**LIGHT MICROSCOPIC STUDIES OF POLLEN GRAINS BY ACETOLYSIS METHOD**

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**Abstract:**

The present investigation deals with pollen morphological study of flowering plants from Ramshej Fort, Mhasrul, Nasik, Maharashtra during the period of January 2016 to December 2016. Fresh flowers from different plants collected early in the morning before anthesis. Collected pollen grains treated by acetolysis technique and observed under light microscope for pollen morphological studies. The purpose of this survey work was to collect the pollen materials from different plants growing in the field and study of different pollen parameters such as shape, size, colpi and exine ornamentation.

Pollen grains from plants such as *Parthenium hysterophorus*, *Alternanthera cicelis*, *Hibiscus panduriformis*, *Plumbago zeylanica*, *Malvastrum spp.*, *Lantana camera*, *Argyrea species*, *Cassia occidentalis*, *Brassica campestris*, *Laukea armenosa*, *Cordia spernum*, *Helichrysum*, *Merremia species*, *Vernonia cineraria*, *Tridax procumbens*, *Zenia pinnata*, *Smetha spp.*, *Ribes unistis*, *Desmodium scoparius*, *Macropeltium uniflorum*, *Euphorbia geniculata*, *Ageratum conyzoides*, *Peristrophe paniiculata*, *Borahavia diffusa*, *Ipomea marginata*, *Sondhus oleaceus*, *Oxalis conicularata*, *Syngredela nudifora*, *Euphorbia hirta*, *Cantarium meyeri* were collected.

Total 30 types of pollen grains from different plants collected. Out of these pollen types 7 belonging to Asteraceae, 3 from Malvaceae, 3 from Convolvulaceae, 3 from Fabaceae, 2 from Euphorbiaceae and one from Amaryllidae, Plumbaginaceae, Verbenaceae, Caesalpinaceae, Sapindaceae, Zygophyllaceae, Boraginaceae, Acanthaceae, Oxalidaceae and Gentianaceae family respectively. Among the collected taxa 9 shows trizonocolporate type, 4 shows polyzono heterocolporate type and one exhibits tetrazonocolporate type of pollen grains. Tetrazonocolporate type of pollen grain is found in *Argyrea* species.

On the basis of pollen parameters the present investigated taxa like *Parthenium hysterophorus*, *Plumbago*, *Lantana camera*, *Argyrea spp.*, *Cassia occidentalis* and *Brassica campestris* having tricolporate pollen grains may be considered in advanced status whereas *Acacia auriculiformis* has primitive status in scale of evolution. The present study is useful in the preparation of a complete pollen calendar in different seasons of the area under investigation. The colporate structures could be noticed very clearly in acetolysed pollen grains. The prepared pollen slides of the taxa investigated may be used as reference slides for identifying the pollen grains captured from air.

**INTRODUCTION**

Palynology, the science of pollen obtained real impetus after the discovery of the microscope. This is logical because the pollen grains are extremely tiny particles comparable to dust particles which cannot be seen by the naked eye. Pollen grain come in an infinite variety of shapes with complex surface ornamentation and occurs on almost every surface in nature. Discovery of microscope by Robert Hooke in 1665 was a landmark in the development of science particularly palynology. Subsequent improvement in microscopy accelerated the study of pollen grains especially finer structure of pollen wall and its varied ornamentation patterns.

The pollen grains are smallest unit of the plants, which contain so many characters of taxonomic & phylogenetic importance. The shape & size of the pollen grains, germinal furrows & the number of germ pores are important taxonomic features, which are taken into consideration in classification of plants.

Light microscopic studies give information on the extreme subsurface morphology & not the extreme surface of pollen grains. In recent years the scope of pollen morphology has widened with advent of scanning electron microscopy (SEM) & with regards to unipalynous taxa, particularly the understanding of finer morphology is of fundamental of exine surface & ornamentation pattern of the pollen grains.

Now a days study of pollen is an important area of research. Various pollen morphological features such as symmetry, shape, apertural pattern and exine configuration are very conservative features for the taxonomic assessment of the plant. Moreover some plants growing in the surroundings cause respiratory troubles or allergy in human beings, the pollen grains of which are responsible for allergy.

Pollens are ubiquitous in nature unlike other plant parts they are highly resist to decay they occur buried deep in rocks ground surface water and air indoor and outdoor including the upper atmosphere. Besides this pollen find their way through nasal and oral cavity to the digestive tract of humans and animal causing various degrees of discomfort. Pollen has the longest geological history as they are well preserved in rock as old as 400 million years. On account of these unique characters pollen and spores are often referred as nature’s fingerprint of plants (Bera et al., 2007: Singh, 2004).

Pollon biology encompasses pollen production, their transfer to the stigma or pollination and details of pollen-pistil interaction leading to fertilization and seed set. Any break in these sequential events affects seed and fruits.
MATERIAL AND METHODS

a) Collection of pollen grains and identification of plant species

Fresh flowers of different plant species collected early in the morning before anthesis from Ramshej Fort, Mhasrul, Nashik, Maharashtra during the period of January 2016 to December 2016. Plants such as Parthenium hysterophorous, Alternanthera cicis, Hibiscus panduriformis, Plumbago zelenica, Malvastrum spp., Lantana camera, Argyreaya species, Cassia occidentalis, Brassica campestris, Launaea samenosa, Cordospermum heicabum, Merremia species, Vernonia cineraria, Tridax procumbens, Zenia pinnata, Smilcea spp., Rubiulus tenestris, Desmodium scoparius, Macroptilium uniflorum, Euphorbia geniculata, Agremum conyzoide, Peristrope paniculata, Borahavia diffusa, Ipomea marginata, Sconchus oleraceus, Oxalis coniculata, Synedrela nudiflora, Euphorbia hirta, Cantarium meyeri were collected and pollen morphology was studied by acetolysis method (Erdtman, 1952).

b) Preparation of glycerin jelly

Pollen from the known plant is shaken on to a microscope slide or the anthers are placed on a slide and a drop of ether is added to disperse the pollen. Any visible particles that are larger than the pollen grains should be removed. Drops of ether are then carefully run over the pollen from a pipette. This will dissolve any oil in the pollen and carry it to one side where it can wipe off or where the solution can be absorbed by the tissue. Then two drops, one of warmed, stained jelly and another of unstained jelly, are placed on the pollen by means of a glass rod. A cover slip is carefully positioned on top, one edge lowered first to avoid trapping air bubbles. The slide is left on a warm plate for about ten minutes. The jelly should be just sufficient to fill the space under the cover slip.

Glycerin jelly is prepared by dissolving seven gm. of gelatin in 42ml of cold distilled water. 50ml of glycerin is added, warmed gently and stirred until it is dissolved; 0.5 gm. of phenol is then added to prevent the growth of mould. To prepare the stained glycerin jelly, 0.1 gm. of basic fuchsin is dissolved in 10ml of alcohol (methylated spirit). This stain is then added drop-by-drop to the glycerin jelly until a clear pink color is produced.

A few hrs. later, when the jelly has finally set, any surplus should be cleaned off with water. The cover slip is then sealed along the edges with clear nail varnish or paraffin wax. Thus treated, the slides will last for many years.

c) Acetolysis of pollen grains (Erdtman, 1952)

The fresh material consisting of full flowers or anthers were placed in test tube, crushed with glass rod in 70% alcohol and then filtered. The sediment left in the test tube after decantation of alcohol is covered with glacial acetic acid, centrifuged and the sediment covered with fresh acetolysis mixture prepared by mixing 9 parts of acetic anhydride and 1 part of concentrated sulphuric acid, the latter being put drop by drop. The tube with the mixture is placed in a hot water bath until the pollen grains become brownish black. The test tube was then cooled and centrifuged and this centrifuged acetolysis mixture was decanted and again centrifuged with glacial acetic acid and then decanted. This procedure was repeated 2-3 times with distilled water. After decanting water, 50% glycerin was added and centrifuged, small quantity of glycerin jelly was placed on warming the slide, gently pollen sample was added from test tube; it was then covered with cover slip, the slide thus was sealed with paraffin wax and then kept for microscopic observation.

The microphotographs of the pollen grains were taken by a microscope (Make Olympus and Model –CX 2 li and Number 12M268). The terminology of pollen is followed from Bhattacharya et al. (2009), Erdtman (1952), Faegri (1964), Knepp (1965) and Moore and Webb (1978).
RESULT AND DISCUSSION

The present investigation deals with pollen morphological studies of flowering plants growing at Ramshej fort, Nashik during the period of January 2016 to December 2016. Fresh flowers from different plants collected early in the morning before anthesis. Collected pollen grains treated by acetolysis technique and observed under light microscope for pollen morphological studies. The purpose of this survey work was to collect the pollen materials from different plants growing in the field and study of different pollen parameters such as shape, size, colpi and exine ornamentation. Total 30 types of pollen grains from different plants collected which are tabulated in Table – 1 and Photoplates I – V, Photo no. -30. Out of these pollen types 7 belonging to Asteraceae, 3 from Malvaceae, 3 from Convolvulaceae, 3 from Fabaceae, 2 from Euphorbiaceae and one from Amaranthaceae, Plumbaginaceae, Verbenaceae, Caesalpinaceae, Brassicaceae, Sapindaceae, Zygophyllaceae, Boraginaceae, Acanthaceae, Asteraeae, Oxalidaceae and Gentianaceae family respectively.

The present study is useful in the preparation of a complete pollen calendar in different seasons of the area under investigation. A pollen calendar is useful for allergy clinics (Tilak, 2012). Pollen calendar is compiled based on data and knowledge obtained from field botanical survey of the area under investigation combined with data from aeropalynological survey (Agashe, 2012).

Thus the taxonomic assessment of the species investigated is possible considering the pollen parameters. However, the data from other field of study like cytological, serological, biochemical and immunological might strengthen this taxonomic assessment of the species. On the basis of pollen parameters the present investigated taxa like *Parthenium hysterophorus, Plumbago, Lantana camara, Argyra spp., Cassia occidentalis and Brassica campestris* having tricolporate pollen grains may be considered as in advanced status whereas *Acacia auriculiformis* has primitive status in scale of evolution. The colporate structures could be noticed very clearly in acetolysed pollen grains. The prepared pollen slides of the taxa investigated may be used as reference slides for identifying the pollen grains captured from air. Aerobiologists reported that the pollen grains of *Alstonia scholaris, Catharanthus roseus, Acacia auriculiformis, Moringa oleifera, Carica papaya, Mangifera indica* and *Brassica campestris* are allergenic in nature (Chakroborty et al., 2005, Ghosh et al., 2007; Talukdar et al., 2012).

Plant pollen is one of the most common causes of seasonal allergic disease worldwide. Mango flower pollen has allergic effects on animal (Talukdar et al., 2012). The pollen grains showed the prominent spines throughout the surface of the exine and are subspheroidal in shape and tricolpate with three germinal apertures, which are common characteristic features of advanced dicot plants.
## Table – 1 – Light microscopic study of pollen of different plant

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Plant Name</th>
<th>Family</th>
<th>Description of pollen grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Parthenium hysterophorus</td>
<td>Asteraceae</td>
<td>Pollen grains are trizonocolporate, subspheroidal and exinespinulose.</td>
</tr>
<tr>
<td>2</td>
<td>Vernonia cineraria</td>
<td>Asteraceae</td>
<td>Pollen grain are oblate spheroidal to occasionally prolate spheroidal, trizonocolporate and echinocolpate with 15 lacunae perforate microreticulate.</td>
</tr>
<tr>
<td>3</td>
<td>Vernonia cineraria</td>
<td>Asteraceae</td>
<td>Pollen grains are isopolar large medium size prolate spheroidal sublet. Pollen grains are 3 colporate colpus long the endopertures are lalongate. The exine is thick sexine is sub chinolopate.</td>
</tr>
<tr>
<td>4</td>
<td>Tridax procumbens</td>
<td>Asteraceae</td>
<td>Porate spinulose spheroid shape radial symmetry.</td>
</tr>
<tr>
<td>5</td>
<td>Zinnia pinnata</td>
<td>Asteraceae</td>
<td>Pantoporate echinate radial symmetry.</td>
</tr>
<tr>
<td>6</td>
<td>Ageratum conyzoides</td>
<td>Asteraceae</td>
<td>Pollen grains are isopolar large medium size prolate spheroidal sublet. Pollen grains are 3 colporate colpus long the endopertures are lalongate. The exine is thick sexine is sub chinolopate.</td>
</tr>
<tr>
<td>7</td>
<td>Synedrella nudiflora</td>
<td>Asteraceae</td>
<td>Colpate striate surface bilateral symmetry.</td>
</tr>
<tr>
<td>8</td>
<td>Hibiscus panduriformis</td>
<td>Malvaceae</td>
<td>Pollen is very large, round multiporate and exine is echinate with long pointed spines.</td>
</tr>
<tr>
<td>9</td>
<td>Malvastrum coromandelianum</td>
<td>Malvaceae</td>
<td>Pollen is small tricolpate with echinate exine.</td>
</tr>
<tr>
<td>10</td>
<td>Sidamy sorensis</td>
<td>Malvaceae</td>
<td>Pollen is small yellow round tricolpate with reticulate exine.</td>
</tr>
<tr>
<td>11</td>
<td>Argyreia species</td>
<td>Convolvulaceae</td>
<td>Monocolpate echinate radial symmetry.</td>
</tr>
<tr>
<td>12</td>
<td>Merenia species</td>
<td>Convolvulaceae</td>
<td>Colpate echinate equatorial shape is prolate sub prolate polar shape circular grain colpi usually as long exinesexine finely reticulate nexine thinner than sexine thickness medium.</td>
</tr>
<tr>
<td>13</td>
<td>Ipomea marginata</td>
<td>Convolvulaceae</td>
<td>Monocolpate echinate radial symmetry.</td>
</tr>
<tr>
<td>14</td>
<td>Smithia</td>
<td>Fabaceae</td>
<td>Pollen is medium to large size spheroidal to oblate spheroidal in shape colpi zonal.</td>
</tr>
<tr>
<td>15</td>
<td>Desmodium corpiurus</td>
<td>Fabaceae</td>
<td>Pollen is small yellow round tricolpate with reticulate exine.</td>
</tr>
<tr>
<td>16</td>
<td>Macroptilium uniflorum</td>
<td>Fabaceae</td>
<td>Square in center polyps in the form pollinia grains group are 16.</td>
</tr>
<tr>
<td>17</td>
<td>Euphorbia geniculata</td>
<td>Euphorbiaceae</td>
<td>Tricolpate echinate equatorial shape is prolate sub prolate polar shape circular grain colpi usually as long exinesexine finely reticulate nexine thinner than sexine thickness medium.</td>
</tr>
<tr>
<td>18</td>
<td>Euophorbia hirta</td>
<td>Euphorbiaceae</td>
<td>Pollen grains are mostly prolate or elongated tectumspilate, granulate reticulate or micro echinate pollen grain are spheroidal prolate triangular subprolate.</td>
</tr>
<tr>
<td>19</td>
<td>Alternantheratenella</td>
<td>Amaranthaceae</td>
<td>Pollen grain are mostly prolate or elongated tectumspilate, granulate reticulate or micro echinate pollen grain are spheroidal prolate triangular subprolate.</td>
</tr>
<tr>
<td>20</td>
<td>Plumbagozeylanica</td>
<td>Plumbaginaceae</td>
<td>Pollen class tricolpate sub transverse to semi transverse exinesexine thicker than exine aperture small to long eliptic acute ends reticulate in shape bilateral symmetry.</td>
</tr>
<tr>
<td>21</td>
<td>Lantana camera</td>
<td>Verbenaceae</td>
<td>Tricolpate when triangular and some are square when 4 colporate.</td>
</tr>
<tr>
<td>22</td>
<td>Cassia occidentalis</td>
<td>Caesalpinaceae</td>
<td>Pollen are small round to triangular and tricolpate with smooth exine</td>
</tr>
<tr>
<td>23</td>
<td>Brassica napus</td>
<td>Brassicaceae</td>
<td>Pollen are small yellow, round and tricolpate with reticulate exine</td>
</tr>
<tr>
<td>24</td>
<td>Cardiospermum hallocacabum</td>
<td>Sapindaceae</td>
<td>Pollen is symmetrical isopolar colporate and porate pollen monoporate and monoporate.</td>
</tr>
<tr>
<td>25</td>
<td>Tribulusterrestris</td>
<td>Zygophyllaceae</td>
<td>Pollen grains are usually radially symmetrical or polar three polyporate taptorate.</td>
</tr>
<tr>
<td>26</td>
<td>Boerhaviarepensvaridiffusa</td>
<td>Nyctaginaceae</td>
<td>Spheroidal pantoporate and the sexine as tubuliferous and spinulose exine is thick reticulate.</td>
</tr>
<tr>
<td>27</td>
<td>Oxalis corniculata</td>
<td>Oxalidaceae</td>
<td>Pollen type tricolpate surface pattern reticulate shape prolate polar shape circular grain arrangement monad.</td>
</tr>
<tr>
<td>28</td>
<td>Centauriummeyeri</td>
<td>Gentianaceae</td>
<td>Pollen grain sculpturing is striate reticulate three colpate pollen grain striate reticulate exine sculpturing.</td>
</tr>
<tr>
<td>29</td>
<td>Peristrophe paniculata</td>
<td>Acanthaceae</td>
<td>Monoporate oblate radial symmetry.</td>
</tr>
<tr>
<td>30</td>
<td>SonchusOleraceus</td>
<td>Asteraceae</td>
<td>Shape in polarview is spheroidal aperture type lacunae pollen class trizonocolpate sculpturing is echinate.</td>
</tr>
</tbody>
</table>
Plate No. -1. – Pollen morphology of some Asteraceae family members

1) Parthenium hysterophorous

2) Launea sarmonosa

3) Vernonia cineraria

4) Tridax procumbens

5) Znna pinnata

6) Ageratum conyzoides
Plate No. - 2. – Pollen morphology of Asteraceae, Malvaceae & Convolvulaceae family members

7) *Synedrella nudiflora*

8) *Hibiscus panduriformis*

9) *Malvastrum*

10) *Sida mysorehensis*

11) *Argyreaya species*

12) *Merremia species*
Plate No. - 3. – Pollen morphology of Convolvulaceae, Fabaceae & Euphorbiaceae family members

13) Ipomoea marginata

14) Smithea

15) Desmodium scorpiurus

16) Macroptilium uniflorum

17) Euphorbia geniculate

18) Euphorbia hirta
Plate No. - 4. – Pollen morphology of different family members

19) Alteranthera cicilis
20) Plumbago zelanica

21) Lantana camera
22) Cassia occidentalis

23) Brassica Camprstris
24) Cordospermum heliicabum
plate No. - 5. – Pollen morphology of different family members

25) *Tibulus tenestris*

26) *Borhavia difusa*

27) *Oxalis conculata*

28) *Centaurium meyeri*

29) *Peristrophe puniculata*

30) *Sonchus Oleraceus*
REFERENCES


