

Morphological Study of Pollen Grains of Seven Species of the Family Ranunculaceae

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Abstract: A morphological study of pollen grain has been conducted for seven different species of the family Ranunculaceae, four of them belonging to the genus *Ranunculus*. As for the three remaining species, they belong to different genera, namely *Delphinium ajacis*, *Adonis dentatus* and *Nigella arvensis*. The common phenotype among the studied genera was tricolpate, with two other less common phenotypes recorded: tetracolpate and polycolpate. As for the shapes of pollen grains, they varied in genera between oblate-spheroidal to prolate-spheroidal. The size of pollen grains in the studied genera varied between small and medium. The studied genera have appeared to be similar in the length of the polar axis, with the exception of *R. muricatus* and *N. arvensis*, which recorded the highest values for the polar axis, and the same is true with the equatorial axis. The lengths of the colpi are close among the studied species, except for *D. ajacis*, which recorded the lowest length of the colp. The variation in wall thickness was not significant among the studied species except for *R. muricatus* and *N. arvensis*, which recorded the highest values of wall thickness. The ornamentation in pollen grain walls varied between fine reticulate and reticulate to macroreticulate for most of the studied species except for *N. arvensis*, which had granulate ornamentation.

Keywords: Ranunculaceae, Pollen morphology, Pollen ornamentation, Pollen shape

Introduction

Palynology is an important avenue for researchers in taxonomy and geology, as it has characteristics that help to separate species and genera within the same family. For instance, the morphological study of pollen grain helped in separating the species and genera of the family Polygonaceae and creating new genera that are separated from other genera, after its species being falling under the rank of species within other genera (Al-Rubaie, 2008).

The study of pollen grains for the species and genera of the family Convolvulaceae in Iraq also contributed to solving many taxonomic problems and establishing a taxonomic key to separate the species of each genus (Al-Edany & Al-Mayah, 2000). The family Ranunculaceae includes about 50 genera and about 2000 species, and it is one of the large families that is widespread in the northern hemisphere and in the southern temperate regions of the globe (Mabberley, 1987).

This family has attracted the attention of many researchers from all fields, including the study of Nowick & Skvarla (1983) of the pollen grain morphology of the species of the genus *Helleborous*, in which the *Trizonocolpate* genus was recorded and pollen grain forms varied between spheroidal and suboblate to subprolate form. Cappelletti & Poldini (1984) were interested in studying the characteristics of the seeds of the European species belonging to the genus *Aconitum*, which gave important taxonomic characteristics that helped in establishing a taxonomic key to separate the species of the studied genera.

Lee (1988) presented a morphological study of pollen grain for a number of endemic species in Korea. The genus *Megaleranthis* belonging to the mentioned family was among the said genera. In addition, the skin characteristics of species of the genus *Clematis* were studied by Shi & Li (2003) used skin characteristics to separate the studied genera.

Three types of pollen grains were recorded in the genera and species of the family Ranunculaceae. The common types were tricolpate and tetracolpate, which are rare types recorded in one species of the family. In respect of the third type which is pantoporate, was recorded only in *Thalictrum isopyroides* (Perveen & Qaiser, 2006).

The genus *Clematis* has been extensively studied in terms of cytology and morphology of pollen grain in Iran by Sheidai et al. (2009). The researchers recorded the chromosomal numbers $2n=2x=16$ and $4x=32$, and they also determined the pollen grain pattern in the species of this genus, which is tricolpate. In North Cyprus, pollen grain morphology has been studied for a number of endemic species, *Delphinium casey* belonging to the family Ranunculaceae was among them, which was distinguished by tricolpate (Yildiz et al., 2009). Emadzade et al. (2010) presented a molecular, morphological and taxonomic study of several genera of the family Ranunculaceae, especially under the rank Ranunculeae.

Heiss et al. (2011) were interested in studying the characteristics of seeds for 15 species belonging to the genus *Nigella* by observing the characteristics of seeds for 15 species belonging to this genus and how to use these characteristics in distinguishing species, especially the characteristics of the electron microscope. In Pakistan, Javed et al. (2012) presented a morphological study of pollen grain with an anatomical study of the genus *Clematis*. Both studies have contributed to solving many taxonomic problems of these species.

The morphological characteristics of pollen grain, whether under an optical or an electron microscope, have contributed to the distinction of three species belonging to the genus *Ranunculus*, namely *R. psilostachis*, *R. bulbosum* and *R. sardous*, which are similar in appearance. The colpet length and width, the length and width of the polar axis, in addition to the thickness of the outer wall played a significant role in the separation process (Kallajxhiu et al., 2015). In Iran, the pollen grain characteristics of 16 species of the genus *Consolida* have been studied by Pakravan (2016), where zonocolpate (pollen with one colpus arranged in equatorial zone) was recorded in different forms between euprolate (shape of pollen grain based on ratio between polar axis and equatorial axis) to subprolate (shape of pollen grain based on ratio between polar axis and equatorial axis) and the ornamentation was

characterized by micro-echinate. The researcher emphasized that these characteristics were not sufficient to solve the taxonomic problems. Isik et al. (2019) focused on the pollen grain characteristics of the species of the trib Nigelleae, especially the species of the genus *Nigella*, with four other genera belonging to this trib, which amounted to five species of the genus *Nigella* and six species belonging to other races. The researchers have recorded the trizonocolpate (pollen with three colpi arranged in an equatorial zone) type with forms of pollen grain oblate to spheroidal.

The current study aims to study the pollen grain characteristics of separated species of the family, four of which belonging to the genus *Ranunculus*: *R. cornutus*, *R. trichophyllus*, *R. dasycarpus* and *R. muricatus* with three different species of other genera: *Delphinium ajacis*, *Adonis dentatus* and *Nigella arvensis* in Basrah.

Materials and Methods

The current study included seven species belonging to the family Ranunculaceae, four of which belonging to the genus *Ranunculus* and three belonging to other genera in Basrah Province, as shown in Table 1. The study based on herbarium samples preserved in the herbarium area of Basrah University and some fresh samples collected from different areas of Basrah Province. The characteristics of pollen grains were studied under an optical microscope, as the method of Actolysis was adopted, which is described in Al-Mayah (1983). The characteristics were also studied under an electron microscope, as the herbal samples were sent to the Scanner Electron Microscope Unit of the College of Pharmacy, University of Basrah. Measurements were recorded for a number of pollen grains ranging from 20-25, and the terms contained in Erdtman (1952), Walker & Doyle (1975) and Moore & Webb (1978) were used.

Results and Discussion

The family Ranunculaceae in Iraq lacks palynological study, so this study was conducted for the purpose of identifying the characteristics of pollen grains for some species of this family and knowing their role in separating species and genera. The current study recorded three pollen grain types, namely: tricolpate, tetracolpate and polycolpate. The first type was recorded in most of the studied species, which are *R. cornutus* L., *R. trichophyllus* Chaix., *R. muricatus* L., *Delphinium ajacis* L., *Adonis dentatus* Del. and *Nigella arvensis* L., as shown in the Figures 1-3 and Table 1, while the second type was recorded in *R. muricatus* L. in addition to the first type. The third type was recorded only in *R. dasycarpus* (Stev.) Boiss. as demonstrated in Plate 1.

Tricolpate is the common type among the studied species, but in general, it is among species in this family, and this is consistent with what was mentioned by Kallajxhiu et al. (2015), Pakravan (2016) and Isik et al. (2019) that the common type in members of the family Ranunculaceae is trizonocolpate.

The dimensions of the polar axis and the equatorial axis varied among the studied species, as shown in Table 1. The highest average length of the polar axis was

recorded in *R. muricatus*. It is amounted to 38.25 μm , while the lowest average length of the polar axis was in *R. trichophyllus* as it is amounted to 22.25 μm .

At the same time, the case with the dimensions of the equatorial axis, as the difference in the average length of the equatorial axis was clear among the studied species. The highest average length of the axis was recorded in *R. muricatus*, which recorded 37.5 μm , while the lowest rate in the length of the equatorial axis was evident in *R. trichophyllus*, which amounted to 24 μm , as indicated in Table 1.

The variation in the average lengths of the two axes between the studied species is reflected in the polar axis/ equatorial axis (P/E) value, from which the shape of the pollen grain is determined. The shape of oblate-spheroidal appeared in four species, as shown in Table 1, and this is consistent with what was mentioned by Sheidai et al. (2009), with most pollen grain shapes of the genus *Clematis* of the family Ranunculaceae, it was the above mentioned shape, while the remaining three species shown in Table 1 were with prolate-spheroidal pollen grain, and this shape was also recorded by Perveen & Qaiser (2006) and Isik et al. (2019).

The average length of the colpi differed among the studied species, as shown in Table 1 and Plates 1 & 2. The longest colpi were recorded in *R. dasycarpus*, with the average of 25 μm , and the shortest in *D. ajacis* with the average of 10.5 μm . The variation between the length of colpi for the studied species is an obvious thing because it firstly belongs to different genera, and secondly because the variation in the length of the colpi depends on the size of the pollen grain (Figures 1, 2 & 3).

The wall thickness appeared to be similar among the studied species except for *N. arvensis* and *R. muricatus*, which recorded the highest average wall thickness with values of 3.5 and 3.2 μm , respectively (Table 1). The wall dimensions recorded in this study were similar in their values to those recorded by Javed et al. (2012).

The ornamentation was seen through electron microscope varying between fine reticulate in *D. ajacis* (Plates 1-4), which was also recorded in the genus *Helleborous* by Nowicke & Skvarla (1983) and the reticulate in the species *R. cornutus*, *R. muricatus* and *R. trichophyllus*, while it was macroreticulate ornamentation in *R. dasycarpus* and uniquely with granulate in *N. arvensis*. This result complies with what was demonstrated by Isik et al. (2019) when studying some of the family's genera, including the genus *Nigella*.

The variation in the size of the pollen grain was not significant. The pollen grain was seen in most species of medium size, except for one species whose pollen grains were of small size (Table 1). The studied species belonging to different genera, and any variation in pollen grain characteristics appears to be a natural matter because of the independence of each genus from the other genera. The species of one genus also differ among themselves, but this difference is sometimes not enough to separate the species, as the value of pollen grain qualities is very limited within the species of one genus (Pakravan, 2016).

Electron microscopy results show that the colpi have differed in the shape of their edges, some of them were narrow colpus with an edge coiled inward, as in *R. dasycarpus*, *R. muricatus*, *R. cornutus* and *R. trichophyllus*, while the remaining species had a wide colpet and an open, non-folding edge as shown in the Plate 4.

The similarity of the first four species in the shape of the colpi and their edges is normal because they belong to one genus.

Table 1: The characteristic of pollen grains of different species of the family Ranunculaceae (Micrometer).

Species	Pollen type	Polar axis	Equatorial axis	P/E 100	Colpus length	Exine thickness	Pollen shape	Pollen size
<i>A. dentatus</i>	Tricolpate	17.5-22.5 (23.5)	20-25 (22.5)	105.6	12.5-15 (13.43)	1.25-2.5 (2.12)	Prolate-spheroidal	Small
<i>D. ajacis</i>	Tricolpate	17.5-35 (27.7)	17.5-34.7 (27.5)	99	7.5-12.5 (10.5)	1.25-2.5 (1.8)	Oblate-spheroidal	Medium
<i>N. arvensis</i>	Tricolpate	23.5-42.5 (36)	30-45 (35)	102.8	12.5-30 (21.5)	2.5-5 (3.5)	Prolate-spheroidal	Medium
<i>R. cornutus</i>	Tricolpate	12.5-37.5 (26)	20-42.5 (25.5)	101.9	2.5-17.5 (13.3)	1.25-2.5 (1.5)	Prolate-spheroidal	Medium
<i>R. dasycarpus</i>	Tricolpate and hexazonocolpate	22-35 (27)	20-47.5 (29)	93	7.5-30.7 (25)	1.25-3.75 (1.8)	Oblate-spheroidal	Medium
<i>R. muricatus</i>	Tricolpate and tetracolpate	35-45 (38.5)	34.5-52.5 (37.5)	99	12.5-25 (19.2)	2.8-4.5 (3.2)	Oblate-spheroidal	Medium
<i>R. tricophyllus</i>	Tricolpate	15-25 (22.2)	15.6-25 (24)	92.7	10-12.5 (11.6)	1.25-2.5 (1.82)	Oblate-spheroidal	Medium

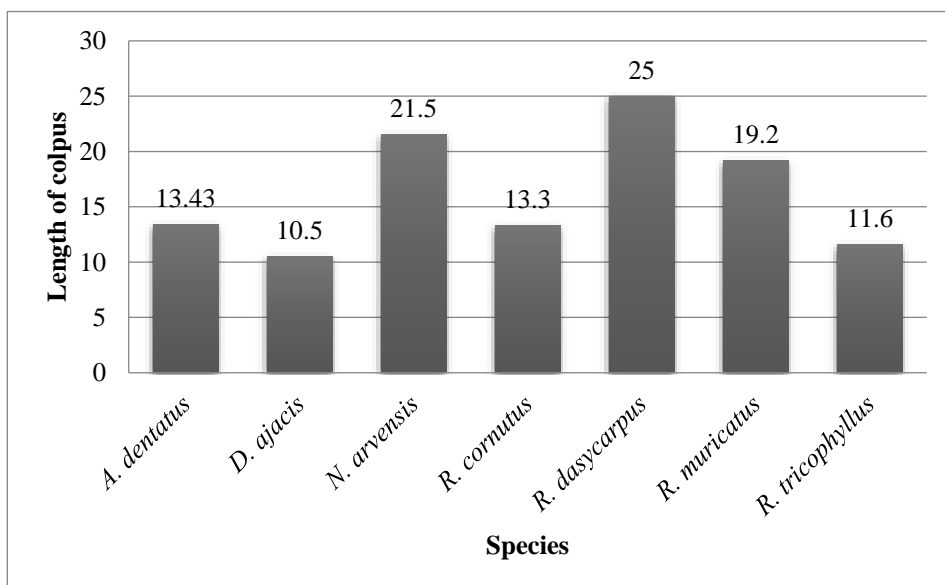


Figure 1: Variation in the dimensions of the colpi among the studied species. (micrometer).

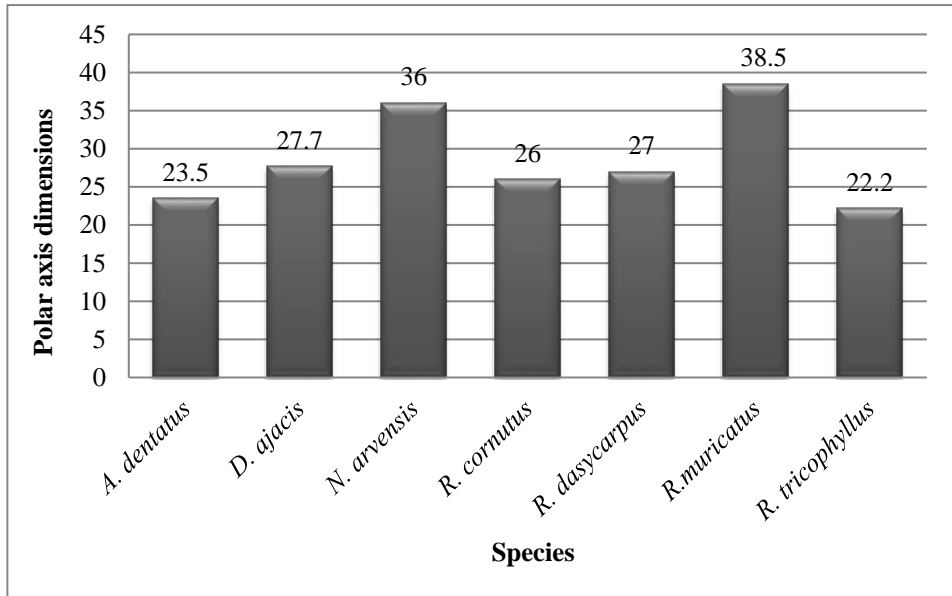


Figure 2: Variation in the dimensions of the polar axis among the studied species. (micrometer).

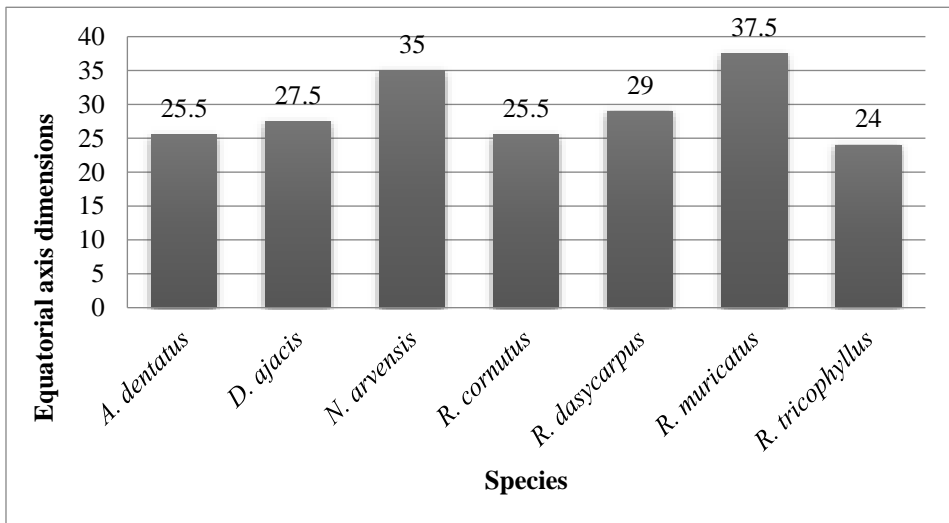


Figure 3: Variation in equatorial axis dimensions among the studied species (micrometer).

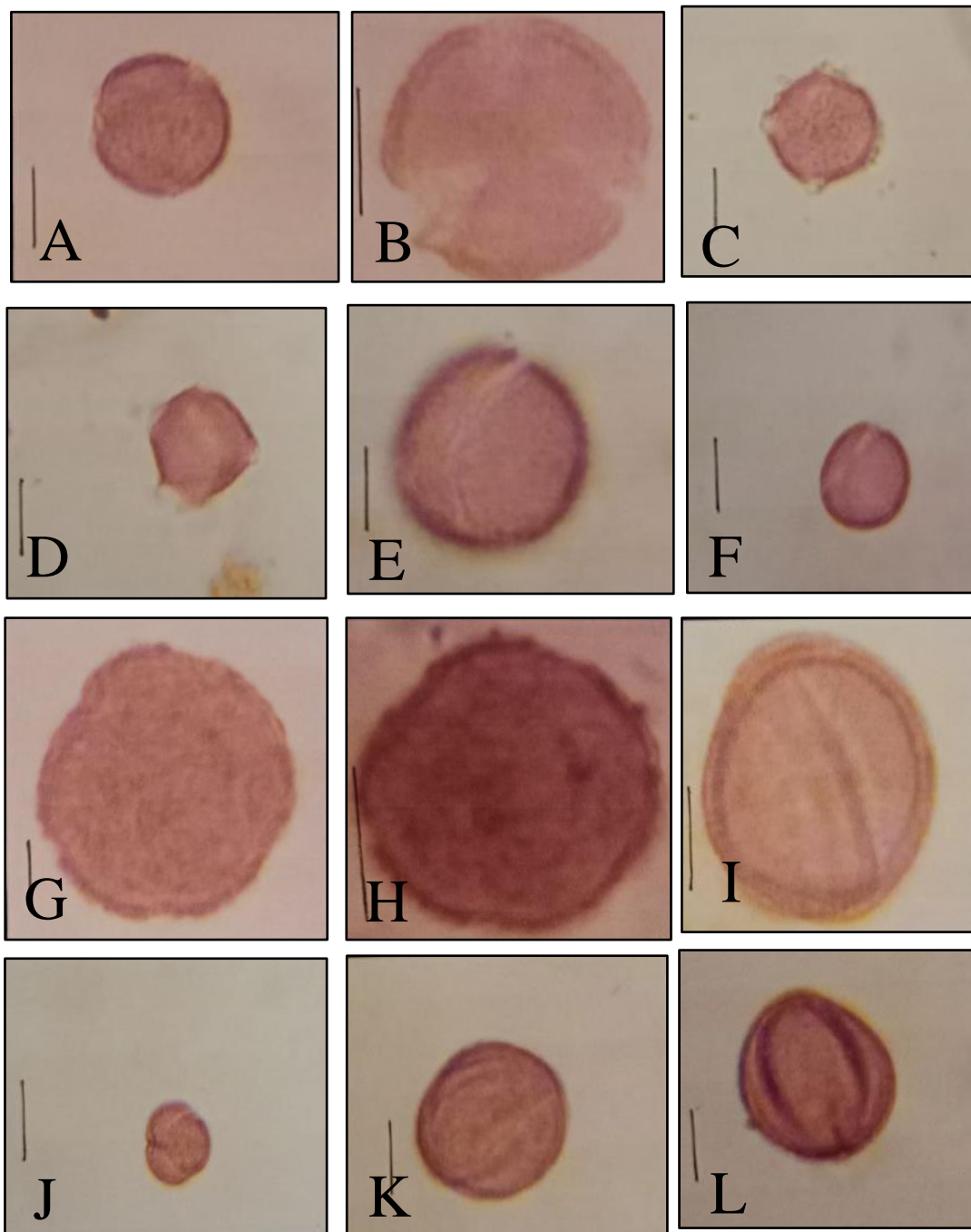


Plate 1: A and B represent the tricolpate in the polar view of pollen of type, C, *R. tricophyllus* and D represents the tetracolpate type, E, *R. muricatus* and F showing the colpi grain of *R. tricophyllus*, G and H represent polycolpate in *R. dasycarpus*, tricolpate in K and L, A. *dentatus*, equatorial view and furrows in pollen of *R. conutus* (Scale 10 μ m).

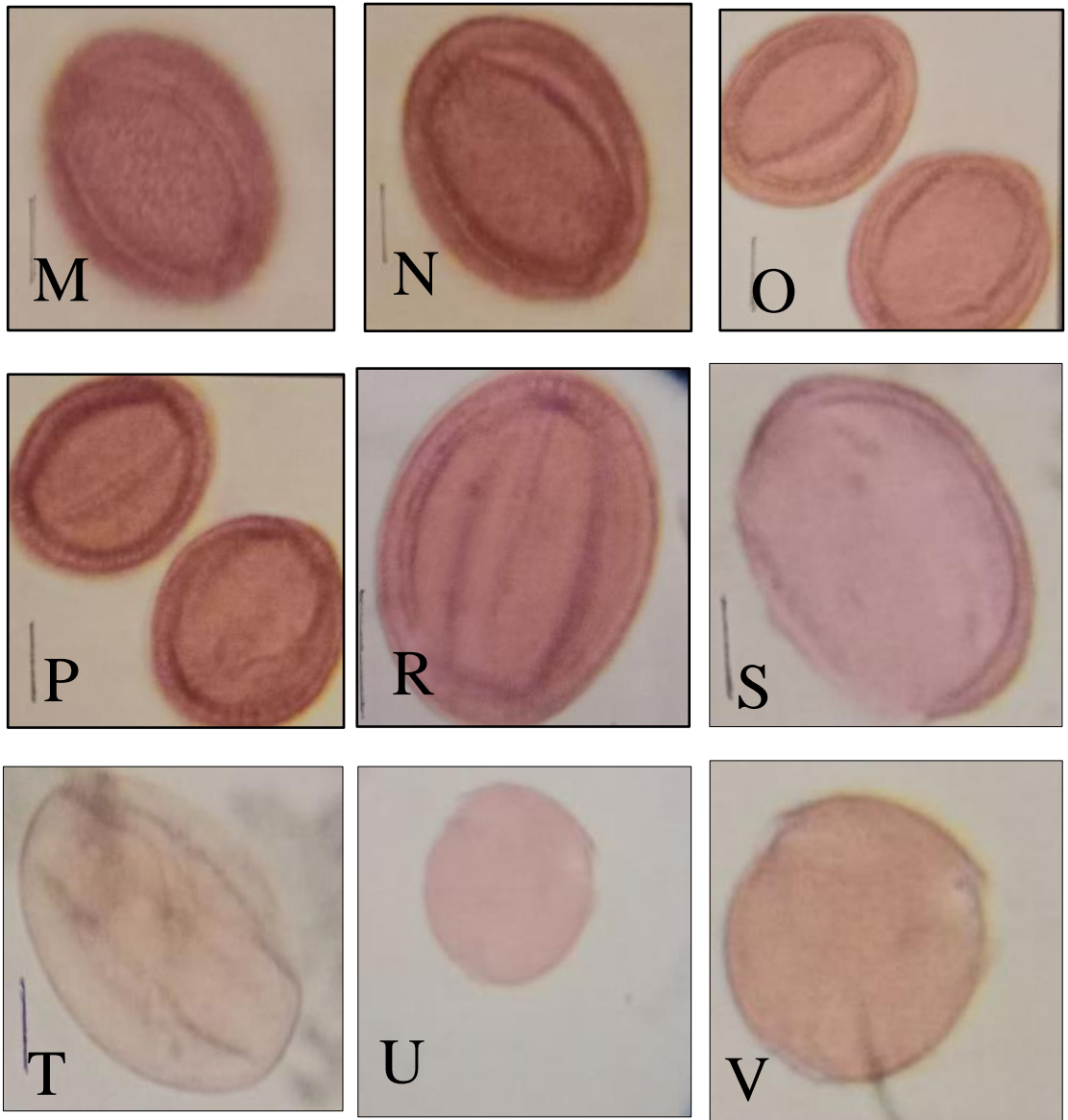


Plate 2: M and N show the pattern of pollen grain of *D. ajacis* in equatorial view with colpi, O, P and R equatorial view with colpi in pollen of *A. dentatus* with the illustration of the wall in the picture (R). S and T equatorial view of *N. arvensis* pollen, U and V tricolpate in the polar view in pollen of *N. arvensis* (Scale 10 μ).

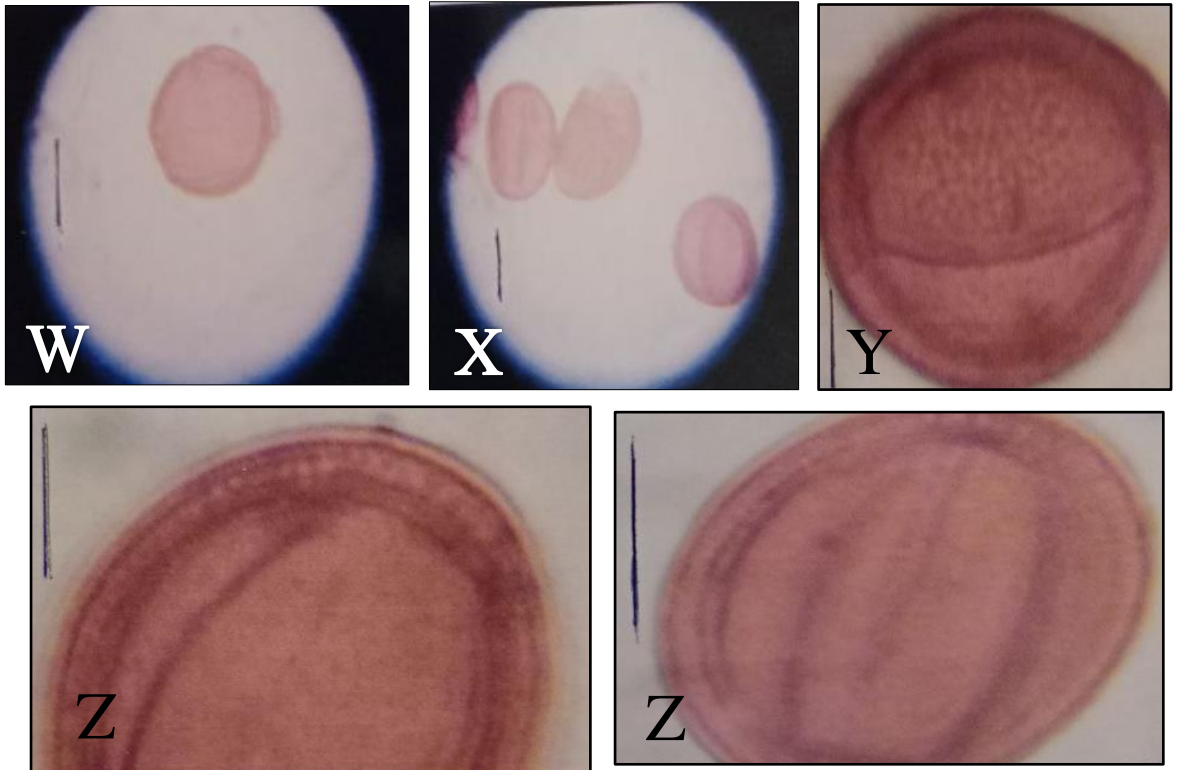


Plate 3: W and X different images of pollen of *R. muricatus*. Granulate decoration in pollen of type Z, *R. tricophyllus* represents an enlarged section in the pollen wall of *N. arvensis* showing the plan in the wall (Scale 10 μ).

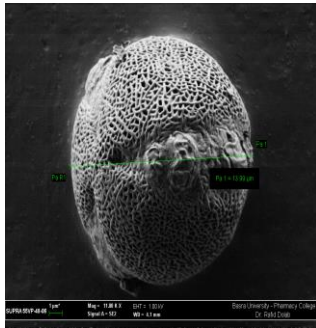
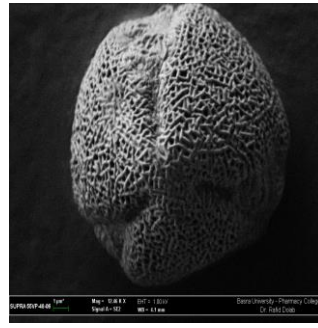
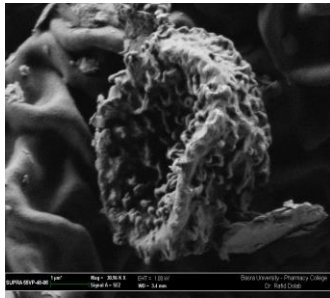
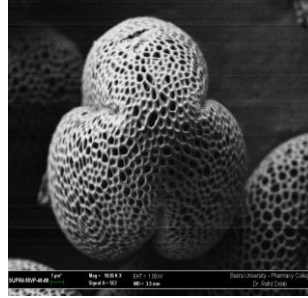
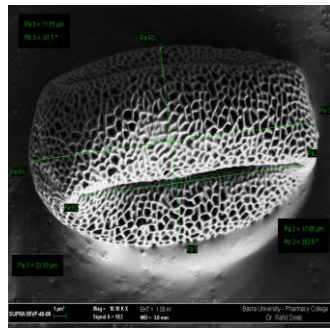
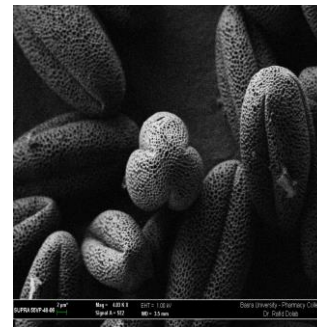
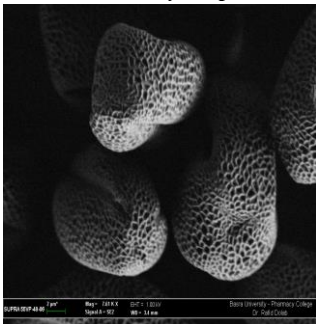
*A. dentatus**D. ajacis**N. arvensis**R. cornutus**R. dasycarpus**R. muricatus**R. tricophyllus*

Plate 4: Scanning electron micrographs of pollen grain in the studied species showing general shape and ornamentation.

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