Pollen Morphology and Fertility of Some Species in Taung Wyne area, Mawlamyine Township

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Abstract

Pollen morphological characters of 10 species belonging to 6 genera of three families were investigated from Mawlamyine Township and examined by light microscopy. The aim of the present investigation is to study the pollen morphology such as pollen shape, size, and exine ornamentation for the taxonomic assessment of the groups of plants. The pollen specimens were collected from December 2019 to May 2020 from Mawlamyine University Township. Standard methods of pollen collection, storage, and preparation were employed by Erdmann's method. The collected specimens contained four species in the family of Convolvulaceae, three species in the family of Lamiaceae, and three species in Verbenaceae. The pollen of these families was recorded and observed by examining the aperture type, number, shape, size, and ornamentation. There are polyporate, colpate, colporate and porate. Aperture types are 4 species of polyporate (Ipomoea carnea, coccinea, hederacea and aquatica), 4 species of colporate (*Clerodendrum indicum*, *villoscum stachytarpheta*, vitex), 1 species of colpate (*Duranta repens*), and 1 species of prolate (*Ocimum tenuiflorum*). The pollen images for each species were presented by their equatorial view and polar view. The pollen production, pollen fertility, and sterility were counted. The high pollen production occurred in *Clerodendrum villoscum* Blume. The most pollen fertility was found in Ipomoea aquatica Forsk. and sterility in *Stachytarpheta indica* Vahl. This finding would be applied to the classification and identification of the plant.

Keywords Pollen, Monads, Colpate, Colporate, Porate, Aperture, Evolutionary

Introduction

Palynology is a branch of botany special studying pollen and spores (Erdtman, 1952). Palynology was introduced by (Hyde and Williams, 1944) to formally include all work with pollen and spores. Palynology is broadly defined as the study of pollen and spores from both living and fossil seed plants. It comes from the Greek verb ″palynein″ meaning 'to strew; to spread; to distribute′ that many pollen grains and spores are easily carried by the wind (Agashe & Caulton, 2009).

Pollen grains are extremely tiny particles comparable to dust particles, which cannot be seen by the naked eye. Pollen is a male gamete produced in the anther of flowers to carry out the gametophytic generation of flowering plants. Pollen plays a key role in plant reproduction. The pollen morphology was studied on their size, shape, and ornamentation. Certain sites in the pollen wall may represent relatively weak areas that are ruptured when the pollen tube emerges during germination. This weak area generally has a thin exine deposition and is referred to as an aperture.

Furrows-like aperture when sited at one of the two poles of the grain is referred to as a sulcus whereas if such apertures are located along the equator and their number per grain is two or more, they are termed as colpi (singular: colpus). A round, pore-like aperture is known as an ulcus (plural: sulci; Walker and Doyle 1975).

The pollen grain wall comprises two layers, the outer layer is called exine and the inner layer is the intine. The exine is divided into two layers: the outermost sexine and the unsculptured underlying nexine. The exine, or outer layer, is composed of a

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high decay-resistant chemical called sporopollenin. The Convolvulaceae and Verbenaceae are eurypalynous families having a large variety of pollen morphotypes (Erdtman, 1952). Convolvulaceae generally has pantoporate, an echinate type of pollen. Lamiaceae is stenophyllous (Halbritter, 2008). In the present study, pollen grains of Lamiaceae are generally colpate, either tri-tetra or hexacolpate. Verbenaceae pollen varies from tri-tetracolpate to tricolporate type.

The present investigation on palynological studies of the Convolvulaceae, Lamiaceae, and Verbenaceae of Mawlamyine Township was undertaken to find out the pollen morphology to provide the valuable pollen characters that can be used in classification and identification of the plant.

Materials and Methods

To collect the pollen of mature and fresh flowers there were a collection of specimens from Mawlamyine Township. Plant collection and preservation techniques are used for making herbarium specimens. The collection was carried out from December 2019 to May 2020. The pollen of each species was stored in glass vials with 1cc of glacial acetic acid and the specimen was labeled. The pollen sample was examined by using a light microscope and imaged by an MI 5X camera directly from the eyepiece of the microscope.

Measurements were based on 20 pollen grains per sample; values of polar axis length (PV) and equatorial diameter (EV) were measured and recorded. Pollen aperture was observed directly. The pollen terminology was used for the description with the help of Erdtman, (1952 & 1969), and Paldat, (2005). Punt et al., (2007) and Halbritter et al., (2009 & 2018).

Determination of pollen production

In determining pollen productivity were collected the mature and indehiscent anthers from plants while they were in peak blooming season. One anther was crushed in a glass vial and dispersed in 50 drops of 50% glycerine. One drop of the mixture was put on the slide and covered with a 22 μm cover glass. The number of pollen grains in this area was counted with an average often drops for each species. For each species under study, the pollen grain size was measured for 30 randomly chosen grains.

Determination of pollen fertility

Pollen fertility was determined using the acetocarmine glycerine staining technique as suggested by (Shivanna and Rangaswami, 1992). The slides were kept for 30 minutes for better staining and then examined under the microscope. Fully stained pollen grains were counted as fertile and partially stained or lightly stained or unstained was counted as sterile. At least 50 grains were examined per flower. Pollen fertility was calculated by using the following formula

$$\% \text{ of pollen fertility} = \frac{\text{Number of fertile pollen grains} \times 100}{\text{Total number of pollen grains}}$$

Results

The pollen morphology of 10 species from belonging to 6 genera of the family Convolvulaceae, Lamiaceae, and Verbenaceae has been investigated. These families are arranged alphabetically.

Scientific name - ***Ipomoea aquatica*** **Forsk. (Figure. 1)**
Myanmar name - Ka-zun
Family - Convolvulaceae

Polyporate, pantoprolate, spheroidal, very large 115 - 122 μm in diameter; pores circular; 7.5 - 12.5 μm in diameter; interporal space 4 - 7.5 μm thick; exine 3 - 5μm thick; sexine thicker than nexine; sculpturing echinate; spine 7.5 – 9 μm in length, interspinal space 5 - 7.5 μm, spine straight, the tip pointed, basal cushion absent and pollen fertility 96%.

Scientific name - ***Ipomoea carnea*** **Jacq. (Figure. 2)**
Myanmar name - La-thar-pan
Family - Convolvulaceae

Polyporate, pantoprolate, spheroidal, very large, 125 - 137 μm in diameter; pores circular; 10 - 12.5 μm in diameter; interporal space 5 - 7.5 μm; exine 2.5 - 3.5 μm thick; sexine thicker than nexine; sculpturing echinate; spine 7.5 - 10 μm in length, intrespinal space 13 - 15 μm wide, spine straight, the tip pointed, basal cushion absent, and pollen fertility 94%.

Scientific name - ***Ipomoea coccinea*** **L. (Figure. 3)**
Myanmar name - Myat-lay
Family - Convolvulaceae

Polyporate, pantoprolate, spheroidal, very large, 137 - 142 μm in diameter; pores circular; 15 - 17.5 μm in diameter; interporal space 2 - 4 μm; exine 3 - 4 μm thick; sexine thicker than nexine; sculpturing echinate; spine 6.5 - 9 μm in length, interspinal space 10 - 12 μm; spine straight, the tip pointed, basal cushion present, and pollen fertility 93%.

Scientific name - ***Ipomoea hederacea*** **Jacq. (Figure. 4)**
Myanmar name - Ye- kazun
Family - Convolvulaceae

Polyporate, pantoprolate, spheroidal, very large, 150 - 162 μm in diameter; pores circular 12.5 - 2.5 μm in diameter; interporal space 5 - 10 μm thick; exine 5 - 7.5 μm thick; sexine thicker than nexine; sculpturing echinate; spine 6 - 8 μm in length, interspinal space 7.5 - 15 μm, the tip pointed, basal cushion present, and pollen fertility 92%.

Scientific name - ***Clerodendrum indicum*** **L. (Figure. 5)**
Myanmar name - Nga-yan-padu
Family - Lamiaceae

Tricolpate, spheroidal, very large, 105 – 125 μm in diameter; amb circular; ¾ way up to the pole, colpi longic colpate, 75 – 90 x 9 – 10 μm in length and breadth; exine 1.25 –1.85 μm thick, sexine thicker than nexine; sculpturing microechinate, spine about 1.85 μm in length, interspinal space 4 – 8 μm and pollen fertility 90%.

Scientific name - ***Clerodendrum villoscum*** **Blume. (Figure. 6)**
Myanmar name - Phet-Kha
Family - Lamiaceae

Tricolpate, prolate, large, 58 – 67 x 70 - 87 µm in length and breadth; amb circular; colpi ¾ way up to the pole, 48 - 55 x 7.5 – 12 µm in length and breadth; exine 2.5 - 3.75 µm thick, sexine thicker than nexine; sculpturing microechinate, spine 0.5- 1.25 µm in length, interspinal space 4-5 µm and pollen fertility 73%.

Scientific name - ***Ocimum tenuiflorum* L. (Figure. 7)**
Myanmar name - Kala-pinsein
Family - Lamiaceae

Hexacolpate, prolate, large, 78 – 80 x 51 - 55 µm in length and breadth; amb circular; colpi longicolpate, 12 - 22 x 5 -10 µm in length and breadth; exine 4 - 6 µm thick, sexine thicker than nexine, sculpturing distinctly reticulate, the lumina heterobrochate, 5 - 12.5 µm in width, muri simplibaculate, 1 µm wide and pollen fertility 78%.

Scientific name - ***Duranta repens* L. (Figure. 8)**
Myanmar name - Bokadaw-myet-hkon
Family - Verbenaceae

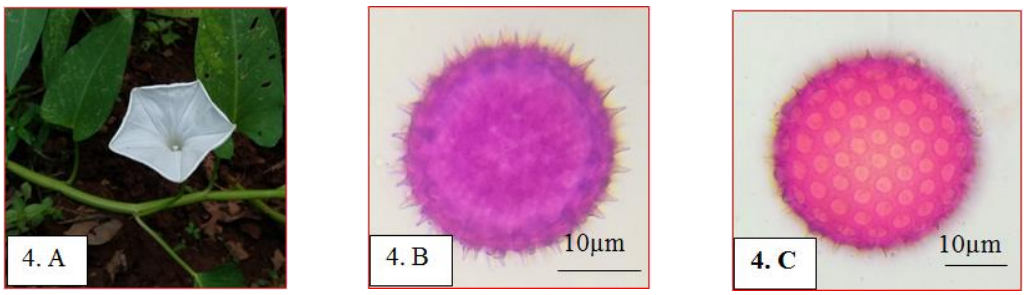
Tricolporate, isopolar, oblate, medium, 28 – 30 x 45 - 48 µm in length and breadth; amb rounded triangular; colpi ½ way up to the pole, 11 - 13 x 3 - 5 µm in length and breadth; pori lolongate, about 7 - 9 µm in length and breadth; exine 1 - 1.5 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate and pollen fertility 83%.

Scientific name - ***Stachytarpheta indica* Vahl (Figure. 9)**
Myanmar name - Aseik-taya
Family - Verbenaceae

Tricolpate, isopolar, oblate, large, 73 - 80 x 88 - 95 µm in length and breadth; amb rounded-triangular; colpi ½ way up to the pole, 50 - 60 x 25 - 30 µm in length and breadth, margin thickened by the fusion of the rounded elevations (verrucate) formed in a single row along the margin; exine about 5 µm thick, sexine thicker than nexine; sculpturing verrucate, 5 -7.5 µm in diameter and pollen fertility 66%.

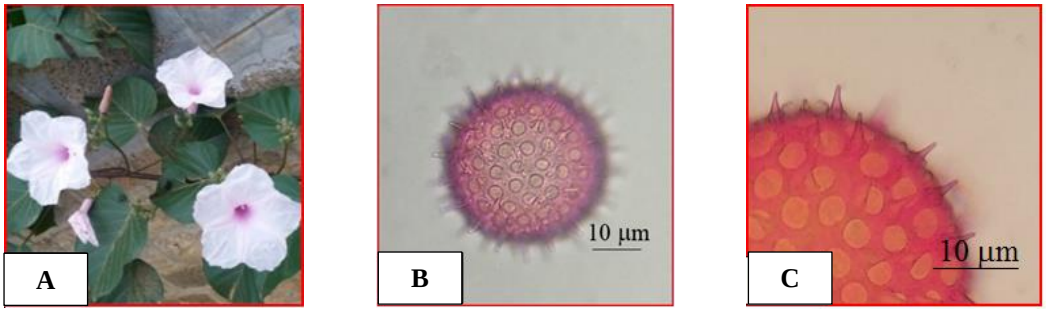
Scientific name - ***Vitex trifolia* L. (Figure. 10)**
Myanmar name - Kyaun- ban
Family - Verbenaceae

Tricolpate, isopolar, prolate, medium, 40 - 47 x 32 - 35 µm in length and breadth; amb rounded triangular; ¾ way up to the pole, colpi longicolpate, 24 - 31 x 4.5 - 6 µm in length and breadth; exine 1.5 - 2.5 µm thick, sexine as thick as nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 0.75 - 1 µm in width, the muri simplibaculate, 0.5 - 1 µm wide and pollen fertility 89%.



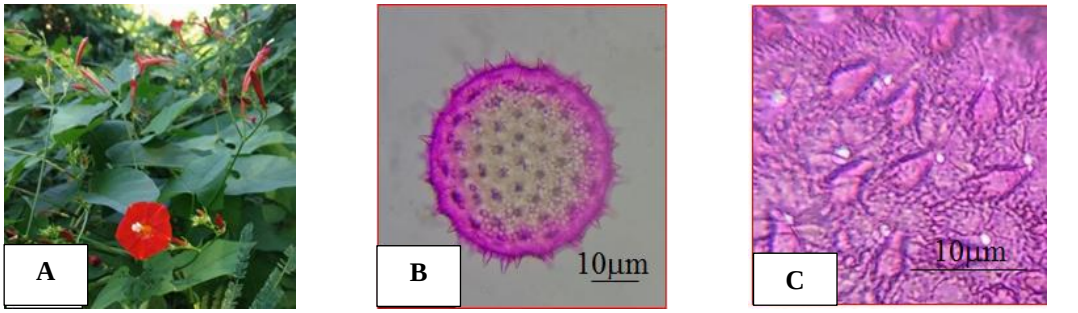
A. Inflorescence B. Polar view C. Pollen equatorial view

Fig. 1. *Ipomoea aquatica* Forsk.



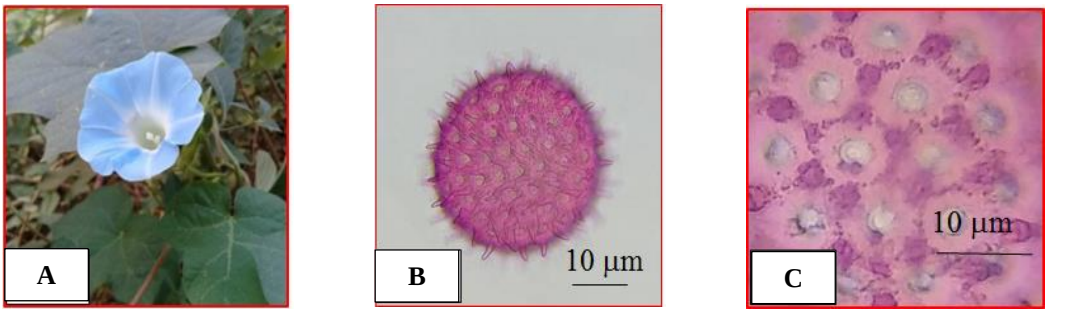
A. Inflorescence B. Polar view C. equatorial view

Fig. 2. *Ipomoea carnea* Jacq.



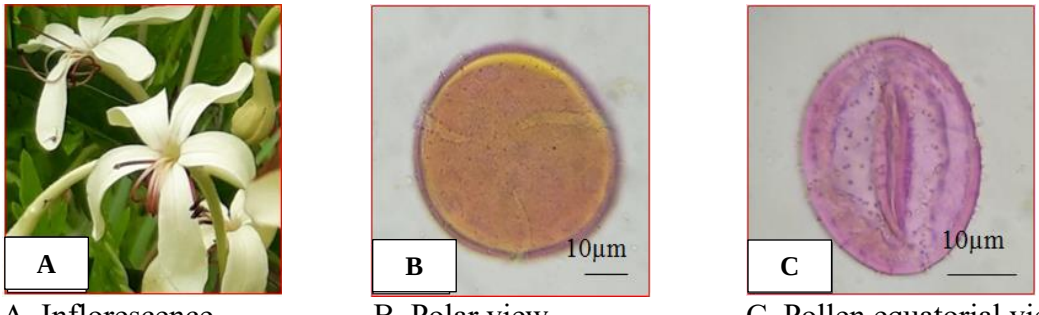
A. Inflorescence B. Polar view C. Pollen equatorial view

Fig. 3. *Ipomoea coccinea* L.



A. Inflorescence B. Polar view C. Pollen equatorial view

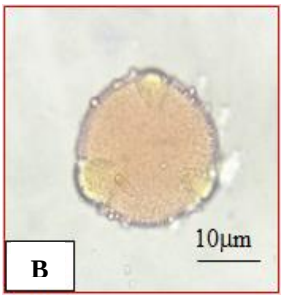
Fig. 4. *Ipomoea hederacea* Jacq.



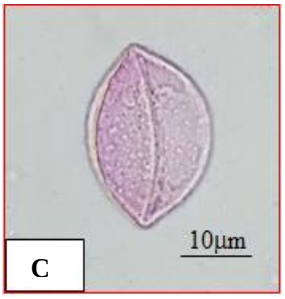
A. Inflorescence B. Polar view C. Pollen equatorial view

Fig. 5. *Clerodendrum indicum* L.

A. Inflorescence



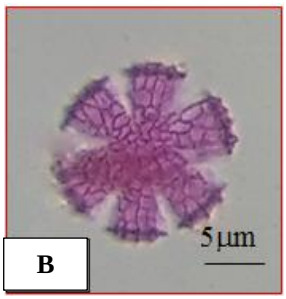
B. Polar view



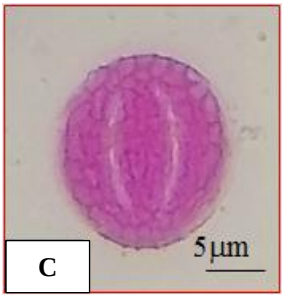
C. Pollen equatorial view

Fig. 6. *Clerodendrum villoscum*

A. Inflorescence



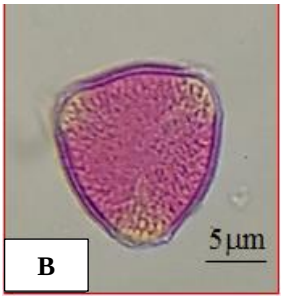
B. Polar view



C. Pollen equatorial view

Fig. 7. *Ocimum tenuiflorum* L.

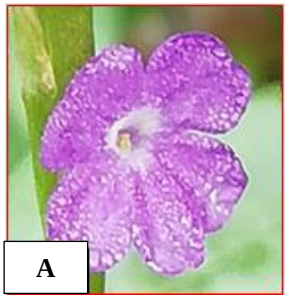
A. Inflorescence



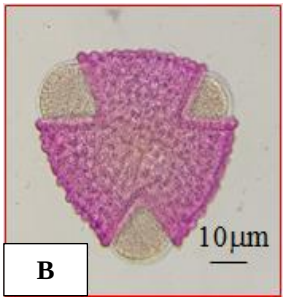
B. Polar view

C. Pollen equatorial view

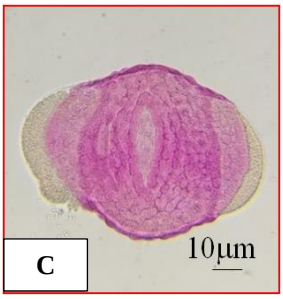
Fig. 8. *Duranta repens* L.



A. Inflorescence

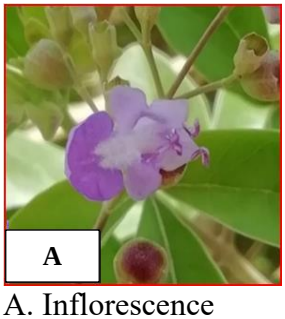


B. Polar view

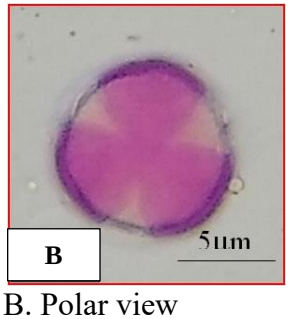


C. Pollen equatorial view

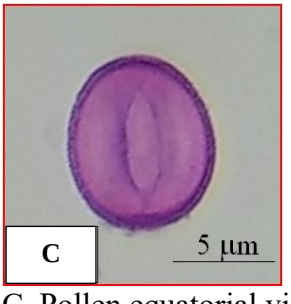
Fig. 9. *Stachytarpheta indica* Vahl



A. Inflorescence



B. Polar view



C. Pollen equatorial view

Fig.10. *Vitex trifolia* L.

Pollen Production

Pollen production of the 10 species studied, in most species the anther dehiscence through longitudinal dehiscence. The entomophily and anemophily modes of pollination were occurred in all species. The total pollen production is directly corrected to the number of anthers per flower and large anther has higher rate of pollen production. The results were shown in Table 1 and Figure 11.

Table 1. Pollen production of collected species

No.	Scientific name	Mode of pollination	Mode of anther	No. of anther/ flower	Average of pollen/ anther	Average of pollen/ flower
1.	*Ipomoea aquatica* Forsk.	ANE	LD	5	141	705
2.	*Ipomoea carnea* Jacq.	ANE	LD	5	66	330
3.	*Ipomoea coccinea* L.	ANE	LD	5	173	865
4.	*Ipomoea hederacea* Jacq.	ANE	LD	5	60	300
5.	*Clerodendrum indicum* L.	ENT	LD	4	1865	7460
6.	*Clerodendrum villoscum* Blume	ENT	TD	4	5897	23588
7.	*Ocimum tenuiflorum L.*	ENT	LD	4	155	620
8.	*Duranta repens* L.	ENT	LD	4	324	1296
9.	*Stachytarpheta indica* Vahl	ENT	LD	2	40	80
10.	*Vitex trifolia* L.	ENT	TD	4	1467	5868

ANE-Anemophily, ENT-Entomophily, TD-Transverse Dehiscence, LD-Longitudinal Dehiscence

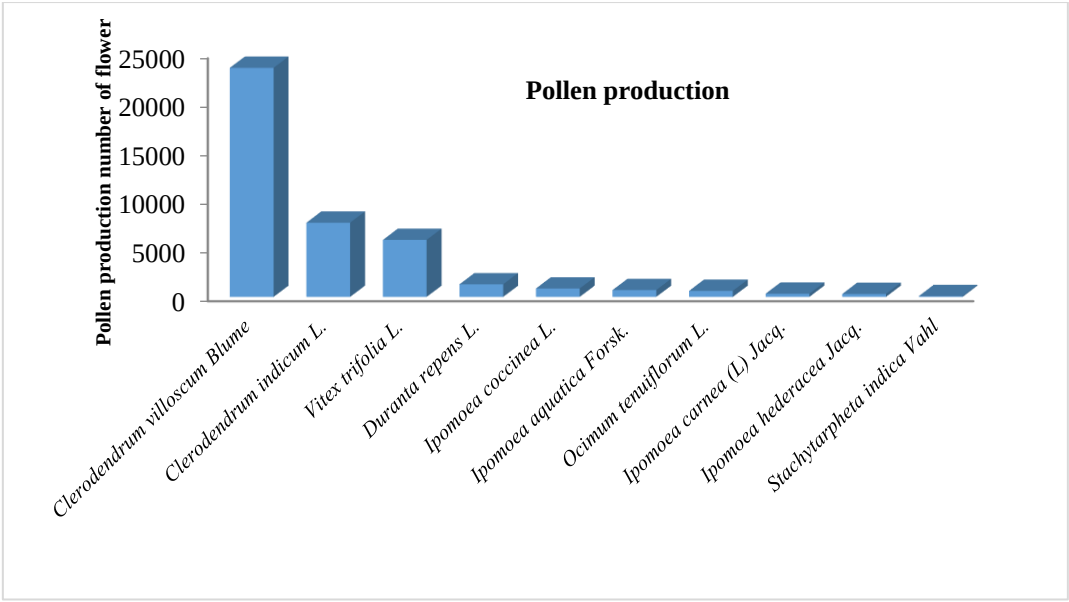


Fig. 11 Histogram showing pollen production of collected species

Pollen Fertility

Pollen fertility was determined by using acetocarmine-glycerine stain. Pollen fertility data was found in *Ipomoea aquatica* was having the height while the least value was shown in *Stachytarpheta indica*. The result was shown in Fig. 12.

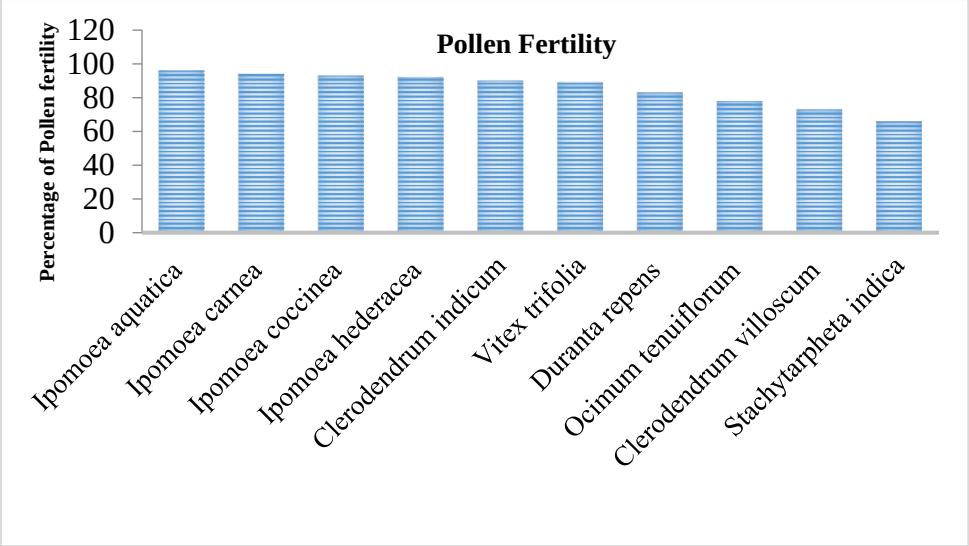


Fig. 12 Histogram showing pollen fertility of collected species

A tentative scheme of evolution of pollen apertures in collected species is presented in Fig.13.

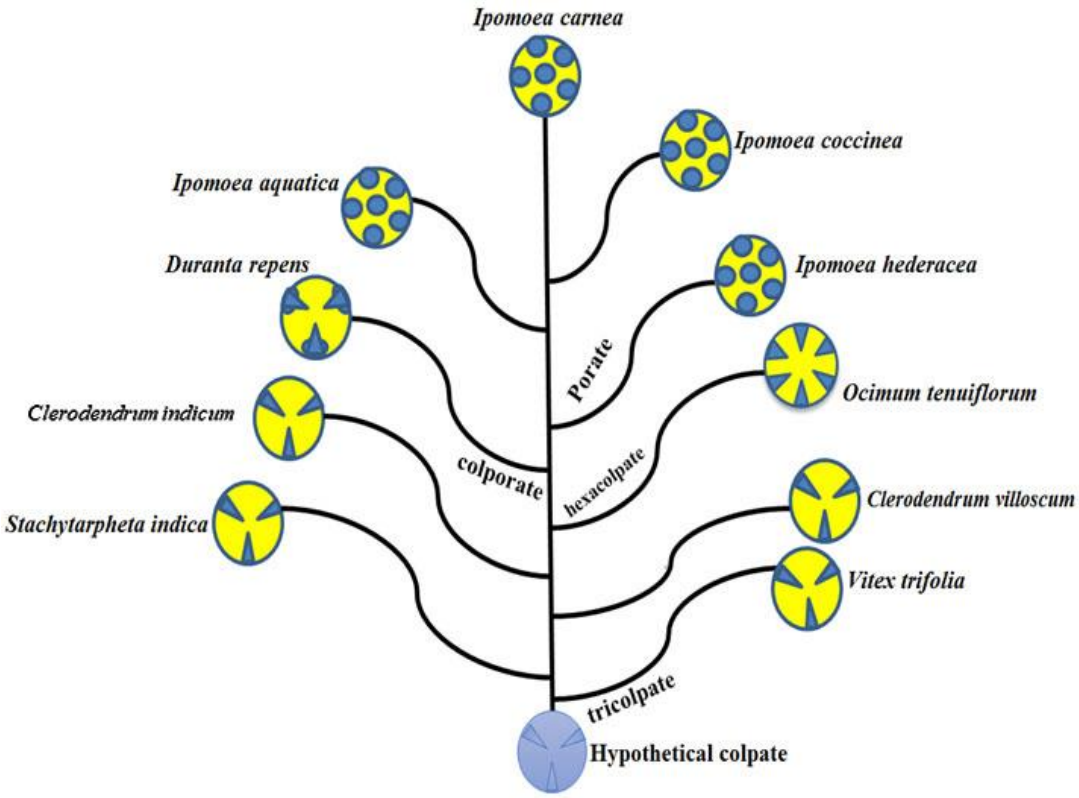


Fig. 13 Tentative scheme of evolution of pollen aperture in collected species

Discussion and Conclusion

In the present study, the pollen grain morphology of 10 species belonging to 6 genera of 3 families has been studied. These families were found a wide range of pollen morphology features. The microscopic observation under the light microscope revealed that the pollen's morphological character such as type of aperture, shape, size, and sculpture. Pollen production, fertility, and sterility were also conducted.

In the present study, different aperture types of pollen grains are found in all the species which include colpate, colporate, and porate. (Takhtajan, 1980) state that tricolpate is primitive and pantoporate is advanced character. In the present results, the pollen grains of colpate have occurred in all species of the family Lamiaceae. Colpate and colporate grains were found in the family Verbenaceae. In Convolvulaceae, all pollen grain was porate aperture. Therefore, Lamiaceae is a primitive character and Convolvulaceae is an advanced character.

The different shapes namely spheroidal, subprolate, prolate, and oblate were observed in the present study. The shapes of pollen grains are spheroidal in all species in Convolvulaceae and *Clerodendrum indicum*, in Lamiaceae. Pollen grains shape prolate in *Clerodendrum villoscum*, *Ocimum tenuiflorum* in Lamiaceae, and *Vitex trifolia* in Verbenaceae. *Duranta repens*, and *Stachytarpheta indica* in Verbenaceae.

According to this study, very large size pollen grains are found in Ipomoea aquatic, Ipomoea carnea, Ipomoea coccinea, Ipomoea hederacea, and *Clerodendrum indicum*. Pollen grains of large size are according to *Clerodendrum villoscum*, *Ocimum tenuiflorum*, and *Stachytarpheta indica*. Medium size pollen grains are found in *Duranta repens* and *Vitex trifolia*. The pori shape is circular and lolongate in all studies. Pori circular occurred in all species of Convolvulaceae Pori lolongate seen in Duranta repens. The rest species are not possessed pori. The colpi largest occurred in *Clerodendrum indicum*.

Exine sculptures are varied. Echinate sculptures are Convolvulaceae. Microechinate occurred in the genus *Clerodendrum*, distinctly reticulate observed in *Ocimum tenuiflorum* and *Vitex trifolia*. Obscurely reticulate occurred in *Duranta repens* and verrucate in *Stachytarpheta indica*. Sexine as thick as nexine was found in *Vitex trifolia* and the rest species were sexine thicker than nexine. The muri were simplibacculate and *lumina heterobrochate*. The size of muri and lumina are variable.

Pollen production has occurred in various numbers of all species. The largest number of *Clerodendrum villoscum* is 23588 grains and the smallest number of *Stachytarpheta indica* is 80 grains. In this study, the highest pollen sterility was found in *Stachytarpheta indica* and the lowest pollen sterility in *Ipomoea aquatica*. The highest pollen fertility has occurred in *Ipomoea aquatic* and the lowest pollen fertility in *Stachytarpheta indica*.

The pollination of the study species was anemophily and entomophily. Anemophily pollination has occurred in the family Convolvulaceae. The rest family Lamiaceae and Verbenaceae were entomophily pollination. The pollen characters have been important provide the identification and classification of flowering plants. So, it is hoped that the present research will support useful information for further studies on pollen morphology.

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