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The micromorphological investigation of pollen grains of some important allergenic plants in Kermanshah (West of Iran)

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Abstract: Allergenic pollens are found in gardens, fields and green spaces around cities. In Kermanshah, located in the west of Iran, seven allergen pollen samples were collected directly from nature and studied according to the Erdtman acetolysis method by using Light Microscope (LM) and Scanning Electron Microscope (SEM). In this study, atmospheric pollens were also examined by the Durham method. The results showed that the pollen grains were small, medium, and large in terms of size, and Triporate, Pantoporate, Colpate and Tricolporate in terms of aperture. In addition, they were Striate, Perforate, Macroechinate, Microechinate, and Psilate with regard to exine ornamentation; however, the echinate type was more common. In general, Prolate to a spherical shape, medium-sized, perforate, and echinate pollen characteristics are popular in the important allergenic plants. Therefore, due to the human's manipulations in nature and also the extensive size of gardens and fields, there are many types of weeds in Kermanshah Province which are significant in terms of being allergenic.

Key words: Aeropalynology; Allergy; Durham method; Erdtman; SEM.

Introduction

Kermanshah province is located between 36" and 33' to 15" and 35' northern latitude and 24" and 45' to 3" and 48' eastern longitude of the prime meridian. Aerobiological studies examine organisms in the air that include the sources, dispersion and effects of these aspects on other organisms (1). Pollen analysis generally began in the early 19th century. Information obtained from European palynology banks includes data from pollen stations in different countries (2, 3). There are palynology stations in Asian countries, including India and Russia. This issue has become increasingly important in many developed countries. Nowadays, there are many sites for allergies and allergenic pollens. In Moscow, pollen calendars have been prepared for atmospheric pollens for many years (4, 5, 6, 7, 8). Aerial pollen research has also been widely carried out in Turkey (9, 10, 11). Aerial pollen studies in Turkey and Pakistan showed that the most allergenic pollens belonged to the species Cupressaceae, Pinaceae, Poaceae, Plantaginaceae, Moraceae, Amaranthaceae, Aceraceae, Juglandaceae, Betulaceae, Salicaceae and Polygonaceae (9, 10, 11). Studies in Turkey have shown that the exine ornamentation of pollen grains in the studied species is the genus Carthamus from the Echinate family Asteraceae (12). It has also been reported that pollen grains in Asteraceae are usually spheroidal or slightly prolate and tricolporate in terms of aperture status and are often echinate regarding their exine ornamentation (13, 5, 14). In addition, it has

been suggested that pollen exine ornamentation in many species of the Asteraceae family is Echinate (15). Ranjbar et al. reported that pollen grains in the genus Cuscuta are Echinate, Microreticulate, and Macroreticulate, and the species of this genus are often Colpate (16). The morphological characteristics of pollen grains of many studied species have been investigated by Medicago and Onobrychis and it has been shown that pollen grains in the studied species are prolate with reticulated ornamentation (4, 17). In another study, the pollen morphology of 40 species from 13 genera of the Chenopodiaceae family from Pakistan was examined by light and electron microscopy and it was indicated that the pollen grains of all the studied species were spherical, polyporate and had echinate ornamentation (18). Pollen research in Nigeria also provided a list of plants that emit spores and pollens in different months (19). In addition, in North America, a number of allergenic pollen grains have been studied morphologically based on the plant's status in terms of tree, shrub, and weeds. The worst type of allergenic pollen for humans has also been mentioned in this study (20). In a study conducted in the Mediterranean area for 8 years, the most frequent allergenic pollen belonged to Poaceae, Oleaceae and Asteraceae, respectively (21). In Iran, Shafiee studied 18 atmospheric pollen in Tehran using the Durham method (22). In his book, Ghanati also studied the morphology of pollens and noted the effects of pollutants on atmospheric pollens (23). Also in Khuzestan province of Iran, certain allergenic weeds have been studied and it was shown that the pollen of *Salsola kali* L. was 80% allergenic compared to other allergens and *Prosopis* sp., among the trees, was 60% allergenic among the study population (24). In other regions of Iran, investigations of allergenic pollen include: In Islamabad Gharb County, 54 allergenic species belonging to 43 plant families were studied using the Erdtman method with the light microscope, the pollens and spores present in the atmosphere were also investigated by the Durham method (25).

In Kermanshah, 56 allergenic pollens were directly collected from nature and examined by a light microscope using the Erdtman method. The morphological characteristics of 6 pollen grains were also examined by scanning electron microscope (SEM). In this study, pollen grains were classified as macroreticulate, microreticulate, granulate, psilate, echinate (macroechinatemicroechinate) and striate in terms of ornamentation. Masoumi et al. investigated the morphological characteristics of different allergenic pollens and atmospheric pollens collected by the Durham method in two stations of Kerend and Kermanshah in the final report of investigation project of aerial palynology of Kermanshah Province (26, 27, 28).

Rashidi examined 50 allergenic pollen samples under the light microscope and 6 samples by scanning electron microscopy (SEM), 10 of which belonged to Kermanshah (29). Badri (2017) found more than 20 atmospheric pollen by Durham method and examined 28 allergenic pollens using Erdtman Method and 6 samples using scanning electron microscopy (SEM). These studies were carried out mostly in the Hasan Abad area of Islamabad Gharb County in Kermanshah province. Furthermore, some pollens from Kermanshah County have also been taken into account (30). The purpose of this study was to investigate the morphology of some important allergenic pollen grains in Kermanshah and to compare the results with previous investigations and to complete the information concerning the pollen allergens of Kermanshah province.

Materials and Methods

In this study, Kermanshah County, especially the College of Agriculture and Natural Resources of Razi University Campus was selected to study pollens in the air, and the pollens were directly collected nature and herbarium of Kermanshah Razi University (RUHK) (Table 1). Durham method (1946) was used for aerial pollen studies (31) and the samples were stained with Safranine (Figure 4). Samples were also taken directly from nature by the acetolysis method (according to Erdtman, 1945) (32).

In this study, the dimensions of 7 pollen grains (taken from nature or herbarium) were examined using a scaled eyepiece with a magnification of 400 times of the HM-LUX3 light microscope (Table 2). Using a Dino Capture mounted on a light microscope, the aperture feature (hole or groove) and exine ornamentations of pollen grains were photographed (Fig. 4). The necessary measurements were also taken from both the polar view and the equatorial view. The pollen grains were first inserted into the sputter coater. The SEM (VEGA-TESCAN model from the Czech Republic) was used to prepare electron images with a magnitude of 1000-10000. These images can reveal details on the walls, such as the type of exine ornamentation, the structure of apertures (Fig. 1,2,3) (Table 3-7).

Results

Morphological characteristics of important pollen grains

Carthamus oxycantha M.B.

Pollen grain was single, medium ($26.84\pm1.59 \mu m$), tricolporate, of orate type, spherical or spherical prolate, circular outline from polar view, polar axis dimensions ($23.59, 25.58, 29.70 \mu m$), prolate or spheroidal outline from equatorial view, equatorial axis dimensions ($14.19, 26.84, 30.40 \mu m$), polar to equatorial ratio P/E = 1.04,

No	Family	Name	Herbarium code	Date
1	Asteraceae	Carthamus oxycantha M.B	89	21 Aug 2017
2	Asteraceae	Xanthum strumarium L.	2645	21 Aug 2017
3	Caesalpiniaceae	Caesalpinia gilliesii (Hook.) Dietr	111	24 Oct 2017
4	Amaranthaceae	Chenopodium vulvaria L.	2888	14 Oct 2017
5	Convolvulaceae	Convolvulus arvensis L.	2768	13 May 2017
6	Cuscutaceae	Cuscuta campestris Yunck.	432	23 April 2018
7	Fabaceae	Arachis hypogaea L.	2889	13 May 2017

Table 2. Pollen characteristics of the species studied by light microscope.

No	Name	Equatorial axis (E)	Polar axis (P)	P/E	Pollen shape	Pollen size (µm)	Exine ornamentation
1	C. oxycantha	47.43±6.46	35.05±3.56	1.34	prolate	medium	Microechinate
2	X. strumarium	26.84±1.59	25.58 ± 2.47	1.04	spheroidal	medium	Microechinate
3	C. gilliesii	40.51±4.89	37.60±10.91	1.07	spheroidal	medium	Striate
4	C. vulvaria	19.17±2.52	18.23 ± 2.48	1.05	spheroidal	small	Microechinate
5	C. arvensis	54.50±4.80	49.50±3.85	1.10	spheroidal	large	Macroechinate- perforate
6	C. campestris	$25.98{\pm}6.75$	24.10 ± 2.78	1.07	spheroidal	medium	Psilate
7	A. hypogaea	30.89±4.87	28.40±3098	1.08	spheroidal	medium	Psilate

Table 3. Specifications of some pollen grains examined by scanning electron microscopy (SEM).

№	Name	Aperture	Exine ornamentation	Aperture ornamentation	Mode of Muri	Width of muri (µm)	Lumina size (µm)
1	C. gillisii	Tricolporate	Perforatemicrostriate	Tuberculate	Continuous	0.5-2.5	0.1-0.6
2	A. hypogaea	Triporolate	echinate- microechinate	Tuberculate	Discontinuous	0.5-1.0	0.05-0.3
3	C. oxycantha	Tricolporate	macroechinate- perforate	multituberculate	Continuous	0.1-0.4	0.05-0.2
4	C. vulvaria	Polyporate	Micro echinate- perforate	Echinate	Continuous	0.10.3	0.05 -0.1
5	C. arvensis	Tricolpate	Macroechinate- tuberculate	Tuberculate	Discontinuous	0.4-4.0	0.6-1.4
6	C. campestris	Tricolpate	Perforate – scabrate	multituberculate	Continuous	0.3-1.4	0.1-0.7
7	X. strumarium L.	Tricolporate	Microechinate- perforate	Perforate	Continuous	0.1-0.3	0.05 -1.50

Table 4.	Classification	of pollen	grains	according to	their size.
Table 4.	Clubbilloution	or pomen	Siums	uccording to	f then bize.

Small	Medium		Large			
Chenopodium vulvaria L.	Arachis hyp	ogaea L.	Convolvulus arvensis L.			
	Caesalpinia gilliesii (Hook.) Dietr.					
	Carthamus oxycantha M.B.					
Cuscuta campestris Yunck.						
Xanthum strumarium L.						
Table 5. Classification of pollen grains according to their shape.						
Sp	Spheroidal Prolate					
Arachis	Arachis hypogaea I Carthamus orycantha M B					

Arachis hypogaea L.	Carthamus oxycantha M.B.
Caesalpinia gilliesii (Hook.) Dietr.	
Chenopodium vulvaria L.	
Convolvulus arvensis L.	
Cuscuta campestris Yunck.	
Xanthum strumarium L.	

Table 6. Classification of pollen grains according to their exine ornamentation of the wall pollen grain.

Striate	Microechinate	Macroechinate - Perforate	Psilate
Caesalpinia gilliesii (Hook.) Dietr.	Carthamus oxycantha M.B.	Convolvulus arvensis L.	Arachis hypogaea L.
	Chenopodium vulvaria L.		Cuscuta campestris Yunck.
	Xanthum strumarium L.		

Table 7. Classification of pollen grains according to kinds of aperture (porate and colpate).

Triporate	(Pantoporate)	(Colpate)	Zonocolpate	(Colporate)
Cuscuta campestris Yunck.	Chenopodium vulvaria L	Convvulvulus arvensis L.	Arachis hypogaea L.	<i>Caesalpinia gilliesii</i> (Hook.) Dietr.
				Carthamus oxycantha M.B.
	•			Xanthum strumarium L

echinate-perforate pollen grain exine ornamentations, wall thickness, width of muri = $(0.1-0.4) \mu m (0.0-10.40 \mu m)$ and Lumina size = $(0.05-0.2) \mu m (0.0-05.20 \mu m)$ (Fig. 1 A, B, C, D).

Xanthium stromarium L

Pollen grain was single, medium ($26.84\pm1.59 \mu m$), tricolporate of orate type, spherical or spherical prolate, circular outline from polar view, polar axis dimensions ($23.59, 25.58, 29.70 \mu m$), prolate or spheroidal outline from equatorial view, equatorial axis dimensions ($24.19, 26.84, 30.40 \mu m$), polar to equatorial ratio P/E = 1.04, echinate- microclimate pollen, short echinate exine or-

namentations, wall thickness, width of muri: $(0.0-10.30 \ \mu m)$ and diameter of pores (Lumina size) $(0.05-1.50 \ \mu m)$ (Fig. 2 I, J, K, L).

Caesalpinia gilliesii (Hook.) D. Dietr.

Pollen grain was single, spheroidal, medium $(40.51\pm4.89 \ \mu\text{m})$, tricolporate, lobed circular outline from polar view, spheroidal outline from equatorial view, polar axis dimensions (30.38-37.60-4328 μ m), equatorial axis dimensions (36-.41-40.51, 44.97 μ m), polar to equatorial ratio P/E = 1.07, striate, perforate, and tricolporate exine ornamentations, width of muri: (0.50-2.50 μ m) and diameter of pores (Lumina size)



Figure 1. Micrograph of pollen grains examined by Scanning Electron Microscope (SEM): A,B,C,D: *C. oxycantha*; A- The outline of pollen grains from a equatorial view that is seen lacuna (2000x); B- Another common form of pollen grains (2000x); C- lacuna with large magnification (2000x); D- Exine ornamentation of pollen with high magnification, which is characterized by echinate and perforate (10000x). E,F,G,H: *C. gilliesii*; E- The outline of several pollen grains with low magnification (6000x); F- The outline of pollen grains with greater magnification (2000x); G- The enlarged figure is a part of the pollen grains where the colpus is well seen (10000x); H- the exine ornamentation within colpus (3000x).

(0.10-1.60 µm) (Fig. 1 E, F, G, H).

Chenopodium vulvaria L.

Pollen grain was single, spheroidal, small (19.17±2.52 μ m), perforate the number of which reaches 80, the diameter of pores about 1, colporate, circular outline from polar view and equatorial view, polar axis dimensions (14.00-18.23, 20.62 μ m), equatorial axis dimensions (15.56- 19.17- 22.5 μ m), polar to equatorial ratio P/E = 1.05, microclimate and perforate exine ornamentations, width of muri: (0.10-0.30 μ m) and diameter of holes (Lumina size) (0.05-0.10 μ m) (Fig. 3 Q, R).

Convolvulus arvensis L.

Pollen grain was single, spheroidal, almost balloonshaped, large (54.5±4.8 μ m), Colpate, three lobes outline from polar view and spheroidal from equatorial view, polar axis dimensions (41-49.5-57.8 μ m), equatorial axis dimensions (47-54.5-63.6 μ m), polar to equatorial ratio P/E = 1.10, Macroechinate and echinate exine ornamentations which can be seen at the surface of pollen grain and at the edges of circular surface, width of muri: (0.4 -40.00 μ m) and diameter of pores (Lumina size) (0.60-1.40 μ m) (Fig. 2 M, N, O, P).

Cuscuta campestris Yunck

Pollen grain was single, spheroidal, medium $(25.98\pm6.75 \ \mu\text{m})$, Colpate, three lobes outline from polar view and spheroidal from equatorial view, polar axis dimensions $(20.43-24.10-26.67 \ \mu\text{m})$, equatorial axis dimensions $(22.38-25.98-34.49 \ \mu\text{m})$, polar to equatorial ratio P/E = 1.07, microclimate- perforate exine ornamentations, width of muri: $(0.30 \ -1.40 \ \mu\text{m})$ and diameter of pores (Lumina size) $(0.10-0.70 \ \mu\text{m})$ (Fig. 3 S, T, U).

Arachis hypogaea L.

Pollen grain was single, spheroidal, medium $(25.40\pm1.5 \ \mu\text{m})$, 3-zonocolpate, spheroidal outline from polar view and equatorial view, polar axis dimensions



Figure 2. Micrograph of pollen grains examined by Scanning Electron Microscope (SEM): I,J,K,L: *X. strumarium*; I- The outline of pollen grains from a equatorial view with low magnification (2000x); J- The outline of pollen grains from a polare view (2000x); K- Pollen surface pore (2000x); L- Exine ornamentation microechinate (10000x); M,N,O,P: *C. arvensis*; M- The outline of pollen grains from a polare view that is spheroidal (2000x); N-Exine ornamentation macroechinate and perforate that is seen at pollen level at the edges of circular surfaces (2000x); O- Exine ornamentation pollen with a larger magnification seen on the edge of the circular area corresponding to the pollen surface (10000x); P- Exine ornamentation pollen with larger magnification, which can be seen echinate (10000x);



Figure 3. Micrograph of pollen grains examined by Scanning Electron Microscope (SEM): Q,R: *C. Vulvaria*; Q- Exine ornamentation of pollen microechinat and perforate with 400x (10000x); R- The outline of pollen grains that is spheroidal (2000x); S,T,U: *C. campestris*; S- The outline of pollen grains from a polar view which has 3-colpate (2000x); T- The outline of pollen grains from a polar view (2000x); U- Exine ornamentation of pollen that is microechinat-perforate (10000x); V,W,X: *A. hypogea*; V- The outline of pollen grains from a equatorial view which has 2-colpate (2000x); W- The outline of pollen grains from a polar view (2000x); X- Exine ornamentation of pollen that is striate -perforate (10000x).

(20.24-46.24-10.67 μ m), equatorial axis dimensions (34.49-22.38-25.98 μ m), polar to equatorial ratio P/E = 1.08, Striate, tilted and perforate exine ornamentations, width of muri: (0.50 -1.00 μ m) and diameter of pores (Lumina size) (0.30-0.05 μ m) (Fig. 3 V, W, X).

Discussion

The Asteraceae is one of the largest families in Iran and includes many weeds. In terms of pollen grain characteristics, echinate exine ornamentation is common in many of their pollen grains. From this family, two



Figure 4. Images obtained from Light Microscope (LM) for pollen grains of studied species prepared by Erdtman acetolysis method: A- Pollen grain image *C. oxycantha*; B,C: Pollen grain image *X. strumarium*: B- The outline of pollen grains from a equatorial view with echinate ornaments; C: The outline of pollen grains from the polar view with Orate is clear; D,E: - Pollen grain image *C. gilliesii*; D- The outline of pollen grains from the polar view with colpi are clear; E- The outline of pollen grains from an equatorial view with striate ornaments; F- Pollen grains *C. vulvaria* from an equatorial view microechinate ornament is clear; G- The outline of the pollen *C. arvensis* the porates are clearly; H- The outline of pollen grains from a equatorial view *C. campestris*; I- The pollen colpus that is seen on the flank *C. campestris*; J- Pollen grain image *A. hypogea* from a equatorial view and polar view that is spheroidal; scale = 20 µm.



Figure 5. The image of pollen grains collected from the Kermanshah region. Images obtained from Light Microscope (LM) images for pollen grains of species examined by Durham's method: A- *C. gilliesii* (400x), Kermanshah, 25 April 2018; B, C- *Chenopodium* sp. (400x), Kermanshah, 28 Aug 2017; D- Convolvulaceae (400x), Kermanshah, 26 June 2018.

species of important weeds from this area were studied. Carthamus oxycantha is one of the important weeds of Kermanshah farms and grows wildly in many farms and rangelands of Kermanshah province. This plant is of great interest to honeybees because of its nectar and pollen. In terms of pollen characteristics, the size of their pollens was slightly different from that of Turkey (12) and Iran (Noor Abad, Lorestan) (29, 33), which is due to the different climatic conditions. Comparison of the morphological characteristics of pollen grain of plants of the Asteraceae family in the Kermanshah region with other researches in this field including Asteraceae pollen atlas (34) showed that these pollens are prolate, medium-sized, tricolporate with echinate ornamentation. Another weed species of the Asteraceae family in the study was Xanthum strumarium, which produces many pollens in late fall. The pollen of this plant is spherical and triangular from polar view, Prolate, and circular from equatorial view with echinate exine ornamentations.

Also, the pollen characteristics such as pollen grain size of this species were almost similar in the author's research and other studies. It should be noted that using the Durham method, atmospheric pollens in Kermanshah area include pollen of Tragopogon graminifolius DC., Taraxacum officinale (L.) Weber ex F.H. Wigg and ornamental species such as Calendula officinalis L., Gazania rigens (L.) Gaertn., Tagetes erecta L. and agricultural species Helianthus annuus from Asteraceae family; in terms of morphological characteristics, these pollens were highly similar to studied species of this family which were directly harvested from nature. Caesalpinia Gilliesii, from the Caesalpinaceae family, was studied. The shape of the pollen grain in this genus is spheroidal, tricolporate with striate ornamentations. Studies in Kermanshah on Gleditsia capsica and Cercis siliquastrum have shown that the different genera of this family have many similarities in shape, aperture and ornamentation (33). Also, pollen samples of Caesalpina gilliesii were observed on the surface of slides in the Durham method. From the Chenopodiaceae family, Chenopodium vulvaria L. was investigated. The pollen grains were pantoporate and spheroidal. In this species, the number of pores is about 30 to 40, and these pollen characteristics are in line with research in Pakistan on Chenopodiaceae, which is now integrated with Amaranthaceae (10). In addition, research in the Ural region of Russia on the pollen of Chenopodium sp., the number of pollen pores has been reported to be 30-40 (26). In Chenopodium sp. from Kermanshah, the pollen was spheroidal, small, multi-porous and having similar characteristics to the genus Chenopodium sp. studied in Kermanshah province and exine ornamentation of pollen grain was microechinate microclimate and polyporate in both genera. However, other studies conducted in Mexico on Atripelix species shown that the ornamentation is monotuberculate (23). Research in Pakistan on 40 species of this plant family provided similar pollen characteristics (10). In the species obtained from aerial pollens by the Durham method, many pollens from the Amaranthaceae family were observed. From the Convolvulaceae family, the Convolvulus arvensis species was studied; the pollen was classified as spheroidal in shape and large in size. Also, exine ornamentations of pollen grains are perforated and macroechinate. Studies in South America indicated that the shape of pollen is spheroidal, tricolporate and the ornamentation are echinate (35). In a study of 9 species of genus Convolvulus, it was indicated that pollen grains are tricolporate with echinate ornamentation (14). In the studied samples, the pollen grains had many similarities with the above researches. In the species obtained from aerial pollens by Durham method, some pollen of Convolvulus arvensis L. were found on the slides. The species of Cuscuta campestris Yunck from the Cuscutsceae family were studied. Its pollen was spheroidal in shape, and medium in size (26.97 μ m) and polar to equatorial axis ratio was 1.07, the exine ornamentation of pollen grain was microechinate which is similar to the research done by Ranjbar et al. (16). From the Fabaceae family, Arachis hypogaea L. species was investigated where the pollen grains were spheroidal, and 3-zonocolpate and the exine ornamentation was perforated and Striate. Whereas, investigations by Chaturvedi on such species were reported to have perforated with stretched-edge exine ornamentations (36). Some researchers (37-40) suggested the microspores in mid-uninucleic stage for

androgenesis.

Fabaceae family vary widely in the morphology of pollen grains and have different types of orate Tricolporate pollen grains or perforated Tricolporate (2, 3 or 4 colpi) perforated.

The pollen grain of 7 plant species belonging to 6 families was studied which were very different in terms of morphology. The shape of pollen grain in the studied species varied from prolate to spheroidal. The pollen grain size in the study area was small, medium and large; the medium size was more common in most species. In terms of pollen grains' aperture status, Pantoporate, Colpate, and Tricolporate statuses were observed. The exine ornamentations were Striate, Echinate, Macroechinate, Microechinate, and Psilate. The exact details of exine ornamentations of pollen grains were observed by using SEM and the results were: Striate, perforated, Striate and tilted, Microechinate, perforated, Macroechinate, macrotuberculate, and sandpaper-perforated. In the Durham atmospheric pollen method, the allergenic pollen grains of different plant families in the study area were observed.

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