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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 grain from plants such as *Parthenium hysterophorous ,Alternanthera cicilis Hibiscus panduriformis ,Plumbago zelanica , Malvastrum spp. ,Lantana camera ,Argyreaya species ,Cassia occidentalis, Brassica campestris ,Laiunea sarmenosa Cordiospermum heicabum ,Merremia species ,Vernonia cineraria ,Tridax procumbens ,Zenia pinnata ,Smithea spp. ,Ribulus tenestris, Desmodium scopiurus ,Macroptilium uniflorum ,Euphorbia geniculata, Ageratum conyzoides ,Peristrophe paniculata ,Borrahavia diffusa ,Ipomea marginata , Sonchus oleraceus ,Oxalis coniculata , Synedrela nudiflora ,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 Amaranthaceae, Plumbaginaceae, Verbenaceae, Caesalpinaceae, Brassicaceae, Sapinadaceae, Zygophylacaeae, Boraginaceae, Acanthaceae, Steraceae, Oxalidaceae and Gentiaceae 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 *Argyreya* 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 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.

Pollen 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).

Pollen 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

set. Pollen biological studies are a prerequisite for any program aimed at optimization and improvement of the yield of crop plant. Pollination ecology is also a part of pollen biology which involves the study of various aspects dealing with efficient pollination (Chauhan, 2006). Pollen biotechnology is one of the techniques employed to study pollen biology for crop production and improvement. Pollen biotechnology is one of the most challenging areas of plants reproductive biology and plays an important role in crop improvement programs (Perveen, 2006).

No survey is carried out with respect to pollen morphological studies from Ramshej Fort, Mhasrul, Nasik, Maharashtra. Therefore this work will be useful for preparation of pollen calendar.By considering immense importance of pollen morphological studies in relation to allergy, present work is undertaken for light microscopic studies of different pollen grains

MATERIAL AND METHODS

a) Collection of pollen grains and identification of plants 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 cicilis Hibiscus panduriformis ,Plumbago zelanica ,Malvastrum spp. ,Lantana camera ,Argyreaya species ,Cassia occidentalis, Brassica campestris ,Laiunea sarmenosa Cordiospermum heicabum ,Merremia species ,Vernonia cineraria ,Tridax procumbens ,Zenia pinnata ,Smithea spp. ,Ribulus tenestris, Desmodium scopiurus ,Macroptilium uniflorum ,Euphorbia geniculata, Ageratum conyzoides ,Peristrophe paniculata ,Borrahavia diffusa ,Ipomea marginata , Sonchus 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 fuchsine 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 later 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), Kremp (1965) and Moore and Webb (1978).

RESULT AND DISCUSSION

The present investigation deals with pollen morphological studies of flowering plants growing atRamshej fort,Mhasrul, 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, Caesalpinacaea, Brassicaceae, Sapinadaceae, Zygophylacaeae, Boraginaceae, Acanthaceae, Asteraceae, Oxalidaceae and Gentiaceae 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). Almost all the plants such as *Argyeaya* species, *Cassia occidentals, Brassica campestris* shows 3-colporate type of pollen grains (Keshavarzi *et. al.,* 2012). Earlier Agashe (1975) reported the pollen morphology of *Parthenium hysterophorus* by light microscopic studies

In the previous study (Pal, 1992; Pal *et al.*, 1993a, 1993b) it has been shown that the taxa having tricolporate pollen grains reveals their advanced status whereas taxa with polyad or colpate type of pollen grains shows its primitive status in the evolutionary scale. Thus the taxonomic assessment of the Brassicaceae is valuable diagnostic features in species delimitation.*Brassica campestris* having tricolporate pollen grains may be considered as in advanced status whereas *Cassia occidentalis* has primitive status in scale of evolution. The *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. *Parthenium hysterophorus* flower pollen has allergic effects on animal.

Among the collected taxa 9 shows trizonocolporate type, 4 shows polyzonoheterocolporate type and one exhibits tetrazonocolporate type of pollen grains. Tetrazonocolporate type of pollen grain is found in *Argyreya* species.All the taxainvestigated presently are characterized by more or less radially symmetrical, oblate to prolate type of pollen grains. Pollen morphologically varies from lalongate-circular to lolongate type. The colpal margin of *Cassia occidentals,Hibiscus panduriform* is associated with exinal thickening. Structurally the exine of 18 species is crassinexinous with clearly discernible tegillate organization and the exine surface of the grains varies from faintly reticulate to reticulate type.

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).

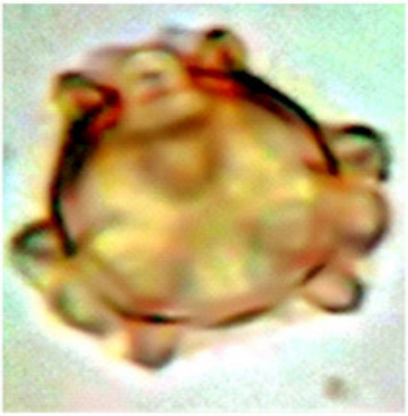
Plant pollen is one of the most common causes of seasonal allergic disease worldwide. Mango flower pollen has allergic effects on animal (Talukdar *et al.*, 2012). The pollen grains showed the prominent spines throughout the surface of the exine and are subspheroidal in shape and tricolpate with three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

Table – 1 – Light microscopic study of pollen of different plant

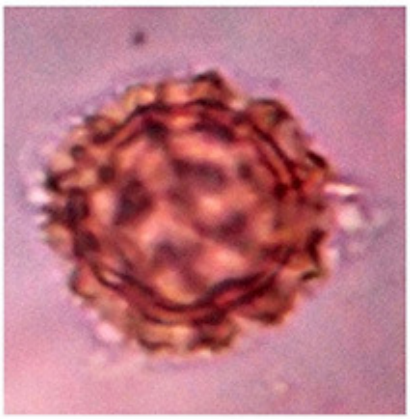
Sr. No	Plant Name	Family	Description of pollen grains
1	Partheniumhysterophorus	Asteraceae	Pollen grains are trizonocolporate, subspheroidal and exinespinulose.
2	Vernonia cineraria	Asteraceae	Pollen grain are oblate sheroidal to occasionally prolatesheroidal ,trizonocolporate and echinocolpate with 15 lacunae perforate microreticulate
3	Vernonia cineraria	Asteraceae	Pollen grains are isopolar large medium size prolatesperoidal sublet. Pollen grains are 3 colporatecolpus long the endopertures are lalongate. The exine is thick sexine is sub chinolopate.
4	Tridaxprocumbens	Asteraceae	Poratespinulous spheroid shape radial symmetry
5	Zinnia pinnata	Asteraceae	Pantaporatespinolousexine radial symmetry
6	Ageratum conyzoides	Asteraceae	Prolatespinolour radial symmetry.
7	Synedrellanudiflora	Asteraceae	Colpate striate surface bilateral symmetry.
8	Hibiscus panduriformis	Malvaceae	Pollen is very large, round multiporate and exine is echinate with long pointed spines.
9	Malvastrumcoromandelianum	Malvaceae	Pollen are small tricolpate with echinateexine
10	Sidamysorensis	Malvaceae	Pantoporateechinate radial symmetry.
11	Argyreia species	Convolvulaceae	Pollen is small yellow round tricolpate with reticulate exine.
12	Merremia species	Convolvulaceae	Micro echinateperporatetecatemicrospines.
13	Ipomoea marginata	Convolvulaceae	Pantaporateporesechinate radial symmetry.
14	Smithia	Fabaceae	Monocolpateexine obscure sub oblate bilateral symmetry
15	Desmodiumscorpiurus	Fabaceae	Pollen is medium to large size spheroidal to oblate spheroidal in shape colpi zonal.
16	Macroptiliumuniflorum	Fabaceae	Square in center polyads in the form pollinia grains group are 16.
17	Euphorbia geniculata	Euphorbiaceae	Tricolpatecolpi clearly distinct exine rather thick sexine, shape prolatesperpidal grain medium size
18	Euophorbiahirta	Euphorbiaceae	Tricolpate reticulate equatorial shape is proate sub prolate polar shape circular grain colpi usually as long exinesexine finely reticulate nexine thinner than sexine thickness medium.
19	Alterantheratenella	Amaranthaceae	Pollen grain are mostly prolate or elongated tectumspilate, granulate reticulate or micro echinate pollen grain are spheroidal prolate triangular subprolate
20	Plumbagozeylanica	Plumbaginaceae	Pollen class tricolpate sub transverse to semi transverse exinesexine thicker than exineaperature small to long eliptic acute ends reticulate in shape bilateral symmetry.
21	Lantana camera	Verbenaceae	Tricolpate when triangular and some are square when 4 colporate.
22	Cassia occidentalis	Caesalpinaceae	Pollen are small round to triangular and tricolpate with smooth exine
23	Brassica napus	Brassicaceae	Pollen are small yellow, round and tricolpate with reticulate exine
24	Cardiospermumhalicacabum	Sapindaceae	Pollen is symmetrical isopolarcolporatecolpate and porate pollen moncolpate and monoporate.
25	Tribulusterrestris	Zygophyllaceae	Pollen grains are usually radially symmetrical or polar three polyporatepantaporate.
26	Boerhaviarepensvardiffusa	Nyctaginaceae	Spheroidal pantoporate and the sexine as tubuliferous and spinulosesexine is thick reticulate.
27	Oxalis corniculata	Oxalidaceae	Pollen type tricolpate surface pattern reticulate shape prolate polar shape circular grain arrangement monad.
28	Centauriummeyeri	Gentianaceae	Pollen grain sculpturing is striate reticulate three colpate pollen grain striate reticulate exine sculpturing.
29	Peristrophepuniculata	Acanthaceae	Monoporate oblate radial symmetry.
30	SonchusOleraceus	Asteraceae	Shape in polarview is spheroidal aperture type lacunate pollen class trizonocolpate sculpturing is echinate.

Plate No. -1. – Pollen morphology of some Asteraceae family members

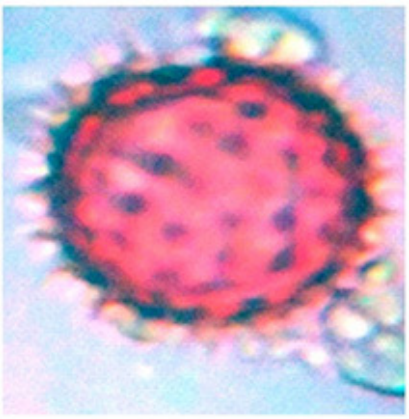
1) *Parthenium hysterophorous*



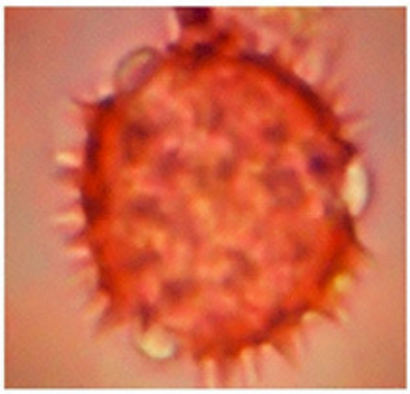
2) *Launea sarmonosa*



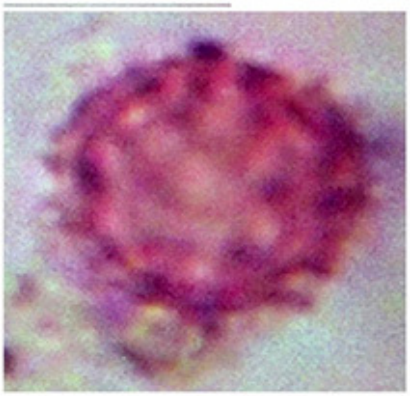
3) *Vernonia cineraria*



4) *Tridax procumbens*



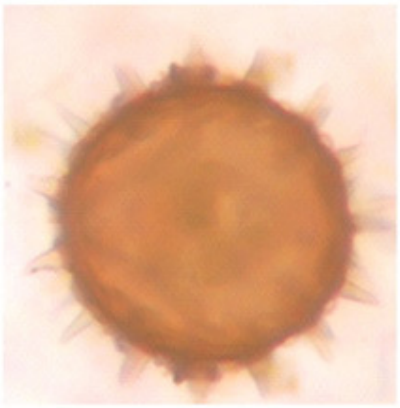
5) *Zenia pinnata*



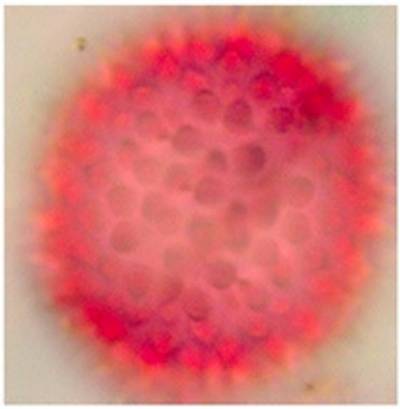
6) *Ageratum conyzoides*

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

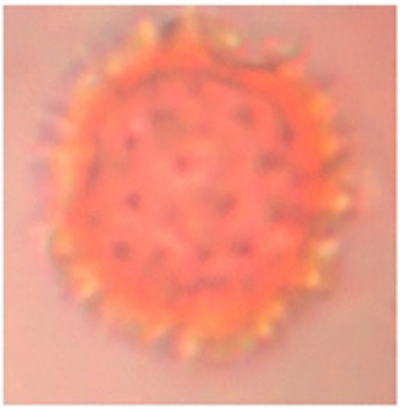
7)Synedrella nudiflora



8) Hibiscus panduriformis



9) Malvastrum

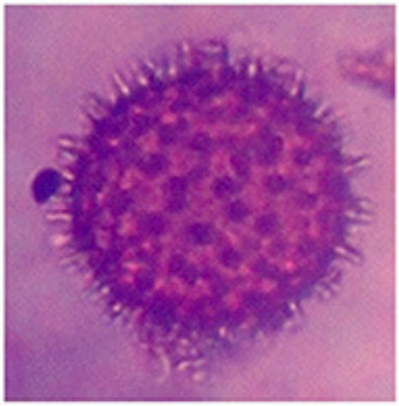


10) Sida mysorehsis

11) Argyreaya species

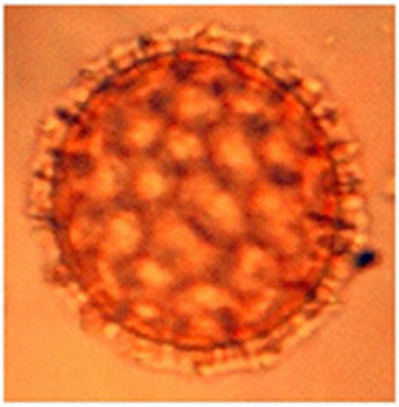
12) Merremia species

Plate No. - 3. – Pollen morphology of Convolvulaceae, Fabaceae & Euphorbiaceae family members



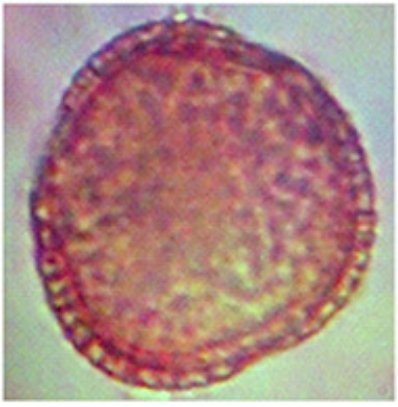
13) *Ipomoea marginata*

14) *Smithea*



15) *Desmodium scorpiurus*

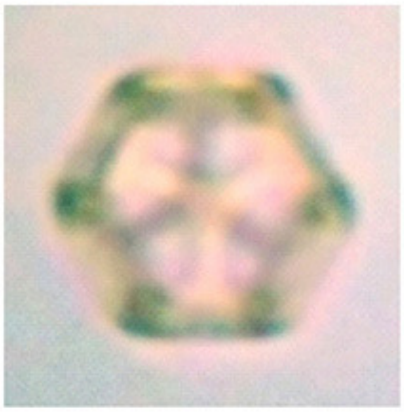
16) *Macroptilium uniflorum*



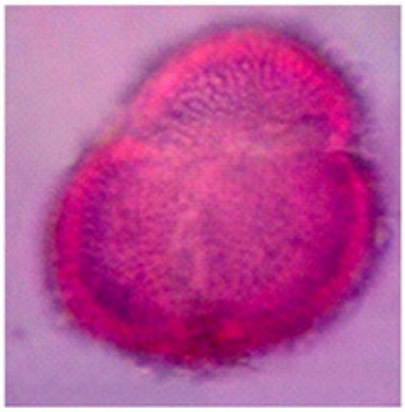
17) *Euphorbia geniculate*

18) *Euophorbia hirta*

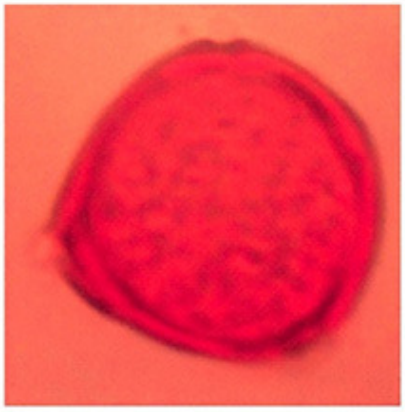
Plate No. - 4. – Pollen morphology of different family members



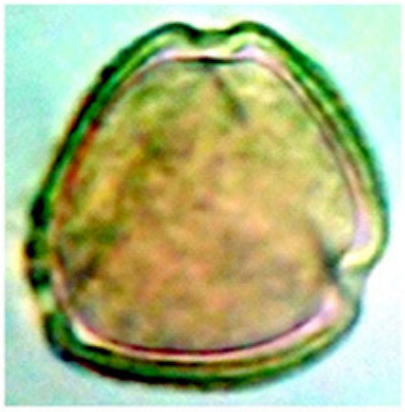
19) Alteranthera cicilis



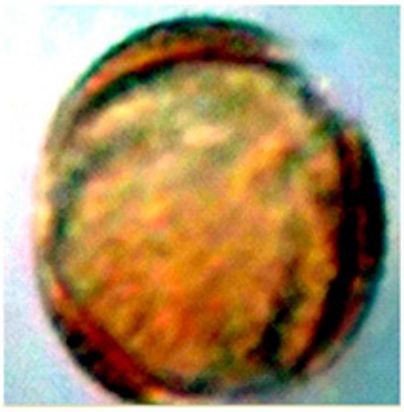
20) Plumbago zelanica



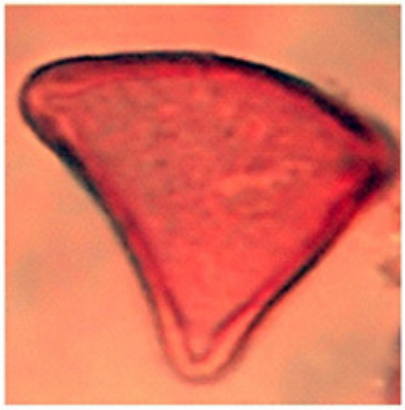
21) Lantana camera



22) Cassia occidentalis

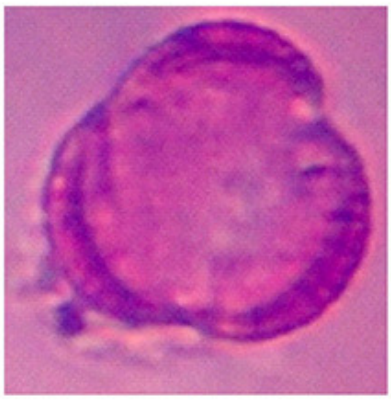


23) Brassica Camprstris

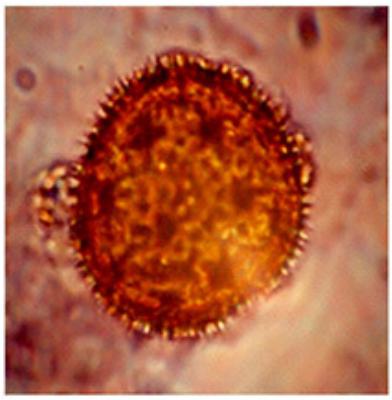


24) Cordospermum heliicabum

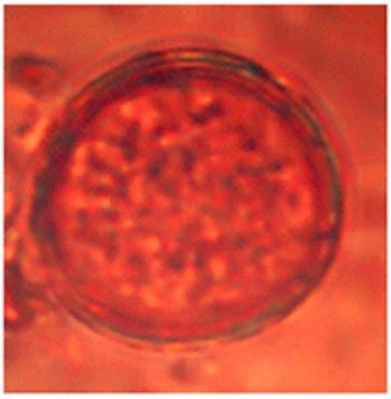
plate No. - 5. – Pollen morphology of different family members



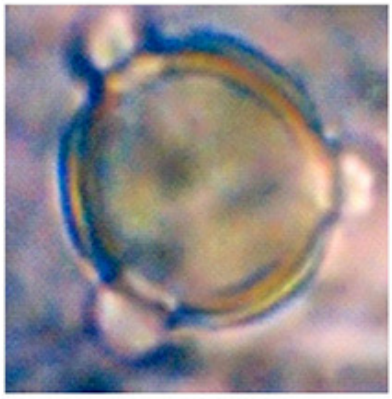
25) *Tibulus tenestris*



26) *Borrhavia diffusa*

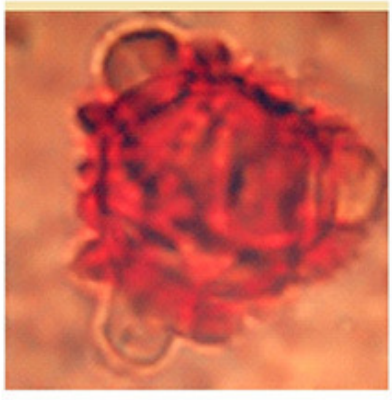


27) *Oxalis conculata*



28) *Centaurium meyeri*

29) *Peristrophe puniculata*



30) *Sonchus Oleraceus*

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