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PALYNOLOGICAL STUDY OF THE SELECTED SPECIES IN CERTAIN GENERA OF THE FAMILY COMPOSITAE (ASTERACEAE)

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SUMMARY

The contemporary study examined several wild species of dicotyledons developing in the Samawa Desert in Southern Iraq. The various characteristics of these species' assessment used a light microscope to isolate the species from each other and better utilize their qualities. Based on the collected field samples, four species belonging to the family Asteraceae reached identification, i.e., *Aaronsohnia factorovskyi*, *Anvillea gracinii*, *Anthemis maritima*, and *Calendula arvensis*. The botanical characteristics, such as shape, size, apertures, pole type, apocolpium, mesocolpium, wall thickness, and exine sculpturing, also gained evaluation in the study. The examined profiles of different species varied greatly, according to the results. The nature of the polarity of the pollen grains determines the pollen grains' polarity, which, in turn, determines the shape of apertures and isopolar to take apertures zono-colporate. The overall shape of the pollen grains, determined by the ratio between the polar axis to the equatorial axis P/E, included prolate spheroidal and oblate spheroidal. By measuring the axis length, the study showed that the species' pollen grains were within a medium range. As for exine sculpturing, the study revealed one type of sculpturing, i.e., echinate in the genera *Aaronsohnia factorovskyi*, *Anvillea gracinii*, and *Anthemis maritima*, while the genus *Calendula arvensis* was devoid of decorations.

Keywords: Compositae (*Asteraceae*), botanical traits, pollen grains, *A. factorovskyi*, *A. gracinii*, *A. maritima*, *C. arvensis*

Key findings: The study showed some botanical traits, such as spines, are closely related to species and taxa belonging to the family Compositae. Most studied species contained the outer wall layer of the pollen on spines of different lengths and shapes.

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INTRODUCTION

Compositae (Asteraceae) is the second-largest family in the Magnoliophyta division, and worldwide, with well-developed members, including 1100 genera and 20,000 known species (Ming, 1999). Most Asteraceae species are herbaceous; however, shrubs and even trees, make up a significant part of the family. Numerous members of the Asteraceae family have commercial value as weeds, ornamentals, medicines, and green foods (Olorode, 1984). According to Moore and Webb (1978), the taxonomy of angiosperms heavily relies on pollen grain traits, and the same is employed to identify the evolutionary relationships in the family Asteraceae.

The floristic and climatic characteristics of geological formations that have undergone extensive study using pollen grains used prehistoric and contemporary plants (Erdtman, 1943; AL-Amery and Abu-Serag, 2015). Prasad (1963) made the first successful attempt to classify crop plants using pollen characteristics. Past studies also contributed to understanding the pollen grain's morphology and the significance of pollen architecture in tracing phylogeny (Patel and Datta, 1958). Some other studies also claimed that the Asteraceae family species' pollen grains were spheroidal, echinate, or spinate and had round apertures (Perveen, 1999; Adediji, 2005; Mbagwu *et al.*, 2009).

The macro-morphological characteristics alone do not considerably determine the family's tribal classification, particularly for species that have historically been challenging to define due to extremely varied macro-morphological characteristics. This conclusion also received support from the presence of multiple taxa, including *Brachylaena*, *Eremothamnus*, *Gundelia*, *Moquinia*, and *Tarconanthus*, which are unclassifiable into a single tribe (Wortley *et al.*, 2007) due to the inconsistent distribution of high macro-morphological features in the genus *Adenocaulon*, which is difficult to assimilate into tribes, and family Asteraceae has proven also challenging to categorize (Panero and Funk, 2008).

Ecological specialization was the source of uneven macro-morphological level features, such as apomixes occurrence in the family under an environmental adaptation process. Except for the Arctic and Antarctica, the Asteraceae family has a high degree of adaptation due to its apomictic character (Zagorski *et al.*, 2020). According to Jeffrey (2007), these parameters also cause alterations in the macro-morphology and even affect the number of chromosome sets, ranging from $2n = 2$ to $2n = 120$. Therefore, permanent distinguishing characteristics are necessary for the proper Asteraceae family classification.

Pollen also exhibits such a character stability type (Wortley *et al.*, 2012). Past studies demonstrated pollen has served as a data source for categorizing the Asteraceae (Almosawi and Aldobaissi, 2022). In the revision of *Gundelia* and Warioina's phylogenetics, it considered the characteristics of pollen grains. In addition, Robinson *et al.* (2008) created the new genus *Tarlmounia* based on pollen morphological characteristics and revitalized the genus *Strobocalyx* from *Gymnanthemum* and *Vernonia*. A study conducted by Meo and Khan (2006) in Qatar examined the Arabic studies on the Family Compositae (Asteraceae). The study included a total of 17 species from 16 genera.

Similarly, Preveen (1999) conducted an Iraqi-level study focusing on the Family Compositae (Asteraceae)'s 56 species. Their research aimed to elucidate the key characteristics of this plant family. Numerous scholars have conducted taxonomic investigations on various genera within the family as part of their comprehensive analysis of pollen characteristics. This study aims to investigate additional aspects, especially pollen shape and variety and wall structural information. The only consideration of macro-morphological qualities does not possess significant discriminatory power in determining the tribal classification of a family, especially in the case of species that have historically posed challenges in their definition due to their highly diverse macro-morphological characteristics.

MATERIALS AND METHODS

Samples collection, preservation, and diagnosis

The study of pollen grains' consideration relied on newfound flowers collected directly from the field during ground visits in the Samawa Desert, Southern Iraq, where the genera are widespread. The studied samples bore diagnoses using the researcher's personal information and the classification keys.

Preparation of microscopic slide

Taking a mature anther bud and putting it on a microscopic slide included adding a drop of the dye. As the anthers opened, removing the pollen grains used an anatomy needle. Removing the anthers' residue followed by placing the cover slide lightly, and the slide was ready for examination.

Preparation of dye

The safranin-jelly-glycerin dye, as reported by Sass (1958), served the purpose of imaging some samples only, and its preparation is as follows: Adding 10 g of gelatin material to 60 ml of distilled water leaving the mixture for two hours before adding 70 ml of glycerin and a few drops of safranin, then mixed. Later, add 0.5 g of phenol and put the mixture in a water bath until homogenization to dissolve the pieces of phenol. In this study, the dye used in examining samples and taking measurements was safranin-glycerin dye for easy movement of the cover slide upon examination and smooth moving of the sample and study from all sides. Preparing the dye continued by adding one size of safranin to six volumes of glycerin.

Microscope slide study

The microscope slide examination used an optical microscope, with measurements taken using an ocular micrometer after the calibration procedure. All the recorded measurements ensued under the Lente 40× except for measuring wall thickness, and the

dimensions of the spines and spinules and width of the apertures taken used the oil lens 100×. The pollen grains photographed under the oil lens had a magnification force that sustained retraction later using a Zeiss light microscope and a Canon digital camera.

Zoom power calculation

The zoom strength of the vaccines' calculation relied on the following formula:

Zoom force = Eyepiece power × objective lens power × camera zoom power (if found).

RESULTS

The current study showed that under scrutiny, genera pollen grains of the family Compositae differed by several points; hence, studying several axes (qualities). The obtained differences among these pollen grains were as follows:

Polarity

In the family Compositae, the pollen grains' release from the anthers is in the form of monads, dyads, or polyads clustered as a mass within an installation called the pollen sac. However, in the presented study, the family Asteraceae genera showed that pollen grains were monad, symmetrical, and isopolar (Figure 1).

Type

Based on the types of apertures in the family Compositae, the pollen grains are divided into seven groups. Initially, C 0, which is the function of the unknown grain, the shape of the pollen, the C1 denotes the pollen whose apertures are a thin area of the outer shell called leptoma, and the C2 denotes tricotomocolpate. The rest of the four are either simple apertures, which are either colpate or porate, or indicate compound apertures, which are either colporate consisting of the outer aperture in colpi and the inner aperture oral and pororate may be the outer and inner aperture in a pore form. The study

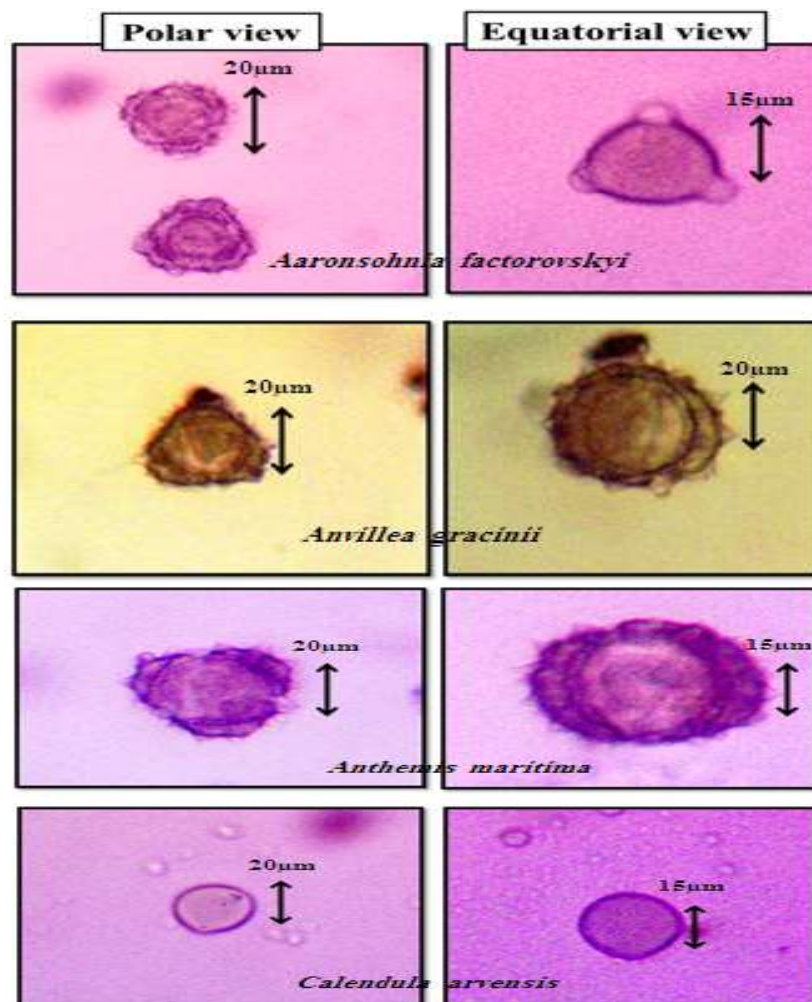


Figure 1. The pollen grains of taxa, Asteraceae by light microscope (400×).

showed that pollen grains in all the genera were of colpate pattern arranged around zono-colpate (Table 3).

Shape

The relevant study of pollen grains commenced by observing the shape of the external boundaries of the two views by taking measurements for the polar and equatorial axes. The polar axis was the axis that connects the poles of the pollen to the equatorial view, while the equatorial axis was the axis that extends along the equator. By evaluating the ratio between the polar axis to equatorial P/E, the overall shape of the pollen attained definition as spherical, prolate, or oblate (Table

1). The ratio of the polar axis to the equatorial axis P/E has identified two pollen shapes in the family Compositae:

- a. Prolate spheroidal as in *Calendula arvensis* and *Anthemis maritima* (Table 3) and
- b. Oblate spheroidal as in *Aaronsohnia factorovskyi* and *Anvillea gracinii* (Table 3).

In the polar view of the genera, the pollen grains appeared circular, and in the equatorial view, these occurred as elongate spherical.

Size

Based on the estimated longest axis measured in pollen grains, the determined size was within the range specified between the tiniest

Table 1. The shapes of pollen based on P/E value.

Shape classes	P/E	100.P/E
Per oblate	<8/4	<50
Oblate	4/8-6/8	50-75
Sub spheroid	6/8-8/6	75-133
Sub oblate	6/8-7/8	75-88
Oblate spheroid	7/8-8/8	88-100
Prolate spheroid	8/8-8/7	100-114
Sub prolate	8/7-8/6	114-133
Prolate	8/6-8/4	133-200
Per prolate	>8/2	>200

Table 2. Size of pollen grains of different types of plants.

No.	Size of pollen grain	Measurement
1	Very small	Smaller than 10 µm
2	Small size	10–25 µm
3	Medium size	25–50 µm
4	Large size	50–100 µm
5	Very large	100–200 µm
6	Gigantic size	Larger than 200 µm

Table 3. Quantitative and qualitative features of taxa, *Asteraceae* pollen grains.

No.	Taxon	Polar view (µm)	Equatorial view		Aperture (µm)			Apolcolpium (µm)	Mesocolpium (µm)	Wall (µm)	P/E	Shape	Size	Exine sculpturing
			Polar axis	Equatorial axis	Colpus		Ora							
					Length	Width								
1	<i>Aaronsohnia factorovskyi</i>	39.4-42.1 (40.5)	36.2-38.5 (38.1)	37.0-39.1 (38.3)	15.3-18.1 (16.5)	4.4-4.9 (4.7)	1.5-1.8 (1.7)	5.3-6.2 (5.8)	23.1-25.5 (24.3)	2.8-3.1 (3.0)	0.99	Oblate spheroidal	Medium	Echinate
2	<i>Anvillea gracinii</i>	40.1-44.5 (40.5)	32.8-33.5 (33.1)	36.1-37.5 (37.4)	15.4-17.1 (16.4)	2.2-3.1 (2.6)	1.7-2.5 (2.3)	13.7-15.5 (14.5)	32.2-33.1 (32.5)	2.2-2.8 (2.5)	0.88	Oblate spheroidal	Medium	Echinate
3	<i>Anthemis maritime</i>	33.8-36.5 (35.5)	27.5-29.5 (27.9)	25.9-27.5 (26.1)	11.6-15.2 (14.3)	2.5-3.3 (3.1)	1.9-2.3 (2.1)	11.1-14.5 (13.3)	21.1-23.5 (22.3)	2.5-3.1 (2.7)	1.06	Prolate spheroidal	Medium	Echinate
4	<i>Calendula arvensis</i>	29.6-32.4 (30.2)	22.2-24.7 (23.5)	17.1-19.5 (18.2)	11.9-15.8 (14.7)	2.8-3.7 (3.6)	1.7-1.9 (1.8)	5.7-6.8 (5.9)	30.2-33.6 (32.1)	2.1-2.6 (2.3)	1.29	Prolate spheroidal	Medium	Psilate

and gigantic (Table 2). In measuring the length of the axes, the study further revealed that the species' pollen grains were within the range of medium in the family Compositae (Table 3).

Apertures

The apertures were compound - zono-3-colporate, with a short and narrow colpus and a lalongate oral. The width of the colpus increased at the equator (hole area or ora). The genera of the family Compositae have distinctions based on the width and length of the colpus in each genus. The lowest colpus length was evident in the *Anthemis maritime* (14.3 μm), with the shortest width recorded in the genus *Anvillea gracinii* (2.6 μm). The highest values for length and width of the colpus resulted in the genus *Aaronsohnia factorovskyi* (16.5 $\mu\text{m} \times 4.7 \mu\text{m}$) (Figure 2 and Table 1). The inner aperture (ora) was lalongate with a diameter of 2.3 μm in the genus *Anvillea gracinii* and 1.7 μm in the genus *Aaronsohnia factorovskyi* (Table 3).

Apocolpium

It is the polar spot represented by the space confined between the ends of the colpus at each pole. Its determination can proceed by measuring the distance between the opposite copus at each pole parallel to the equator.

However, there was no apocolpium in pantoaperturate and syncolpated pollen. The highest measurement rate for the apocolpium appeared as 14.5 μm in the genus *A. gracinii*, while the lowest value was in the genus *A. factorovskyi* (5.8 μm) (Table 3).

Mesocolpium

It is the largest area between two colpus, as determined from the straight line measurement, which links the two adjacent colpus at the equator. The highest value for distance between two contiguous colpus (mesocolpium) of the genus *A. gracinii* was 32.5 μm , while the lowest value came from the genus *A. maritime* (22.3 μm) (Table 3).

Wall thickness

The pollen always has surrounding two wraps - inner cellulose, called intine, and an outer cuticle, known as exine. The outer shell of the pollen consists of endexine and ectexine, and the latter consists of the foot layer, Columbia, and tectum. The highest value for the outer layer thickness of the pollen grain wall was visible in the genus *Aaronsohnia factorovskyi* (3.0 μm), with the lowest value recorded in the genus *Calendula arvensis* (2.3 μm) (Figure 2 and Table 3).

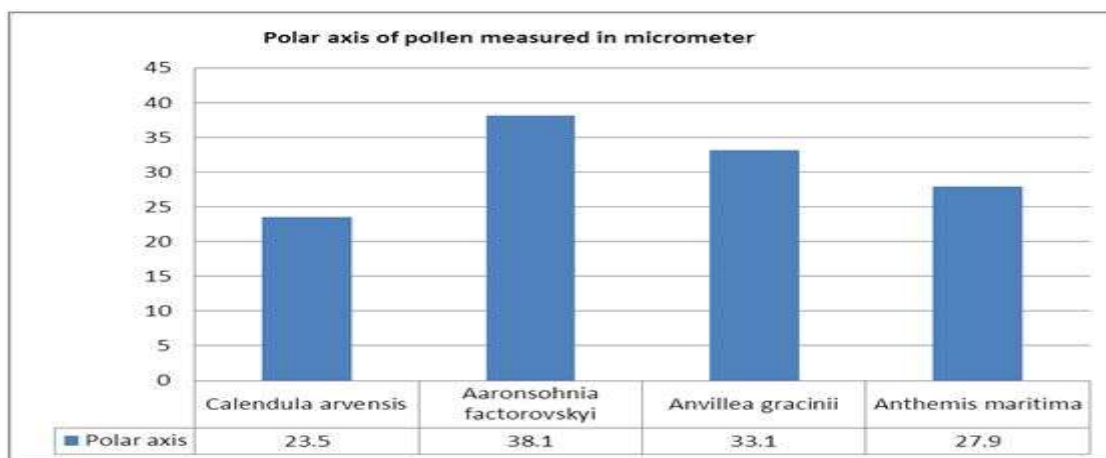


Figure 2. Variability in polar axis of pollen of the species.

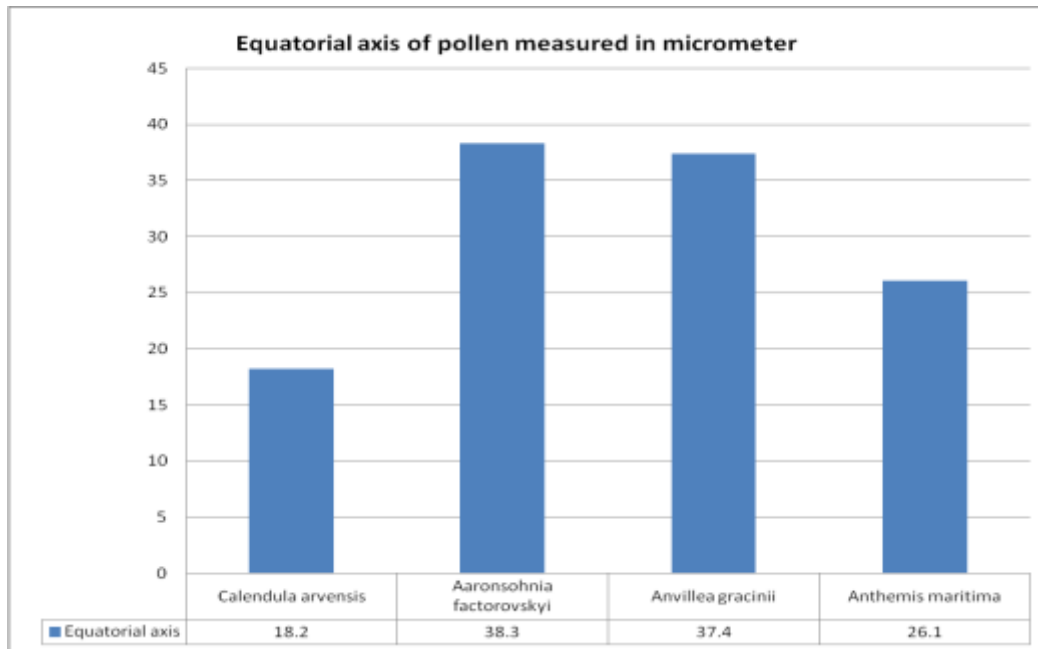


Figure 3. Variability in equatorial axis of pollen of the species.

Exine sculpturing

If the surface dish is free of ornamentation or sculpturing and contains spores with a smooth exterior, the pollen is psilate, and the term itself calls the unclear pollen decorative under a light microscope. However, if they contain granules, the sculpturing is called granulate, and if it has warts, then it is verrucate. By containing the spines, it is an echinate or spinate, and a spinule if containing spinules. If there are air sacs, it is a saccate. If it contains drumstick-shaped pila, the sculpturing is called pilate (Table 3).

The study also showed two types of ornamentation: a) Psilate - in genus *Calendula arvensis*, if the surface exine is free of sculpturing or spines (Figure 2) and b) Echinata - this type has the pollen grains further dividing into two groups depending on the nature of the spines: b1) Pollen grains with spines that are conical as in the genus *Anthemis maritima* or a spine may be composed of two broad-based parts as in the genus *Anvillea gracinii*, and b2) Pollen grains with spinules, and in this group, the spinule is not sharp and is closer to the granules, and the

genus *Aaronsohnia factorovskyi* showed this type of spinules.

Equatorial axis

An observation further showed that the longest equatorial axis of the pollen grain manifested in the genus *A. factorovskyi*, ranging from 37.0 to 39.1 μm , with an average of 38.3 μm . However, the shortest equatorial axis of the pollen grain appeared in the genus *C. arvensis*, ranging from 17.1 to 19.5 μm , with an average of 18.2 μm , indicating a significant difference in length of the equatorial axis (Figure 3 and Table 3).

Polar axis

In these pollen grains, the longest polar axis emerged in the genus *A. factorovskyi*, ranging from 36.2 to 38.5 μm , with an average length of 38.1 μm . However, the shortest polar axis surfaced in the genus *C. arvensis*, ranging from 22.2 to 24.7 μm , with an average length of 23.5 μm , indicating considerable differences in polar axis lengths (Figure 2 and Table 3).

DISCUSSION

The classification of angiosperms and relationships among them depends greatly on the architecture of the pollen grains (Moore and Webb, 1978). Palynology is crucial for more than just taxonomy; it has also shown to be helpful in genetic and evolutionary research, forensic science, allergy research, tracing the evolutionary history of specific species and taxa, paleo-climate research, and the investigation of past human impacts on vegetation (Erdtman, 1943).

In several wild species of dicotyledons, the wide range of pollen qualities has undergone studies in the Samawa Desert, South Iraq. Observations have indicated that the desert's vegetation was minimal due to the diminishing rainfall, contributing little vegetation. Pollen studies have become one of the constant foundations of taxa separation and the best mechanism for isolating similar species in other regions. However, it also does not mean dispensing with the morphological studies, as it is the basis for initial isolation. Notably also are the characteristics of pollen grains considered as micro-morphological features, in addition to being stable traits to a large extent; hence, their utilization to isolate the different Taxa (Aipeisova *et al.*, 2023; Hussain *et al.*, 2023). These parameters included apertures, grain type, shape, size, wall thickness, and exine sculpturing. These qualities have shown importance for classification, but with varying degrees.

The study also confirmed that, on average, the size of the pollen grain was of medium type and had elongated spherical in the equatorial view and a circular shape in the polar view, and these results were in analogy with the findings of Perveen (1999), who reported that the size of the taxa was medium; however, in the equatorial view, the shape of the pollen was oval-circular, while lobed square in the polar view. It might be due to variations in the distance rate between the end of the colpi and the end of the colpus, with this study also revealing that his research does not rely solely on the external manifestations of the pollen grains.

The pollen grains of all studied genera in the family Compositae were monad, symmetrical, and isopolar. Past studies also authenticated these parameters as the general characteristics of the family Asteraceae (Erdtman, 1943). This study showed that the family's pollen grains have compound apertures and zono-colporate. Also, the genus *Aaronsohnia factorovskyi* differed by having spinulus in this family. Clark *et al.* (1980) findings showed the importance of this spinulus, although the first study on the nature of the spines and the wording of the terms exine and intine came from Jassim and Najr (2003) and Alam (1987). The thoughtful species bore differentiation based on the exine sculpturing with other qualities. *Anthemis maritime* has acute conical spines.

The general description of the pollen grains coincides with what they found; however, the size of pollen in the current study was superior, which may be due to the dry samples showing the size of smaller pollen than that of field samples (Al-Joboury *et al.*, 2017), or maybe because the size sustained influences from the method of preparation (Agrawal, 1983). In particular, the results of the pertinent study were identical to those acquired by Al-Dobaissi (2008). *Anvillea gracinii* exhibited characteristics of spikes being sharp and composed of two broad-based parts, and this description corresponds to the findings of Garg and Sharma (2007). The differentiation between species depending on the shapes of spines was also a portion in past studies of Hufford (1995), explaining the differences with illustrations. The genus *Calendula arvensis* was free of sculpturing or spines (Rowley *et al.*, 1981). Findings by Al-Dobaissi (2017) may correspond to this current study and the general description of the pollen grains.

The presented study explained the importance of pollen grain features in isolating different taxa even at the variety level, particularly the characteristics of pollen shape, size, and sculpturing, as well as other features like aperture (Table 3). These results were consistent with previous studies, highlighting the significance of pollen studies in isolating Asteraceae taxa (Abou-El-Hamd *et al.*, 2012).

Moreover, pollen volume also received influences from various conditions, such as moisture ratio and the pollen detection phase, with recent studies using scanning electron microscope (SEM) relying on these qualities, including exterior wall sculpturing, ring diameter, cover, and the nature of its sculpturing. The results have shown agreement with past studies (Sadeq, 2019).

The latest results also suggested that the upcoming study should include the anatomical study of pollen grains, with the need to use a scanning electron microscope for the researcher to accurately describe the exact parts of the pollen and the surface sculpturing of the wall, capturing clear images to open up new prospects in the science of pollen. Iraqi studies had not addressed, in detail, pollen grains of more than one family, and an emphasis is necessary for more broad classification studies of the pollen.

CONCLUSIONS

Studying the pollen grains is highly valuable for morphological characteristics, which can benefit solving various interactions between species in terms of morphological traits. Therefore, studying pollen grains is essential in taxa segregation.

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