

## Palynological Studies in the Family Plumbaginaceae from Jordan and Iraq

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ABSTRACT. Pollen morphological variation of representative species belonging to four polymorphic genera of the family Plumbaginaceae were investigated by using LM, SEM and TEM. Three groups of exine type of pollen grains were observed: finely reticulate, coarsely reticulate and gemmate - echinate. TEM revealed three layers as the exine *i.e.* tectum, columellae and foot layer, the tectum have additional structures such as spines or perforations. Intine forms a continuous layer. This emphasizes that pollen polymorphism is associated with other floral variable characteristics.

The order Plumbaginales, contains the single family Plumbaginaceae, mostly or semi-arid zones of the Old World. This family is represented in Jordan and Iraq by four polymorphic genera, *Acantholimon*, *Limonium*, *Plumbago* and *Psyllios-tachys*.

This family was placed by Hallier (1868-1932) in the Centrospermae, while other taxonomists *e.g.* Wettstein (1862-1931) and Rendle (1865-1938) related Plumbaginaceae with the order Primulales (Lawrence 1951). Boissier (1879), and Bentham and Hooker (1873-1876) showed taxonomic disagreements with reference to generic delimitation within the Plumbaginaceae. Hutchinson (1926-1934) considered the Plumbaginaceae to be one of two families in his Primulales. In order to avoid taxonomic confusion many authors showed that the evidence favors its separation in a distinct order, more advanced than the Primulales with both orders derived from the Centrospermae or their ancestors.

The occurrence of heterostyly in Plumbaginaceae was first noticed by Müller (1868). The relationship between pollen dimorphism and heterostyly in the family is observed by Baker (1948a,b, 1953a,b, 1954, 1966), Iversen (1940), Erdtman (1952, 1966, 1969), Vuilleumier (1967), Bokhari (1972) and Richards (1987).

The present investigation adds new information about the pollen morphology within four species belonging to four different genera of Plumbaginaceae, as mentioned above, by using Light (LM), Scanning electron (SEM) and Transmission electron (TEM) microscopy.

### Materials and Methods

The material used for pollen morphological investigations is listed in Table 1. Mature anthers were obtained from flowers collected on field trips and from dried specimens kept in the Herbaria of the Jordan Natural History Museum, Department of Biological Sciences, Yarmouk University and the National Harbarium of Iraq (BAG), Baghdad, Abu-Ghraib, Iraq.

Samples of pollen grain were analyzed, with Scanning, Transmission electron and Light microscopes.

1. Erdtman's improved acetolysis method (1960) was used to acetolyse the mature pollen grains and their characters were studied as listed in Table 1. Measurements and observations by light microscope were for an average of 10-15 grains for each species.
2. For Scanning electron microscopy mature anthers were selected from dry specimens, then pressed or squeezed. The pollen grains were then fixed on a clean stub with double-sided sellotape. Stubs were then transferred into a rotary coating apparatus and coated under vacuum with gold, silver and palladium. The mounted specimens were then examined in a Zeiss Scanning electron microscope at a voltage of 20-25 kV at the Geology Department, Jordan University. Photographs were taken at different magnifications.
3. Transmission electron microscopy was used for the study of four species of genera fixed in glutaraldehyde, in phosphate buffer (pH 7.4) and then fixed at 0.2% Osmium tetroxide dissolved in phosphate buffer. Epon-Araldite resin was used for embedding (Glauert 1979). The selections were cut with Ultratome-E and thin sections contrast-stained with uranyl acetate and lead citrate. (A Zeiss microscope was used for the examination).

**Table 1.** Pollen grain characters of selected species of Plumbaginaceae

Species	Collections from	Shape of grain	Type of pollen	Sculpturing surface	Ektexine thickness (µm)	Pollen grain size (µm)		
						Polar axis	Equatorial axis	P/E
<i>Acantholimon caryophyllaceum</i> Boiss	Iraq only	nearly spheroidal	tri-colporate	finely reticulate	10-12	75	68	1.1
<i>Limonium thouinii</i> (Viv.) O. Kuntze	Jordan & Iraq	nearly spheroidal	tri-colporate	coarsely reticulate echinate	4.5-6	42	38	1.1
<i>Psylliostachys spicata</i> (Willd.) Nevski	Jordan & Iraq	nearly spheroidal	tri-colporate	coarsely reticulate echinate	5-6	36	32	1.1
<i>Plumbago europaea</i> L.	Jordan & Iraq	ellipsoidal oblong	tri-colporate	gemmate-echinate	6-7.5	64	44	1.4

### Results

The morphological characteristics of the pollen grains for four different species of Plumbaginaceae are shown in Table 1. The pollen grains of all species are tricolporate, elliptical to spheroidal in shape and in the P/E ratio (polar axis/equatorial axis). (Pls. 1a,c, 2a,c, 3a,c, & 4a,c). The size of the pollen grains was in the range 36-75  $\mu\text{m}$  length, 32-68  $\mu\text{m}$  width.

Ektexine thickness of species ranged from 4.5  $\mu\text{m}$  to 12.0  $\mu\text{m}$ . The shape may be variable (P), polar view from ellipsoidal-oblong equatorial view (E) circular to oblong. Examination of the exine surface of all the species under the SEM gave very good diagnostic characters. These exine surfaces showed gemmate-echinate to coarsely reticulate sculpturing (Pls. 1a,b, 2a,b, 3a,b & 4a,b). It was possible to differentiate them according to the fine details of various kinds of sculpturing (Pls. 1b, 2b, 3b & 4b). In the gemmate type sharp large pointed echinae were shown by SEM (Pl. 4a & b) and in the reticulate type, fine scattered verrucae were seen (Pl. 1a & b).

Sporoderm stratification, which was investigated with the Transmission electron microscope (TEM), was a further attempt to achieve additional evidence on the pollen wall of different species of this family. In brief, three layers can be differentiated: a roof-like covering termed tectum, supported by a series of vertical and horizontal rods columellae, which in turn reside on a foundation termed a foot layer. Frequently, the tectum may have additional structures such as spines or perforations. An endexin in some species, e.g. *Plumbago europaea* L. (Pl. 4e), was not clearly recognized and in others found as a thin, slightly irregular fragmented layer e.g. *Limonium thouinii* (Pl. 2d & e). Intine forms a continuous well-defined layer (Pls. 2d,e & 4d,e).

### Discussion

The Plumbaginaceae is euryalynous. It shows genera of a widely variable range of morphological features, especially with regard to the habit, form, leaf-shape, inflorescence-type and floral structures (heterostyly). The members of this family are related to each other in having a 5-styled pistil, and the unilocular and uniovulate ovary.

In respect to plant taxonomy, the family Plumbaginaceae stands apart, allied by some of its characteristics to the Primulaceae or, by others to the Centrospermae (near the Phytolaccaceae and Plantaginaceae) (Post & Dinsmore 1932-1933).

It may however, be, that this conclusion will be changed. In fact, pollen - morphologically there is a smooth transition from certain Linaceae, perhaps via the tropical shrub genus *Aegialitis*, to the main body of the Plumbaginaceae, the Plumbaginoidae. The rest of the family, the Armerioideae, differ more in their pollen grains and constitute a fairly well - separated taxon, the "Armeriaceae" (Erdtman 1969).

In sporoderm stratification, pollen grains of *Ceratostigma wilmottianum* (Plumbaginaceae), with 30 colpi surrounding 12 pentagonal areas, are particularly like some of the pollen types encountered in the Linaceae. That these ideas concening the relationships of the Plumbaginaceae were not reached may perhaps be due to the attachment of undue importance to the stamens and their position (the Plumbaginaceae have epipetalous stamens) (Erdtman 1969).

Plumbaginaceae is the important example among the classic examples of heterostyly in Angiosperm plants, such as Primulaceae, Polygonaceae, Boraginaceae, Oxalidaceae, Linaceae and Lythraceae (Baker 1948a, 1953a,b, 1954 & 1966).

In this study, pollen is most variable both in terms of shape, exine sculpturing and in sporoderm stratification as well.

Pollen grain characters of Plumbaginaceae were examined by (LM) listed in Table 1. The nearly spherical shape of pollen were calculated by  $P/E = (1.1)$  as shown in *Acantholimon caryophyllaceum*, *Limonium thouinii* and *Psylliostachys spicata* (Pls. 1,2 & 3), while the elliptical shape,  $P/E = (1.4)$ , is shown in *Plumbago europaea* L. (Pl. 4).

The fine reticulum of the exine surface showed very good diagnostic characters for separating the taxa or combining them into two groups. First, the *Psylliostachys* group, comprising *Acantholimon caryophyllaceum* (Pl. 1a & b) and *Limonium thouinii* (Pl. 2a & b), with which *Psylliostachys spicata* (Pl. 3a & b) seems more or less similar and related, having finely or coarsely reticulate-echinate sculpturing. Second, the *Plumbago* group, which comprise one species, *Plumbago europaea* (Pl. 4a & b), which had gemmate-echinate sculpturing and more advanced than the first group (Saad 1961 and Erdtman 1969). From the above results it seems that the genera of Plumbaginaceae are heterogeneous, which strongly supports the idea that the Plumbaginaceae is from a eurypalynous family (Erdtman 1969 and Weber El-Ghobary 1987).

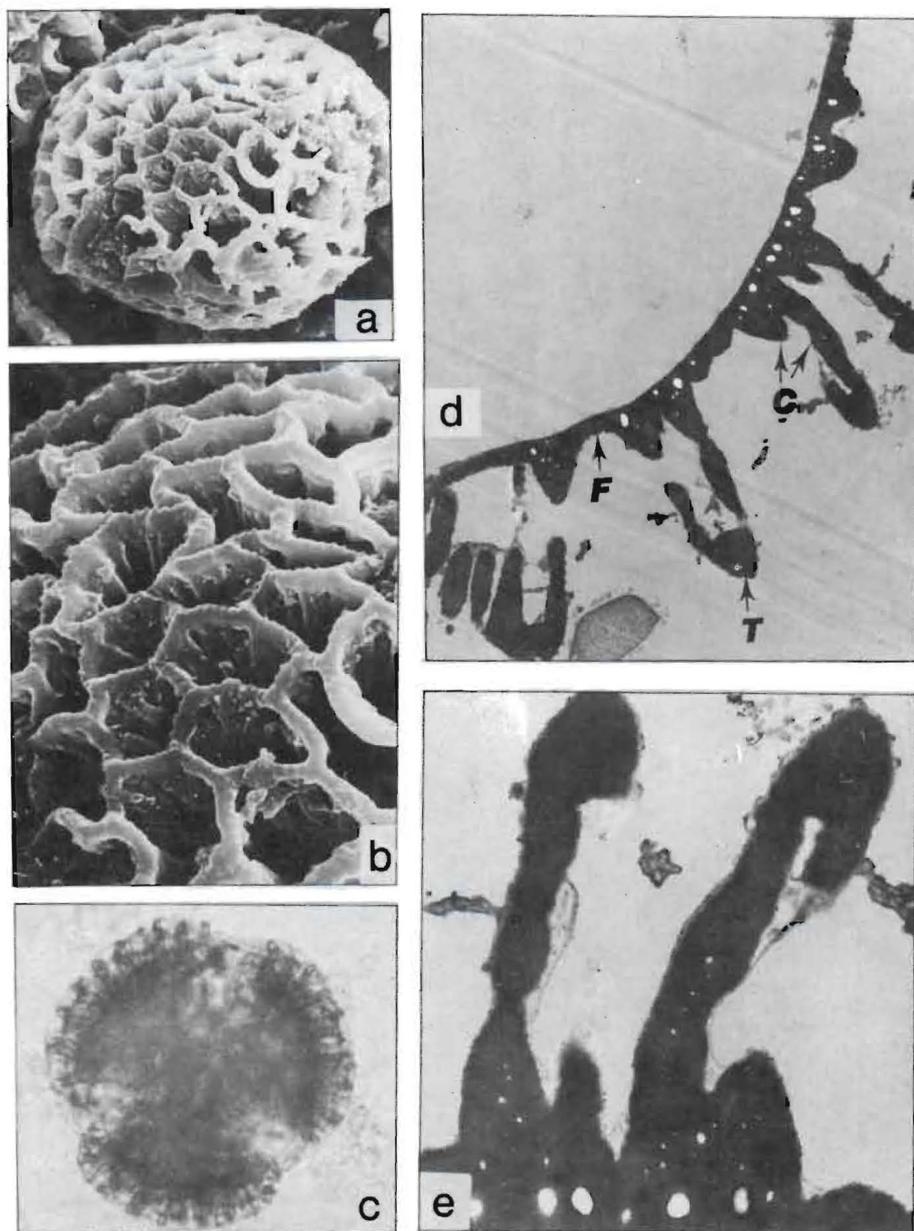
TEM micrographs of non-acetolysed grains of specimens revealed that the three general layers of the ectexine (tectum, columellae and foot layer) are present and so the intine layer (Pls. 2d & 4d), with the exception of *Acantholimon*

*caryophyllaceum* (Pl. 1d & e), in which it is not visible. The absence of intine and endexin layers within species may be due to preservation for a long period of time which caused the dryness in the pollen grains as the specimen was taken from the herbarium. A gradual increase in the thickness of the intine was seen from *Psylliostachys spicata*, *Plumbago europaea* to *Limonium thouinii* (Pls. 3e, 4e & 2e).

The present investigation concerns the morphology of pollen of some species of the family Plumbaginaceae in Jordan and Iraq and to emphasize the systematic study of these species. Our data about the heteromorphic floral and pollen grain characters support the views of Boissier (1879), Post (1932-1933), Feinbrun-Dothan (1978), Erdtman (1969) and Weber El-Ghobary (1987), about the exine's reticulation and clarifying the relationships in these taxa.

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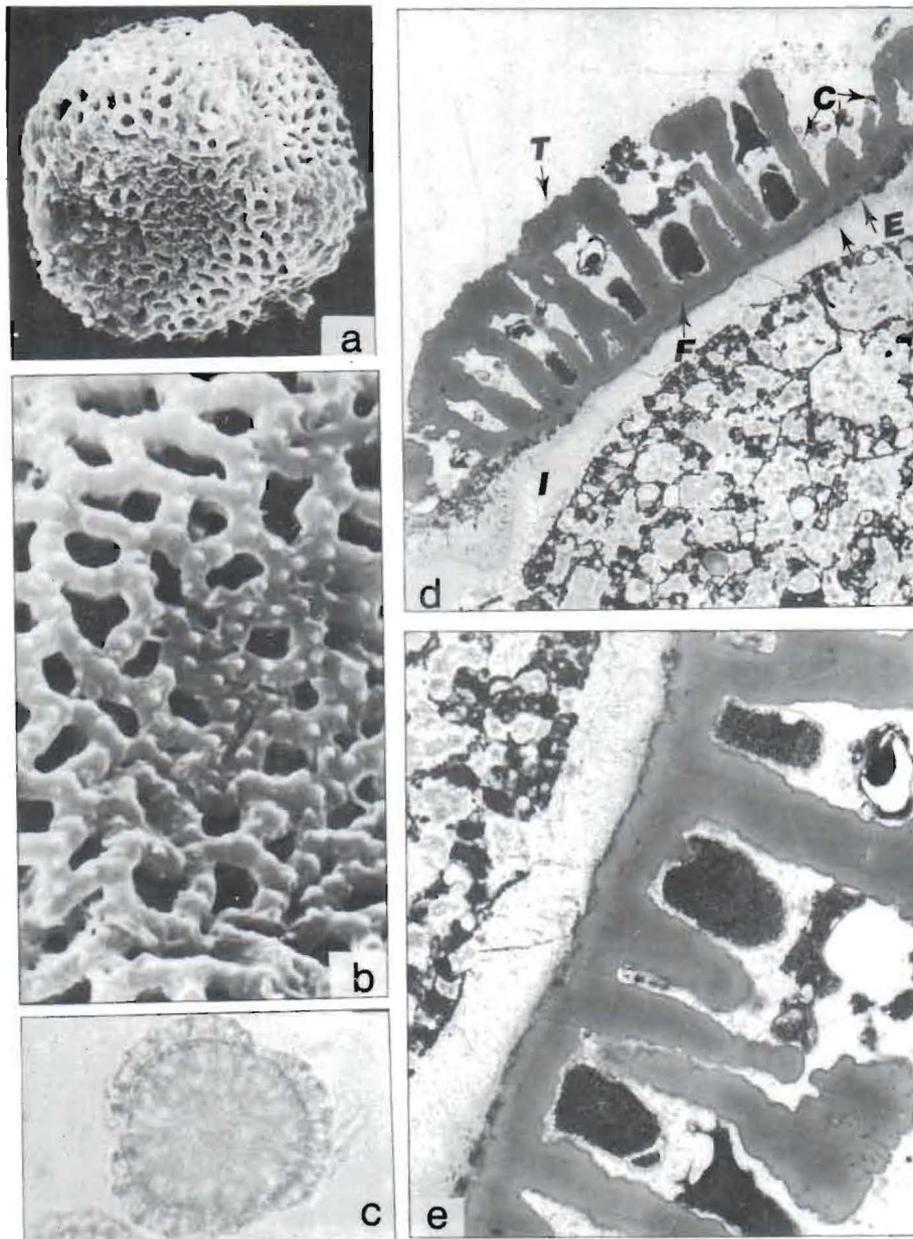
**Plate 1.** SEM, TEM, and LM micrographs of *Acantholimon caryophyllaceum*.

(a) General view of pollen by SEM (1125X).

(b) Exine stratification of pollen by SEM (4937X).

(c) General view of pollen by LM (625X).

(d&e) TEM micrographs of pollen (5000X & 7500X) showing the tectum (T), Columella (C) and foot layer (F).



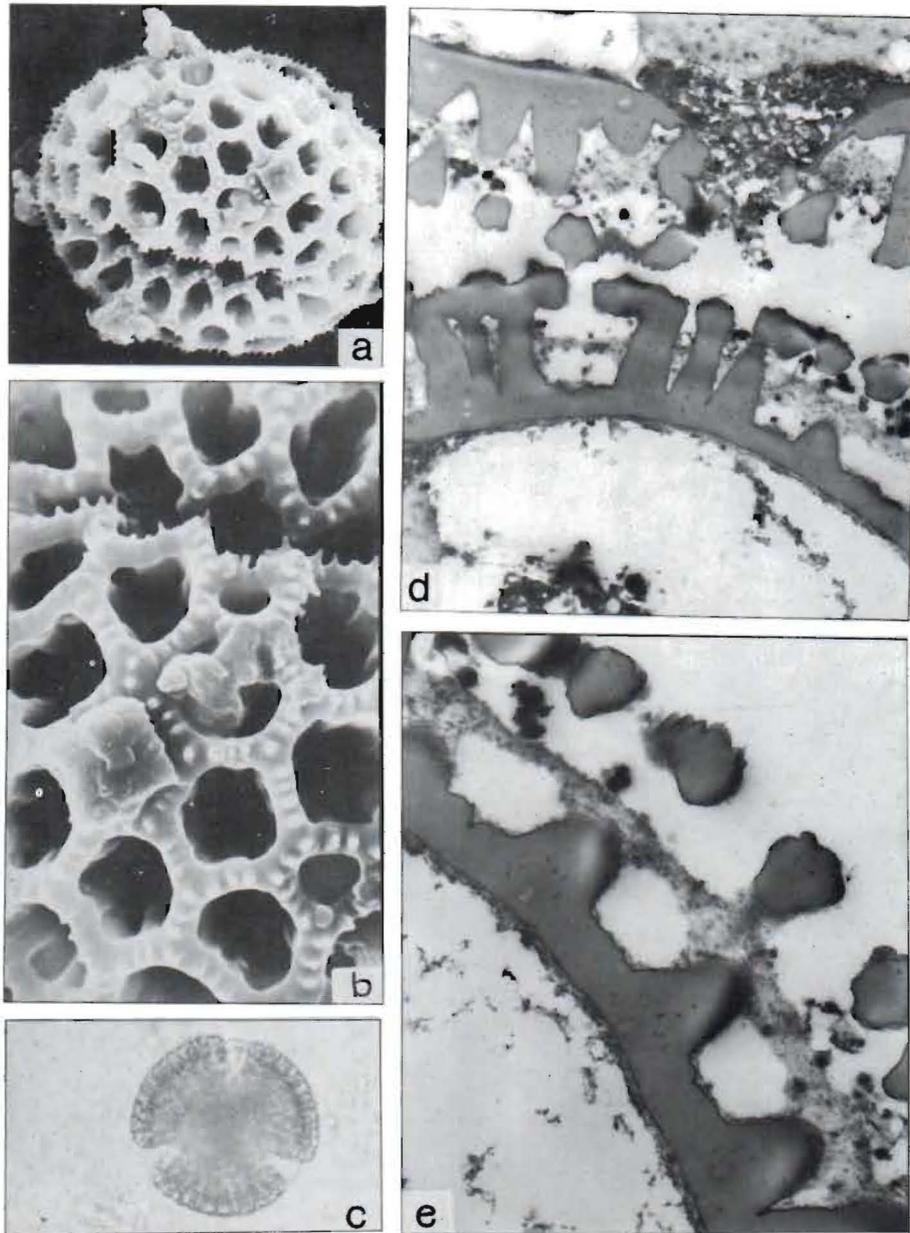
**Plate 2.** SEM, TEM, and LM micrographs of *Limonium thouinii*.

(a) General view of pollen by SEM (1320X).

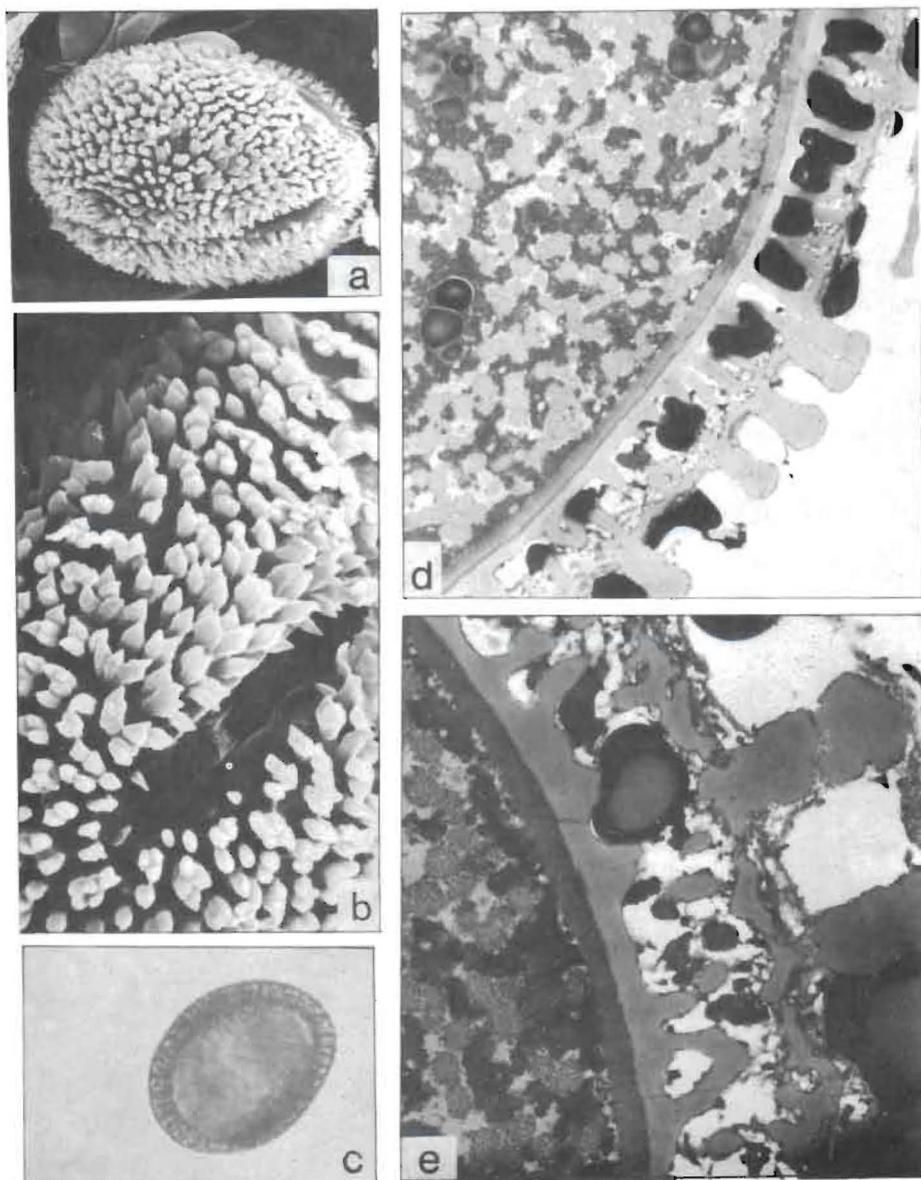
(b) Exine stratification of pollen by SEM (4200X).

(c) General view of pollen by LM (600X).

(d&e) TEM micrographs of pollen (4800X & 6000X) showing the tectum (T), columella (C) and foot layer (F), endexine (E) & the intine (I).



**Plate 3.** SEM, TEM, and LM micrographs of *Psylliostachys spicata*.  
 (a) General view of pollen by SEM (1298X).  
 (b) Exine stratification of pollen by SEM (4425X).  
 (c) General view of pollen by LM (590X).  
 (d&e) TEM micrographs of pollen (3540X & 5900X).



**Plate 4.** SEM, TEM, and LM micrographs of *Plumbago europaea*.

- (a) General view of pollen by SEM (1200X).  
 (b) Exine stratification of pollen by SEM (4800X).  
 (c) General view of pollen by LM (600X).  
 (d&e) TEM micrographs of pollen (4500X & 6000X).

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## دراسة المظهر الخارجي والتشريحي لحبوب اللقاح في العائلة البلمبوجينيبي في الاردن والعراق

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أظهرت الدراسة التي تمت بواسطة المجهر الالكتروني الماسح والناقل والمجهر الاعتيادي لأربعة أنواع مختلفة من حبوب اللقاح لنباتات تعود لأربعة أجناس مختلفة ضمن العائلة البلمبوجينيبي المثلة في الفلورا العراقية والاردنية ، إختلافاً واضحاً في هذه الأنواع من حيث الشكل والتشريح الداخلي والسطح الخارجي كما أكدت الدراسة الحالية لهذه الأنواع من حبوب اللقاح .

### طريقة العمل :

- ١ - أُجُذت العينات من الحقل مباشرة أو من معشب متحف التاريخ الطبيعي الاردني ومعشب كلية العلوم - قسم علوم الحياة وكذلك من المعشب الوطني العراقي .
- ٢ - تمت دراسة الانواع المذكورة في الجدول رقم (١) لحبوب اللقاح بواسطة المجهر الضوئي الاعتيادي والمجهر الالكتروني الماسح والناقل .

### نتيجة البحث والمناقشة :

بيّنت دراسة الصفات المورفولوجية لحبوب اللقاح في الجدول رقم (١) أن هذه العائلة تعتبر تصنيفياً غير متجانسة، حيث أن شكل وتركيب حبوب (أو غبار) اللقاح لأنواعها المختلفة تميل إلى عدم التشابه فهي إما كروية، إهليلجية أو بيضوية كما أن لكل حبة ثلاث فتحات أو أكثر، أما زخرفة السطح الخارجي فتتراوح ما بين الشبكي والشوكي . إذ أن مختلف الدراسات التصنيفية الحديثة للنباتات تعتمد على شكل، حجم، زخارف السطح الخارجي أو عدد ونوع طبقات الغلاف الخارجي والداخلي بواسطة المقاطع التشريحية لحبوب اللقاح، وتتم هذه الدراسة عادة بواسطة المجهر الضوئي الاعتيادي والمجهر الإلكتروني الماسح والناقل . فقد أظهرت الدراسات السابقة التي قام بها كل من :-  
أردتمان ١٩٦٩ والغباري ١٩٨٧ لدراسة عائلة البلمبوجينيسي ومدى علاقتها بالعوائل المقاربة لها بواسطة الطرق المذكورة أعلاه، وقد جاءت دراستنا هذه مطابقة لهما تماماً كما تم دراسته سابقاً .