

Micro-Morphological Study of Eleven Taxa of the Apiaceae Family in Salah Al-Deen, Iraq

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Abstract

The present study examines the morphological characteristics of pollen grains from eleven taxa belonging to different genera within the Apiaceae family that grow wild in Salah Al-Deen governorate, Iraq, using a light microscope: Hoffmann, *Zosima absinthifolia* (Vent.). The current study results show that all pollen grains of the studied taxa are monads and isopolar, all of which are tricolporate. The study includes the morphological characteristics of the pollen grains in terms of the pollen grain's shape and size, the pores' dimensions, the exine's thickness, and its ornamentation. The sizes of the pollen grains range from small in the two taxa *C. carvi* and *C. papillaris*, while being medium in seven species, namely *C. maculatum*, *T. viscidulum*, *S. iberica*, *Z. absinthifolia*, *M. secacul*, *H. amplifolium*, and *O. daucoides*, and large in the two species of *T. latifolia* and *L. strigosa*. They were oblong in all the studied species except *C. carvi*, *O. daucoides*, and *H. amplifolium*, and also showed differences in the shape of both the equatorial and polar views. The findings from the study hold significant taxonomic value for the isolation of the species examined.

Keywords: polar axis, pollen, Tricolporate, Umbelliferae

دراسة مظهرية دقيقة لأحد عشر جنساً من العائلة الخيمية في صلاح الدين، العراق

رقية مظهر الحشموي ونجلاء مصطفى العبيد

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المستخلص:

تناولت الدراسة الحالية دراسة حبوب اللقاح لأحدى عشرة مرتبة عائدة لأجناس مختلفة من العائلة الخيمية Apiaceae النامية برياً في العراق وهي *Cachrys Lisaea strigosa*, *Malabaila secacul*, *Conium maculatum*, *Hellenocarum amplifolium* Boiss, *papillaris* Boiss, *Carum carvi* L. *Scandix iberica* M., *Trigonoscidium viscidulum* Boiss, *Turgenia latifolia* (L.) Hoffm, (Mill.) Boiss, *Orlaya daucoides* (L.) *Zosima absinthifolia* (vent) باستخدام المجهر الضوئي. وقد تبين من نتائج الدراسة الحالية ان حبوب لقاح الانواع المدروسة جميعها كانت مفردة Monads ومتمائلة الاقطاب isopolar، وجميعها ذات طراز ثلاثي الاخاديد والتقوب Tricolporate كما اوضحت النتائج انها من نوع Zonocolpate اي لا تمتد الاخاديد من القطب الى نهاية القطب ولا تلتقي نهاية الاخاديد مع بعضها وتضمنت الدراسة الصفات المظهرية لحبوب اللقاح من ناحية شكل وحجم حبة اللقاح وابعاد التقوب وسمك الغلاف الخارجي والزرخفة السطحية وتراوحت احجام حبوب اللقاح بين الصغيرة في النوعين *C. carvi* و *C. papillaris*، بينما كانت متوسطة الحجم في سبعة مراتب هي *C. maculatum* و *T. viscidulum* و *S. iberica* و *Z. absinthifolia* و *M. secacul* و *H. amplifolium* و *O. daucoides* وكبيرة الحجم في النوعين *T. latifolia* و *L. strigosa*، في حين كانت ذات شكل متطاول في جميع الأنواع المدروسة عدا *C. carvi* و *O. daucoides* و *H. amplifolium*، كما اظهرت اختلافات في شكل كل من المنظرين الاستوائي والقطبي، وكان للنتائج المستحصل عليها من الدراسة اهمية تصنيفية جيدة في عزل الانواع المدروسة.

الكلمات المفتاحية: العائلة المظلية، حبات اللقاح، ثلاثية التقوب، المحور القطبي

Introduction

The first to point out the importance of pollen morphological characteristics in taxonomic studies was Brown (1811) through his study of the Protoceae family (Erdtmann, 1971). He pointed out that pollen grains have important characteristics that make them of great benefit and importance in taxonomic studies, especially after the invention and development of the scanning electron microscope (SEM) and the transmission electron microscope (TEM), which had a significant impact on the development of this science and the use of precise morphological characteristics in isolating and identifying genera and species and solving many complex and ambiguous taxonomic problems. These characteristics include the shape and size of the pollen grain, spines, and surface decorations, in addition to the pores and their number in a single grain, the furrow (sulcus), and the composition of the grain wall. Davis & Heywood (1973) indicated that a family whose species are characterized by pollen grains with fixed morphological characteristics is called stenopalynous, and these characteristics may be limited to a specific family and not others. Cerceao-Larrival & Ronald-Heydacker (1976) divided the family Apiaceae based on the shape of pollen grains using scanning electron microscopy (SEM) into five types: the rhomboidal type, which is the most primitive type, in which the pollen grain is small and thin-walled; the

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semicircular type, which is similar to the first type in characteristics; the ovoid type, in which the pollen grain is large and thick-walled; the oblong type, which is the most common among the family members, in which the pollen grain is thick-walled; and the equatorial type, which is the most complex in shape and structure, in which the pollen grain becomes winged due to the elongation of the outer wall (exine).

Researchers have been interested in studying the morphology of pollen grains of the Apiaceae family with technological progress and taking advantage of these precise phenotypic characteristics in isolating species. Among these studies are the study of Erdtman (1952) and the study of Ting et al. (1964). This study included the morphological characteristics of pollen grains of species from the secondary family Hydrocotyloideae. (Punt, 1984)

showed through his study of pollen grains of 55 genera of the Apiaceae family in northern Europe that Apiaceae pollen grains are characterized by the presence of internal and external lines, and their external shape is characterized by the presence of polar. Another important study is the study of BALDEMİR et. al. (2018), as he was interested in studying the shape of the pollen grains of the family and explained that they were triangular to semi-oval in the polar view. The study of (Halbritter et. al., 2018) showed that the studied family's pollen grains were tricolporate. The study (Bakr, 2011) focused on studying 4 species belonging to the two genera *Scandix* and *Smyrniun* in northern Iraq, and the study (Al-Newani et. al., 2018) used a scanning electron microscope (SEM) for 12 species belonging to the Apiaceae family. The study (Al-Taie, 2022) focused on the morphology of pollen grains of species belonging to the two genera, *Torilis* and *Gramosciadium*. Here, we report for the first time information on the micro-morphological features of eleven genera of the Apiaceae family found in the Salah Al-Deen area to collect as much information as possible for future taxonomic research and to differentiate between the genera under consideration.

Materials and Methods

The current study of pollen grains relied on fresh samples in the flowering and fruiting stages collected during field trips during the period from the beginning of February 2023 to March 2024. Surveys were conducted for Apiaceae species in different locations in Salah Al-Deen Governorate, including Yathrib, Tikrit, Al-Alam, Sharqat, Al-Dhuluiya, and Baiji. The studied plant species were diagnosed based on the Flora of Iraq, which includes the Apiaceae family (Ghazanfar & Edmondson, 2013). To confirm the diagnosis, specialized professors working in the Iraqi herbaria that were visited were consulted, especially the Iraqi National Herbarium in Abu Ghraib. The study of pollen grains was based on the acetolysis method, which was indicated by Willson & Goodman (1964) with some modifications. As for the glycerin gel, it was (Sass, 1958) in the following proportions: (Glycerin 35ml, Distal water 30ml, and Gelatin 5gm).

1. Some mature flower anthers preserved in 70% ethyl alcohol are placed inside a watchglass containing a few drops of distilled water. The mature anthers are crushed with a fine dissecting needle to extract pollen grains, and then the excess anthers are removed from the watchglass.
2. Then, the watch glass is placed on a hot plate at 60°C until the water dries.
3. After that, 4-5 drops of acetolysis solution prepared from 9 parts of concentrated hydrochloric acid and 1 part of concentrated sulphuric acid are added. The pollen grains are covered with the solution and heated gently on a hot plate at a temperature of 70°C until the solution turns brown.
4. Several drops of 100% absolute ethyl alcohol were added to the hot mixture. The solution is observed to move towards the edge of the watch glass and is dried with dry paper. Then, several drops of the solution containing the pollen grains are transferred with a clean dropper specific for each type to a glass slide.
5. A few drops of the previously prepared glycerin gel are added to the glass slide and then covered with a cover slide. Pollen grains are photographed using a camera mounted on a compound light microscope at a power of 40. Polar and equatorial axis measurements are taken, and the P/E value is calculated in addition to measuring the thickness of the outer grain wall using an Ocular 100 micrometer at a power of 100X.

Results and Discussion

The results of the current study of the studied species show that all pollen grains are monads, and isopolar, but the variation begins to be clear in shape, size, exine, and surface ornamentation. It is found that pollen grains are of the Tricolporate type in all studied species, and pollen grains of all species are of the Zonocolate type, meaning that the furrows in them do not extend to the end of the pole, and the ends of the grooves do not meet each other. The results of the current study are consistent with what Altaie & Al-Ani (2020) mention in describing

some species of the Apiaceae family in terms of the nature of the pollen grain, its shapes, sizes, and wall thickness.

According to the criteria provided by Erdtman (1952) to determine the size of pollen grains, their sizes ranged in the polar view between small, medium, and large. The species *C. papillaris*, *T. viscidulum*, *S.iberica*, *Z. absinthifolia*, *M. secacul*, *H. amplifolium*, and *O. daucoides* have medium pollen grains, ranging in length from 25-50 micrometers. The small-sized pollen grains are recorded by the species *C maculatum*, and *C. Carvi at a* rate ranging between (10-25) micrometers. As for the large- sized pollen grains, they are seen in the two species *L. strigosa* and *T. latifolia* (Table 1).

Species	Polar axis p	Equatorial axis E	P / E	External wall thickness rating	pores dimesnions	Distance between gaps	Pollen shape in tropical view view	Pollen shape i n polar view
<i>C. maculatum</i>	15-20 (16.6)	12-14 (12.6)	1.31	1-2 (1.3)	5-6 (5.3)	5-7 (5.6)	Elongated	Triangular
<i>C. papillaris</i>	30-38 (35.5)	20-25 (23.6)	1.50	1-2 (1.3)	3-4 (3.3)	5-6 (5.3)	Elongated	Triangular
<i>C. carvi</i>	15-18 (15.6)	10-14 (12.6)	1.23	1-2 (1.6)	2-3 (2.3)	4-5 (4.3)	Sub- elongated	Triangular
<i>T. latifolia</i>	48-54 (51.3)	34-35 (34.6)	1.48	1-2 (1.6)	5-7 (5.6)	4-5 (4.6)	Elongated- constricted	Triangular
<i>T. viscidulum</i>	25-27 (26.3)	18-20 (18.6)	1.41	1-2 (1.3)	3-4 (3.6)	4-5 (4.3)	Elongated- constricted	Triangular
<i>S. iberica</i>	38-40 (39.3)	20-22 (20.6)	1.71	1-2 (1.6)	5-7 (6.3)	6-8 (6.6)	Elongated- constricted	spherical
<i>Z. absinthifolia</i>	28-32 (29.3)	18-20 (19.3)	1.51	(1)	5-6 (5.3)	4-5 (4.6)	Elongated- constricted	Circular
<i>M. secacul</i>	28-34 (32.6)	22-25 (22.5)	1.60	(1)	3-5 (3.6)	5-8 (6)	Elongated- constricted	Circular
<i>L. strigosa</i>	50-55 (53.3)	34-38 (37.3)	1.42	2-3 (2.6)	10-14 (12.6)	6-8 (6.6)	Elongated- constricted	Circular
<i>H. amplifolium</i>	23-28 (25.3)	20-23 (20.6)	1.22	2 (2)	2-3 (2.6)	4-5 (4.6)	Sub- elongated	Circular
<i>O. daucoides</i>	26-28 (26.6)	20-23 (21)	1.26	(1)	5-8 (7)	4-6 (5)	Sub- elongated	Triangular

The average length of the polar axis and the average length of the equatorial axis show a clear variation between the species, as the highest average length of the polar axis is 53.3 micrometers and the equatorial axis is 37.3 micrometers in the species *L. strigosa*. The lowest average length of the polar axis is 15.6 micrometers in the species *C. carvi*, while the lowest average length of the equatorial axis is 12.6 micrometers in the species *C. papillaris* and *C. carvi*. The values of the rest of the species range between these two averages.

As for the ratio between the polar and equatorial axes P/E, the highest rate is 1.71 micrometers in the species *S. iberica*, while the lowest rate is 1.22 micrometers in the species *H. amplifolium*. The values of the rest of the species range between these two limits (Table 1 and Plates 1,2).

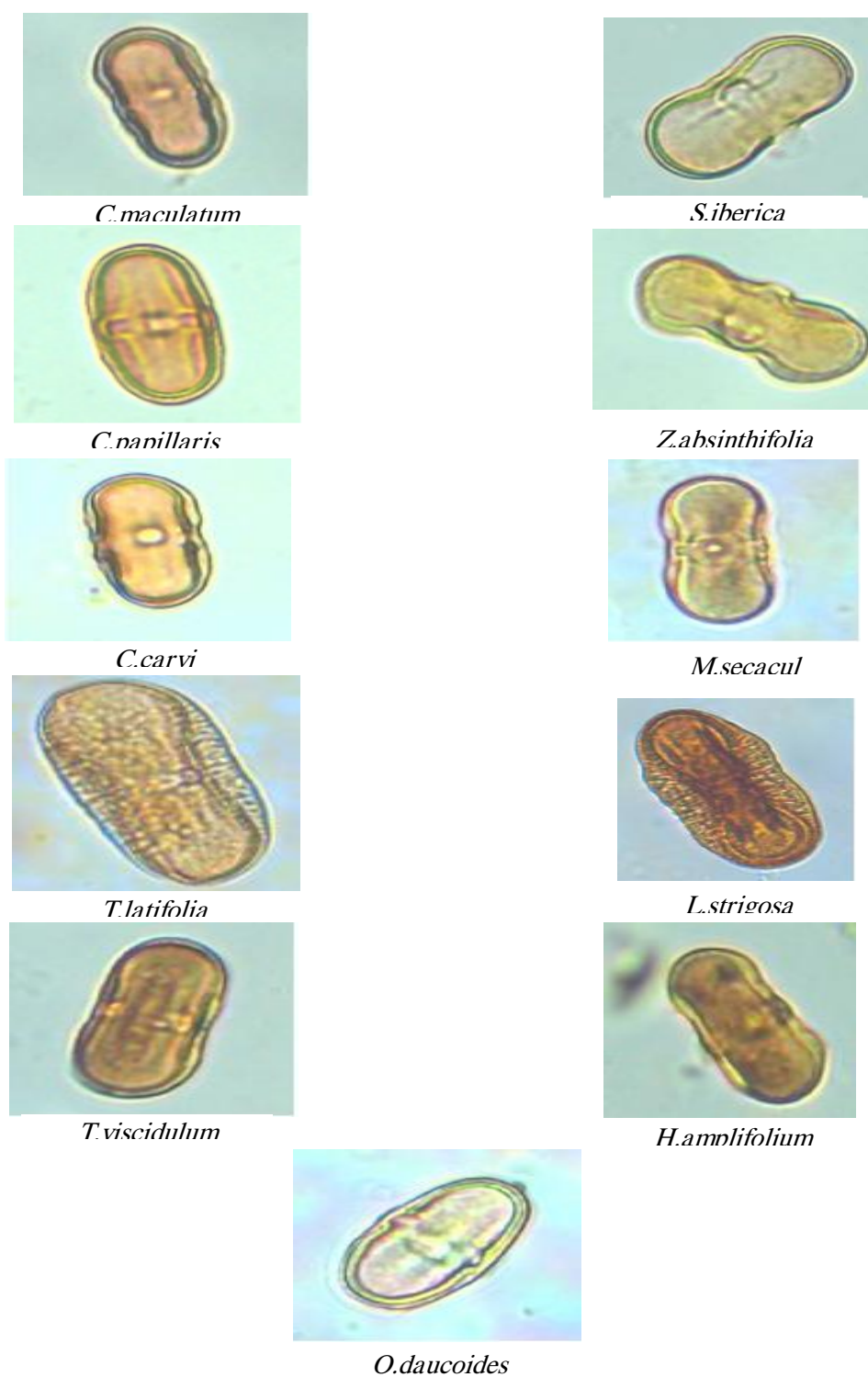
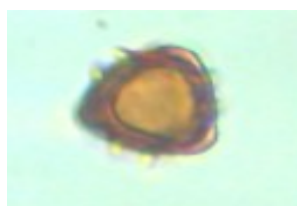
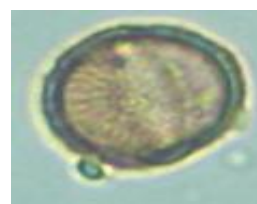


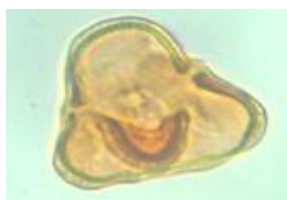
Plate 1: Variations in dimensions and shapes of pollen grains in the equatorial view of the studied species



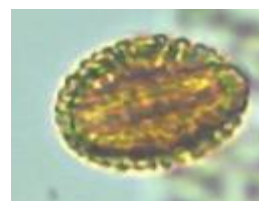
C. maculatum



S. iberica



C. papillaris



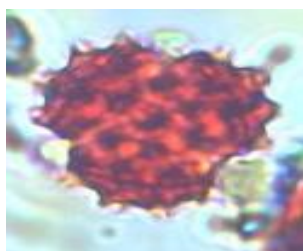
Z. absinthifolia



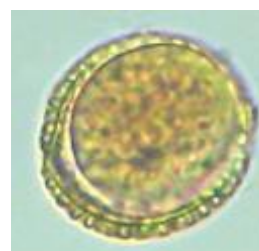
C. carvi



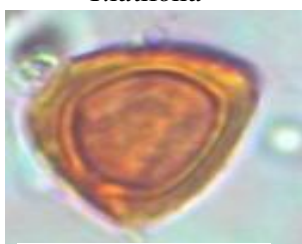
M. secacul



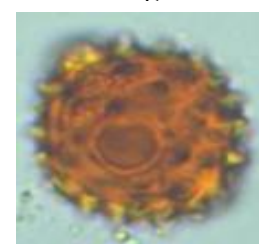
T. latifolia



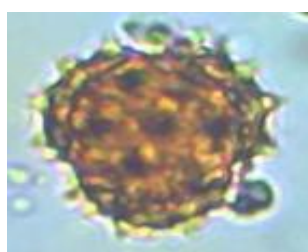
L. strigosa



T. viscidulum



H. amplifolium



O. daucoides

Plate 2: Variations in dimensions and shapes of pollen grains in the polar view of the studied species

It is also found that there is a difference in the shapes of pollen grains, and that pollen grains vary in the same species in their polar and equatorial views. In terms of the shape of pollen grains in the equatorial view, they are divided into three groups: a group with elongated pollen grains, as in the species *C. maculatum* and *C. papillaris*, a group with elongated pollen grains constricted in the middle, as in *M. secacul*, *Z. absinthifolia*, *T. viscidulum*, *S. iberica*, and *T. latifolia* and *L. strigosa* confirming to [16] and a group with sub-elongated pollen grains, as in the species *H. amplifolium*, *C. carvi*, and *O. daucoides* in agreement with (Özköket. al., 2022).

In terms of pollen grain shape, the species in the polar view are divided into two groups: a group with triangular pollen grains, which includes the species *C. maculatum* and *C. papillaris*, *C. carvi*, *T. latifolia*, *T. viscidulum*, and *O. daucoides*. The second group has circular pollen grains and includes the species *S. iberica*, *L. strigosa*, *M. secacul*, *Z. absinthifolia*, and *H. amplifolium*. This agrees with Birjees et. al. (2022).

The results of the study show a difference in the dimensions of the aperture opening, as the largest is in the species *L. strigosa*, which averages 12.6 micrometers, and the smallest is recorded in the species *C. carvi*, with an average of 2.3 micrometers, and the dimensions of the other species range between these two averages. The results also show a difference in the distance between every two adjacent pores, as the highest average is 6.6 micrometers in the species *S. iberica* and *L. strigosa*, and the lowest average is 4.3 micrometers in the species *C. Carvi* and *T. viscidulum*. It is also found that there is a difference in the shapes of pollen grains, and that pollen grains vary in the same species in their polar and equatorial views. In terms of the shape of pollen grains in the equatorial view, they are divided into three groups: a group with elongated pollen grains, as in the species *C. maculatum* and *C. papillaris*, a group with elongated pollen grains constricted in the middle, as in *M. secacul*, *Z. absinthifolia*, *T. viscidulum*, *S. iberica*, and *T. latifolia* and *L. strigosa* confirming to [16] and a group with sub-elongated pollen grains, as in the species *H. amplifolium*, *C. carvi*, and *O. daucoides* in agreement with (Özköket. al., 2022).

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The studied species also vary in the thickness of the outer pollen wall. The highest average thickness is 2.6 μm in the pollen walls of *L. strigosa*, and the lowest is 1 μm in the pollen walls of *O. daucoides*, *M. secacul*, and *Z. absinthifolia*. Values for other species ranged between these two averages.

The surface decoration of pollen walls appears in different patterns, and accordingly, species are divided into four groups: a group with smooth surfaces, including *C. maculatum*, *C. carvi*, *C. papillaris*, and *T. viscidulum*; a group with granular pore-like (foveolate) surface decoration in *S. iberica*; a group with reticulated surface decoration, such as *L. strigosa*, *M. secacul*, and *Z. absinthifolia*; and a group with spiny surface decoration, such as *O. daucoides*, *H. amplifolium*, and *T. latifolia*. This is consistent with Demirpolat et al. (2021).

Wall decorations contribute to the distinction between the studied species, as the fine morphological characteristics of pollen grains are of great taxonomic importance due to the chemical nature of the wall and the resistance of its coatings to environmental conditions and decomposition over the years.

Conclusions

The results of the current study demonstrate that the quantitative and qualitative characteristics of pollen grains, observed using a compound light microscope, are taxonomically significant. Pollen grains are Tri sporangial in all studied species, and are of the iso polar type. Their sizes range from small to medium and large. The study demonstrates the importance of pollen grain size and shape, and the presence or absence of the polar and equatorial axes, in identifying the studied species. Furthermore, the thickness of the outer wall and the presence of surface decorations provide clear evidence for distinguishing and isolating the studied species.

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