Chapter 7

Investigations on pollinaria and pollen grains
7. INVESTIGATIONS ON POLLINARIA AND POLLEN GRAINS

The palynological investigations in six experimental taxa comprised of study of two distinguishing features of the male gametophyte. As all the plant species belong to the sub family Cynanoideae (Asclapidiodeae) of the family Asclepiadaceae; where the pollens were aggregated in a specialized structure called pollinaria (pollinia), hence the investigations involve two fold studies of the pollinaria and the pollen grains.

At first the different morphological features of the pollinaria in each individual plant were studied separately. The measurements were taken with the help of ocular micrometer using a compound microscope (10×10x). Data were presented in Table No. X. For palynological studies polliniferous materials were collected from fresh flowers of healthy potted plants. The study of the pollen grains have been done after crashing the pollinia. Pollen preparations were made following the acetolysis method of Erdtman (1952).

Micromorphological characteristics associated with pollinaria of Calotropis gigantea Br.; Calotropis procera Br.; Calotropis acia Hamilt, Asclepias curassavica Linn., Hoya parasitica Wall. and Hoya arnottiana Wight. have been thoroughly investigated. These studies included pollinaria orientation, shape, colour, size, position of pellucid margin, pollinium attachment, size of the translator, caudicle wing and corpusculum. Measurements were taken with the help of ocular microscale using the low power of compound microscope.

The whole pollinarium is composed of three main parts – at the top of each anther cleft lies the chief part called corpusculum; where from toothed translator
Table No. X: Detail analysis of different morphological features of pollinaria in *Calotropis gigantea* Br., *Calotropis procera* Br., *Calotropis acia* Hamilt., *Asclepias curassavica* Linn., *Hoya parasitica* Wall. and *Hoya arnottiana* Wight.

<table>
<thead>
<tr>
<th>Name of the taxa</th>
<th>Shape of the pollinia</th>
<th>Colour of the pollinia</th>
<th>Attachment of the pollinia</th>
<th>Length of the pollinia μm±SE</th>
<th>Breadth of the pollinia μm±SE</th>
<th>Translator length μm±SE</th>
<th>Translator breadth μm±SE</th>
<th>Corpusculum length μm±SE</th>
<th>Corpusculum breadth μm±SE</th>
<th>Caudicle length μm±SE</th>
<th>Caudicle breadth μm±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. gigantea</em> Br.</td>
<td>Oblong pendulous stalked</td>
<td>Yellowish green, waxy</td>
<td>Basal</td>
<td>458.60 ±0.291</td>
<td>201.01 ±0.026</td>
<td>63.3 ±0.248</td>
<td>22.6 ±0.222</td>
<td>188.6 ±1.028</td>
<td>60.1 ±0.820</td>
<td>150.0 ±0.002</td>
<td>50.24 ±0.436</td>
</tr>
<tr>
<td><em>C. procera</em> Br.</td>
<td>Oblong pendulous stalked</td>
<td>&quot;</td>
<td>&quot;</td>
<td>450.60 ±0.226</td>
<td>200.03 ±0.076</td>
<td>58.4 ±0.262</td>
<td>20.2 ±0.028</td>
<td>169.02 ±0.024</td>
<td>53.7 ±0.007</td>
<td>110.22 ±0.072</td>
<td>43.04 ±0.028</td>
</tr>
<tr>
<td><em>C. acia</em> Hamilt</td>
<td>Oblong pendulous stalked</td>
<td>&quot;</td>
<td>&quot;</td>
<td>408.60 ±0.008</td>
<td>198.0 ±0.207</td>
<td>58.0 ±0.201</td>
<td>20.7 ±0.201</td>
<td>143.07 ±0.019</td>
<td>48.8 ±0.126</td>
<td>130.27 ±0.201</td>
<td>49.26 ±0.129</td>
</tr>
<tr>
<td><em>A. curassivica</em> Linn.</td>
<td>Obliquely ovoid, pendulous, stalked</td>
<td>Olive green</td>
<td>&quot;</td>
<td>320.0 ±0.126</td>
<td>125.02 ±0.101</td>
<td>75.5 ±0.027</td>
<td>25.26 ±0.002</td>
<td>260.70 ±0.126</td>
<td>120.2 ±0.112</td>
<td>180.02 ±0.025</td>
<td>72.4 ±0.024</td>
</tr>
<tr>
<td><em>H. parasitica</em> Wall.</td>
<td>Elliptical erect, pedicelled</td>
<td>Reddish brown</td>
<td>Ventral lateral</td>
<td>270.6 ±0.026</td>
<td>120.20 ±0.024</td>
<td>38.7 ±0.28</td>
<td>20.6 ±0.661</td>
<td>120.02 ±0.111</td>
<td>29.8 ±0.122</td>
<td>57.6 ±0.126</td>
<td>30.02 ±0.124</td>
</tr>
<tr>
<td><em>H. arnottiana</em> Wight</td>
<td>Elliptical, erect, pedicelled</td>
<td>&quot;</td>
<td>&quot;</td>
<td>296.2 ±0.028</td>
<td>131.02 ±0.025</td>
<td>35.8 ±0.026</td>
<td>20.7 ±0.078</td>
<td>134.09 ±0.124</td>
<td>35.1 ±0.220</td>
<td>65.6 ±0.029</td>
<td>37.06 ±0.022</td>
</tr>
</tbody>
</table>
Fig. No. 19 Pollinaria of *Calotropis gigantea* Br. (4×10x)
(a) Corpusculum, (b) Caudicle, (c) Pollinia, (d) Pellucid margin

Fig. No. 20 Pollen grain of *Calotropis gigantea* Br. (10×40x)

Fig. No. 21 Pollinaria of *Calotropis procera* Br. (4×10x)
(a) Corpusculum, (b) Caudicle, (c) Pollinia, (d) Pellucid margin

Fig. No. 22 Pollen grain of *Calotropis procera* Br. (10×40x)

Fig. No. 23 Pollinaria of *Calotropis acia* Hamilt. (4×10x)
(a) Corpusculum, (b) Caudicle, (c) Pollinia, (d) Pellucid margin

Fig. No. 24 Pollen grain of *Calotropis acia* Hamilt. (10×40x)
come out and its arms were called as caudicles; from each of which hang waxy masses of aggregated pollens called pollinia.

7.1 Morphological features of pollinaria

7.1.1 Pollinaria in *Calotropis gigantea* Br.

In *Calotropis gigantea* Br. pollinia were found pendulus, oblong, waxy and stalked. The colour of the pollinia have been observed as yellowish green; the attachment of the pollinia in all the three species of *Calotropis* under study was found on basal position. Pellucid germinating mouth was observed on the lower portion of the outer margin of the pollinia. In *Calotropis gigantea* Br. the caudicle was light brown in colour and the corpusculum was dark brown in colour and winged. The size of pollinia was observed as 458.60(±0.291) μm in length and 201.01(±0.026)μm in breadth. The measurement of translator was found 63.3(±0.248) μm and 22.6 (±0.222) μm as length and breadth respectively. The length and breadth of the corpusculum were observed as 188.6(±1.028) and 60.1(±0.820)μm respectively. In *Calotropis gigantea* Br. the length of caudicle was found as 150.0(±0.002)μm and breadth as 50.24(±0.436) μm. These measurements are shown in Table No. X; Fig. No. 19a, b, c, d.

7.1.2 Pollinaria in *Calotropis procera* Br.

In *Calotropis procera* Br. colour and attachment of the pollinia were observed to be similar as in *Calotropis gigantea* Br. The caudicle was also light brown in colour and the corpusculum was dark brown in colour. Position of pollinaria
Fig. No. 25 Pollinaria of *Asclepias curassavica* Linn. (4×10x)
(a) Corpusculum, (b) Caudicle, (c) Pollinia, (d) Pollen grain

Fig. No. 26 Pollen grain of *Asclepias curassavica* Linn. (10x40x)

Fig. No. 27 Pollinaria of *Hoya parasitica* Wall. (10x40x)
(a) Corpusculum, (b) Caudicle, (c) Pollinia, (d) Pellucid margin

Fig. No. 28 Pollen grain of *Hoya parasitica* Wall. (15x40x)

Fig. No. 29 Pollinaria of *Hoya arnottiana* Wight. (10x40x)
(a) Corpusculum, (b) Caudicle, (c) Pollinia, (d) Pellucid margin

Fig. No. 30 Pollen grain of *Hoya arnottiana* Wight. (15x40x)
was found basal and pellucid germinating mouth was observed on the lower portion of the outer margin of the pollinia. The length and breadth of the pollinia were observed as 450.60(±0.226)μm and 200.03(±0.076)μm respectively. The translator was found 58.4(±0.262)μm in length and 20.2(±0.028)μm in breadth. Corpusculum was measured as 169.02 (±0.024)μm and 53.7(±0.007)μm in length and breadth respectively. Like wise the caudicle was found 110.22(±0.072)μm in length and 43.04(±0.028)μm in breadth. Their measurements were shown in Table No. X and Fig. No. 21 a, b, c, d.

7.1.3 Pollinaria in Calotropis acia Hamilt.

In Calotropis acia Hamilt., the pollinaria were found as pendent, oblong, waxy and stalked. The colour of the pollinia were yellowish green and attachment of the pollinia were found on basal position. Pellucid germinating mouth was observed on the lower portion of outer margin of the pollinia. The length and breadth of the pollinia in Calotropis acia Hamilt. were observed as 408.60(±0.008)μm and 198.0(±0.207)μm respectively. Translator arms were found as 58.9(±0.202)μm long and 20.7(±0.201)μm wide tapering towards the corpusculum. The corpusculum length was found as 143.07 (±0.019)μm and 48.8(±0.126)μm as wide. The caudicle was 130.27(±0.201)μm long and 49.26(±0.129)μm wide. The Table No. X shows the different measurements of pollinaria in Calotropis acia Hamilt. (Fig. No. 23 a, b, c, d).
7.1.4 Pollinaria in *Asclepias curassavica* Linn.

In *Asclepias curassavica* Linn, the colour of pollinaria was slightly different and observed as olive green. They were waxy and obliquely ovoid in shape. Like *Calotropis* species, here also the pollinia were pendulus and stalked. The colour of caudicle and corpusculum were observed as light brown. The pollinium were attached on basal position to the translator arms and bear two pollinia on each anther. Over all 10 pollinia were found in the flower. The pellucid germinating mouth was observed on the inner margin. The measurement of pollinia were found as 320.00 (±0.126) μm long and 125.02 (±0.101) μm wide. The translator arms were 75.5 (±0.027) μm long and 25.26 (±0.002) μm wide. The length and wide of corpusculum were found as 260.70 (±0.126) μm and 120.2 (±0.112) μm respectively. Caudicles were 180.02 (±0.025) μm long and 72.4 (±0.024) μm wide. Caudicles were light brown and the corpusculum were dark brown in colour. The measurements of different features of pollinaria in *Asclepias curassavica* Linn. were shown in Table No.X (Fig. No. 25a, b, c, d).

7.1.5 Pollinaria in *Hoya parasitica* Wall.

Significant differences were observed in the features of pollinaria of two species of *Hoya*. Pollinia were erect, elliptical and pedicelled. These were waxy in nature and reddish brown in colour. Pollinium were attached ventrolaterally to the translator arm. Pellucid layer was observed covering the whole external margin of the pollinia. The translator arms were 38.7 (±0.028) μm long and 20.6 (±0.661) μm wide; where as pollinia were 270.6 (±0.026) μm long and 120.20 (±0.024) μm wide.
They were attached by the light brown caudicle to the dark brown corpusculum. The corpusculum length were observed as 120.02 (±0.011) μm and wide as 29.8(±0.122)μm. The caudicle were 57.6(±0.126)μm long and 30.02 (±0.124) μm wide. The size of pollinaria in *Hoya parasitica* Wall. is smaller than the species of *Calotropis* and *Asclepias* of the present investigation. The measurements of different features of pollinaria in *Hoya parasitica* Wall. are shown in Table no. X (Fig. No. 27 a, b, c, d).

7.1.6 Pollinaria in *Hoya arnottiana* Wight.

In *Hoya arnottiana* Wight. Pollinia were erect, elliptical and pedicelled like *Hoya parasitica* Wall. Colour of the pollinia was reddish brown. They were found to be waxy in nature. The pollinium was ventrolaterally attached to the translator arm. Distinct exterior pellucid margin was observed that covered complete external margin of the pollinia. The size at the pollinium was almost similar to that of *Hoya parasitica* Wall. It was 296.2(±0.028)μm long and 131.02(±0.025)μm wide. Translator arms were found as 35.8(±0.026)μm long and 20.7(±0.078) μm wide. Corpusculum length and breadth were found as 134.09(±0.124) μm and 35.1(±0.220)μm respectively. The measure of caudicle were found as 65.6(±0.029)μm long and 37.06(±0.022)μm wide. The measure of all the morphological features of both the species of *Hoya* under study were almost identical. However, pollinaria in *Hoya Parasitica* Wall. was found to be slightly smaller than those of *Hoya arnottiana* Wight. in all respect. Table No.X shows the
measurement of different features of pollinaria in *Hoya arnottiana* Wight. (Fig No. 29 a, b, c, d).

### 7.2 Morphological features of pollen grains

As the family *Asclepiadaceae* is a stenopalynous or eupalynous, Nair (1970) the pollens were constant or similar in all the species. Stenopalynous taxa are generally considered to be very natural and the experimental taxa bear such type of pollens which were constant in characteristics.

The pollen grains in all the species were found to be trizonocolporate with a spheroidal shape at polar view. The exine was more thicker than the intine. There were three distinct furrows with crescent shape apertures. The exine surface was filled up with minute dots. In *Calotropis gigantea* Br. the polar diameter (P) and equatorial diameter (E) were found as 32.60 (±0.242)μm and 31.91(±0.131)μm respectively. The P/E (Polar diameter: equatorial diameter) ratio percentage were 102.16. The colpa length was found as 22.60 (±0.162)μm. Pore diameter varied from 0.8 to 1.5 (±0.212)μm and the exine thickness was found as 2.72(±0.042)μm. The exine thickness at aparture were as 3.27(±0.279)μm. Table No. XI and Fig. No.20 show the different features of pollen grains in *Calotropis gigantea* Br.

In *Calotropis procera* Br. the shape and type were same to the *Calotropis gigantea* Br.; only the size were slightly differing. The pollen grains of *Calotropis procera* Br. were prolate, spheroidal and large in size. Polar diameter was 32.5(±0.222)μm and equatorial diameter was 30.5(±0.660)μm. The ratio of polar diameter and equatorial diameter were found as 106.88. The colpa length was
Table No. XI: Detail analysis of pollen morphology in *Calotropis gigantea* Br., *Calotropis procera* Br., *Calotropis acia* Hamilt., *Asclepias curassavica* Linn., *Hoya parasitica* Wall. and *Hoya arnottiana* Wight.

<table>
<thead>
<tr>
<th>Name of the Plants</th>
<th>Pollen size and shape</th>
<th>Polar range of diameter µm±SE</th>
<th>Range of equatorial diameter µm±SE</th>
<th>P/E x100</th>
<th>Colpa length µm</th>
<th>Exine thickness µm</th>
<th>Exine thickness at operature µm</th>
<th>Pore diameter µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. gigantea Br.</td>
<td>Prolate spherical and large size</td>
<td>32.60 (±0.242)</td>
<td>31.91 (±0.131)</td>
<td>102.16</td>
<td>22.60 (±0.162)</td>
<td>2.72 (±0.042)</td>
<td>3.27 (±0.272)</td>
<td>0.8 (±0.212)</td>
</tr>
<tr>
<td>C. procera Br.</td>
<td>&quot;</td>
<td>32.5 (±0.222)</td>
<td>30.5 (±0.660)</td>
<td>106.88</td>
<td>21.65 (±1.26)</td>
<td>2.40 (±0.272)</td>
<td>3.00 (±0.222)</td>
<td>0.8 (±0.521)</td>
</tr>
<tr>
<td>C. acia Hamilt.</td>
<td>&quot;</td>
<td>30.0 (±0.625)</td>
<td>28.2 (±0.248)</td>
<td>106.38</td>
<td>22.47 (±1.111)</td>
<td>2.22 (±0.129)</td>
<td>2.85 (±0.016)</td>
<td>1 (±0.026)</td>
</tr>
<tr>
<td>A. curassavica Linn.</td>
<td>Prolate elliptic, obtuse and large</td>
<td>25.20 (±0.005)</td>
<td>25.01 (±0.021)</td>
<td>100.75</td>
<td>20.50 (±0.026)</td>
<td>1.65 (±1.126)</td>
<td>2.53 (±0.017)</td>
<td>1 (±0.006)</td>
</tr>
<tr>
<td>H. parasitica Wall.</td>
<td>Prolate triangular, acute and medium size</td>
<td>12.12 (±0.026)</td>
<td>11.56 (±0.121)</td>
<td>104.84</td>
<td>10.60 (±0.076)</td>
<td>1.56 (±0.116)</td>
<td>2.20 (±1.220)</td>
<td>1.1 (±0.126)</td>
</tr>
<tr>
<td>H. arnottiana Wight</td>
<td>&quot;</td>
<td>12.96 (±0.055)</td>
<td>12.25 (±0.695)</td>
<td>105.79</td>
<td>10.65 (±0.028)</td>
<td>1.75 (±0.272)</td>
<td>2.25 (±1.726)</td>
<td>1.3 (±0.127)</td>
</tr>
</tbody>
</table>
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21.65(±1.26)μm and the pore diameter ranges between 0.8 to 1.5 (±0.521)μm. The exine thickness was observed as 2.40(±0.272)μm and the exine thickness with aperture was observed as 3.00(±0.222)μm. The exine surface was filled up with minute dots. Table No. XI and Fig. No. 22 show the measurements of different features of pollen grains of *Calotropis procera* Br.

The pollens of *Calotropis acia* Hamilt. were also same only the size were slightly differing. The polar diameter were found 30.0(±0.625)μm and equatorial diameter 28.2(±0.248)μm (Fig. No. 24). The *P/E ratio per cent were calculated as 106.38. The colpa length was found as 22.47(±1.111)μm and the range of pore diameter was found between 1.0 to 1.8(±0.026)μm. The exine thickness was measured as 2.22(±0.129)μm and the exine thickness at aperture was 2.85(±0.016)μm. The pollens of *Calotropis acia* Hamilt. were large prolate and spheroidal in size and exine was thick like the other two species of *Calotropis* and sculptured. The Table No. XI and Fig. No. 24 show the data on different pollen measurements.

Pollens of *Asclepias curassavica* Linn. were prolate spheroidal but structure were some what smaller. The polar diameter (P) found as 25.20(±0.005)μm and equitorial diameter (E) were as 25.01 (±0.021)μm. The P/E ratio percentage were found as 100.75. Colpa length was measured as 20.5(±0.026)μm and the range of pore diameter was found in between 1 to 1.5 (±0.006)μm. The exine thickness was found as 1.65(±1.126)μm and at aperture level the value was measured as

\[ * \text{P/E} = \frac{\text{Polar diameter}}{\text{Equatorial diameter}} \]
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2.53(±0.017)μm. The measurement of different features of pollens of *Asclepias curassavica* Linn. are mentioned in the Table No. XI and shown in Fig. No. 26.

The pollens of *Hoya parasitica* Wall. were comparatively much smaller. They were also prolate spheroidal in shape. The surface view of mature grains were triangular with broadly rounded apices. The measurement of polar diameter (P) and equatorial diameter (E) were found as 12.12(±0.026)μm and 11.56(±0.121)μm respectively. The P/E% (percentage) was calculated as 104.84. Colpa length was 10.60(±0.076)μm and pore diameter was found as 1.1 to 2.0(±0.126)μm. The exine thickness was found as 1.56(±0.116)μm and exine thickness at aperture region was observed as 2.20(±1.220)μm.

*Hoya parasitica* Wall. showed smaller dimensions in size in respect of all the criteria of pollen as compared to other species studied. The recorded data are presented in the Table No. XI. Pollen grain is shown in Fig. No. 28.

In case of *Hoya arnottiana* Wight. the pollen shape size and other characters were like *Hoya parasitica* Wall. only size and measurements were slightly differing. Polar diameter (P) and equatorial diameter (E) were found as 12.96(±0.055)μm and 12.25(±0.695)μm respectively and their ratio per cent were found as 105.79. Colpa length and the range of pore diameter were observed as 10.65(±0.028)μm and 1.2 to 2.3(±0.127)μm respectively. The exine thickness was measured as 1.75(±0.272)μm and exine thickness at aperture region was found as 2.25(±1.726)μm. All the recorded data are presented in the Table No. XI. Pollen grain is shown in Fig. No. 30.