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Pollen Grain Variation among Some Trees of Fabaceae in New Damietta, Egypt

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Abstract

The current study aims at investigating the morphology of pollen grain for eleven woody species of Fabaceae cultivated in New Damietta, Egypt. The pollen grains were prepared according to Erdtman's acetolysis method, described and photographed using light microscopy (LM). The results showed variations in their qualitative and quantitative features. Differences in pollen shape (subprolate, prolate, prolate-spheroidal, oblate-spheroidal), size (medium to large), pollen arrangement (monads or polyads), amb (circular, irregular, elliptic or triangular), polarity (isopolar, heteropolar), type of apertures (porate, colporate, inaperturate) and exine ornamentation patterns (psilate, verrucate, granulate, reticulate and psilate-perforate) are characterized. Electrophoretic (SDS-PAGE) examination of total pollen protein content of four species (*Acacia ehrenbergiana*, *Cassia glauca*, *Delonix regia*, *Leucaena leucocephala*) revealed distinct protein bands. The results of this study is of great importance in understanding the pollen types of some allergy producing species.

Keywords: Egypt, Fabaceae, New Damietta, Pollen grains morphology.

Introduction

The Fabaceae (or Leguminosae) family is among the most varied families, with members found in almost all ecological systems on the earth (Bahadur et al., 2022). The third largest angiosperm family, with species that are morphologically, physiologically, and environmentally diverse (APG III 2009; Schwarz et al., 2015; Werner et al., 2015). Cercidoideae, Detarioideae, Duparquetioideae, Dialioideae, Caesalpinioideae, and Papilionoideae are the six subfamilies of the Fabaceae. The biggest subfamily, Papilionoideae, has 30 tribes, 455 genera, and over 12,000 species (LPWG, 2017). It is the most widespread, and its members are adaptable to a wide range of habitats.

Fabaceae is eurypalynous family (Luz et al., 2013), with vastly diverse pollen characters (Pavlova & Manova, 2000; Taia, 2004; Lashin 2006; Liao et al., 2022). Among the numerous studies that explain the pollen morphology of Fabaceae species: Erdtman (1952), Barth et al., (1976), Graham et al., (1980), Banks & Lewis, (2009), Luz et al., (2013), Antonio-Domingues et al., (2018), Talebi, et al., (2020), Soares et al., (2022). In previous studies, a wide range of pollen types and variations in pollen morphology among Fabaceae species, demonstrating the importance of describing pollen grains for the identification of species, as well as to aid in the resolution of taxonomic issues within the family.

Airborne pollen is a frequent component of atmospheric aerosols and high quantities of pollen grains in the atmosphere cause allergy symptoms in many people (Taia, 2020). So, it is essential to know the types of allergic pollens that are widespread in the region's environment as well as their concentration throughout the seasons (Tütüncü & Dane, 2012). Pollen allergy, in particular, has a significant influence on public health because of its high incidence and related expenses. Allergic illnesses such as asthma and rhinitis can affect up to 30% of the population in industrialized regions (Asher et al., 2006), and their incidence appears to be increasing (D'Amato et al., 2007).

Thus, the purpose of this study was to characterize and describe the pollen morphology of specific species of Fabaceae family, conduct a literature review of Fabaceae palynology, enhance morphological knowledge, contribute to taxonomic study, and examine some allergy producing species.

Materials And Methods

Sampling:

The current study collected polleniferous material from eleven woody species representing Fabaceae family in the examined region and determined the variety in pollen characteristics. Plant species have been identified according to POWO, (2022). Plant species were collected from several locations in New Damietta (Figure 1). Table (1) contains a list of all the collected species. The plants were photographed in the field then carefully gathered, dried for further analyses. Pollen slides were kept in Damietta University Herbarium, Faculty of Science, Botany and Microbiology Department.

Microscopy:

Light microscopy

Erdtman's acetolysis teqnique was used to prepare pollen grains for the light microscope (LM) (Erdtman 1960). For each sample, measurements are based on at least 15 pollen grains. The morphological characteristics of various pollen grains were extensively investigated. Photomicrographs of pollen grains were acquired at 100x magnification with 16x eyepieces using a Motic SFC-200FL microscope equipped with an EGY-CAM camera. The terminology used in identifying and describing pollen grains follows Faegri and Iversen (1989), Erdtman (1952), and Punt et al. (2007).

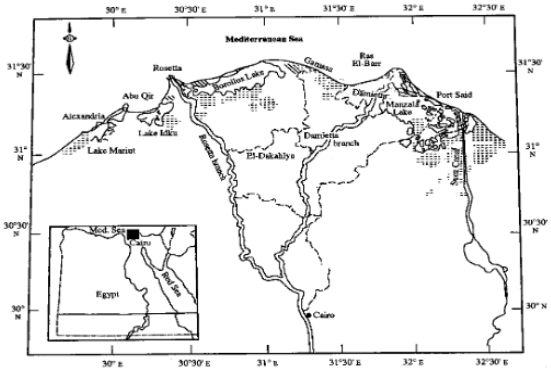

Figure (1): Location map of New Damietta City

SDS-PAGE Analysis

Pollen total proteins were extracted by homogenizing 100 mg frozen pollen grains with 50 mM Hepes buffer (pH 7.4) containing 5 mM β-mercaptoethanol and 5 mM phenylmethylsulfonyl fluoride (PMSF). The extracted samples were then centrifuged at 12,000 rpm for 10 min at 4 ◦C to get rid of the debris. The protein concentration in the extracts was determined as described by Bradford (1976). Each assay included 200 μL of Bradford protein reagent (BioRad Laboratories, Hercules, CA, USA), 50 μL of extract and 750 μL of water. The proteins were resolved in SDS-PAGE as described by Laemmli (1970) using the BioRad Mini Protean 3 (BioRad, Hercules, CA, USA). The resolving gel contained 11% acrylamide and the stacking gel was composed of 5% acrylamide. Fifteen microgram of protein samples was loaded onto each lane. The SDS gels were stained with brilliant blue R-250 and then de-stained with 20% methanol. After completing the destaining, the gels were dried. The identity of protein bands was determined by their molecular weights based on the loaded protein ladder (PageRuler™ Prestained Protein Ladder, Thermo Fisher Scientific, Cat. No. 26616).

Table (1): List of selected Fabaceae species with their localities.

Taxon	Geography coordinates	Life form	Life span
Acacia ehrenbergiana	31°26□22.90"N 31°40□56.05"E	Evergreen small tree	Perennial
Acacia saligna	31°26□20.35"N 31°40□57.87"E	Evergreen shrub or small tree	Perennial
Cassia alata	31°26□23.71"N 31°40□59.68"E	Evergreen tree	Perennial
Calliandra emarginata	31°26□21.89"N 31°40□59.68"E	Evergreen small tree	Perennial
Cassia fistula	31°26□21.85"N 31°41□00.17"E	**Deciduous** tree	Perennial
Cassia glauca	31°26□29.55"N 31°41□00.36"E	**Deciduous** tree	Perennial
Cassia nodosa	31°26□21.89"N 31°40□59.68"E	**Deciduous** tree	Perennial
Cassia tora	31°26□28.80"N 31°41□01.14"E	**Deciduous** shrub or small tree	Perennial
Delonix regia	31°26□21"N 31°40□59"E	**Deciduous** tree	Perennial
Leucaena leucocephala	31°26□23.53"N 31°40□59.61"E	Evergreen shrub or small tree	Perennial
Tipuana tipu	31°26□06.60"N 31°41□04.13"E	**Deciduous** tree	Perennial

Principal component analysis

Principal component analysis (PCA) ordination was performed using the program PAST version (4.04). The characteristics used for PCA included: Pollen unit, polarity, pollen shape, aperture type, exine sculpture, polar axis, equatorial diameter and pollen size.

Results

The palynological data of the examined species are listed alphabetically by genus and species, abbreviations and measurements are placed within brackets, and all data are reported in table (3).

Pollen grains morphology

***Acacia ehrenbergiana* Hayne.** Pollen grains isopolar, subprolate in equatorial view, large in size with mean dimensions of (44.46 - 55.4) µm. The grains are inaperturate, exine psilate, exine thickness 1.6 µm, P/E ratio 1.25, outline in polar view rounded.

***Acacia saligna* Labill.) H.L. Wendl.** Pollen grains isopolar, subprolate in equatorial view, medium in size with mean dimensions of (36.8 - 48.5) µm. The grains are inaperturate, exine psilate, exine thickness 1.46 µm, P/E ratio 1.32, outline in polar view circular to semi-rounded.

***Cassia alata* L.** Pollen grains isopolar, prolate-spheroidal in equatorial view, medium in size with mean dimensions of (29 - 29.9) µm. The grains are tricolporate, exine psilate, exine thickness 1.59 µm, P/E ratio 1.03, outline in polar view circular.

***Calliandra emarginata* Benth.** Pollen grains heteropolar, prolate in equatorial view, very large in size with mean dimensions of (68 - 114) µm. Polyads calymmate, with one end rounded and the opposite end tapered, composed of 8 pollen grains, being one central pollen grain surrounded by seven peripheral heteromorphic pollen grains, one of the peripheral has a conical and tapered shape. The grains are tetra- to penta-porate, exine verrucate, exine thickness 2.2 µm, P/E ratio 1.676, outline in polar view irregular- elliptic.

***Cassia fistula* L.** Pollen grains isopolar, oblate-spheroidal in equatorial view, medium in size with mean dimensions of (33.8 - 35.9) µm. The grains are tricolporate, exine granulate, exine thickness 2.8 µm, P/E ratio 0.94, outline in polar view circular to subtriangular.

***Cassia glauca* L.** Pollen grains isopolar, prolate-spheroidal in equatorial view, medium in size with mean dimensions of (37 - 37.75) µm. The grains are tricolporate, exine psilate, exine thickness 2.03 µm, P/E ratio 1.02, outline in polar view circular- elliptic.

***Cassia nodosa* L.** Pollen grains isopolar, prolate-spheroidal in equatorial view, medium in size with mean dimensions of (27.7 - 30.5) µm. The grains are tricolporate, exine psilate, exine thickness 1.84 µm, P/E ratio 1.1, outline in polar view triangular.

***Cassia tora* (L.) Roxb.** Pollen grains isopolar, prolate-spheroidal in equatorial view, medium in size with mean dimensions of (25.59 - 27.5) µm. The grains are triporate, exine psilate, exine thickness 2 µm, P/E ratio 1.072, outline in polar view circular- elliptic.

***Delonix regia* (Boj. ex Hook) Raf.** Pollen grains isopolar, subprolate in equatorial view, medium in size with mean dimensions of (33.3 - 36.3) µm. The grains are tricolporate, exine reticulate, exine thickness 4.56 µm, P/E ratio 1.09, outline in polar view circular.

***Leucaena leucocephala* (Lam.) de wit.** Pollen grains isopolar, prolate-spheroidal in equatorial view, medium in size with mean dimensions of (31.31 - 34.39) µm. The grains are tricolporate, exine psilate-perforate, exine thickness 2.97 µm, P/E ratio 1.098, outline in polar view circular.

***Tipuana tipu* (Benth.) Kuntze.** Pollen grains isopolar, prolate-spheroidal in equatorial view, medium in size with mean dimensions of (25.2 - 27) µm. The grains are tricolporate, exine psilate, exine thickness 1.55 µm, P/E ratio 1.07, outline in polar view triangular.

Pollen protein analysis

Electrophoretic (SDS-PAGE) analysis of total pollen protein content revealed several different protein bands. Two bands with molecular weights of 45 and 72 kDa in *Acacia ehrenbergiana*, four bands with molecular weights of 32, 55, 64, 95 kDa in *Cassia glauca*, two bands with molecular weights of 20 and 55-250 kDa in *Delonix regia*, two intensive bands with molecular weights range between 28-34 kDa and 40-95 kDa in *Leucaena leucocephala*.

Table (2): Representation of protein bands and their equivalent molecular weight (KDa) of some selected species of Fabaceae.

Scientific name	MWT (KDa)
A. ehrenbergiana	45
	72
C. glauca	32
	55
	64
	95
D. regia	20
	55-250
L. leucocephala	28-34
	40-95

PLATE (A)

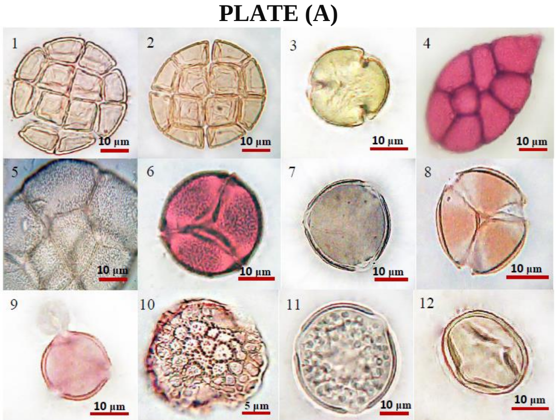

PLATE (A): Light micrographs of pollen grains of A. ehrenbergiana (1), A. saligna (2), C. alata (3), C. emarginata (4,5), C. fistula (6), C. glauca (7), C. nodosa (8), C. tora (9), D. regia (10), L. leucocephala (11), T. tipu (12).

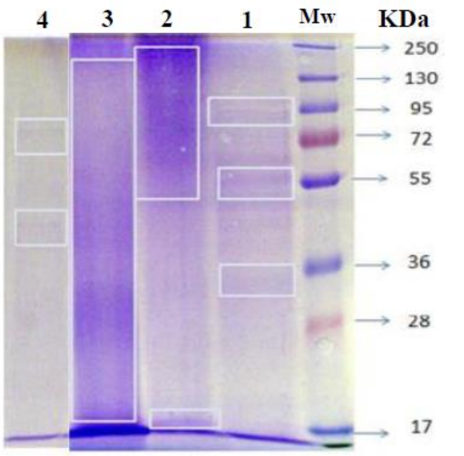

Figure (2): SDS-PAGE protein profiling of pollen (Molecular weight of bands assigned based on the similarity with molecular weight marker). (1- *C. glauca*., 2- *D. regia*, 3- *L. leucocephala*, 4- *A. ehrenbergiana*).

The palyno-morphological characteristics of 11 species belonging to 6 genera of the Fabaceae family are shown in Table 1. Photographs of the examined species were collected in (Plate B). Variations in all of the examined species have been reported using both quantitative and qualitative features. Polar and equatorial diameter (Figure 3), aperture types (Figure 4), P/E ratio (Figure 5), and exine thickness (Figure 6).

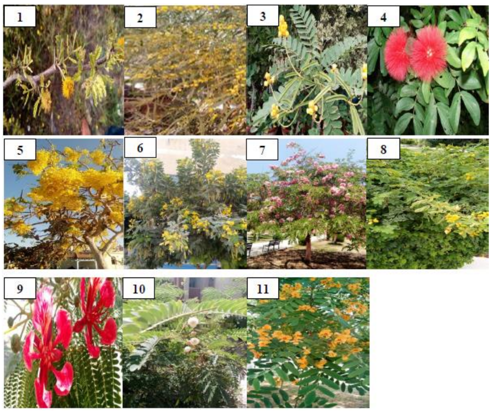

PLATE (B): Plant species of family Fabaceae in New Damietta, (1) *A. ehrenbergiana*, (2) *A. saligna*, (3) *C. alata*, (4) *C. emarginata*, (5) *C. fistula*, (6) *C. glauca*, (7) *C. nodosa*, (8) *C. tora*, (9) *D. regia*, (10) *L. leucocephala*, (11) *T. tipu*.

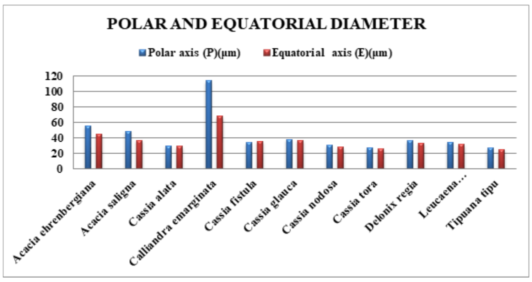

Figure (3): Polar and equatorial diameter variations of some selected Fabaceae species.

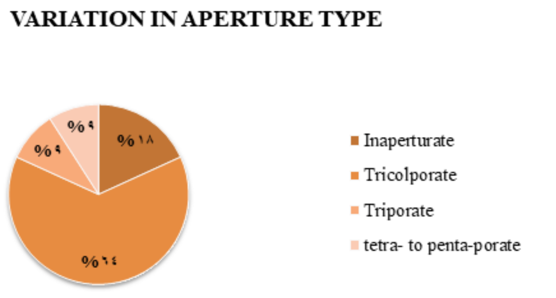

Figure (4): Aperture variations of selected Fabaceae species

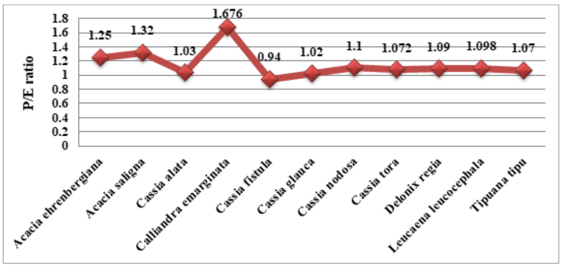

Figure (5): P/E index of some selected Fabaceae species

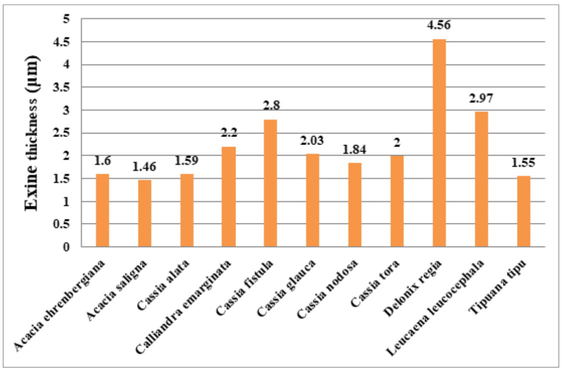

Figure (6): Variation in exine thickness of some selected Fabaceae species.

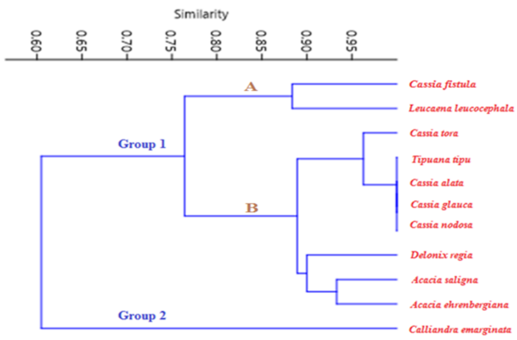

Figure (7): Dendrogram based on the palynological characters of some selected Fabaceae species.

Table (3): PCA of some selected species of Fabaceae

PC	Eigenvalue	% variance
1	11.3712	84.516
2	1.71377	12.738
3	0.151943	1.1293
4	0.0955564	0.71022
5	0.0887068	0.65931
6	0.0232064	0.17248
7	0.0101139	0.075171
8	3.27776E-34	2.4362E-33

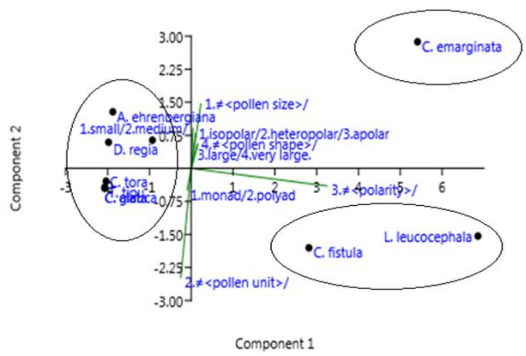

Figure (8): PCA scatter biplot of selected species of Fabaceae

Discussion

Light microscopic examinations of 11 species from the Fabaceae family in New Damietta province provided useful information on pollen morphological features. Pollen shape, size, polarity, polar and equatorial outlines, exine thickness, number of apertures, and exine ornamentation are the most important morphological aspects of the species studied. The pollen grains from Fabaceae family presented in this study show variation in terms of pollen unit, size, amb, shape, polarity, exine thickness, and ornamentation. These variations were also identified in the descriptions of certain species in results of NIEZGODA et al., (1983), Maghni (2020), Deshmukh et al., (2014), Khan et al., (2021) and Ullah et al., (2022).

The results showed that the shape of pollen grain is subprolate, rounded or circular for *A. ehrenbergiana*, *A. saligna*. This result confirms that stated by Al-Watban et al., (2013) and Rajurkar et al., (2013) with 16 monads which was similar to that revealed by Caccavari and Dome (2000). *Delonix regia* has subprolate, tricolporate and reticulate exine which was in agreement with the results of Orijemie, (2017) and Ullah et al., (2022) but it conflicted with that was described by Antonio-Domingues et al., (2018) who revealed oblate-spheroidal shape. Prolate-spheroidal shape recorded in *Cassia alata*, *Cassia glauca*, *Cassia nodosa*, *Cassia tora*, *Leucaena leucocephala and*

Table (4): The pollen morphological characters of the examined taxa (values in parentheses represent average lengths)

Taxa	Polar axis (P)(µm)	Equatorial axis (E)(µm)	P/E ratio	Pollen Size	Pollen Unit	Shape in Equatorial view	Shape in Polar view	Polarity	Aperture condition	Exine thickness (µm)	Exine sculpture Pattern
Acacia ehrenbergiana	50.7-58.5 (55.4±3.3)	27.3-54.6 (44.5±10.5)	(1.25) 1.07-1.27	Large	Polyad	Subprolate	Rounded	Isopolar	Inaperturate	1.6	Psilate
Acacia saligna	44-52 (48.5±2.9)	28-48 (36.8±5.8)	(1.32) 1-1.85	Medium	Polyad	Subprolate	Circular to Semi-rounded	Isopolar	Inaperturate	1.46	Psilate
Cassia alata	27.9-32.3 (29.9 ±1.7)	27.9-29.4 (29± 0.69)	(1.03) 1-1.09	Medium	Monad	Prolate Spheroidal	Circular	Isopolar	Tricolporate	1.59	Psilate
Calliandra emarginata	100-130 (114±9.8)	60-70 (68±4.1)	(1.676) 1.43-1.86	Very large	Polyad	Prolate	Irregular-Elliptic	Heteropolar	Tetra- to penta-porate	2.2	Verrucate
Cassia fistula	27.9-37 (33.8±2.3)	31.2-39 (35.9± 2.7)	(0.94) 0.8-1.13	Medium	Monad	Oblate Spheroidal	Circular to subtriangular	Isopolar	Tricolporate	2.8	Granulate
Cassia glauca	35.3-41.2 (37.75 ± 2)	35.3-38.2 (37±1.4)	(1.02) 1-1.08	Medium	Monad	Prolate Spheroidal	Circular-Elliptic	Isopolar	Tricolporate	2.03	Psilate
Cassia nodosa	23.4-35.1 (30.5±2.9)	23.4-35.1 (27.7±2.8)	(1.1) 0.86-1.3	Medium	Monad	Prolate Spheroidal	Triangular	Isopolar	Tricolporate	1.84	Psilate
Cassia tora	26.2-27.8 (27.5±0.7)	23.2-27.72 (25.59±1.7)	(1.072) 1-1.2	Medium	Monad	Prolate Spheroidal	Circular-Elliptic	Isopolar	Triporate	2	Psilate
Delonix regia	32.5-42.5 (36.3±4.5)	30-42.5 (33.3±4.4)	(1.09) 1-1.3	Large	Monad	Subprolate	Circular	Isopolar	Tricolporate	4.56	Reticulate
Leucaena leucocephala	29.4-38.2 (34.4±3.03)	26.46-38.2 (31.31±3.7)	(1.098) 1-1.19	Medium	Monad	Prolate Spheroidal	Circular	Isopolar	Tricolporate	2.97	Psilate-Perforate
Tipuana tipu	22.05-30.8 (27± 3.8)	19.2-32.34 (25.2±4.7)	(1.07) 0.88-1.4	Medium	Monad	Prolate Spheroidal	Triangular	Isopolar	Tricolporate	1.55	Psilate

Tipuana tipu. Prolate shape for *C. emarginata*, oblate-spheroidal for *C. fistula* which disagrees with that stated by Ullah et al., (2022). Also, the psilate exine was found in seven species, *A. ehrenbergiana*, *A. saligna*, *C. alata*, *C. glauca*, *C. nodosa*, *C. tora* and *T. tipu*. Verrucate, granulate, reticulate and psilate-perforate patterns are represented by one species only as follow *C. emarginata*, *C. fistula*, *D. regia* and *L. leucocephala* respectively. In comparison with previous studies: *L. leucocephala* has tricolpate and subpsilate to scabrate exine sculpturing (Aftab & Perveen, 2006 ; Bahadur et al., 2022**)**, this was not supported by our findings. The tricolporate pollen grains are found in seven species, *C. alata*, *C. glauca*, *C. nodosa*, *C. fistula*, *D. regia*, *L. leucocephala* and *T. tipu*. Porate type is found in two species, *C. emarginata* and *C. tora*. Inaperturate pollen grains are found in *A. ehrenbergiana*, *A. saligna* which corresponded with the results of Maghni, (2020). But it disagrees with that reported by Al-Watban et al., (2013) since *Acacia* species have colporate pollen grains. Qualitative and quantitative characteristics indicated that some of the species were compatible with previous studies and exhibit similarities in traits, demonstrating the genus' consistency.

The cluster analysis (Dendrogram) at 60% similarity showed two distinct groups of species (Groups 1 and 2) in Figure (7); Group (1) includes ten species divided into two groups (A & B) at 75% similarity, group (A) shows ≈ 88% similarity and consists of two species: *C. fistula* and *L. leucocephala*; group (B) contains the reminder of species and shows ≈ 88% similarity was further divided into two groups. The first group contained five species at 95% similarity: *C. tora*, *T. tipu*, *C. alata*, *C. glauca* and *C. nodosa*. The second group included three species: *A. ehrenbergiana*, *A. saligna*, *D. regia*. Group 2 comprises of one species: *C. emarginata*. This grouping was also reflected in the PCA. The pollen morphology of the Fabaceae taxa studied demonstrated high consistency, with just minor variations across species.

The results identified an allergenic protein band with a molecular mass of 45 KDa in *A. ehrenbergiana*, which was consistent with a previous result of (Shamsbiranvand, et al., 2014). This band was also detected in *L. leucocephala*, suggesting that the 45 kDa protein identified in this study could be a candidate for being one of the proteins responsible for the allergy in this species. There were six major allergenic proteins in *Phoenix dactylifera* with molecular mass ranging from 12 to 94 kDa bands (Kwaasi et al., 1993; Asturias et al., 2005). Among these proteins, there was a 55 kDa band discovered in *Cassia glauca*, *Delonix regia*, *Leucaena leucocephala*. The 20-kDa band was also identified in *D. regia*. Protein bands of 28-30 kDa were also identified in *L. leucocephala*. According to the findings *A. ehrenbergiana*, *C. glauca*, *D. regia*, and *L. leucocephala* contain molecular weight related proteins which may cause allergies in these plants, but further research utilizing immunoblotting analysis could be required to confirm these results.

Taxonomic key based on pollen micro-morphology

1- Pollen in polyads **2**
-Pollen in Monads... **4**
2- Polyads with one tapered end, composed of 8 grains ....***Calliandra emarginata***
-Polyads with uniform ends, composed of 16 grains **3**
3- Polyads medium sized (44-52µm x 38–48µm), ...***Acacia saligna***
-Polyads large in size (50.7-58.5µm x 27.3-54.6µm), ...***Acacia ehrenbergiana***
4- Pollen grains oblate spheroidal or subprolate **5**
- Pollen grains prolate spheroidal **6**
5- Pollen grains oblate spheroidal, exine granulate ....***Cassia fistula***
- Pollen grains subprolate, exine reticulate ..... ***Delonix regia***
6- Pollen grain triporate .... ***Cassia tora***
-Pollen grain tricolporate **7**
7- Exine sculpture is psilate **8**
- Exine sculpture is psilate- perforate ....***Leucaena leucocephala***
8- Exine thickness more than 1.8 µm ....***Cassia glauca* & *Cassia nodosa***
- Exine thickness less than 1.8 µm ....***Cassia alata* & *Tipuana tipu***

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الملخص العربي

عنوان البحث:

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تهدف الدراسة الحالية إلى التحقيق في مورفولوجيا حبوب اللقاح لأحد عشر نوعا خشبيا من الفَصيلة القَرْنيّة المزروعة في مدينة دمياط الجديدة ، تم اعداد شرائح حبوب اللقاح وذلك بعد صباغتها طبقا لطريقة **(Erdtman 1960)** ودراسة الصفات البالينولوجية المختلفة وتصويرها باستخدام المجهر الضوئي ، وأظهرت النتائج تباين في خصائص حبوب اللقاح النوعية والكمية ومن خلال هذه الدراسة أمكن وضع مفتاحا يمكن بواسطته التعرف والتفرقة بين هذه النباتات التي تم دراستها. بالإضافة الي دراسة بعض حبوب اللقاح المسببة للحساسية عن طريق فصل البروتين في هذه العينات باستخدام الفصل الكهربائي **(SDS-PAGE analysis)** لإجمالي محتوى بروتين حبوب اللقاح في أربعة أنواع ، وكانت النتيجة هي ظهور **Protein bands** ذات أوزان جزيئية تتراوح بين ٢٠-٢٥٠ كيلودالتون في الاربعة الانواع.