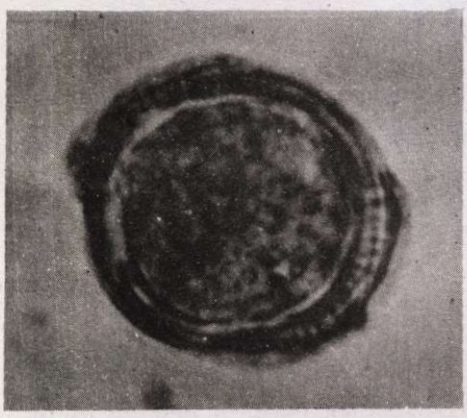
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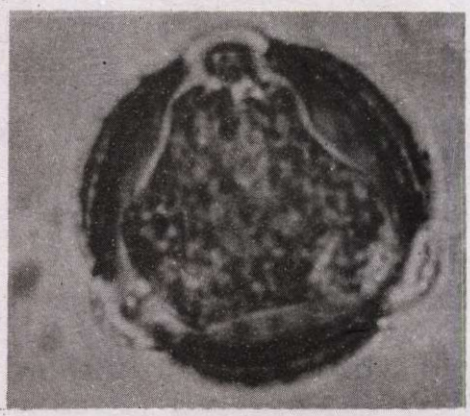
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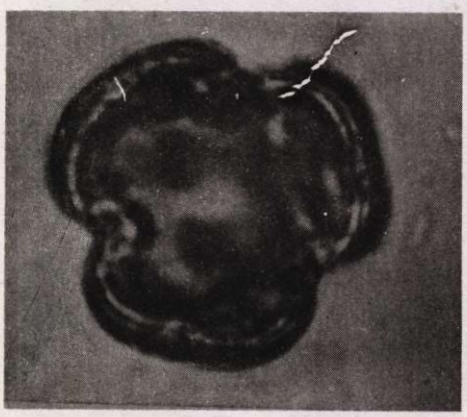
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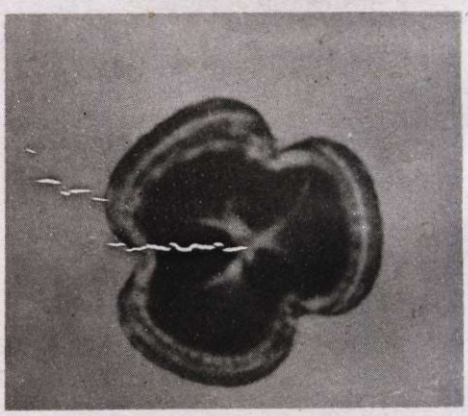
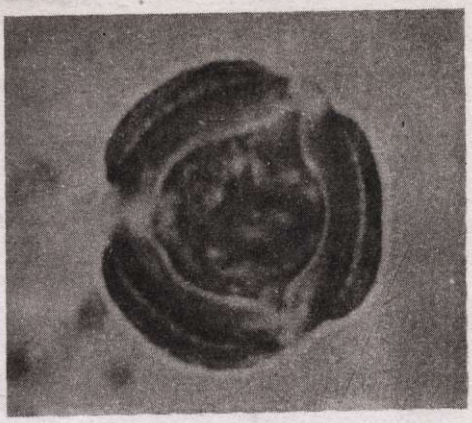

(f)

Plate 1. (a) *Artemisia amygdalina* (b) *A.biennis*
(c) *A.brevifolia* (d) *A.desertorum*
(e) *A.dubia* (f) *A.gmelinii*

(g)



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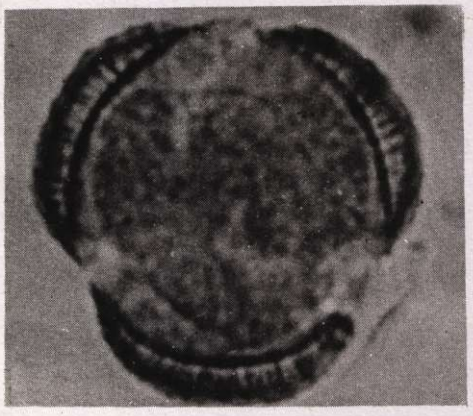
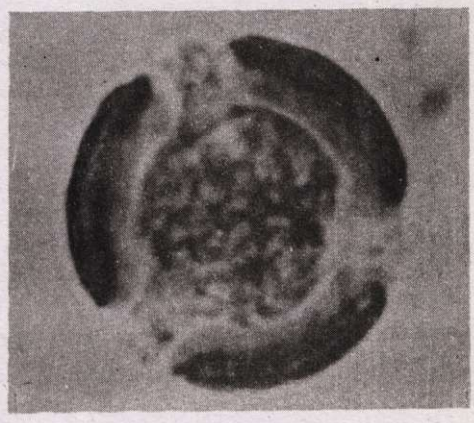


(i)



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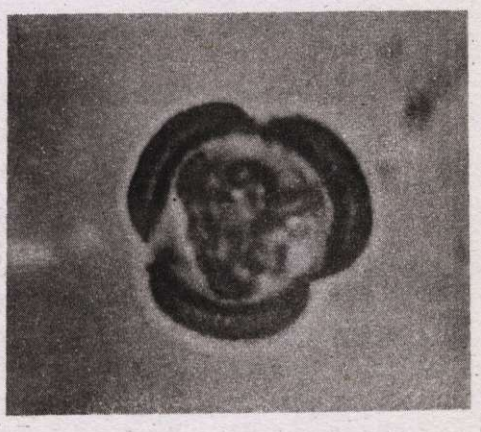

(l)

continued
Plate 1.

(g) *Ajaponica*
(h) *A.laciniata*
(i) *A.moorcroftiana*
(j) *A.roxburghiana*
(k) *A.scoparia*
(l) *A.sieversiana*

Table 1. Comparative study of qualitative and quantitative characters in each species of *Artemisia* Linn.

| | Class | Shape in polar view | Shape in equatorial view | 'P' | 'E' | 'P/E' | No. of colpi | Exine thickness | Pore diameter | Sculpturing |
|---|---|---|---|---|---|---|---|---|---|---|
| *Artemisia amygdalina* | Zonocolpate | Spherical | Broadly spherical | 24.13 | 20.13 | 1.2 | 3 | 5 | 2.5 | Smooth |
| *Artemisia biennis* | Zonocolpate | Spherical | Broadly oblong | 19.75 | 16 | 1.24 | 3 | >2.5 | Not clear | Smooth |
| *Artemisia brevifolia* | Zonocolpate | Spherical | Spherical | 20.5 | 18 | 1.14 | 3 | 5 | 5 | Smooth |
| *Artemisia desertorum* | Zonocolpate | Lanceolate or spherical | Oblong | 16.88 | 13 | 1.31 | 3 | 5 | Not clear | Coarse |
| *Artemisia dubia* | Zonocolpate | Wavy spherical | Spherical to oblong | 21.63 | 18.5 | 1.19 | 3 | 2.5-5 | 2.5 | Coarse |
| *Artemisia gmelinii* | Zonocolpate | Spherical | Oblong to lanceolate | 25.13 | 20.5 | 1.23 | 3 | 2.5 | 5-7.5 | Coarse |
| *Artemisia japonica* | Zonocolpate | Spherical | Lanceolate to spherical | 19.13 | 15.88 | 1.20 | 3 | 2.5 | Not clear | Coarse |
| *Artemisia laciniata* | Zonocolpate | Spherical | Broadly ovate | 19.13 | 16 | 1.20 | 3 | 5-7.5 | 2.5 | Smooth |
| *Artemisia moorcroftiana* | Zonocolpate | Spherical | Anguar as hexagonal | 21.75 | 19 | 1.15 | 3 | 2.5-5 | 5 | Coarse |
| *Artemisia roxburghiana* | Zonocolpate | Spherical | Spherical | 21.13 | 17.88 | 1.20 | 3 | 2.5 | 5-7.5 | Smooth |
| *Artemisia scoparia* | Zonocolpate | Spherical | Lanceolate | 19.13 | 16.5 | 1.17 | 3 | 5 | >2.5 | Coarse |
| *Artemisia sieversiana* | Zonocolpate | Spherical | Lanceolate to spherical | 16.38 | 14.38 | 1.14 | 3 | 5 | >2.5 | Coarse |

### *A.sieversiana* Ehrch & Willd.

The smallest sized pollen (16.38μm) with narrow colpi and pore. The exine layers are much broader with a clear columella layer and sharply tapering edges, and pollen shows the minimum value for P/E i.e. 1.14 (Plate 1, Table 1 & 2).

It is observed that pollen shape of *A.amygdalina, A.brevifolia* and *A.roxburghiana* is spherical both in polar and equatorial view, while the pollen of *A.desertorum* becomes oblong equatorially. Similarly polar diameters of *A.biennis, A.japonica, A.laciniata* and *A.scoparia* range from 19.0 μm to 19.99 μm, while those of *A.amygdalina, A.brevifolia, A.dubia, A.gmelinii, A.moorcroftiana* and *A.roxburghiana* exceed 20.0 μm (max. 24.13 μm). At the same time, a third category of *A.desertorum* and *A.sieversiana*, can also be identified where the polar diameter in pollen grains does not exceed 17.0 μm. Pollen grains of *A.brevifolia* mostly showed spherical shape as it carried the least 'P/E' ratio (av. 1.14).

Table 2. The comparative study of polar diameter, equatorial diameter (μm) and 'P/E' ratio of *Artemisia* Linn.

| Species | Polar diameter | | | Equatorial diameter | | | P/E ratio | | |
|---|---|---|---|---|---|---|---|---|---|
| | Av. | Se. | Range | Av. | Se. | Range | Av. | Se. | Range |
| *Artemisia amygdalina* | 24.13 | 0.58 | 20.0-27.5 | 20.13 | 0.38 | 17.5-22.5 | 1.20 | 0.03 | 1.0-1.57 |
| *Artemisia biennis* | 19.75 | 0.44 | 17.5-22.5 | 16.0 | 0.28 | 15.0-17.5 | 1.24 | 0.04 | 1.0-1.5 |
| *Artemisia brevifolia* | 20.5 | 0.43 | 17.5-22.5 | 18.0 | 0.23 | 17.5-20.0 | 1.14 | 0.02 | 1.0-1.29 |
| *Artemisia desertorum* | 16.88 | 0.36 | 15.0-20.0 | 13.0 | 0.34 | 10.0-15.0 | 1.31 | 0.03 | 1.16-1.5 |
| *Artemisia dubia* | 21.63 | 0.27 | 20.0-22.5 | 18.5 | 0.53 | 12.0-22.5 | 1.18 | 0.03 | 1.0-1.6 |
| *Artemisia gmelinii* | 25.13 | 0.78 | 20.0-35.0 | 20.5 | 0.78 | 17.5-27.5 | 1.23 | 0.03 | 1.0-1.43 |
| *Artemisia japonica* | 19.13 | 0.27 | 17.5-20.0 | 15.88 | 0.27 | 15.0-17.5 | 1.20 | 0.02 | 1.14-1.33 |
| *Artemisia laciniata* | 19.13 | 0.42 | 17.5-20.0 | 16.0 | 0.38 | 15.0-20.0 | 1.20 | 0.02 | 1.14-1.33 |
| *Artemisia moorcroftiana* | 21.75 | 0.26 | 17.5-22.5 | 19.0 | 0.28 | 17.5-20.0 | 1.16 | 0.02 | 1.0-1.29 |
| *Artemisia roxburghiana* | 21.13 | 0.34 | 20.0-22.5 | 17.88 | 0.45 | 15.0-20.0 | 1.19 | 0.29 | 1.0-1.5 |
| *Artemisia scoparia* | 19.25 | 0.41 | 17.5-22.5 | 16.5 | 0.45 | 15.0-20.0 | 1.14 | 0.02 | 1.0-1.33 |
| *Artemisia sieversiana* | 16.38 | 0.29 | 15.0-17.5 | 14.38 | 0.36 | 12.5-17.5 | 1.14 | 0.02 | 1.0-1.2 |

Av. = Average, Se = Standard Error.
All the values are measured in μm except 'P/E' ratio

Palynology is a generic character, but only few characters remained constant throughout the pollens of these species. Those are pollen class (zonocolpate), number of colpi (tricolpate), shape of pollen in polar view (± spherical). On the other hand, six out of nine characters proved diagnostic for *Artemisia*. Hence, it is worth knowing the pollen features of this group.

## Discussion

Perhaps the most noteworthy feature of the *Artemisia* pollen grains is the minuteness of their spines (Woodhouse, 1935). However, this character can be distinctly studied through Scanning Electron Microscopy (SEM). As the present account is based upon the light microscopic studies, therefore, the character is indistinctly seen as coarsely granular surface, which can hardly be of help in predicting any sort of sculpturing on the pollen wall.

The present studies reveal that the maximum pollen size is 25.13 μm for *A.gmeinii* grain in polar view, while the minimum size is 16.38 μm in *A.sieversiana* grains followed by 16.88 μm of *A.desertorum*. Pollen grains of closely allied species

*Artemisia japonica* and *A.desertorum* showed similarities except that the average size of the former was 16.1 μm and that of later was 19.13 μm (polar diameter). Similarly pollen morphology also provides certain differences e.g. *A.dubia* is distinguished from its closely related species *A.roxburghiana* and *A.moorcroftiana* by having a double columella layer in its exine layer. Not only this, *A.desertorum* which is morphologically very similar to *A.japonica*, differs in pollen morphology especially in polar diameter, equatorial diameter, colpi length and pore diameter, hence the two are different species.

It can be rightly concluded that pollen morphology is a significant character in species delimitation. It is considered supplementary to the general plant morphology and some times surprisingly, plays a critical role. This fact can be effectively elaborated through the following example. Tomšovic (1997), noted that the pollen grains of *Echinops strigosus* Linn. are almost rounded, c. 40 μm in diameter (relatively smaller in the genus) and the ectosexine is simple with unbranched baculae. The species also differ's in the form of the leaves which are deeply divided with strip like segments covered with dense spinules on the upper surface, such leaves are not found in the whole genus. On these bases, he considered this species as a different genus called *Psectra*, containing only one species *Psectra stigosa* (L.) Tomšovic Comb. Nova (syn: *Echinops strigosa* L.).

Ikram and Hussain (1978) reported *Artemisia maritima* Linn. from Neelam valley (Muzaffarabad), Gilgit (Northern Areas) and Kurram valley (NWFP). This species is also cultivated for the manufacture of the drug 'Santonin' by 'Kurram Chemical Company, Rawalpindi'. The present work does not find any authentic specimen from the said area that proves its presence, rather it is probable that *A.maritima* Linn is a misidentified species and is confused with a closely resembling species *A.brevifolia* which is frequently found in that area. The present account discusses pollen grains of *A.brevifolia* and if the pollen material of *A.maritima* becomes available, this taxonomic problem may be resolved effectively.

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