LM And SEM Studies of Pollens of Two Species of *Plumbago* Linn.

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Abstract: *Two species Plumbago selected for LM and SEM investigation of pollens are Plumbago zeylanica Linn. and Plumbago auriculata Lam. Morphological analysis of pollens was made which was based on shape, size, P/E ratio, symmetry, polarity, pollen association, apertures, exine (stratification, thickness and ornamentation). Pollen grains of both taxa are large (pollen size class) with amb more or less tricircular, bilaterally symmetrical, isopolar, monads, trizonocolpate, sexine thickner than nexine, exine reticulate (LM) and verrucate (SEM). Pollen of both taxa differs in their shape in equatorial view, size, exine thickness and verrucae placement on exine. They also showed slight variation in P/E ratio.*

Key words: *Pollen, LM, SEM, Plumbago zeylanica Linn., Plumbago auriculata Lam.*

Introduction

"The great micromorphological diversity of pollen grains amongst the seed plants is not only a marvel of nature but also a significant tool for plant biologists, especially taxonomists and ecologists"

- Grant Downton [1].

Swedish botanist Linnaeus introduces the term 'Pollen'. Rudramuniyappa (1995) defined pollen grains as highly reduced male gametophyte and unique entity utilized by higher plants for their reproduction through which genetic information is transmitted. Erdtman (1952) and Nair (1970 a) called pollen as most vital unit of angiosperm flower both with regard to form and function and represents an essential genetic link between one generation to the next. The study of pollen was started by Wodehouse (1935) with publication of his book the "Pollen grains" This was followed by sigificantl contribution of Erdtman (1952) who published the book "Pollen morphology and plant taxonomy: Angiosperms". Study of pollen grains is called "Polynology". This term was first coined by Hyde and Williams (1945) [2].

Nair (1970 a) stated that it is the most conservative morphological unit with potential value in phylogeny. According to Saad (1972), Nair (1974 c) pollens are least affected by diverse environmental conditions and hence palynological characters are considered as more reliable tool in the study of comparative morphology which leads to conclusion in plant taxonomy, phylogeny and evolution than any other vegetative organ. According to Nair (1980 a) pollen is the core of reproductive machinery [2].

Palynological characters have been used in repositioning of many disputed taxa (Nair, 1980), in interpretation of problems related to the origin and evolution of different groups (Nair, 1980) and in providing classification of angiosperms (Cronquist, 1981) [3].

Bailey and Nast (1943) reported that there are families of dicotyledons in which the pollen is of considerable taxonomic significance in differentiation of not only subfamilies and tribes but also of genera and species [4].

Palynological characters of taxonomic value are shape, size, symmetry of pollen; pollen association; pollen nuclear number; apertural form; exine stratification and sculpturing etc. [3]. The apertural conditions of pollens are considered as strong characters in solving taxonomic problems [4]

The importance of palynological data in taxonomy and phylogeny has been greatly emphasized by many workers like Kuprianova (1948, 1967, 1969); Davis and Heywood (1963); Ornduff (1966); Nowicke and Skvarla (1980); Crompton (1982); Larsen and Larsen (1983); Mathew and Philip (1983); Donoghue and Doyle (1989); Crane (1990); Zavada (1991); Rao et al (1999) [2]

Palynology is an interdisciplinary science and also has direct and scientific importance in Aerobiology, Melissopalynology, Forensic palynology, Biostratography and Geochronology, Paleapalynology, Geothermal alteration, Hydrocarbon exploration, Limnology studies, Archaeopalynology, Copropalynology [5].

Pollen morphology of family Plumbaginaceae has been examined by Chanda (1963), Praglowski and Erdtman (1969), Rao and Shukla (1975), Nowicke and Skvarla (1977), Moore and Webb (1978), Turner and Blackmore (1984) [6].

Pollen grains of *Plumbago* are studied by many previous workers. Nowicke and Skvarla (1977) [7] studied pollens of 20 species and one variety representing nine genera of family Plumbaginaceae including *Plumbago auriculata* Lam., *Plumbago rosea*, *Plumbago scandens*, *Plumbago europaea*. They studied pollen morphology and the relationship of the Plumbaginanceae, Polygonaceae and Primulaceae to the order Centrospermae. Weber–EI Ghobary (1984) [8] studied pollens of *Plumbago rosea* along with pollens of *Aegialities.* Nair and Kothari (1985) [9] investigated pollen morphology of 5 genera and 8 species of plants of family Plumbaginaceae including *Plumbago auriculata, Plumbago indica, Plumbago zeylanica.* Pathak (1994) [10] studied pollen flora of Pune district, Maharashtra which also include study of pollens of *Plumbago zeylanica* Linn*.,* Perveen and Qaiser (2004) [6] studied pollen grains of *Plumbago capensis* and *Plumbago zeylanica* along with other plants of family *Plumbaginaceae* from Pakistan. Rawat's study [11]of Pollen Flora of Doon Valley, Uttarakhand, India also include plant *Plumbago zeylanica* Linn. Shubharani et al (2013) [12] studied pollen morphology of 68 plant species from 39 families of apiculture importance which include *Plumbago zeylanica.* Hirapure et al (2014) [13] studied pollen morphology of *Plumbago auriculata* along with other 8 plants to prepare database useful in criminal investigation of forensic importance. Prabhakar and Ramkrishna (2014) [14] studied pollen morphology of 54 important ethnomedicinal plants of Boath Mandal Forest division in Adilabad district, Telangana State which also include *P. zeylanica* Linn.

Materials and Methods

Pollen preparation for LM (Light Microscopy) study:

Fresh pollen material was obtained from flowers of cultivated plants, For LM studies pollen preparation was made following the acetolysis method suggested by Nair, 1960 [15]. The acetolysed pollen grains were mounted in glycerine jelly and examined under light microscope.

Pollen preparation for SEM (Scanning Electron Microscopy) study:

For SEM study pollens of *P. zeylanica* and *P. auriculata* were suspended in the drop of water separately. Suspended pollens were directly transferred to metallic stub using double sided carbon tape. Then the sample is viewed with FEI Quanta 200 Environmental Scanning Electron Microscope with EDAX system and photomicrographs were taken at different magnifications.

Measurements For palynological study:

Measurements were taken by using LM 52-1712 Digiscope (LCD Digital microscope) of Lawrence and Mayo and FEI Quanta 200 Environmental Scanning Electron Microscope. Mean values of 10 observations with standard deviation were taken for the polar diameter, equatorial diameter, colpus length, exine thickness of pollen.

To explain pollen morphological characters terminology used is in accordance with Nair [16], Chaubal [17], TsChudy [18], Bhattacharya et al [5], Mondal [3].

Results:

***Plumbago zeylanica* Linn**. (Figure 1 and Figure 2)

1. **Shape**

   In equatorial view – Depending upon shape in equatorial view, two types of pollens are observed

   a. Prolate
   b. Sub-oblate to oblate spheroidal, rarely oblate

   Amb – More or less Tricircular

2. **Size**

   Large Pollen grains (Magnae)

   a. In prolate pollen grains – The polar and equatorial diameter and P/E ratio are as follows

Pollen Character	Min.	Max.	Mean ± S.D.
Polar diameter	70.580 µm	81.410µm	76.543 ±5.021 µm
Equatorial diameter	50.010 µm	56.670 µm	54.130 ± 4.308 µm
P/E ratio	1.349	1.668	1.440 ± 0.169

   b. In sub oblate to oblate spheroidal or rarely oblate pollen grains –The polar and equatorial diameter and P/E ratio are as follows

Pollen Character	Min.	Max.	Mean ± S.D.
Polar diameter	57.113 µm	72.493µm	65.406 ± 4.913 µm
Equatorial diameter	74.711 µm	87.350 µm	78.669 ±3.606 µm
P/E ratio	0.662	0.940	0.823±0.082

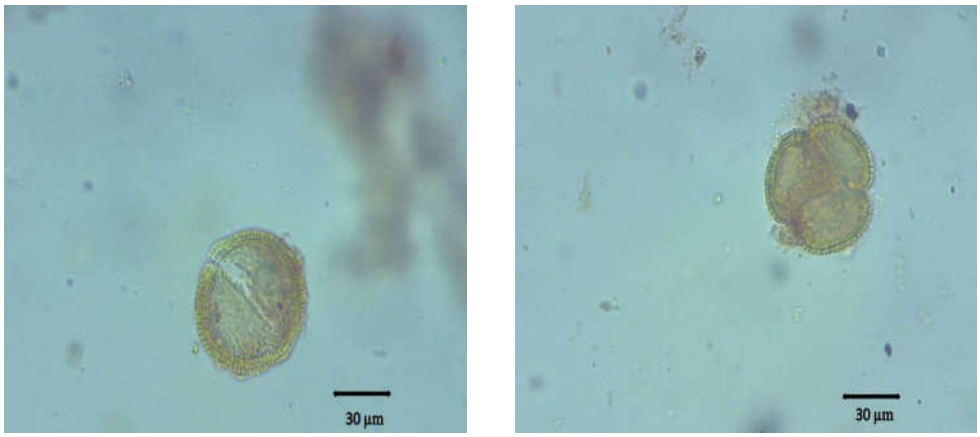
3. **Symmetry**
   Bilateral symmetry
4. **Polarity**
   Isopolar
5. **Pollen association**
   Monads
6. **Apertures**
   Number – Three (tritreme)
   Position – located at equatorial plane (zonotreme)
   Characters – Colpus (colpate) with rounded and dilated apices
   Colpus length – Colpus length in both types of pollens is as follows

Pollen Type	Min. (µm)	Max. (µm)	Mean ± S.D. (µm)
Prolate	55.380	68.090	62.564 ± 8.018
Sub-oblate to oblate spheroidal rarely oblate	40.556	65.043	49.100 ±6.746

   The colpus is long, having length ± 3/4 polar diameter
   Thus pollen grains are trizonocolpate
7. **Exine**
   Exine Stratification -
   Sexine thicker than nexine
   Exine thickness-
   The minimum and maximum exine thickness in pollen of *P. zeylanica* Linn. is 4.479 µm and 8.198 µm respectively. The mean exine thickness is 6.502 ± 1.365 µm.
   Exine ornamentation –
   In LM study exine showed reticulate ornamentation. SEM study revealed verrucate ornamentation of exine. Verrucae occur singly or in clusters and are with rounded or pointed tips. Comparatively verrucae (single or clustered) are closely placed

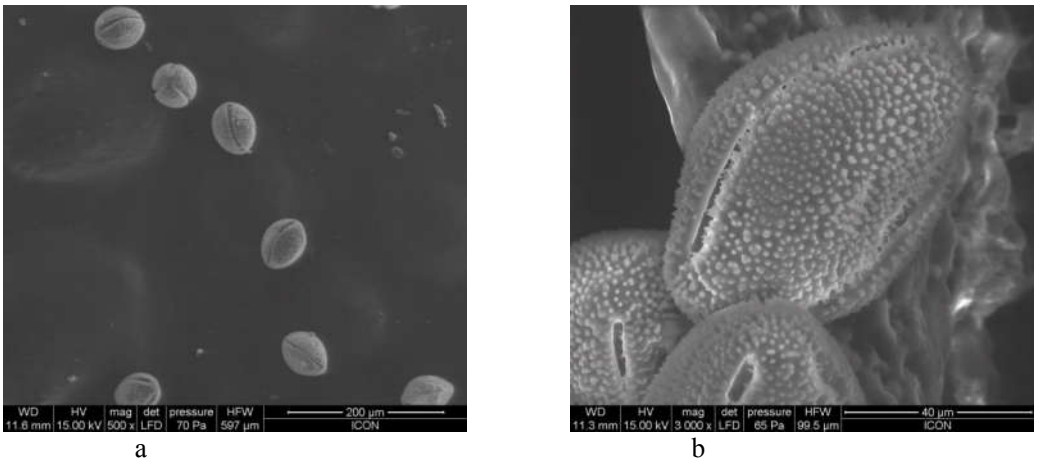
a

b



c d

Figure 1 LM photomicrographs of pollen of *P. zeylanica* Linn. a. Fresh pollen grains in equatorial view (×400x) b. Fresh pollen grain in polar view (×400x) c. Acetolysed pollen grains in equatorial view (×400x) d. Acetolysed pollen grains in polar view (×400x)



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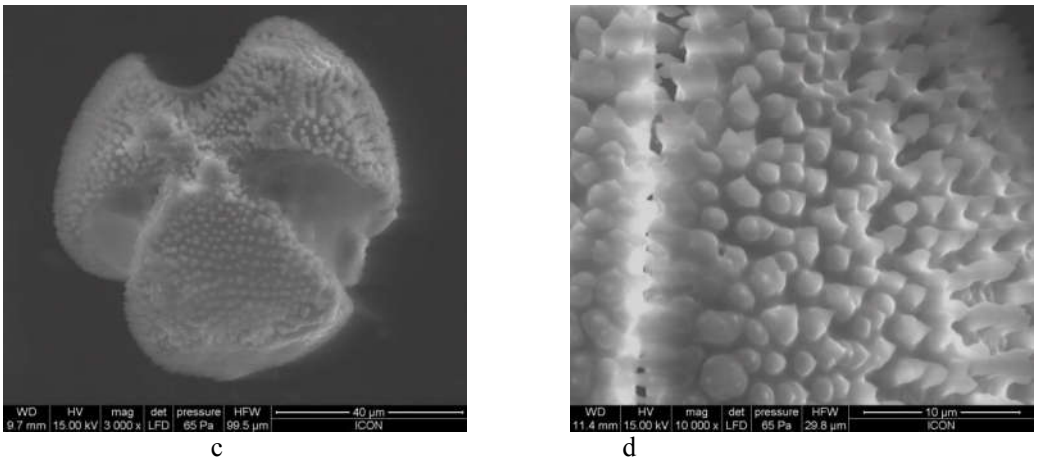

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Figure 2 (a-d) SEM photomicrographs of pollens of *P. zeylanica* Linn. a. Pollen grains (×500x) b. Pollen grain in equatorial view (×3000x) c. Pollen grains in equatorial polar view (×3000x) d. Pollen grains with verrucate ornamentation showing verrucae (occurring singly or in clusters) with rounded or pointed tips and comparatively close arrangement (×10000x)

***Plumbago auriculata* Lam.** (Figure 3 and Figure 4)

1. **Shape**

In equatorial view – Depending upon shape in equatorial view two types of pollens are observed

a. Sub prolate rarely prolate or prolate sheroidal

b. Sub-oblate to oblate spheroidal

Amb – More or less tricircular

2. **Size**

Large pollen grains (Magnae)

a. In sub-prolate, rarely prolate or prolate spheroidal- The polar and equatorial diameter and P/E are as follows

Pollen Character	Min.	Max.	Mean ± S.D.
Polar diameter	62.146 µm	69.750 µm	66.641 ± 3.170 µm
Equatorial diameter	49.780 µm	61.580 µm	54.072 ± 3.308 µm
P/E ratio	1.087	1.387	1.235 ± 0.075 µm

b. In sub-oblate to oblate spheroidal - The polar and equatorial diameter and P/E are as follows

Pollen Character	Min.	Max.	Mean ± S.D.
Polar diameter	56.321 µm	68.916 µm	62.669± 4.674 µm
Equatorial diameter	61.082 µm	74.056 µm	70.212 ± 5.966 µm
P/E ratio	0.849	0.988	0.905 ± 0.046

Pollens of both types of flowers in *Plumbago auriculata* Lam. show only subtle difference in size. Pollens of short styled flowers with long stames are slightly larger that pollens of long styled flowers with short stamen

3. **Symmetry**

Bilateral symmetry

4. **Polarity**

Isopolar

5. **Pollen association**

Monads

6. **Apertures**

a. Number – Three (tritreme)

b. Position – located at equatorial plane (zonotreme)

c. Chartacters - colpus (colpate) with rounded and dilated apices

d. Colpus length – Colpus length in both types of pollens is as follows

Pollen shape	Min.	Max.	Mean ± S.D
Sub-prolate, rarely prolate or prolate spheroidal	50.956 µm	60.440 µm	55.068 ± 4.767 µm
Sub – oblate to oblate spheroidal	35.599 µm	58.371 µm	48.806 ± 4.810 µm

The colpus is long having length ± 3/4 polar diameter

Thus pollen grains are trizonocolpate

7. **Exine**

Exine stratification-

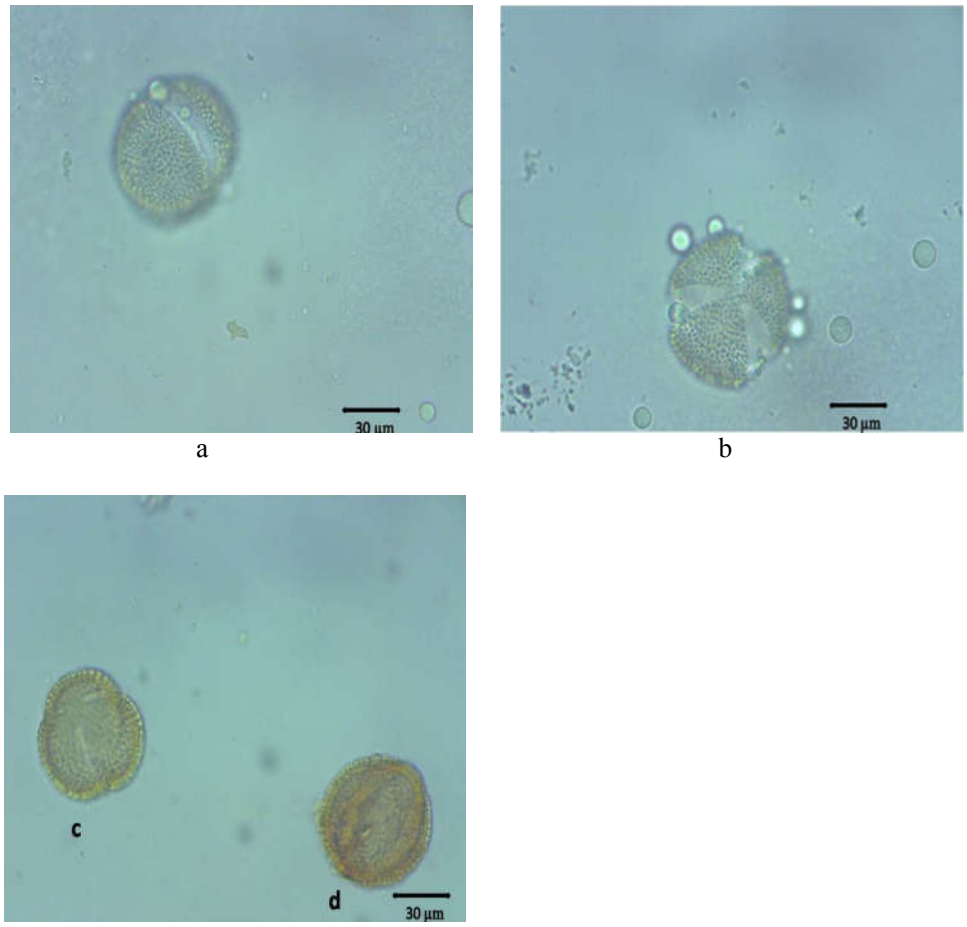
Sexine thicker than nexine

Exine thickness -

The minimum and maximum exine thickness in pollen of *P. auriculata* Lam. is 3.562 µm and 6.266 µm respectively. The mean exine thickness is 5.269 ± 0.859 µm.

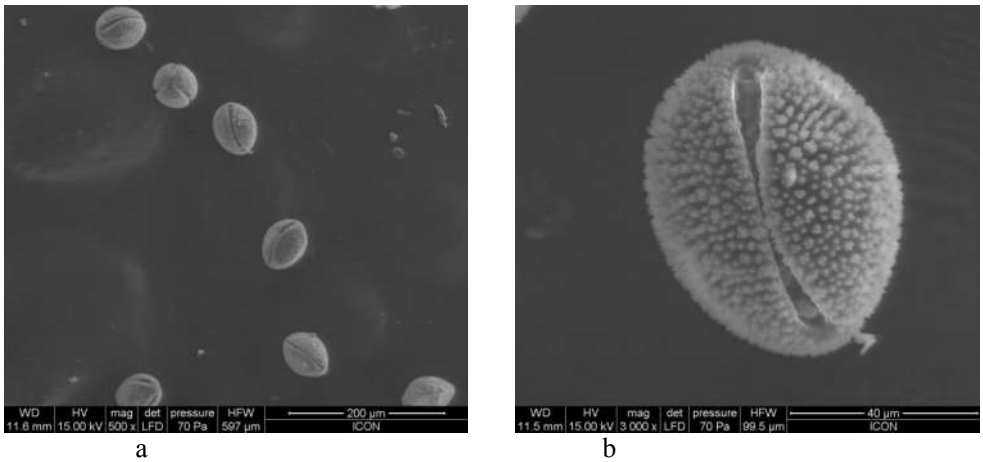
Exine ornamentation -

In LM study exine showed reticulate ornamentation SEM. study revealed verrucate ornamentation of exine. Verrucae occur singly or in clusters and are with rounded or pointed tips. Comparatively veruccae (single or clustered) are distantly placed.



a b

Figure 3 LM photomicrographs of pollen of *P. auriculata* Lam. a. Fresh pollen grains in equatorial view (×400x) b. Fresh pollen grain in polar view (×400x) c. Acetolysed pollen grains in polar view (×400x) d. Acetolysed pollen grains in equatorial view (×400x)



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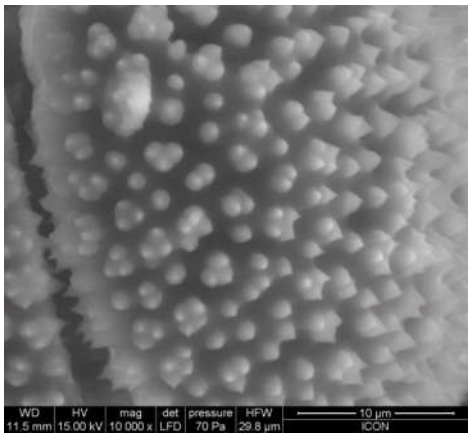

d

Figure 4 (a-d) SEM photomicrographs of pollens of *P. auriculata* Lam. a. Pollen grains (×500x) b. Pollen grain in equatorial view (×3000x) c. Pollen grains in polar view (×3000x) d. Pollen grains with verrucate ornamentation showing verrucae (occurring singly or in clusters) with rounded or pointed tips and comparatively distant arrangement (×10000x)

Discussion

Pollen morphological analysis was made to study pollens in detail and their significance in plant taxonomy.

LM and SEM study of pollen of both plants *P. zeylanica* Linn. and *P. auriculata* Lam. revealed same morphological characters except size, shape, exine thickness and verrucae placement in exine ornamentation.

1. Shape-

   Slight differences in the shape of pollen grains in equatorial view are observed. In *P. zeylanica* Linn. two types of pollen shape observed are 1) Prolate 2) Sub-oblate to oblate spheroidal, rarely oblate. In *Plumbago auriculata* Lam. two types of pollen shape observed are 1) Sub-prolate, rarely prolate or prolate spheroidal 2) sub-oblate to oblate spheroidal.

   Pathak [10], Rawat [11] reported the sub-prolate shape of pollen of *P, zeylanica* Linn. Prolate to sub-prolate and prolate spheroidal shapes of pollen of *P. zeylanica* Linn are observed by Shubharani et al [12], Prabhakar and Ramakrishna [14] respectively. Perveen and Qaiser [6] reported oblate spheroidal to sub-oblate shape of pollen of *P. zeylanica* Linn. We did not observe sub-prolate, prolate spheroidal shape in pollen of *P. zeylanica* Linn. but we additionally observed oblate shape.

   Perveen and Qaiser [6], Hirapure et al [13]reported oblate spheroidal and prolate shape of pollen of *P. auriculata* Lam. respectively. We observed all these shapes in pollen of *P.auriculata* Lam. and additionally we observed sub-prolate, prolate – spheroidal and sub-oblate shape. Bhattacharya et al [5] reported sub-oblate to sub-prolate shape of pollen in family plumbaginaceae.

   Amb of pollen in both the plants is more or less tricircular

2. Size -

   Pollen grains in both plants *P. zeylanica* Linn. and *P. auriculata* Lam. belongs to pollen size class large (Magnae). But pollen grains of *P. zeylanica* Linn. are larger than pollen grains of *P. auriculata* Lam.

   Our observations of range and mean of polar diameter and equatorial diameter of *P. zeylanica* Linn. and *P. auriculata* Lam. show considerable variations with those recorded by Perveen and Qaiser [6]. Considerable variations in the range and mean of polar and equatorial diameter of pollen of *Plumbago zeylanica* Linn. are observed with those recorded by Pathak [10] while slight variations in them are found with those recorded by Rawat [11]

3. P/E ratio-

   Besides pollen size, P/E value proved to be useful character of systematic value [19]. The P/E ratios in *P. zeylanica* Linn. are in the range of 1) 1.349 to 1.668 with mean 1.440 ± 0.169 (in

prolate pollen) and 2) 0.662 to 0.940 with mean 0.823 ± 0.082 (in sub- oblate to oblate spheroidal or rarely oblate pollen)

The P/E ratios of *P. auriculata* Lam. are in the range of 1) 1.087 ± 1.387 with mean 1.235 ± 0.075 (in sub-prolate, rarely prolate or prolate – spheroidal) 2) 0.849 to 0.988 with mean 0.905 ± 0.046 (in sub-oblate to oblate-spheroidal). Slight variation is found in P/E ratio of pollen in both taxa. Perveen and Qaiser [6] recorded P/E ratios 0.63 and 0.95 in *P. zeylanica* Linn. and *P. auriculata* Lam. respectively.

4. Symmetry -

Pollen grains of both species of *Plumbago* are bilateral symmentrical. The present observation conforms totally with record of Shubharani et al [12] in *P. zeylanica* Linn. But Prabhakar and Ramakrishna [14] reported radial symmentry in pollen of *Plumbago zeylanica.* Perveen and Qaiser [4] reported radially sysmmetrical pollen in Plumbaginaceae

5. Polarity –

Pollen grains of both species of *Plumbago* are isopolar. Our observation of polarity in pollen of *P. zeylanica* Linn. is in accordance with observation of Prabhakar and Ramakrishna [14].

6. Pollen association –

The pollen grains do not remain attached in tetrad at maturity. Hence single pollen grains i.e. monads are observed in both plants at maturity.

7. Apertures

Pollen grains in both the plants are trizonocolpate. Our observation of trizonocolpate pollen in *P. zeylanica* Linn. is in the conformity of Rawat [11]. Tricolpate pollen in *P. zeylanica* Linn. are also reported by Pathak [10], Perveen and Quaiser [6], Shubharani et al [12] and Prabhakar and Ramakrishna [14].

Tricolpate pollens in *P. auriculata* Lam. are also reported by Perveen and Qaiser [6] but Hirapure et al [13] reported colporate pollen in *P. zeylanica* Linn. and *P. auriculata* which is not in conformity with our observation.

Colpus in both the taxa are long with ± 3/4 polar diameter and has rounded and dilated apices. The colpus length in *P. zeylanica* is in the range of 1) 55.380 µm to 68.090 µm with mean 62.564 ± 8.018 µm (in prolate pollen) 2) 40.556 µm to 65.043 µm with mean 49.100 ± 6.746 µm (in sub-oblate to oblate spheroidal, rarely oblate to oblate spheroidal, rarely oblate pollen). The colpus length in *P. auriculata* is in the range of 1) 50.956 µm to 60.440 µm with mean 55.068 ± 4.767 µm (in sub-prolate rarely prolate or prolate spheroidal) 2) 37.599 µm to 53.371 µm with mean 48.806 ± 4.810 µm (in sub-oblate to oblate spheroidal).

Perveen and Qaiser [6] reported colpus length range 35 to 42.5 µm with mean 40 and range 5 to 6 µm with mean 5.4 µm in *P. zeylanica* Linn. and *P. auriculata* Lam. respectively. Thus our observations of colpus length show variations with those observed by Perveen and Qaiser [6].

Our observation of rounded and dilated apices of colpus in pollen of *P. zeylanica* Linn. demonstrated variation in the observation of Rawat [11] who investigated colpus with mildly acute ends.

8. Exine

Exine patterns are worthwhile in recognizing the different species of genera [4].

Exine Stratification-

The pollen wall, sporoderm is stratified i.e. layered [5]. It consists of two layers 1) Intine – It is inner layer and usually destroyed during acetolysis 2) Exine – It is outer and acetolysis resistant layer. Morphologically exine is stratified into two distinct layers 1) outer sculptured sexine and 2) inner non-sculptured nexine [20].

In pollen of both plants sexine is thicker than nexine. Our finding of sexine thicker than nexine in *P. zeylanica* conforms the findings of Pathak [10], Rawat [11], Perveen and Qaiser [6].

Exine thickness –

Exine of pollen of *P. zeylanica* Linn. is thicker than exine of pollen in *P. auriculata* Lam.

Our observation of exine thickness of pollen of *P. zeylanica* Linn. show slight variations with those recorded by Pathak [10], Rawat [11], Perveen and Qaiser [6]. But our

observation of exine thickness of pollen of *P. auriculata* Lam. show considerable variation with those recorded by Perveen and Qaiser [6].

Exine ornamentation –

We observed reticulate exine ornamentation in LM study and verrucate exine ornamentation in SEM study in both taxa. Verrucae occur singly or clustered and are with rounded or pointed tips. Verrucae (single or clustered) are comparatively closely placed in *P. zeylanica* Linn. and comparatively distantly placed in *P. auriculata* Lam.

However, pollen grains of both taxa are variously suggested to have pilate to synpilate, pilate or bacculate exine ornamentation by Nair and Kothari [9], Pathak [10], Perveen and Qaiser [6]respectively.

Our observation of verrucate pollen in *P. zeylanica* Linn. conforms the findings of Prabhakar and Ramakrishna [14]and in *P. auriculata* Lam. conforms the findings of Nowicke and Skvarla [7] but Hesse et al [21] reported clavate exine ornamentation in *P. auriculata* Lam. Datta [4], Kubitzki[22] and Mondal [3] reported verrucate pollens in Plumbaginaceae

Our observation of reticulate exine ornamentation of pollen of *P. zeylanica* Linn. in LM study is in consonance with findings of researchers Rawat [11] and Shubharani et al [12].

Taxonomic significance of pollen morphological characters

Pollen micromorphological characters are helpful to delineate the two species of *Plumbago*

Keys to *Plumbago* Species

1. **Key based on pollen size**

Pollen grains larger (76.543 ± 5.021 µm × 54.130 ± 4.308 µm [polar diameter × equatorial diameter] and 65.406 ± 4.913µm × 78.699 ± 3.606 µm)

-Plumbago zeylanica

Pollen grains smaller (66.641 ± 3.170 µm × 54.072 ± 3.308 µm and 62.669 ± 4.674 µm × 70.212 ± 5.966 µm)

-Plumbago auriculata

2. **Key based on verrucae placement on exine**

Comparatively verrucae (single or clustered) of exine are closely placed

-Plumbago zeylanica

Comparatively verrucae (single or clustered) of exine are distantly placed

-Plumbago auriculata

3. **Key based on Exine thickness**

Exine comparatively thick (6.502 ± 1.365 µm)

-Plumbago zeylanica

Exine comparatively thin (5.269 ± 0.859 µm)

-Plumbago auriculata

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