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Pollen Morphology of Solanaceae in Jordan

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Abstract: 15 solanacean species were investigated palynologically by LM and SEM. Most of the examined species have 3-zonocolporate pollen class, except four species. They were, *Hyoscyamus aureus* L. (4-loxocolporate), *Lycopersicon esculentum* L. (4-loxocolporate), *Mandragora autumnalis* L. (4-loxocolporate) and *Nicotiana tabacum* L. Polar measurements ranges between (11.5-45.5) μm , *Solanum dulcamara* L. has the smallest polar measurements (11.5-13 μm), While *Datura stramonium* L. has the largest (40-45.5 μm). Equatorial measurements ranges between (11.5-51) μm , *Solanum dulcamara* L. (11.5-13.5 μm) also has the smallest equatorial measurements while *Datura stramonium* (48-51 μm) has the largest. P/E ratio ranges between (0.83-1.32), *Datura stramonium* L. has the smallest ratio (0.83-0.89), while *Nicotiana tabacum* L. (1.32-1.21) has the largest. Pollen outline has been recognized, the equatorial view of all species examined was elliptic except two were circular (*Datura stramonium* L. and *Solanum dulcamara* L.); the polar view was angular for all solanacean species except three were circular (*Datura stramonium* L., *Solanum dulcamara* L. and *Withania somnifera* L. Dunal); P/E ratio has three views, suberect, subtransverse and semitransverse. The morphology of ectoaperture structures (colpi, fastigia and margins) have reflected distinguished variation, three species only exhibited short colpi, while the remaining twelve were not exhibiting, five species have indistinct ectoaperture margin, while the remaining ten have distinct margin. The morphology of endoaperture structures (colpi, costae and margins) have reflected also distinguished variation; seven species have short colpi, while the remaining eight species have long ones; four species have present costae, while the remaining eleven have absent ones; eleven species have indistinct endoaperture margins, while the remaining four have distinct ones.

Key words: Solanaceae, palynology, zonocolporate, endoaperture, ectoaperture

INTRODUCTION

Solanaceae in Jordan is widely distributed wildly and cultivated, morphological evidence sometimes to depend upon alone is not sufficient to delimit solanacean species which made this palynological study is necessary, to investigate some aspects related to pollen class, polar and equatorial measurements, P/E ratio, pollen outline, endoaperture and ectoaperture structures, to see whether some palynological variations among the examined species will exist and will be relied on for such differentiation^[1-9].

The basic structure of pollen grains is well-known and many publications gave descriptions and keys for those studied as guidelines for the others. The palynological terms and expressions used by different researchers and investigators sometimes are making confusion and misleading, because each one is oriented to express his results by special terms, at many times not used by previous investigators. The ectoaperture and endoaperture of pollen grains are considered as a complex

structures constituting highly determine parts such as the colpi, costae and fastigia; these structures are vary among species^[10-18]. The colpi are bounded by external margin which appears as halo under LM, this is occurring because of special structure or space like girdle or band formed that separates colpi margins from the other parts of the mesocolpium^[19-21].

Pollen class in some plant families is point of confusion and disagreement between the previous workers dealing with pollen endo-and ecto-apertures, some of them were using colpate term, while others were using colporate, the third group was using colpoidate since of the clear separation which make the area between differentiated sexine and the apertures in form of two wall layers^[22-24].

Echinae or micro-echinae in form of spine-like projections which give the pollen exine an ornamental view may be depend upon as important distinguishing character in delimitation, but even these pollen exine structures are points of variation and contradictory terms, because some investigators described micro-echinae as

scabrae. So the using of SEM dissolved many palynological issues and clarify the palynological structure in focus^[22-26].

Recent investigations in pollen grains were trying to study them as a major component in biological aerosol particles of the atmosphere and their applications, while others were trying to use the pollen grains as new bioindicator for pollution. but the major type of investigations was using the ancient deposited pollen grains as realistic palynological evidence in paleobotany in documentation for extincted plant species in fossil records, because of the very hard outer layer (sporopollenin) which tolerates the harsh environmental conditions^[27-33].

Jordan is located between longitudes 53°40' and 39°E and between latitudes 29°30' and 34°N, this unique location of this country comprises at least four main different phytogeographical elements (mediterranean, irano-teranean, sahara-arabian and sudanian), which makes the biodiversity in Jordanian plants is very high and reflected over the large volume of variations between the wild and cultivated species, especially that solanaceae is highly distributed in different phytogeographical regions in Jordan^[34-41].

The purpose of this study was to use the pollen morphology as palynological evidence in taxonomy of solanaceae in delimitation of solanacean species in Jordan, depending on concrete palynological structures or measurements valid to this purpose since this family has large scale of variation not completely understood, which is helpful also to give an illustrated guide to pollen grains of solanaceae.

MATERIALS AND METHODS

Fresh polliniferous material was collected from fresh specimens belonging to 15 solanacean species by using field collecting tools (field vials and small brushes).

For LM studies, the material was prepared according to acetolysis method of Erdtman^[42] and the improved method by Faegari and Iversen^[43], 4 duplicates for each species were prepared and 6 slides from each were made. LM micrographs and measurements were taken by using Nikon HFX-11 microscope using ocular micrometer scale by glycerin Jelly method.

For SEM studies, the treated pollen grains by acetolysis method were coated by carbon layer first, then by gold layer to increase the conduction and electron yield rates, micrographs were taken by SEM.

RESULTS AND DISCUSSION

All palynological structures and measurements for the examined 15 solanacean pollen grains concerning

pollen class, Polar (P) measurements, Equatorial (E) measurements, P/E range were exhibited in Table 1, while the other results concerning pollen outline; equatorial view, polar view, P/E view, Ectoaperture structures concerning the colpi, fastigia, margins, Endoaperture structures concerning the colpi, costae and pollen margins were exhibited in Table 2 and Fig. 1-6.

By depending on pollen class, it is obvious that this palynological structure cannot be depend upon to delimit solanacean species, because all of them were 3-zonocolporate except four species (*Hyoscyamus aureus* L., *Lycopersicon esculentum* L., *Mandragora autumnalis* Bertol and *Nicotiana tabacum* L.) these findings are typically similar to those exhibited by other investigators^[36].

Polar (P) measurements of pollen grains exhibited large scale of variations, from small size for *Solanum dulcamara* L. (11.5-13 µm), to medium size for *Lycium shawii* L. (25-32.5 µm), to large one for *Datura stramonium* L. (40-45.5 µm). Equatorial measurements of pollen grains exhibited also large scale of variations, from small size for *Solanum dulcamara* L. (11.5-13.5 µm), to medium size for *Lycium europaeum* L. (23-28 µm), to large size for *Datura stramonium* L. (48-51 µm) these measurements are sometimes differ from those got by Bernardello^[36], simply because the measurements depend on the method used; either glycerine jelly, silicon oil, or any other jelly or oil used by the researchers.

P/E ratio of pollen grains exhibited relatively large scale of variations, from small size for *Datura stramonium* L. (0.83-0.89), to medium size for *Lycopersicon esculentum* L. (0.98-1.04), to large one for *Nicotiana tabacum* L. (1.32-1.21). These polar, equatorial and P/E measurements cannot be depend upon as diagnostic evidence in palynological significance (Table 1).

By depending on the pollen outline, it is also obvious that equatorial view for all examined solanacean species is elliptic except two species (*Datura stramonium* L. and *Solanum dulcamara* L.) while the polar view is angular for all species except three were circular (*Datura stramonium* L., *Solanum dulcamara* L. and *Withania somnifera* L. Dunal), P/E view for all species shows three types: subtransverse, semitransverse and suberect.

Pollen ectoaperture and endoaperture structures show the highest degree of variations among the species examined compared to the other diagnostic criteria that can be depend on, three species have short sunken colpi (*Capsicum annuum* L., *Datura stramonium* L. and *Mandragora autumnalis* Bertol), while the remaining twelve species show long sunken colpi, the fastigia is present in nine species, while it is absent in the

Table 1: Plant species examined with relative to their pollen class measurements of polar (P) and equatorial (E) views in μm and P/E ratio

Species	Pollen class	P (μm)	E (μm)	P/E
<i>Capsicum annum</i> L.	3-zonocolporate	23.0-39.0	23.0-30.0	1.00-1.30
<i>Datura stramonium</i> L.	3-zonocolporate	40.0-45.5	48.0-51.0	0.83-0.89
<i>Hyoscyamus albus</i> L.	3-loxocolporate	25.5-38.0	22.0-35.0	1.15-1.09
<i>Hyoscyamus aureus</i> L.	4-loxocolporate	22.5-36.0	20.0-35.0	1.13-1.03
<i>Hyoscyamus reticulatus</i> L.	3-zonocolporate	20.0-34.5	19.0-34.0	1.05-1.01
<i>Lycium europaeum</i> L.	3-zonocolporate	28.0-33.0	23.0-28.0	1.22-1.18
<i>Lycium shawii</i> L.	3-zonocolporate	25.0-32.5	24.0-33.0	1.04-0.98
<i>Lycium schweinfurthii</i> L.	3-zonocolporate	24.0-34.0	22.0-32.0	1.09-1.06
<i>Lycopersicon esculentum</i> L.	4-loxocolporate	20.5-28.0	21.0-27.0	0.98-1.04
<i>Mandragora autumnalis</i> Bertol	4-6 loxocolporate	16.0-24.0	15.0-26.0	1.07-0.92
<i>Nicotiana tabacum</i> L.	4-loxocolporate	29.0-40.0	22.0-33.0	1.32-1.21
<i>Solanum dulcamara</i> L.	3-zonocolporate	11.5-13.0	11.5-13.5	1.00-0.96
<i>Solanum luteum</i> Miller	3-zonocolporate	23.5-29.0	23.5-28.0	1.00-1.04
<i>Solanum nigrum</i> L.	3-zonocolporate	19.0-31.5	19.0-32.0	1.00-0.98
<i>Withania somnifera</i> (L.) Dunal	3-zonocolporate	25.0-29.0	29.0-33.0	0.86-0.88

Table 2: Plant species examined relative to their outline views, ectoaperture and endoaperture structures

Species	Outline			Ectoaperture			Endoaperture		
	E view	P view	P/E view	Colpi	Fastigia	Margins	Colpi	Costae	Margins
<i>Capsicum annum</i> L.	elliptic	angular	suberect	s	-	ind.	1	-	ind.
<i>Datura stramonium</i> L.	circular	circular	subtransverse	s	-	ind.	1	-	ind.
<i>Hyoscyamus albus</i> L.	elliptic	angular	semtransverse	1	+	d.	1	+	d.
<i>Hyoscyamus aureus</i> L.	elliptic	angular	subtransverse	1	+	d.	1	-	ind.
<i>Hyoscyamus reticulatus</i> L.	elliptic	angular	semtransverse	1	+	d.	1	+	d.
<i>Lycium europaeum</i> L.	elliptic	angular	semtransverse	1	+	d.	s	-	ind.
<i>Lycium shawii</i> L.	elliptic	angular	semtransverse	1	+	d.	s	-	ind.
<i>Lycium schweinfurthii</i> L.	elliptic	angular	semtransverse	1	+	d.	1	-	ind.
<i>Lycopersicon esculentum</i> L.	elliptic	angular	semtransverse	1	-	ind.	s	-	d.
<i>Mandragora autumnalis</i> Bertol	elliptic	angular	subtransverse	s	+	d.	1	+	ind.
<i>Nicotiana tabacum</i> L.	elliptic	angular	subtransverse	1	-	d.	s	-	ind.
<i>Solanum dulcamara</i> L.	circular	circular	subtransverse	1	+	ind.	s	-	ind.
<i>Solanum luteum</i> Miller	elliptic	angular	semtransverse	1	+	d.	s	+	ind.
<i>Solanum nigrum</i> L.	elliptic	angular	semtransverse	1	+	ind.	s	-	ind.
<i>Withania somnifera</i> (L.) Dunal	elliptic	circular	semtransverse	1	-	d.	1	-	d.
P: polar	E: equatorial			+: present	-: absent	ind: indistinct	d: distinct	s: short	l: long

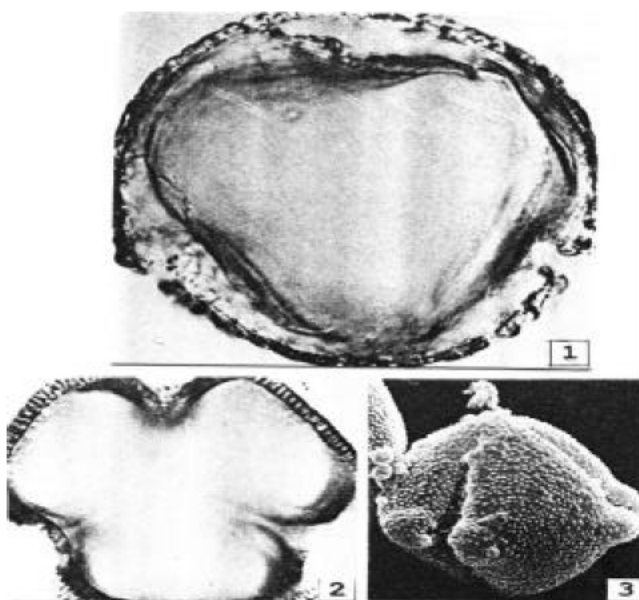


Fig. 1(1-3): 1: LM showing pollen grains of *Datura stramonium* L., polar view (1100 X)
 2: LM showing pollen grains of *Hyoscyamus aureus* L., polar view (1000 X)
 3: SEM showing pollen grains of *Capsicum annum* L., equatorial view (2000 X)

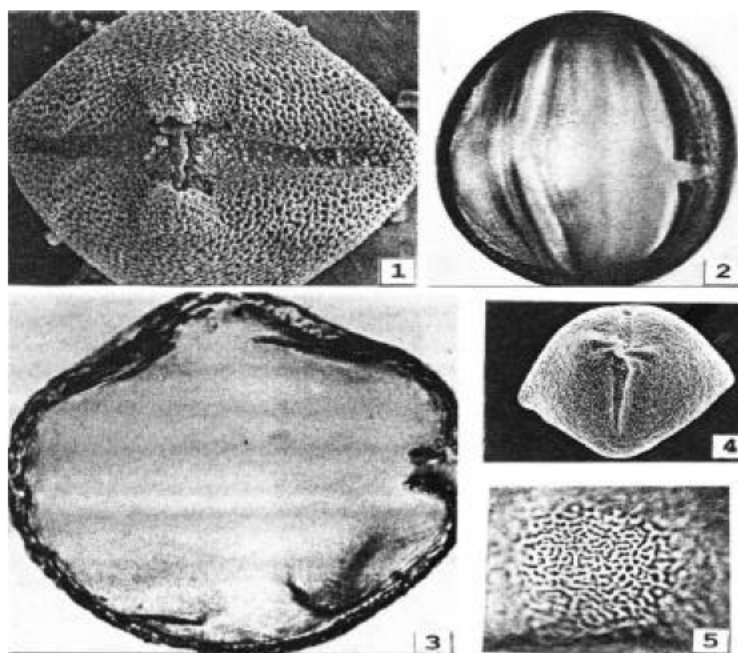


Fig. 2(1-5): 1: SEM showing pollen grains of *Hyoscyamus albus* L., equatorial view (2100 X)
 2: LM showing pollen grains of *Nicotiana tabacum* L., polar view (1000 X)
 3: LM showing pollen grains of *Datura stramonium* L., equatorial view (1200 X)
 4: SEM showing pollen grains of *Solanum nigrum* L., equatorial view (1900 X)
 5: LM showing pollen grains of *Nicotiana tabacum* L., ornamentation in focus (1000 X)

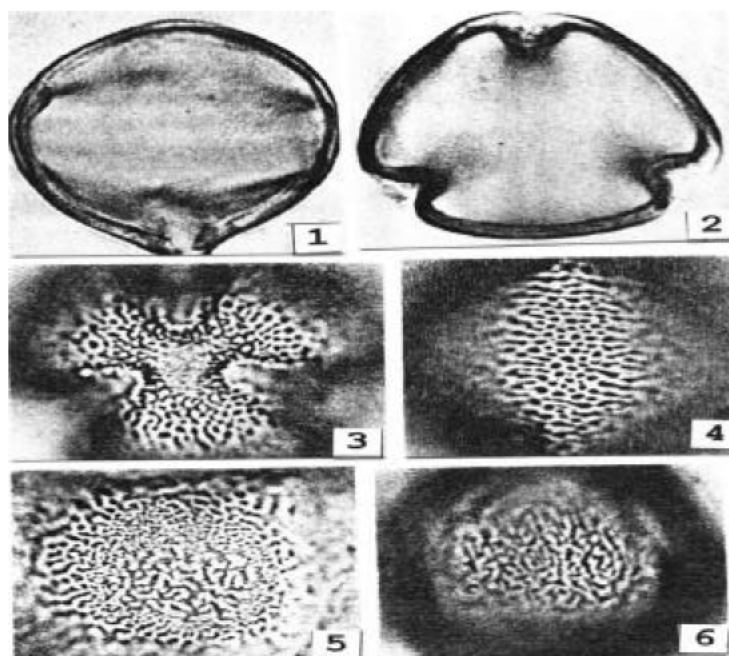


Fig. 3(1-5): 1: LM showing pollen grains of *Lycium europaeum* L., equatorial view (800 X)
 2: LM showing pollen grains of *Solanum luteum* L., polar view (800 X)
 3: LM showing pollen grains of *Hyoscyamus reticulatus* L., polar view, 3-colporate (1000 X)
 4: LM showing pollen grains of *Withania somnifera* (L.)Dunal, reticulum at focus (800 X)
 5: LM showing pollen grains of *Solanum dulcamara* L., ornamentation at focus (800 X)
 6: LM showing pollen grains of *Mandragora autumnalis* Bertol, ornamentation at focus (1000 X)

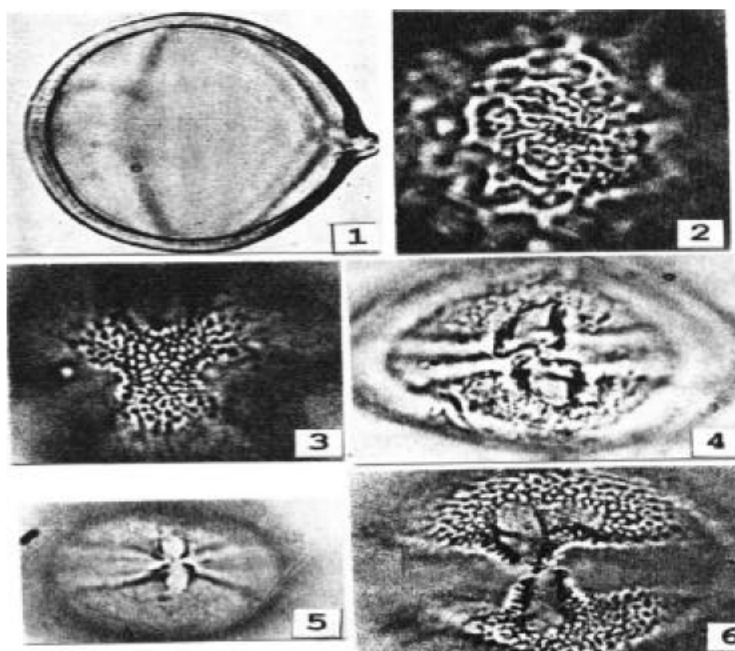


Fig. 4(1-6): 1: LM showing pollen grains of *Lycopersicon esculentum* L., equatorial view (1000 X)
2: LM showing pollen grains of *Lycium schweinfurthii* L., ornamentation at focus (800 X)
3: LM showing pollen grains of *Lycium shawii* L., 3-colporate (900 X)
4: LM showing pollen grains of *Capsicum annuum* L., endoaperture colpi (1200 X)
5: LM showing pollen grains of *Hyoscyamus aureus* L., endoaperture structures (800 X)
6: LM showing pollen grains of *Lycopersicon esculentum* L., endoaperture colpi (1100 X)

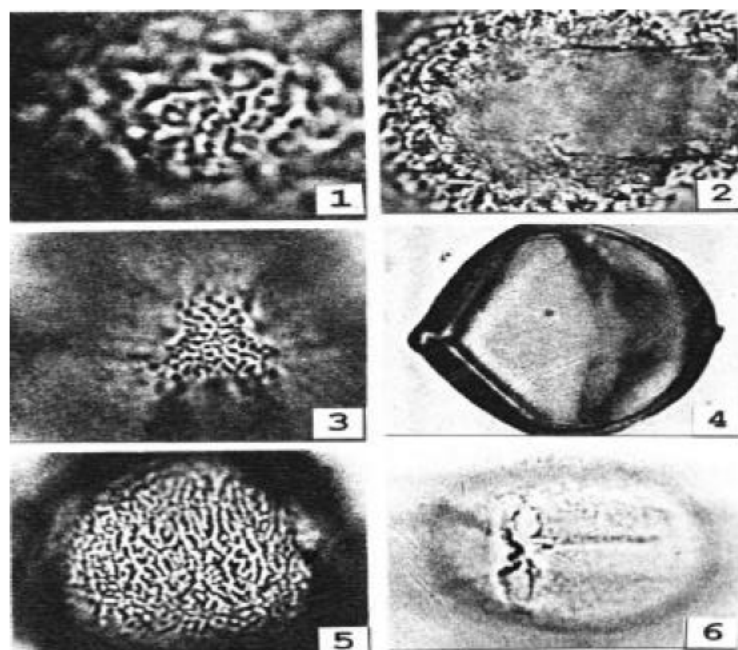


Fig. 5(1-6): 1: LM showing pollen grains of *Datura stramonium* L., endoaperture colpi (900 X)
2: LM showing pollen grains of *Hyoscyamus aureus* L., reticulate ornamentation (900 X)
3: LM showing pollen grains of *Hyoscyamus reticulatus* L., microreticulate ornamentation (1000 X)
4: LM showing pollen grains of *Lycopersicon esculentum* L., equatorial view (1000 X)
5: LM showing pollen grains of *Solanum nigrum* L., endoaperture colpi (1100 X)
6: LM showing pollen grains of *Mandragora autumnalis* L., fastigia visible (1000 X)

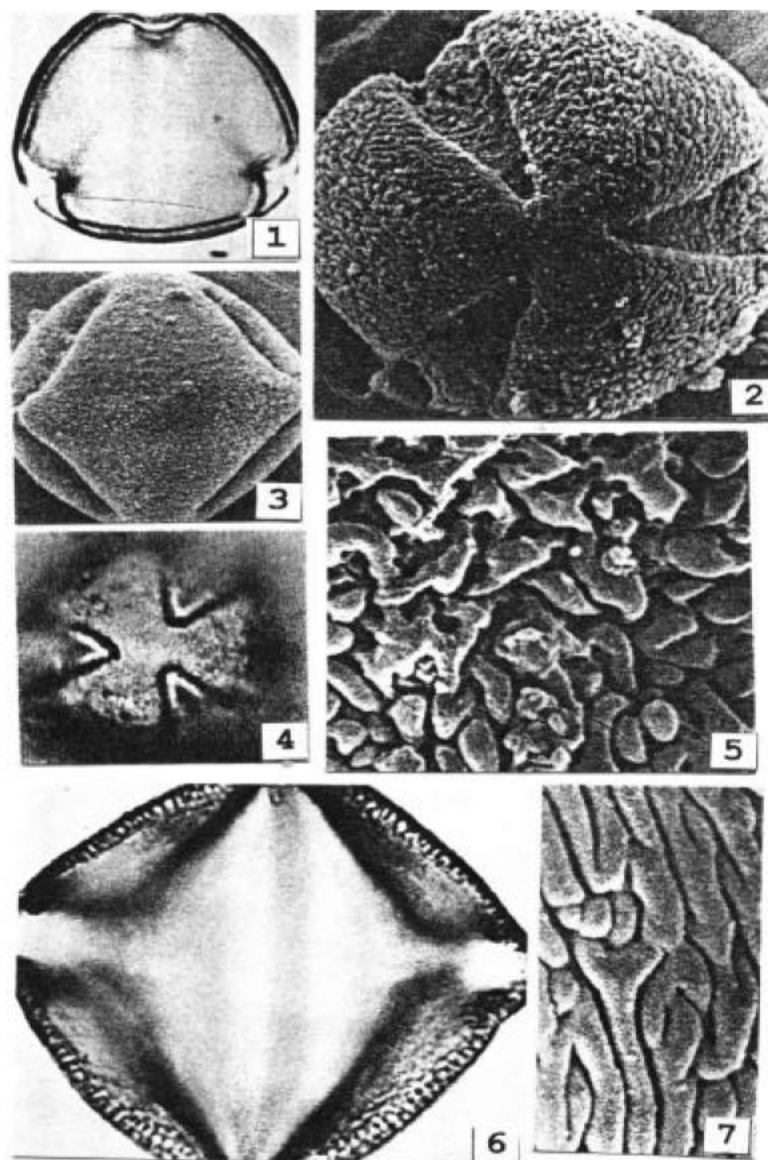


Fig. 6(1-6): 1: LM showing pollen grains of *Withania somnifera* (L.) Dunal polar view (1000 X)
 2: SEM showing pollen grains of *Datura stramonium* L., with ornamentation (10000 X)
 3: SEM showing pollen grains of *Solanum luteum* L., equatorial view (2300 X)
 4: LM showing pollen grains of *Lycium schweinfurthii* L., 3-colporate (1000 X)
 5: SEM showing pollen grains of *Solanum dulcamara* L., endoaperture colpi (1600 X)
 6: LM showing pollen grains of *Lycium shawii* L., colpi ends (1200)
 7: SEM showing pollen grains of *Lycium schweinfurthii* L., endoaperture colpi (1700 X)

remaining six species, ectoaperture margins were indistinct in five of the examined species, while they were distinct in the other ten species.

With respect to pollen endoaperture structures, the colpi were short snken in seven species, while the other eight species were long, endoaperture costae were present in four species, while they were absent in the remaining eleven species, endoaperture margins were indistinct in eleven species, while they were distinct in the

other four ones (Table 2). These results, findings and measurements are very close to those obtained by Moore *et al.*^[1] but fluctuate from those obtained by Bernardello^[36].

The most important palynological structures that can be depend on as concrete diagnostic criteria to depend on (Table 1 and 2) in delimitation of solanacean species are endoaperture and ectoaperture, so it is possible to categorize the examined species into two main categories:

- I. solanacean species with present fastigium in their ectoaperture: *Hyoscyamus albus* L., *Hyoscyamus aureus* L., *Hyoscyamus reticulatus* L., *Lycium europaeum* L., *Lycium shawii* L., *Lycium schweinfurthii* L., *Mandragora autumnalis* Bertol, *Solanum dulcamara* L., *Solanum luteum* Miller and *Solanum nigrum* L.
 - A. with present costae in their endoaperture: *Hyoscyamus albus* L., *Hyoscyamus reticulatus* L., *Mandragora autumnalis* Bertol and *Solanum luteum* Miller.
 - B. with absent costae in their endoaperture: *Hyoscyamus aureus* L., *Lycium europaeum* L., *Lycium shawii* L., *Lycium schweinfurthii* L., *Solanum dulcamara* L. and *Solanum nigrum* L.
- II. solanacean species with absent fastigia in their ectoaperture: the remaining five species.

The using of this palynological study to have some pollen diagnostic criteria as palynological evidence in taxonomy of 15 solanacean species is mainly worthful and can be used, because it is obvious from the results that some of the pollen structures and measurements seen by LM and SEM can be depend upon in this kind of delimitation.

The pollen class, polar measurements, equatorial measurements (μm), P/E ratio, pollen outline (polar view, equatorial view, P/E view), are not palynological criteria to depend on as diagnostic characters, because there is high degree of similarities between the examined species and there is no clear differences in the diagnostic features exhibited.

The ectoaperture and endoaperture features of the pollen grains examined are the only palynological evidences that can be depend on to categorize the solanacean species examined into two main categories; the first category includes those having present fastigia in their ectoaperture, which contains two types; the first type with present costae in their endoaperture, while the second type with absent costae; the second category includes those having absent fastigia in their ectoaperture which includes the remaining species.

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