

Pollen Morphological and Taxonomical Studies in Some Members of Family Euphorbiaceae

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Abstract

Euphorbiaceae, commonly known as spurge family is largest among the families of the order Malpighiales. Euphorbiaceae is diverse family having great variability in taxonomical and pollen morphological characters. The study deals with the taxonomy and pollen morphology of some species of Euphorbiaceae i.e. Euphorbia milii, Des Moul, Euphorbia pulcherrima, Willd. Ex Klotzsch, Jatropha integerrima Jacq. and Ricinus communis, L. For each species, taxonomical characters like inflorescence, flower, bract colour, fruit type and size, seed and floral formula were described. For Pollen studies shape of pollen, number and type of aperture, size of colpi, thickness of exine and pollen wall ornamentation were included in four plants. During studies smallest pollen were found in Euphorbia pulcherrima Willd. Ex Klotzsch and largest pollen size was found in Jatropha integerrima Jacq. The aperture in three species were tricolpate type but in case of J. integerrima aperture were absent. Exine of E. milii was found to be thickest and J. integerrima was thinnest. Psilate ornamentation of exine is found in three plants but in case of J. integerrima Jacq. ornamentation is reticulate. The taxonomical parameters are important for identifying the plant species. Maximum pollen production was observed in R. communis L. and minimum in E. pulcherrima Willd. Ex Klotzsch. Maximum viable pollen percentage was recorded in Ricinus communis and minimum viable pollen percentage in Euphorbia milii. The variable structure of pollen grains is necessary for the delimitation of species and hence important for taxonomical and biodiversity conservation purposes.

Keywords

Taxonomy, Morphology, Aperture, Exine, Euphorbiaceae, Pollen.

Introduction

Pollen are haploid unit that evolve as a result of the phenomenon of meiosis, and as such are the repository of hereditary characters of the relevant taxa. The morphological characters of the pollen are contained in the exine and are considered to reflect, to a fair degree, the pattern of origin and evolution of the plants, the grains belong to. It is on this principal that evolutionary deliberation of pollen is based, and which forms the subject matter of the present treatise (Nair, 1974). The latex of many Euphorbia species is used medicinally as a purgative, anti-diarrhoealand antibacterial agents. Species are used to treat boils, warts, wounds and other skin disorders. The leaves of several Euphorbia species are used to treat asthma. *Euphorbia pulcherrima* Willd. Ex Klotzschis grown as garden ornamentals all over the world (Thi Thi Htun, 2009)

Euphorbia milii is included in IUCN list of threatened species, according to T. Haevermans (2004).

Scientific Classification of Euphorbiaceae:

Kingdom – Plantae,

Division- Magnoliophyta,

Class – Magnoliopsida

Subclass-Rosidae,

Order-Malpighiales,

Family- Euphorbiaceae.

By analysing pollen from well dated sediment cores, scientists can obtain records of changes in vegetation going back even millions of years. Pollen study is important medically. The main objective of this work will be to study pollen morphology for taxonomical and biodiversity conservation purposes.

Materials and methods

The plant material of *Euphorbia milii*, Des Moul *E. Pulcherrima* Willd. Ex Klotzsch and *Jatropha interrigma* Jacq. and *Ricinus communes* L. were randomly collected from R.G. (P.G.) college Meerut, department of Botany. The flower buds were preserved in FAA. Acetolysis method was used to isolate pollen. The pollen grains from flower bud before anthesis were prepared for standard Acetolysis method proposed by Erdtman (1952). For light microscopy, the pollen grains were mounted in glycerine jelly. Phase contrast microscope was also used to analyze the pollen. The values given were based on the measurements of 25 pollen grains.

Morphological observations, including the pollen structure and pollen size (um) were taken with the help of light microscope power of 40X pollen measured with the help of ocular meter. The study was based on NPC system of pollen classification. We calculated the pollen size (polar axis and equatorial axis), pollen aperture, number, exine thickness and ornamentation. The pollen terminology was based on Punt et al. (1999) and NPC System of pollen morphological characters by P.K.K. Nair (1970). To estimate the number of pollen per flower spore/cell counting method was used. Pollen production was measured with the help of haemocytometer. To observe pollen viability, pollen were stained in acetocarmine and viable pollen were counted. Taxonomical characters were analyzed in the laboratory under light microscope and phase contrast microscope. The pollen structure, size, shape, aperture number and size, exine thickness and ornamentation is observed with the help of light microscope. Pollen grains are released as monad.

Observations

Taxonomy of four plants studies

1. *Euphorbia milii* Des Moul.

It is a succulent climbing shrub, Height 0.7-1.0 metre, Stem bear slender spines up to 3 cm long, root is tap root, leaves are ovate up to 3.5 cm long and 1.5 broad, inflorescence is cyathium. Flower is actinomorphic. A pair of petal like bracts of dark pink colour are present. Five nectary are present on the cyathium. Cyathium is a cup like involucre bearing male and female flower. Male flower consist of 8-10 stamen is long with slender filament, bifid stigma and female flowers is syncarpous and tricarpellary ovary with three bifid stigmas. Stigma are hairy. Placentation is axile. One ovule is present in each ovary.

Fruit is a capsule (plate 1, fig. 1 & 2)

Male flower - Br, K0, C0, A8-10, G0.

Female flower - Br, K0, C0, A0, G(3).

2. *Euphorbia pulcherrima* Willd. Ex Klotzsch

It is a shrub. Height 5-10 metre. Root is tap. Stem is woody, green branched, cylindrical solid milky latex. Leaves are simple alternate, stipulate, petiolate, ovate, entire margin, acute apex, lanceolate stipules, caduceus, unicostate and reticulate venation. Inflorescence is cyathium arranged in axillary clusters. Flower pedicellate, bracteate and unisexual. Central female flower surrounded by many male flowers and all are enclosed in a cup shaped involucre of bracts. Male flower is naked with no perianth, bracteate, represented by a stamen. Stamen is 1 stalked, filament long, jointed anthers 2 celled. Female flower is naked with no perianth. It is bracteate. Gynoecium is tricarpellary, syncarpous, ovary superior, trilobular and axile placentation. Pistil represents the entire female flower, styles three, stigma 3, each bifid. (Plate 1)(fig. 3 & 4)

Male flower - Br, K0, C0, A1, G0.

Female flower - Br, K0, C0, A0, G(3).

3. *Jatropha integerrima* Jacq.

Habit is a tall shrub, branches green and brown with prominent lenticels. Height is up to 6 m. Stem is woody green, hard and cylindrical. Leaves are alternate stipulate, fiddle cordate shape, where there are 3-4 glandular teeth on each side, smooth dark green above and pale or purplish tinged below. Inflorescence terminal corymbose cymes. Flowers are monoecious. Male flowers possess small calyx small cup shaped, imbricate, 5 lobed purplish-red. Petals 5 scarlet red, or pink, twisted, stamens are red, 8-10 in number, monodelphous, 4-5 short, anthers reddish yellow, hastate. Disk 5 lobed female flower. Petals and sepals same as in male flowers, ovary 3-celled, styles 3, bifid, recurved. Fruits is capsule purplish green in colour. Seeds are 3. Flower formula (plate 1 fig. 5 & 6)

Male flower - Br, K0, C0, A8-10, G0

Female flower - Br, K0, C0, A0, G(3)

4. *Ricinus communis* L.

Ricinus communis L. is a tall glabrous annual or sometimes a small perennial tree. Root is tap root. Stem erect herbaceous and woody. Leaf is alternate, broad palmately lobed and veined, lobes usually 7-9, simple serrate, gland-dotted with green or reddish color. Inflorescence is a terminal raceme. Flower is unisexual, monoecious, upper female, lower male, apetalous, hypogynous.

Perianth is present. Perianth is membranous, gamophyllous splitting into 3-5 valvate segments, green. Stamens are 5, rarely numerous, arranged upon 5-8 repeatedly branched partially connate filaments, anthers globose, 2-celled, divergent. Pistilode is absent. Female flower is perianth. Perianth is gamophyllous, 3-5, enclosing the ovary, spatheous, splitting along one side, caduceus. Stamines is absent. Gynoecium is tricarpyllary syncarpous ovary superior, covered with warty prickles, tricolar with axile placentation, one ovule in each locule, styles three short or long, spreading often very large entire, 2-fid or 2-partite, feathery or papillose, red stigmas. Fruit is schizocarpic, capsule or regma with spines, splitting into three cocci, each containing one seed. Seeds are oblong, smooth, testa crustaceous and mottled with a conspicuous knob-like caruncle. (plate 2 fig. 6 & 8)

Flower formula.

Male flower- Br, P5, A5, G0.

Female flower - Br, P3-5, A0, G(3)

Pollen morphology

Pollen morphological characters four species of family Euphorbiaceae were investigated under light and phase contrast microscope.

In *Euphorbia milii* polar axis of pollen is 20-45 μm and equatorial axis is 19-40 μm . In case of *Euphorbia pulcherrima* polar axis pollen is 10-15 μm and equatorial axis is 10-14 μm . In *Jatropha integerrima* polar axis pollen is 42-60 μm and equatorial axis is 40-55 μm . In case of *Ricinus communis* polar axis pollen is 16-19 μm and equatorial axis is 15-19 μm . In case of *Euphorbia milii* length of colpus is 15-18 μm and width is 1.6-2 μm in *Euphorbia pulcherrima* length of colpus is 5-7 μm and width is 0.5-1 μm . In *Jatropha integerrima* colpus are not found. In *Ricinus communis* length of colpus is 2.5-3.2 μm and width is 1.8-2.5 μm but in case of *Jatropha integerrima* aperture is absent.

Exine is 0.5-1.6 μm in thickness in *Euphorbia milii*. Exine is 0.7-1.4 μm in thickness in *E. pulcherrima* is 0.8-1.0 μm in thickness in *Jatropha integerrima*. Exine is 0.8-1.2 μm in thickness of *Ricinus communis*. Pollen is tricolpate and exine ornamentation is psilate type in all three species studied but in case of *Jatropha inegrrima* exine is reticulate. (Table 1) Pollenkitt a yellow sticky substance is seen around the pollen grain.

Pollen germination

Through cotton blue stain 15.38% pollen germination was in *Euphorbia milii*, 6.56% in *Euphorbia pulcherrima*, 18.26% in *Jatropha integerrima* and 40.98 in *Ricinus communis*.

| S.No. | Name of Species | Shape of Pollen | Pollen Size | | Size of colpi | | Exine thickness μm | Ornamentation | No. of aperture |
|-------|---|--------------------|--------------------------|-------------------------------|----------------------|---------------------|-------------------------------|---------------|-----------------|
| | | | Polar Axis μm | Equatorial Axis μm | Length μm | Width μm | | | |
| 1 | <i>Euphorbia milii</i> , Des Moul | Triangular Prolate | 20-45 | 19-40 | 15-18 | 1.6-2 | 0.5-1.6 | Psilate | Tricolporate |
| 2 | <i>Euphorbia pulcherrima</i> Willd. Ex Klotzsch | Prolate Spheroidal | 10-15 | 10-14 | 5-7 | 0.5-1 | 0.7-1.4 | Psilate | Tricolporate |

| | | | | | | | | | |
|---|-----------------------------------|--------------------|-------|-------|---------|---------|---------|------------|--------------|
| 3 | <i>Jatropha integerrima</i> Jacq. | Prolate Spheroidal | 42-60 | 40-55 | - | - | 0.8-1.0 | Reticulate | Absent |
| 4 | <i>Ricinus communis</i> L. | Prolate Spheroidal | 16-19 | 15-17 | 2.5-3.2 | 1.8-2.5 | 0.8-1.2 | Psilate | Tricolporate |

Table- 1 showing pollen morphology of four species of family Euphorbiaceae

Pollen germination

Through cotton blue stain 15.38% pollen germination was in *Euphorbia milii*, 6.56% in *Euphorbia pulcherrima*, 18.26% in *Jatropha integerrima* and 40.98 in *Ricinus communis*.

| S. No. | Name of species | Total number of pollen | Number of pollen germinate | Standard deviation | Percentage |
|--------|---|------------------------|----------------------------|--------------------|------------|
| 1. | <i>Euphorbia milii</i> , Des Moul | 156 | 24 | 14.43 ± 7.184 | 15.38% |
| 2. | <i>Euphorbia pulcherrima</i> Willd. Ex Klotzsch | 259 | 17 | 6.782 ± 4.094 | 6.56% |
| 3. | <i>Jatropha integerrima</i> Jacq. | 104 | 19 | 80.8 ± 9.68 | 18.26% |
| 4. | <i>Ricinus communis</i> L. | 61 | 25 | 58.54 ± 33.20 | 40.98% |

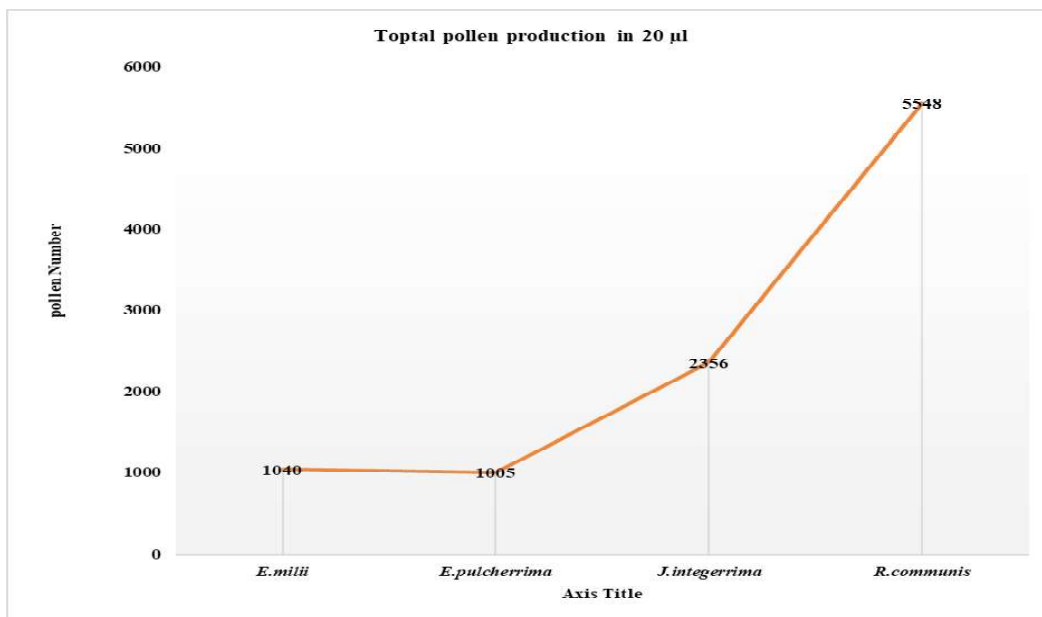
Table2 showing pollen germination

Pollen production

Number of pollen is found to be 1040 on an average in *E. milii*. Number of pollen is found to be 1005 on an average in *Euphorbia pulcherrima*. Number of pollen is found to be 2356 on an average in *Jatropha integerrima*. Number of pollen is found to be 5548 on an average in *Ricinus communis*. (Table – 3) (Graph - 1)

Table (3) showing Pollen production.

| S. No. | Name of species | Number of pollens in 20 µl |
|--------|---|----------------------------|
| 1. | <i>Euphorbia milii</i> , Des Moul | 1040 |
| 2. | <i>Euphorbia pulcherrima</i> Willd. Ex Klotzsch | 1005 |
| 3. | <i>Jatropha integerrima</i> Jacq. | 2356 |
| 4. | <i>Ricinus communis</i> L. | 5548 |



Graph 1 – showing Pollen production

Pollen viability

To test pollen viability 1000 pollen were taken of each species. In case of *Euphorbia millii* 7.9% viable pollen were observed were 79, in *Euphorbia pulcherrima* 29.4%, in *Jatropha integerrima* 15.1% and in *Jatropha integerrima* 45.2% viable pollen were observed.(Table- 4)

Table (4) - Pollen viability test in acetocarmine stain.

| S.No. | Name of species | Viable Pollen | Total pollens | Standard deviation(SD) | Percentage% |
|-------|----------------------------|---------------|---------------|------------------------|-------------|
| 1. | <i>Euphorbia milii</i> | 79 | 1000 | 3.16±1.53 | 7.9% |
| 2. | <i>E. pulcherrima</i> | 294 | 1000 | 21±11.46 | 29.4% |
| 3. | <i>Jatropha interrigma</i> | 151 | 1000 | 3.02±1.47 | 15 |
| 4. | <i>Ricinus communis</i> | 452 | 1000 | 23.78±24.50 | 45.2% |

Plate 1–Pollen morphology

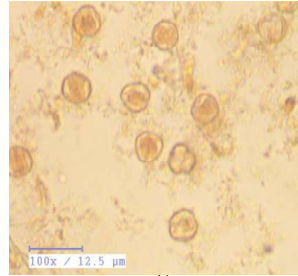


Plate 2 - Pollen morphology



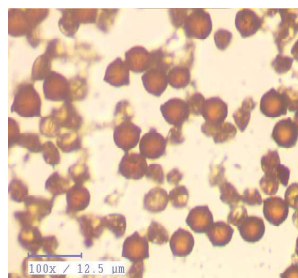
5



6



7



8

Plate 1, Fig- (1)- Showing flower of *E.milii*. Des Moul (2)-showing aperture & shape of pollen of *E. milii* Des Moul.(3)- Showing flower of *E.pulcherrima*, Willd. Ex Klotzsch(4)- Showing aperture & shape of pollen of *E. pulcherrima*, Willd. Ex Klotzsch.

Plate 2, (5)- Showing flower of *Jatropha integerrima*, Jacq. (6)- Showing shape of pollen of *Jatropha integerrima* Jacq. (7) - Showing flower of *Ricinus communis* L. (8). H- Showing aperture & shape of pollen of *Ricinus communis* L. [pollen pictures from phase contrast microscope].

Plate 3 – Pollen germination in vivo

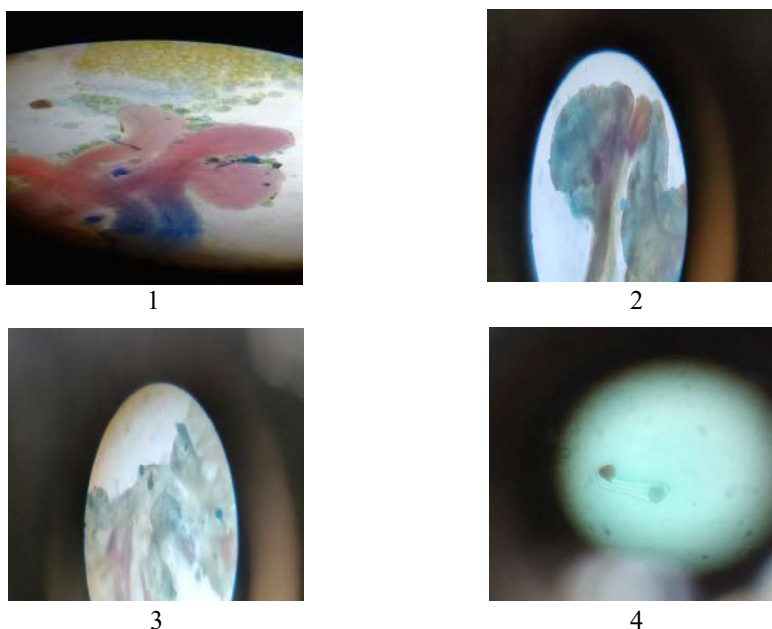


Fig. pollen germination in(1) *Euphorbia milii*(2) *E. pulcherrima*(3) *J.integerrima* and (4) *Ricinus communis*.

Results and conclusion

Pollen morphology characters of four species of family Euphorbiaceae were investigated. Pollen size is largest in *Jatropha integerrima* and smallest in *Euphorbia pulcherrima*. All three species like *Euphorbia pulcherrima*, *Jatropha integerrima* and *Ricinus communis* pollen grains are prolate and spheroidal, but *Euphorbia milii* pollen are triangular and prolate. Exine ornamentation was found to be psilate in all three species *Euphorbia. milii*, *Euphorbia pulcherrima* and *Ricinus communis*. but reticulate type of ornamentation was observed in *Jatropha. integerrima*.

The unisexual flowers are present in all species viz *E. Milii*, Des Moul, *E. pulcherrima*, Willd. Ex Klotzsch, *J. integerrima* Jacq. and *R. communis*. In all four species studied and nectary is present and perianth, Plant taxonomical characters can be helpful in identification of genera and species.

In *Euphorbia pulcherrima*, Willd. Ex Klotzsch *J.Integerrima Ricinus communis*,L. the aperture in all three species studied are tricolporate but in *J.integerrima* aperture is absent. The exine ornamentation was psilate in all three species except *J.integerrima* where it is reticulate type.

The pollen in *E. milii* Des Moulare medium sized, small in *E. pulcherrima* Jacq. and *Ricinus communis* L. and large in *Jatropha integerrima*. Jacq. Pollen size variation is among genera as well as species. The pollen morphology is one of the significant tools in solving some taxonomical problems in the family, genera or species level. It can be concluded that pollen morphology cannot be useful for taxonomical classification but it can be useful to differentiate between species belonging to same genera. According to Paul, E. et al. (2014) pollen analytical studies of these species showed a lot of variations in the types of apertures, shapes, exine pattern, pollen size, colpi length and width.

Pollen kitt a yellow sticky substance is seen around the pollen grain. Presence of pollen kitt shows the entomophilous nature of the plant. Pollen kitt help in insect pollination, due to its sticky nature pollen is attached to the body of the insects.

Maximum pollen germination by cotton blue stain is observed in *Ricinus communis* is 40.98% and minimum in *Euphorbia pulcherrima* is 6.56%. Pollen production was maximum in *Ricinus communis* (L.) and minimum in *Euphorbia pulcherrima* Willd. Ex Klotzsch. Maximum viable pollen were observed in *Ricinus communis* and minimum viable pollen in *Euphorbia milii*. Pollen production was maximum in *Ricinus communis* (L.) and minimum in *Euphorbia pulcherrima* Wild Ex Klotzsch. This investigation is significant in identification of pollen of different and same taxa and for conservation of biodiversity.

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