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PALYNOLOGICAL STUDY OF SOME CULTIVATED SPECIES OF GENUS *HIBISCUS* FROM NORTH WEST FRONTIER PROVINCE (N.W.F.P.) PAKISTAN

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Abstract

Pollen morphology of four species and three cultivars belonging to genus *Hibiscus* of family Malvaceae from North West Frontier Province (N.W.F.P.) of Pakistan were examined by light and scanning electron microscope. Pollen morphology of the family is fairly uniform. Pollen grains are generally radially symmetrical apolar, mostly spheroidal to oblate-spheroidal, pantoporate or polyporate. Tectum uniformly echinate, medium to finely perforated, or punctate with granules or scabrae in between spines.

Introduction

The family Malvaceae comprising of c. 88 genera and c. 2, 3000 species are distributed in tropical, subtropical and temperate regions (Willis, 1973; Mabberley, 1987). In Pakistan it is represented by 19 genera with 94 specific and intraspecific taxa (Abedin, 1979). Palynologically, Malvaceae is stenopalynous family and pollen characters in this family are more or less uniform. Culhane & Blackmore (1988) divided the family into six pollen type, based on number of apertures, grains diameter and spinular morphology. This is also supported by Christensen (1986) that the generic delimitation based on pollen morphology is difficult in this family. However, Saad (1960) considered that that the pollen morphology in the family Malvaceae is quite distinctive which could apparently distinguish between the genera.

Whereas pollen morphology of the family Malvaceae has been studied by Master (1874), Lang (1937), Sayeeduddin *et al* (1942), Erdtman (1952), Nair (1958, 1960, 1962), Saad (1960), Chadhuri (1965), Fryxell & Hashmi (1971) and El Naggar (2004), but the most comprehensive study of the Malvaceae pollen is that of Christensen (1986). In Pakistan, pollen morphology of only few genera of this family has been studied by Siddiqui *et al* (1982, 1984), Tahavi (2000), Hussain (2004) using light microscope. Perveen *et al* (1994; 2007) provided pollen morphology of 42 species belonging to 12 genera and 12 species belonging 3 genera, respectively, from Pakistan using light and scanning electron microscope. In present studies an attempt has been made to provide complete information of pollen morphology of the genus *Hibiscus* growing wild and cultivated as ornamental in N.W.F.P. Pakistan. Present pollen data is based on pollen morphology of four species and three cultivars of the genus *Hibiscus.*

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Materials and Methods

Pollen samples were obtained from the wild plants as well as from cultivated ornamentals at various localities of North West Frontier Province (N.W.F.P.) Pakistan. The pollen grains were prepared for light and scanning electron microscopy by the standard methods described by Erdtman (1952). For light microscope the pollen grains were mounted in stained glycerine jelly and observations were made with a Wetzlar Research Microscope, under (E 40, 0.65) and oil immersion (E100, 1.25) using a 10X eye piece. For SEM studies pollen grains suspended in a drop of water and indirectly transferred with a fine pipette to a metallic stub using double sided cellotape and coated with gold, in a sputtering chamber (Ion-sputter JFC-1100) with coating restricted to 150A^{o}. SEM examination was carried out on a Jeol microscope JSM-T200. The measurements were based on 15-20 readings from each specimen. Various pollen characters viz. pollen class, shape, size, aperture, sporoderm stratification, and exine ornamentation were studies following Huang (1972). Following specimens were investigated *Hibiscus mutabilis* Linn., *Hibiscus rosa- sinensis* Linn. (Common shoe flower), *Hibiscus rosa- sinensis* Linn. (Double), *Hibiscus rosa- sinensis* Linn. (Cooperi), *Hibiscus schizopetalus* (Dyer) Hook. f. and *Hibiscus syriacus* Linn. The terminology used is in accordance with Erdtman (1952), Kremp (1965), Faegri & Iversen (1975), Huang (1972) and Walker and Doyle (1975).

Results

***Hibiscus mutabilis* Linn. (Plate I, Fig.1, 2 and Plate III, Fig. 13, 14)**: Pantoporate, spherical, radial symmetry in polar view, amb spherical to sub spherical. Pollen diameter 133 (148) 154 µm, exine thickness 4 (5) 6 µm, echini height 9 (12) 14 µm, echini base 4 (5) 6 µm wide, echini apexes are 21 (29) 37 µm apart, echini bases are 12 (15) 21 µm apart and pore diameter 3 (4) 5 µm. Number of spines 38 (48) 56. Echinate, spine dimorphic, large and blunt, short with rounded apex and basal cushion. Surface seems to be rough and show number of ridges upon which spines are located. The space between ridges appears to be chambered. Tectum shows variation. it is granulate to striate _ regulate. Central ridges with their spines are appressed to the center of pollen grain while outer ridges with their spines lie above this. Due to this surface appear to be rough.
Voucher No. NB/003/S-05

***Hibiscus rosa-sinensis* Linn. (Common shoe flower) (Plate I, Fig. 3, 4):** Pantoporate, spherical to globose, isopolar, radial symmetry in polar view and bilateral in equatorial view, circular to oval. Pollen diameter of 143 (165) 190 µm, exine 3.5 (4.6) 4.8 µm thick, echini 9 (14) 21 µm high, echini base 5 (7) 9 µm wide, echini apexes 25.3 (37.4) 53 µm apart, inter echini base distance 18 (25) 32 µm, pore diameter 5 (10) 14 µm. Number of spines 24 (35) 46. Number of pores 28 (12) 16. Echinate, echini large in size and spaced widely, easy to count and distinct with blunt apex. Arranged regularly. Central spines which form a ring are somewhat different. Tectum is finely reticulate. Echini strong and resistant to acetolysis, dimorphic with apex blunt, rounded and bifurcated. In some spines the apex is as much wide as base. Tectum perforated and densely granulated between spines. Aperture large and clear.
Voucher No. NB/017/S-05

Table 1: Pollen analysis of genus *Hibiscus*

Sample name	Shape	Size µm	Exine thickness	Tectum	Echini apex	Pore No.	No. of Echini	Class
Hibiscus mutabilis	Spherical	142	5um	Chambered	Blunt & Bifurcated	7	48	1
Hibiscus rosa sinensis (Common)	Globose	165	4.8um	Punctuate	Blunt	13	35	1
Hibiscus rosa sinensis (Double)	Globose	133	6um	Granulate	Rounded	12	35	1
Hibiscus rosa sinensis(Cooperi)	Globose	124	4.6um	Psilate	Blunt	8	29	1
Hibiscus schizopetalous	Spherical	124	3um	Granulate	Blunt	15	34	1
Hibiscus syriacus	Spherical	169	5.2um	Psilate	Blunt/ Acute	8	31	2

Class 1 = Pantoporate, **Class 2** = Polyporate

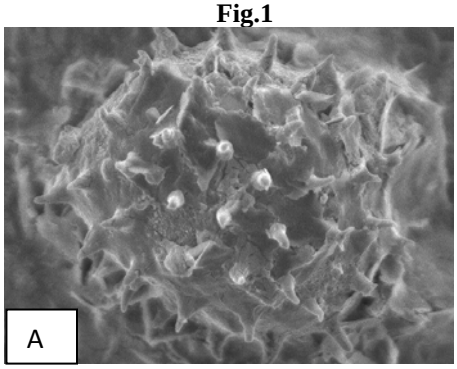
***Hibiscus rosa-sinensis* Linn. (Var. Double) (Plate I, Fig.5, 6 and Plate III, Fig. 15, 16):** Pantoporate, spheroidal, isopolar, radial symmetry in polar view and bilateral in equatorial view. Pollen diameter 131 (133) 156µm, exine 5 (6) 9 µm thick, echini 13 (14) 16 µm high, echini base 5 (7) 8 µm wide, echini apexes are 25 (35) 41 µm apart, echini bases are 14 (19) 25 µm apart and pore diameter 5 (6) 7 µm. Number of spines 27 (35) 42, Number of pores 7 (12) 21. Pores large, scattered, circular to oval, exine thick. Echinate, spines monomorphic with blunt to rounded apex, without basal cushion, spaced widely, directed away from centre, mostly curved, depression were also observed. Tectum perforated, punctuate to granulate. All spines including marginal and central are alike.
Voucher No. NB/006/S-05

***Hibiscus rosa-sinensis* Linn. (Var.Cooperi)** (Plate II, Fig.7, 8 and Plate III, Fig. 17, 18): Pantoporate, spheroidal, Pollen diameter 108 (124) 161 µm, exine 2.3 (5) 6 µm thick, echini 7 (12) 14 µm high, echini base 5 (6) 7 µm wide, spine apexes are 18 (30) 37µm, echini bases are 14 (17) 23µm apart, pore diameter 4 (5) 7µm. Number of spines 21 (29) 35, number of pores 6 (8) 10. Echinate, spines with bulbous or swollen apex, spaced, no basal cushion, central spines not differ too much and hence monomorphic. Tectums show ridges and imbalanced surface but not granulate or punctuate.
Voucher No. NB/022/S-05

***Hibiscus schizopetalous* (Dyer) Hook. f. (Plate II, Fig.9, 10 and Plate IV, Fig. 19, 20):** Pantoporate, spheroidal, Pollen diameter 106 (124) 168µm, exine thickness 2.3 (3) 5µm, echini height 9.2 (12) 14 µm, echini base 6.2 (7) 9.2 µm wide, echini apexes are 25 (33) 39 µm apart, echini bases are 16 (21) 35 µm apart and pore diameter2.3 (3) 7 µm. Number of spines 33(34)43, number of pores 10(21)28, Echinate, spines dimorphic large with curved and blunt apex, short one provided by basal cushion. Tectum perforated but at the margin of pores tectum is very much granulate so that pores not clear enough. Surface between spines is also granulate. Spine arrangement in this case also vary. Spines are spaced widely hence easy to count and measure.
Voucher No. NB/028/S-05

Plate-I

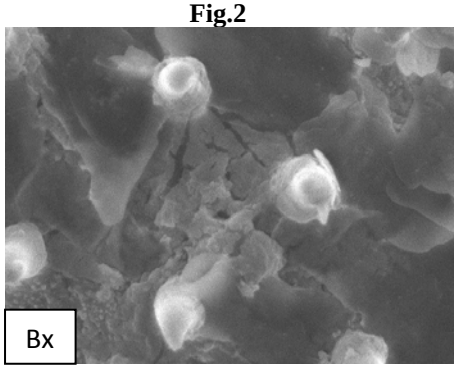
Fig.1



Bar: 25.00µm Mag: x800 *Hibiscus mutabilis*

Entire Pollen View

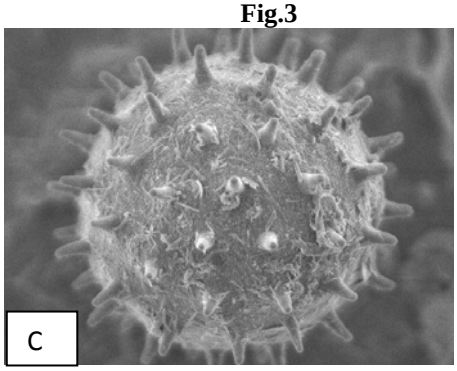
Fig.2



7.41µm Mag: x2700

Exine Pattern

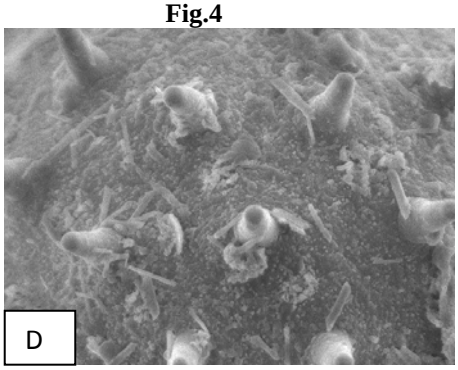
Fig.3



:28.57µm Mag: x700 *Hibiscus rosa-sinensis*

Entire Pollen View

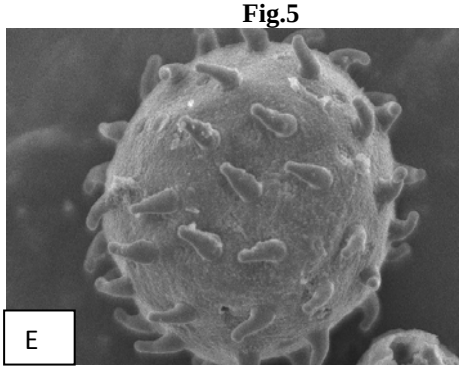
Fig.4



:12.50µm Mag :x1600

Exine Pattern

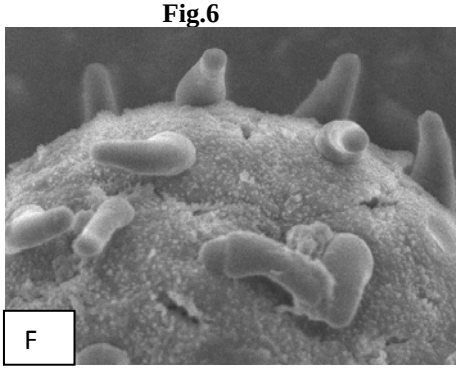
Fig.5



:23.53µm Mag: x850 *Hibiscus rosa-sinensis* (Double)

Entire Pollen View

Fig.6

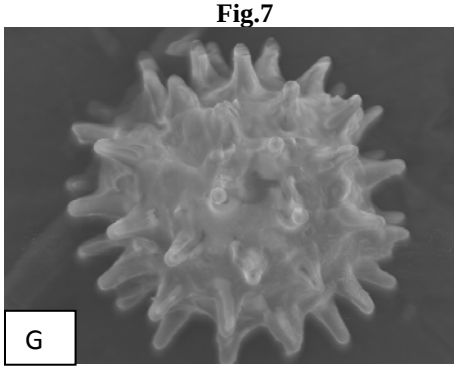


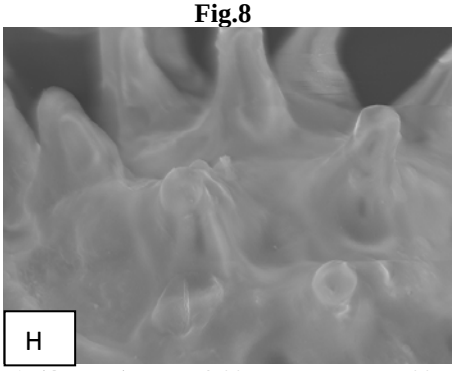
11.11µm Mag: x1800

Exine Pattern

Plate-II

Fig.7 **Fig.8**

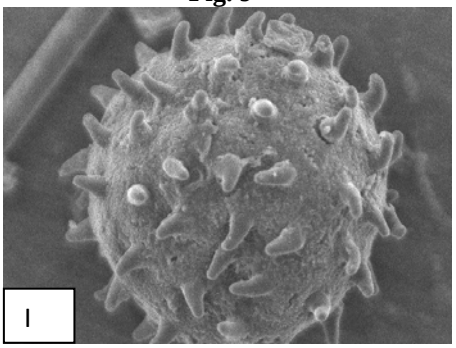


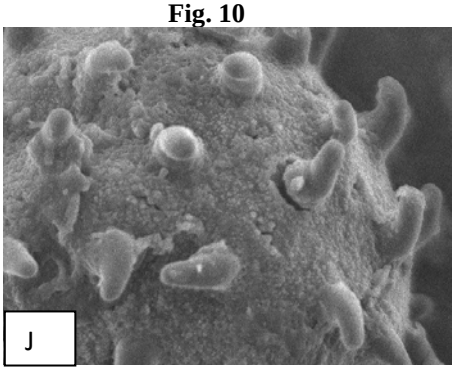


_____:22.22µm Magx9000 *Hibiscus rosa-sinensis* (Cooperi)_____8.00µm Mag :x2500

Entire Pollen View **Exine Pattern**

Fig. 9 **Fig. 10**

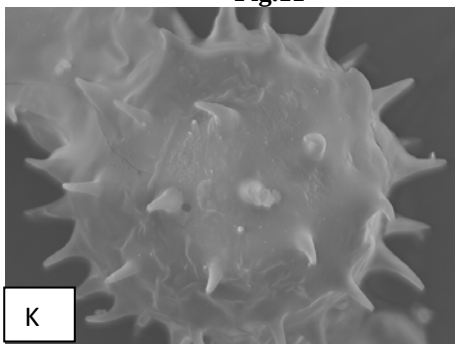


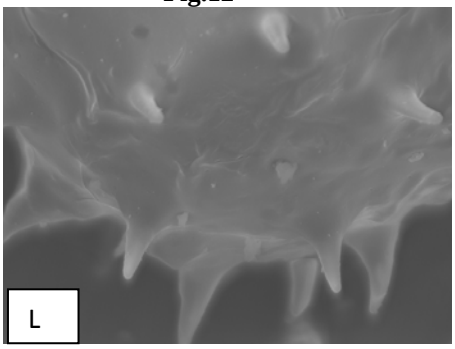


_____:23.53µm Mag: x850 *Hibiscus schizopetalous*_____:12.50µm Mag : x1600

Entire Pollen View **Exine Pattern**

Fig.11 **Fig.12**



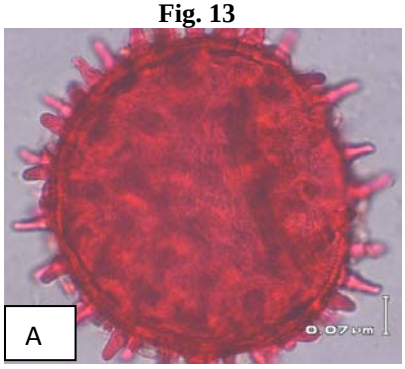


_____:26.67µm Mag: x750 *Hibiscus syriacus* _____:14.29µm Mag: x1400

Entire Pollen View **Exine Pattern**

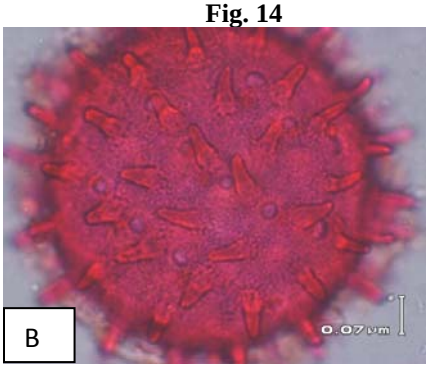
Plate-III

Fig. 13



Polar view 40X.

Fig. 14



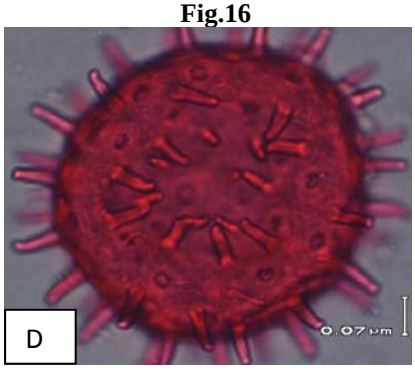
Hibiscus mutabilis

Equatorial view 40X.

Fig.15

Polar view 40X.

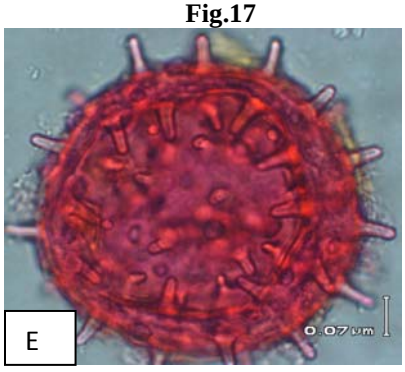
Fig.16



***Hibiscus rosa-sinensis* (Double)**

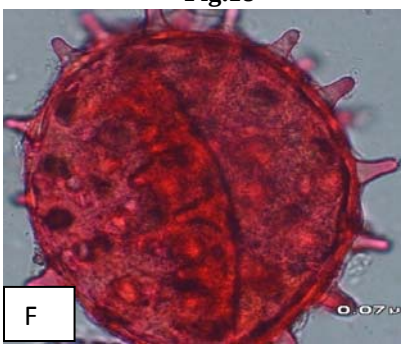
Equatorial view 40X.

Fig.17



Polar view 40X.

Fig.18

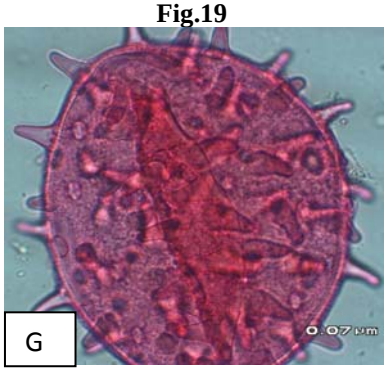


***Hibiscus rosa-sinensis* (Cooperi)**

Equatorial view 40X.

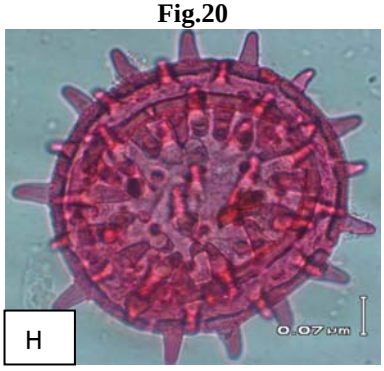
Plate-IV

Fig.19



Equatorial view 40X

Fig.20



Hibiscus schizopetalous **Polar view 40X**

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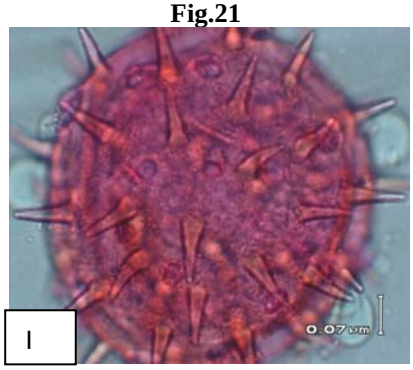
Fig.21



Hibiscus syriacus **Polar view 40X**

Hibiscus syriacus **Linn. (Plate II, Fig.11, 12 and Plate IV, Fig. 21):** Spherical to spheroidal, polyporate, Pollen diameter 159 (169) 186 µm, exine thickness 3 (5) 9 µm, echini height 18 (21) 25 µm, echini base 6 (7) 10 µm wide, echini apexes are 28 (39) 44 µm apart, echini bases are 18 (21) 25 µm apart and pore diameter 7 (9) 11 µm. Number of spines 27 (31) 36, number of pores 6 (7) 12. Echinate, spines large with acute apexes and curved in some. Spines are spaced widely and easy to count and measure. Spines monomorphic. Tectum slightly perforated but smooth and only imbalance ridges.
Voucher No. NB/030/S-05

KEY TO SPECIES AND VARITIES ON THE BASIS OF POLLEN MORPHOLOGY

1. a. Pollen with basal cushions of central spines…… *Hibiscus mutabilis*
   b. Pollen without basal cushions of central spines …... 2.
2. a. Pollen with bulbous apex of echini…….*Hibiscus rosa-sinensis* (Cooperi)
   b. Pollen with acute apex of echini ……*Hibiscus syriacus*
3. a. Spines with irregular arrangement of spines ……. *Hibiscus schizopetalous*

b. Spines with regular arrangement of spines……. 4.

4. a. Pollen with specific central spines ……*Hibiscus rosa-sinensis* (Common)

b. Pollen without specific central spines ……*Hibiscus rosa-sinensis* (Double)

Discussion

Palynologically Malvaceae is a stenopalynous family and pollen characters of this family are more or less uniform confirmed the present findings. Culhane *et al.,* (1988). Pollen grains are generally radially symmetrical, apolar, pantoporate or triporate zonoaperturate. Tectum uniformly echinate, medium to finely perforated or punctate to granulate with scabrae in between spines. These results agree to that of Perveen *et al.* (1994).

The present findings are in accordance with EI Naggar (2004) that pollen grain in Malvaceae are usually spheroidal or globular in outline and are colporate or porate with an echinate sculpture. Spines are evenly distributed over the surface of grain and vary in length, shape, density and apex which vary from pointed, rounded blunt and bulbous to bifurcated. Results of pollen size do not fall within the prescribed range reported for the family that is 30-190 µm by Erdtman (1952).

The pollen diameter of *Hibiscus mutabilis* as measured is 142 µm does not fall in range specified by Pervaiz *et al.* (2005) which is 60-65 µm. Mohammad *et al.* (1996) described pollen size as tool for separating the species and it is proved from present findings which result in differentiation of different cultivars of *Hibiscus rosa-sinensis* by utilizing this taxonomic tool.

Tahavi (2000) arguments that *Hibiscus* pollen are the largest among all the genera of family, contradict our results where the pollen grains of *Malvaviscus arboreus* have the largest pollen size. But their results about tectum, which is uniformly echinate, medium to finely perforate sparsely to densely granulate between spines, confirm the present findings. Pollen morphology of 4 species belonging to 2 genera of the family Malvaceae from Lahore was examined by him. Tahavi (2000) described that pollen morphology of the family is fairly uniform. Pollen grains are generally radially symmetrical, apolar or isopolar, mostly spheroidal to oblate spheroidal rarely sub oblate, pantoporate or triporate, zonoaperturate. Tectum uniformly echinate, medium to finely perforated or punctuate with granules and scabrae in between spines.

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