



## Palynological Study of the Genus *Valerianella* Miller (Valerianaceae) in Kurdistan Region-Iraq

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### Abstract

The present study dealt with the pollen grains of 11 species of the genus *Valerianella* Miller within the family Valerianaceae which have been collected within the field trips during spring and summer season of year 2014 (about 20 trips) from the different districts in Kurdistan of Iraq. From the used characters that had a role in the separation of some species were the pollen shapes, sizes, surface sculpture and the end shapes of the colpi, generally the pollens had a limited role in the species separation. Photographs have been taken for the polar and equatorial views of the pollen grains. The species were *Valerianella chlorostephana* Boiss., *V. coronata* (L.) DC., *V. dactylophylla* Boiss. & Hohen, *V. discoidea* (L.) Lois., *V. kotschyi* Boiss., *V. lasiocarpa* (Stev.) Betcke, *V. muricata* (Stev.) Baxt., *V. oxyrhyncha* Fisch & Mey, *V. pumila* (L.) DC., *V. tuberculata* Boiss. and *V. vesicaria* (L.) Moench.

### Introduction

#### A. Valerianaceae Batsch.

Annuals or perennials. Leaves opposite, exstipulate, entire or dentate to pinnate. Inflorescence a cyme, usually dichotomous. Bracteoles 0. Corolla 5-fid, tube often gibbous or spurred. Flowers white, pink, red or mauve. Stamens 1-3, exserted. Ovary inferior, with 1 fertile and 2 sterile loculi. Fruit an achene (cypsela) [1].

#### B. *Valerianella* Miller

Annual herbs, erect, often branched. Indumentum of retrorse, asperous papillae or puberulence, sometimes more or less glabrous, especially in upper parts of plant. Basal leaves entire to sinuate-dentate, spatulate and cuneate at base or oblanceolate or oblong and more or less sessile. Median leaves with more pronounced teeth. Upper leaves usually with a few very distinct teeth at the base. Inflorescence a contracted cyme. Calyx variously toothed or coronate, regular or irregular, sometimes inflated or absent. Corolla not spurred, regularly 5-fid. Fruit 3-locular with 2 sterile loculi [1].

The ease by which pollen grains can be prepared for study and their morphological diversity makes pollen morphology a taxonomic tool which should be explored by systematists. Pollen data have proven taxonomically useful at all taxonomic levels [2].

The palynological features have provided a wealth of characters that have been important in inferring phylogenetic relationships of plants. In addition, the features of spores and pollen grains can often be used to identify a particular plant taxon [3].

The pollen wall has been a subject of considerable attention, especially in an attempt to establish the evolutionary history of angiosperms. Some families, such as Asteraceae, show different types of pollen grains (eurypalynous), whereas several others have a single morphological pollen type (stenopalynous). Such stenopalynous groups are of considerable significance in systematic palynology [4].

[5] pointed out the pollen morphology of about 25 species belong to 10 genera within the family Valerianaceae, and he mentioned that the pollen grains in Valerianaceae are  $\pm$  similar to those in Dipsacaceae. According to [6] there are 15 species of the genus *Valerianella* in Iraq, while [7] mentioned 13 species. In Pakistan, [8] studied the Pollen morphology of 13 species representing 2 genera of the family Valerianaceae from them *Valerianella muricata* and *V. oxyrhyncha* by using light and scanning electron microscope, also by using the same instruments, [9] mentioned that two major groups of genera could be recognized: the *Valeriana* group which has simple columellae, and the *Valerianella* group which has elongated, branched columellae at the poles. In a comparative study of pollen wall structure by means of light-microscopy, SEM and TEM investigation, [10] showed that the pollen grains of Valerianaceae are known as trizonocolporate to tricolporoidate, the exine surface usually being echinate or microechinate and the sexine exhibiting branched columella, also they could distinguish four different pollen types involving *Valerianella* – type (Type III) which already can be distinguish from the others by its vigorously exine, where the microechinate supractectate structure are slightly pronounced.

## Materials and Methods

The preparation technique for study of pollen morphology was followed through with some modification with the use of a light microscopic [3]. The living material of anthers fixed in a chemical solution, such as FAA, a single anther removed from the fixative material was placed in a drop of water or 50% glycerol (the latter to prevent the material from drying out), and dissected with a scalpel to extrude the pollen grains; the anther wall material removed and a cover slip applied. Then, the pollens stained with safranin instead of acetocarmine.

Specimens have been collected within the field trips during spring and summer season of year 2014 (about 20 trips) from the different districts in Kurdistan of Iraq which involved Amadiya (MAM), Rowanduz (MRO), Sulaimaniya (MSU), Kirkuk (FKI), Arbil (FAR) and Nineveh (FNI) districts (Fig 1). Slides have been prepared for each species which has been collected from the different locations within the studied districts during spring and summer seasons of the year 2014, the data have been taken for (10-15) pollens from each species (Table 1). The slides have examined under Olympus-compound microscope, and photographed by using Sony-digital camera in College of Education/Salahaddin University. The palynological traits that have been used in the study were polar and equatorial axis length, average length of polar axis/equatorial axis, wall thickness, distance between colpi and colpi length and width, and the palynological terms mentioned in [5] are used in this study.

Table 1: Specimens locations of *Valerianella* species which their pollen grains have been studied, and their collection dates with the districts

<i>Species</i>	<i>Districts</i>	<i>Specimen Location</i>	<i>Collection Date</i>
<i>V. chlorostephana</i>	MAM	Gali Balinda	25.4.2014
	MRO	Sakran mountain	1.5.2014
	MSU	Dokan	22.3.2014
<i>V. coronata</i>	MAM	Matina mountain	25.4.2014
	MRO	Safin mountain	15.4.2014
	MSU	Qaradagh	24.3.2014
<i>V. dactylophylla</i>	MAM	Matina mountain	25.4.2014
	MRO	Mirgasour	6.6.2014
	MSU	Tawela	9.5.2014
<i>V. discoidea</i>	MAM	Girbeesh	17.5.2014
	MRO	Piran	6.6.2014
	FNI	Bardarash	30.5.2014
<i>V. kotschyi</i>	MAM	Matina mountain	25.4.2014
	MRO	Bekhal	11.4.2014
	FAR	Daratu	10.3.2014
<i>V. lasiocarpa</i>	MRO	Sakran mountain	1.5.2014
	MSU	Mawat	10.5.2014
<i>V. muricata</i>	MAM	Gali Balinda	25.4.2014
	MRO	Sakran mountain	22.5.2014
	MSU	Tawela	9.5.2014
<i>V. oxyrhyncha</i>	FKI	Kirkuk	7.3.2014
	FNI	Kalaki yasin Agha	19.4.2014
<i>V. pumila</i>	FKI	Perdi	7.3.2014
	FAR	Aski kalak	19.4.2014
<i>V. tuberculata</i>	MRO	Mirgasour	6.6.2014
	MSU	Tawela	9.5.2014
<i>V. vesicaria</i>	MRO	Shaqlawa	8.4.2014
	FKI	Kirkuk	7.3.2014
	FAR	Aski kalak	19.4.2014

## Results and Discussion

In this study, the light microscope was used to observe some traits of pollen grains, including their colors, shapes in the polar and equatorial views, surface ornamentations, length of polar and equatorial axis, sizes, apertures, wall thickness, distance between the colpi, and end shapes of the colpi as well as the side shapes of the polar view.

The pollens of *Valerianella* members were yellow, single, and tricolporate. In the equatorial view, it appeared that they were oblate in *V. chlorostephana*, *V. coronata*, *V. discoidea*, *V. kotschyi*, and *V. oxyrhyncha*, whereas; they were prolate in *V. muricata*, *V. pumila*, and *V. tuberculata*. In some species like *V. dactylophylla* and *V. lasiocarpa*, they were either oblate or prolate. In *V. vesicaria*, the pollens were oblate, spheroid and subspheroid. However, the pollens were spheroid, subspheroid in the polar view.

Concerning the size of pollen grains, and based on [5], we found that the pollen grains have been differed in sizes and they were divided into small, medium and small-medium. In small sizes, the longest axis of the pollen grains was between 10 to 25  $\mu\text{m}$ , in medium, it was between 25 to 50  $\mu\text{m}$  and in small-medium size was 10-50  $\mu\text{m}$ . In addition, in the species of *V. discoidea*, *V. lasiocarpa*, *V. muricata*, *V. oxyrhyncha*, *V. pumila* and *V. tuberculata*, they were small; in *V. kotschyi* and *V. vesicaria*, those were medium and in the rest of studied taxa the sizes were small-medium. While, in the polar views, the shape of the sides for all the pollen grains was convex.

In the current research, it appeared that the surface sculpture (ornamentations) in all species was echinate and micro-echinate but in the species of *V. chlorostephana*, *V. lasiocarpa*, *V. oxyrhyncha*, *V. pumila*, and *V. tuberculata* was only echinate (see Plates 1, 2 and table 2).

It appeared that the distance between colpi was between 7 to 11  $\mu\text{m}$  as a minimum limit in the species *V. pumila*, whilst, this was between 18 to 21  $\mu\text{m}$  as a maximum limit in the species *V. kotschyi*. Furthermore, the colpi length was between 11 to 14  $\mu\text{m}$  as a minimum limit in the species *V. dactylophylla* and *V. oxyrhyncha*; while, in the species *V. tuberculata*, it was between 17 to 20  $\mu\text{m}$  as a maximum limit. In addition, the end shapes of the colpi in all studied species were acute or obtuse except in *V. discoidea*, *V. pumila* and *V. muricata* were acute and in *V. lasiocarpa* was obtuse.

Through this research, the longest axis and end shapes of colpi played an important role in distinguishing of species. The longest axis was used to determine the shapes and sizes of the pollen grains. We observed that the longest axis of the pollen grains of *V. chlorostephana*, *V. coronata*, *V. discoidea*, *V. kotschyi*, and *V. oxyrhyncha* was the equatorial axis; whereas, in *V. muricata*, *V. pumila*, and *V. tuberculata* was the polar axis. However, there was a significant interaction in this trait among *V. dactylophylla*, *V. lasiocarpa* and *V. vesicaria*. We found that the species of *V. chlorostephana*, *V. lasiocarpa*, *V. oxyrhyncha*, *V. pumila*, and *V. tuberculata* had the echinate surface ornamentation; whilst, the others had micro-echinate ornamentation. The end shapes of the colpi, including obtuse and acute, was used to distinguish the species from each other; for example, *V. lasiocarpa* was only obtuse, *V. discoidea*, *V. pumila* and *V. muricata* were only acute; while, others were both obtuse and acute. However, wall thickness and colpi width didn't play a role in separation of species from each other because of the interactions in these traits.

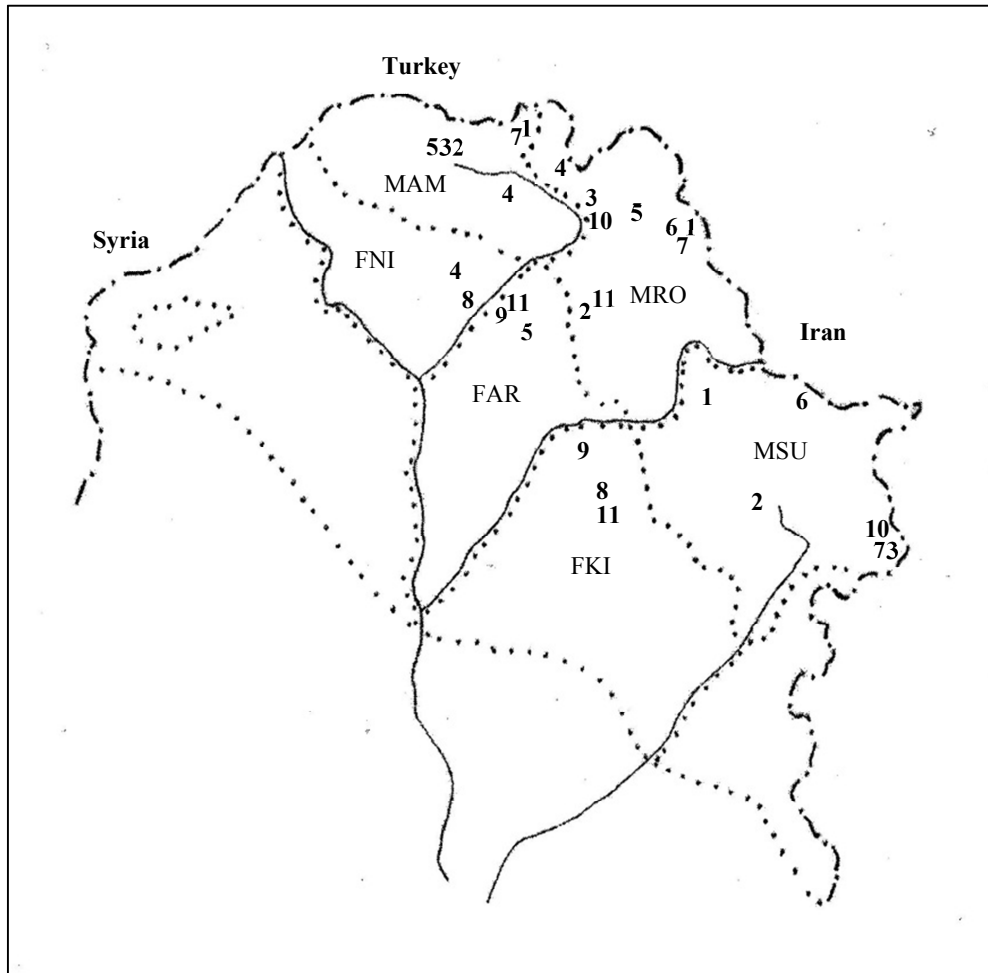


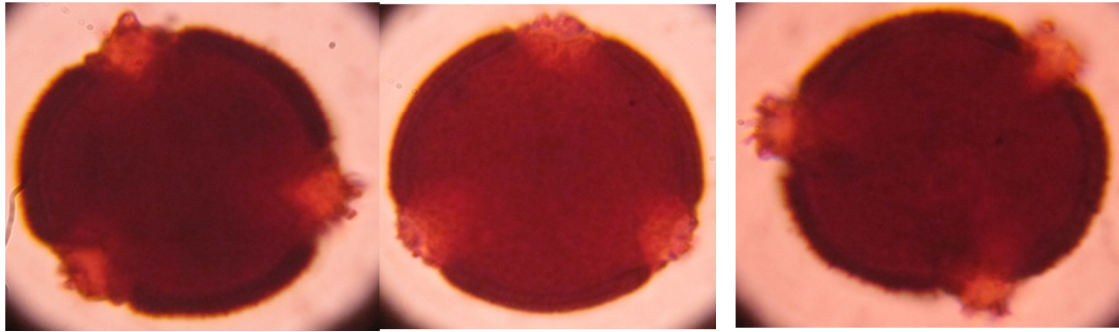
Figure 1: A map of the geographical distribution of the species which their pollen grains have been studied: 1. *V. chlorostephana* 2. *V. coronata* 3. *V. dactylophylla* 4. *V. discoidea* 5. *V. kotschyi* 6. *V. lasiocarpa* 7. *V. muricata* 8. *V. oxyrhyncha* 9. *V. pumila* 10. *V. tuberculata* 11. *V. vesicaria*

Table 2: Pollen grains dimensions of *Valerianella* species in micrometer

Species	Pollen Grains							M±SE
	Polar axis length	Equatorial axis length	Al(P/E)**	Wall thickness	Distance between colpi	Colpi length	Colpi width	
<i>V. chlorostephana</i>	(20-23)21.66*	(23-26)24.33	0.89	(1.5-2.0)1.70	(13-16)14.66	(13-16)14.50	(6-8)7.33	9.05±3.39
<i>V. coronata</i>	(21-24)22.33	(23-25)24.00	0.93	(1.5-2.0)1.76	(15-18)16.33	(14-17)15.66	(7-9)7.66	11.08±3.31
<i>V. dactylophylla</i>	(17-25)22.00	(20-23)21.00	1.04	(1.5-2.5)2.00	(14-17)15.50	(11-14)12.33	(6-7)6.33	8.9±2.86
<i>V. discoidea</i>	(19-22)20.66	(20-24)22.00	0.90	(1.5-2.5)2.05	(14-18)15.75	(15-17)16.00	(7-8)7.50	8.48±2.73
<i>V. kotschyi</i>	(22-24)23.00	(25-28)25.00	0.92	(1.5-2.5)2.00	(18-21)19.66	(16-18)17.00	(8-10)9.00	8.78±2.97
<i>V. lasiocarpa</i>	(18-21)19.75	(17-24)21.75	0.90	(1.5-2.0)1.76	(15-18)16.66	(14-17)15.33	(5-8)6.66	6.9±2.48
<i>V. muricata</i>	(20-23)21.50	(17-20)18.50	1.16	(1.0-2.0)1.50	(8-13)10.75	(15-18)16.50	(7-9)8.00	5.99±2.24
<i>V. oxyrhyncha</i>	(16-21)19.00	(20-23)21.66	0.87	(1.5-2.0)1.70	(15-18)16.33	(11-14)12.66	(6-7)6.33	5.61±2.23
<i>V. pumila</i>	(20-24)21.75	(16-23)19.00	1.14	(2.0-2.5)2.20	(7-11)9.00	(13-16)14.33	(7-8)7.33	4.98±2.09
<i>V. tuberculata</i>	(22-24)23.00	(17-20)18.66	1.23	(2.0-2.5)2.23	(14-17)15.66	(17-20)18.33	(8-9)8.50	5.47±2.14
<i>V. vesicaria</i>	(21-27)24.00	(25-28)26.50	0.90	(1.5-2.0)1.73	(14-19)16.00	(16-19)17.75	(7-9)8.00	5.58±2.48

\* The numbers inside bracts represent the minimum and maximum limit and that outside bracts represent the average.

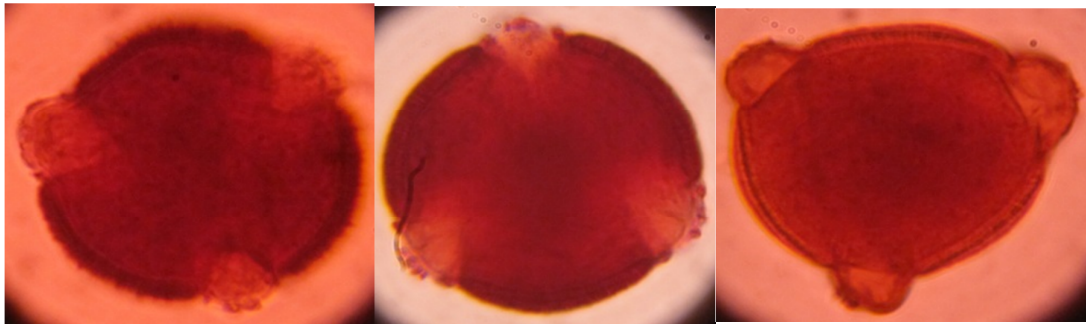
\*\*Al (P/E) = average length (Polar axis /Equatorial axis).



*V. chlorostephana*

*V. coronata*

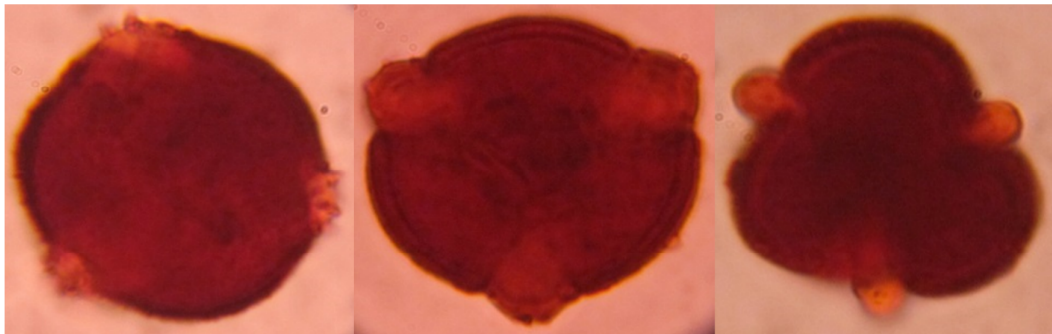
*V. dactylophylla*



*V. discoidea*

*V. kotschyi*

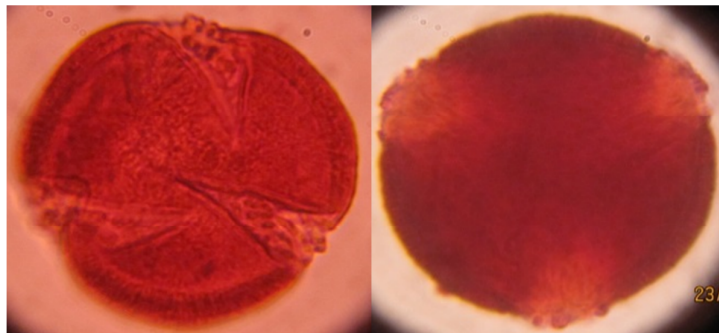
*V. lasiocarpa*



*V. muricata*

*V. oxyrhyncha*

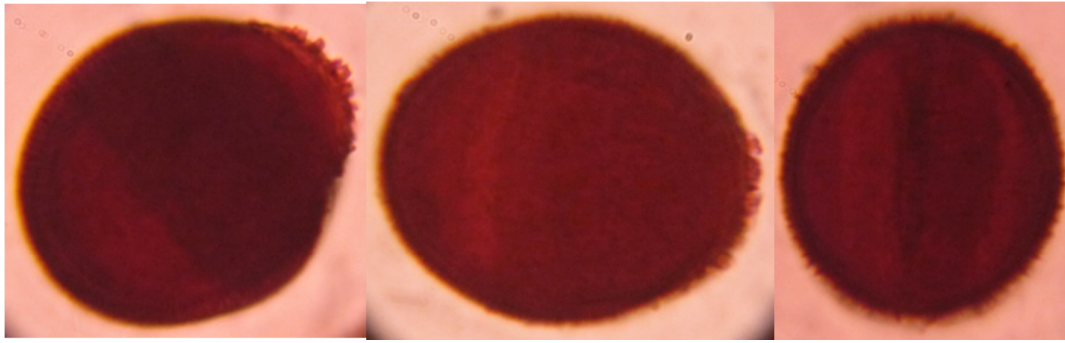
*V. pumila*



*V. tuberculata*

*V. vesicaria*

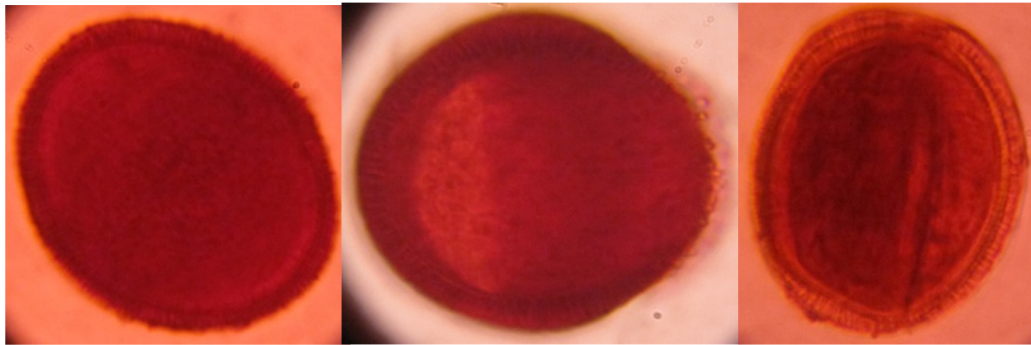
Plate 1: Pollen grains in polar view of *Valerianella* species: x1000



*V. chlorostephana*

*V. coronata*

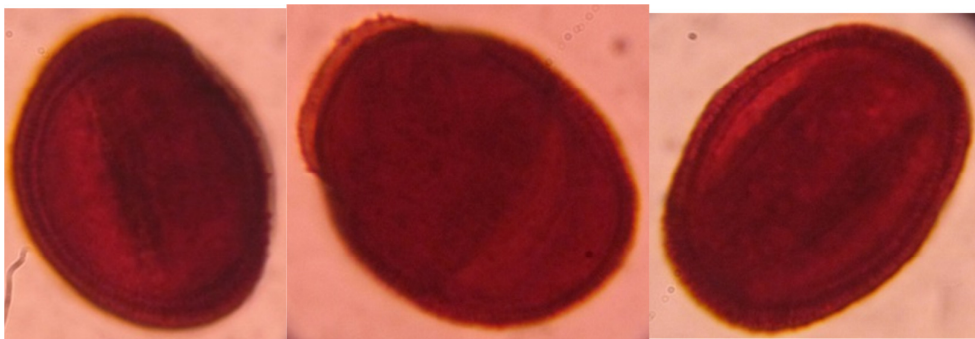
*V. dactylophylla*



*V. discoidea*

*V. kotschy*

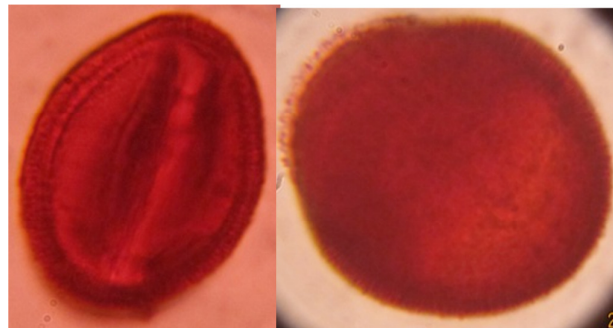
*V. lasiocarpa*



*V. muricata*

*V. oxyrhyncha*

*V. pumila*



*V. tuberculata*

*V. vesicaria*

Plate 2: Pollen grains in equatorial view of *Valerianella* species: x1000

## Conclusion

In this study, some of the pollen grain traits were used as taxonomic data, including their shapes in the polar and equatorial view, their sizes and surface ornamentation in the separation of 11 species of the genus *Valerianella*. As a result, the pollen grains helped us to distinct some species from each other and generally they played a limited role in the species separation. The study is regarded as a first study for the pollen grains of Valerianaceae family in Iraq.

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