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RESEARCH ARTICLE

POLLEN MORPHOLOGY OF SEVEN PLANT SPECIES OF THE ASTERACEAE (COMPOSITAE) FAMILY NATURALLY GROWING IN THE KINGDOM OF SAUDI ARABIA

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ABSTRACT

The current study included investigated pollen grains morphology (mainly exine sculpture) of seven species of the Asteraceae family from the northern and eastern parts of the Kingdom of Saudi Arabia. These plant species included *Artemisia sieberi* L., *Achillea fragrantissima* (Forssk.) Sch. Bip., *Calendula officinalis* L., *Matricaria aurea* Loefl., *Picris abyssinica* L., *Rhanterium epapposum* Oliv. and *Tanacetum santolinoides* D.C. The pollen morphology of seven Asteraceae plant species was investigated with light microscopy. Pollen slides were prepared using Wodehouse technique. Measurements were based on 25 -30 pollen grains per specimen. The pollen grains of the studied plant species are radially symmetric and isopolar. The pollen grains are oblate-spheroidal with the polar axes 18.5–23.4 µm and the equatorial axes 19.8–26.7 µm. The pollen grains of the plant species are tricolporate. The pollen grain of all species has echinate ornamentation. The spines have conical shape with a wide base tapering towards an apical section. The spine length ranges between 2.1–3.5 µm and its width ranges between 2.6–3.8 µm. The overall exine thickness ranges from 3.7 - 5.1 µm, while intine thickness ranges from 0.37-0.81 µm. It is clear that the external surface characteristics of the pollen and the presence of spines are important traits and can be used successfully for species taxonomic classification.

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INTRODUCTION

Taxonomic studies are important in identifying and classifying wild and cultivated plant species, especially those that are considered of economic importance and represent essential components of the natural resources of any country. Therefore, the studies of plant communities and their environmental conditions are necessary for developing the proper scientific programs for maintaining and improving the natural plant cover. Thus, different researchers and taxonomists have been interested in evaluating the morphological, cellular, chemical characteristics of plant species as well as pollen characteristics, etc. in addition to the plants phenotypic characteristics in order to determine the correct plant species classification (Hamad, 1990). Palynology (Pollen science) is an important branch of biological sciences that has received widespread attention by Taxonomists worldwide and contributed in solving major problems in other related sciences such as plant taxonomy, geology and paleobotany (Moore and Webb, 1978). The first to use the term Palynology was Hyde and Williams (1945), which means pollen grain and spores science. The Asteraceae (Compositae) family is the largest family among all vascular plants, with an estimated population of about 950 genera and about 20,000 plant species distributed worldwide and under all environmental conditions.

Asteraceae is one of the first families whose pollen grains were studied, as it was firstly described by Fisher (1890), followed by the comprehensive Wodehouse (1926, 1928a, b, 1935) studies for most of genera of this family using the optical microscope. These studies are considered amongst the most important taxonomical studies. Wodehouse distinguished three patterns of pollen shapes: Psilate (the pollen grain with a completely smooth surface), Echinate (the pollen grain has echinae. Echinae are structures with broad bases and a sharp pointed apexes) or Lophate (Pollen contains lacunae surrounded by Ridges. In this pattern, there are two types of pollen either smooth or called psilolophate or with more or less prominent spines and called echinolophate). The important studies of this family also included those conducted by Erdtman (1952) who studied 400 species, 155 genera as well as the studies carried out by Stepa (1960) and Stix (1960) who covered altogether 235 species that belong to the Asteraceae family. Radford *et al.* (1974) noted that pollen characteristics have a significant taxonomic significance in the determination of taxonomic classes, especially when the Scanning Electron Microscope (SEM) and the Transmission Electron Microscope are used. Skavarla *et al.* (1977) reported that Stix (1960) study is considered one of the most important studies as well as Blackmore *et al.* (1995) study who reported that the number of holes and their locations in pollen grains was important in

determining the classes, families and communities. The Torres (2000) study, which is considered among the latest studies on the Asteraceae, recorded the relationship between the size of the pollen grain and the length of the pistil in the Asteraceae. The pollen has important characteristics that make it very useful in taxonomic studies. These characteristics include the shape and size of the pollen grain, its colors and type ornamentations on its surface, the presence of pores and colpi as well as the number of grooves and pores and their shapes in the surface of the pollen grain (Erdtman, 1971). In the present paper, the pollen morphology, types and sculpture of indigenous plant species in Asteraceae from the northern and eastern regions of Saudi Arabia were studied.

MATERIALS AND METHODS

Pollen material used in the current study was based on fresh pollen samples which had been obtained from seven naturally growing Asteraceae plant species i.e. *Artemisia sieberi* L., *Achillea fragrantissima* Forssk., *Calendula officinalis* L., *Matricaria aurea* Loefl., *Picris abyssinica* L., *Rhanterium epapposum* Oliv and *Tanacetum santolinoides* D.C. collected during field trips in the different parts of the Eastern region of the Kingdom (Table 1) and kept in Ethel alcohol 70%, the method of Al-Mayach, (1983) was followed with some modifications. Three to five mature, unopened flowers were selected from each inflorescence and transferred to a clean glass slide and a drop of water was added at the top. The sepal (calyx), petal (corolla) and pistil were removed. The staminal cylinder was obtained and then transferred to another glass slide and was opened to obtain pollen grains. For light microscopy, the pollen grains were mounted on a glass slide in saffranin stained glycerin jelly and covered with a cover slip sealed with transparent nail polish. Morphological observations of pollen grains were conducted using an OLYMPUS/BX-51 light microscope at King Abdul-Aziz City for Science and Technology (KACST), Natural Sciences Institute, Riyadh, Saudi Arabia. The measurements were taken for 25-30 pollen grains per plant species. Equatorial axis length, polar axis length, thorn length, germination pore diameter and wall thickness of each pollen grain were measured using the Ocular Micrometer and for each parameter, the arithmetic mean has been calculated according to Erdtman (1952) and Reitsma (1970).

RESULTS AND DISCUSSION

The pollen grains of Asteraceae are known to be spherical, slightly flattened or helianthoid. They are principally tricolporate, echinate, and the colpus number and size differs significantly (Skvarla *et al.* 1977 and Wodehouse 1930, 1935). In addition, the Asteraceae is considered to be euryalynous family and has zonocolporate pollen grains (Sachdeva and Malik 1986 and Erdtman 1952). The pollen grains from plants naturally growing on the eastern region of Saudi Arabia that were evaluated in this study exhibited a wide range of differences in size and sculpture that have potential taxonomic value. Pollen grains are isopolar and radially symmetrical. The pollen grains are oblate-spheroidal with the polar axes 18.5–23.4 μm and the equatorial axes 19.8–26.7 μm (Table 2). The mean length of the polar axis and the equatorial axis showed a marked variation between the studied species. It recorded the highest value in the plant species *T. santolinoides*.

It is 23.4 μm for the polar axis and 26.7 μm for the polar axis and lowest in the plant species *A. sieberi* with 18.5 μm the polar axis and 19.8 μm for the equatorial axis (Table 2). As for the ratio between the polar axis length and the equatorial axis length, the studied plant species fall within one group with a ratio of less than one. The present data are consistent with those recorded by Caramiello and Fossa (1994) who claimed that the polar diameter of the pollen grains of plant species belonging to Asteraceae ranges from 17.4 to 36.2 μm and those of the equatorial diameter ranges from 15 to 32.2 μm . The data of the present study indicated that the pollen of the studied species falls within the medium size pollen classification with the exception of the pollen of *A. sieberi*, which falls within the small pollen classification of Erdtman (1971) classification. However, in general, the pollen diameter of species in Asteraceae is considered small Erdtman (1952) classified pollen in accordance to their size into six groups: perminuta (dia. < 10 μm), minuta (dia. 10–25 μm), media (dia. 25–50 μm), magna (dia. 50–100 μm), permagna (dia. 100–200 μm), giganta (dia. > 200 μm). In accordance to this classification, the pollen of the studied species belong to the groups minuta and media groups. The P/E ratio is believed to be taxonomically useful at the plant species level. The P/E ratio of the species under study ranged between 0.95–0.85 (Table 2), thus the pollen is oblate spheroidal (P/E ratio < 1). However, Mumtaz *et al.* (2000) recorded a P/E value of 1.14 to 1.31 in certain *Artemisia* species, and claimed that this characteristic is highly significant.

The mean thickness of the exine of the studied plant species ranged between 3.5 - 5.1 μm , while the intine ranged between 0.37–0.81 μm (Table 3). Table 3 shows that the highest values for the exine and intine are recorded by *A. fragrantissima* (5.1 and 0.81 μm , respectively), while the lowest values were recorded by *R. epapposum* for the exine (3.5 μm) and *T. santolinoides* for the intine (3.7 μm). Huang (1972) reported that the exine thickness of some Asteraceae plant species varied between 1.0 - 3.0 μm , Caramiello and Fossa (1994) reported that the exine thickness varied between 3.1 - 4.6 μm , while Mumtaz *et al.* (2000) recorded an exine thickness of between 2.5 and 5.0 μm in twelve species of Asteraceae family. The data of the present study, which also is in agreement with these findings. The colpus of the studied plant species is short (10.5–13.7 μm) and narrow (3.1–5.1 μm) (Table 3). The highest values were recorded for *M. aurea* and the lowest for *A. sieberi* (Table 3). The number of pores as well as the thickness between pores varied from one plant species to another and ranged between 7 (*C. officinalis*) - 11 (*P. abyssinica*) (Table 3). In general, pollen pores are randomly distributed, thus, thickness between pores are small and ranges between 4.3–6.6 μm (Table 3).

The current study shows that the pollen aperture in the species under study is tricolporate (Table 4). This is consistent with what Karim and Ali (1979) have pointed out. It was noted that generally the aperture of *M. aurea* varied in accordance to the following ratio 1 % tricolporate: 2 % syncolporate: 97 % tricolporate. Nair and Kaul (1965) and Inceoglu (1973) attributed the variations in pollen size and aperture type to heteromorphy in pollen grains. Pollen spines characteristics have a diagnostic value in Asteraceae. Wodehouse (1935) discussed the morphological evolution of spine shape in Asteraceae, and suggested a reduction state from long to minute spines.

Table 1. The investigated plant species of Asteraceae family collections Sites in the eastern region of Saudi Arabia

Plant species	Site	Life form	Habit
<i>A. sieberi</i>	Northeastern	CH	Herb
<i>A. fragrantissima</i>	Central	CH	Per
<i>C. officinalis</i>	Central	HE	Herb
<i>M. aurea</i>	Southeastern	TH	Herb
<i>P. abyssinica</i>	Central	CH	Herb
<i>R. epapposum</i>	Southeastern	CH	Per
<i>T. santolinoides</i>	Southeastern	CH	Herb

Table 2. Pollen measurements of different plant species

Plant species	Polar axis (P)	Equatorial axes (E)	P/E	Pollen shape
	μm	μm		
<i>A. sieberi</i>	18.5	19.8	0.93	Oblate-spheroidal
<i>A. fragrantissima</i>	20.8	24.6	0.85	Oblate-spheroidal
<i>C. officinalis</i>	23.2	25.9	0.90	Oblate-spheroidal
<i>M. aurea</i>	19.6	22.9	0.86	Oblate-spheroidal
<i>P. abyssinica</i>	21.8	23.5	0.93	Oblate-spheroidal
<i>R. epapposum</i>	22.9	24.4	0.95	Oblate-spheroidal
<i>T. santolinoides</i>	23.4	26.7	0.86	Oblate-spheroidal
Mean	21.5	24.0	0.90	Oblate-spheroidal

Table 3. Palynological characters

Plant species	Exine μm	Intine μm	Colpus		No. of pore	Pore	
			Clt μm	Clg μm		Plt μm	Plg μm
<i>A. sieberi</i>	3.8	0.61	3.1	10.5	11.0	4.3	4.3
<i>A. fragrantissima</i>	5.1	0.81	4.3	12.9	9.0	6.4	6.4
<i>C. officinalis</i>	3.7	0.63	4.5	11.4	7.0	6.6	6.6
<i>M. aurea</i>	4.5	0.51	5.1	13.7	9.0	5.6	5.7
<i>P. abyssinica</i>	4.8	0.52	5.0	13.4	11.0	6.2	6.1
<i>R. epapposum</i>	3.5	0.41	4.1	12.3	10.0	5.2	5.2
<i>T. santolinoides</i>	3.7	0.37	4.8	11.7	9.0	6.6	6.6

Table 4. Aperture type, spine, Perforation, and Ornamentation of Pollen of the studied plant species

Plant species	Aperture type	Spine		Perforation number at base	Ornamentation	Ornamentation of inter-spinal area
		Length (μm)	Width (μm)			
<i>A. sieberi</i>	tricolporate	2.1	2.6	8–15	Echinate	Rugulate-perforate
<i>A. fragrantissima</i>	tricolporate	2.3	3.5	8–15	Echinate	Rugulate-perforate
<i>C. officinalis</i>	tricolporate	3.2	3.4	8–16	Echinate	Rugulate-perforate
<i>M. aurea</i>	tricolporate	2.4	2.7	10–16	Echinate	Rugulate-perforate
<i>P. abyssinica</i>	tricolporate	2.8	3.5	10–18	Echinate	Rugulate-perforate
<i>R. epapposum</i>	tricolporate	3.2	3.8	10–15	Echinate	Rugulate-perforate
<i>T. santolinoides</i>	tricolporate	3.5	3.8	8–15	Echinate	Rugulate-perforate

The spinate pollen it is considered to be primitive characteristic compared to spineless pollen. Clark *et al.* (1980) indicated that pollen size, spine length and spine rows between the colpi number varies significantly between plant species in Asteraceae family. These results were in line with the findings of the current study. The data presented in Table 4 showed that spines were present in all pollen wall so fall studied plant species. The spines are usually conical in shape with a wide base tapering to apical portion. The margins are regular and the ends are acute. The spine length ranges between 2.1–3.5 μm in the studied plant species, while their width ranges between 2.6–3.8 μm (Table 4). The pollen grains of *T. santolinoides* have the longest spines and the thickest wall (Table 4), while that of *sieberi* has the shortest spines and the thinnest wall. The spines base in the studied plant species has irregular two seriate perforations. Larger distal holes are recorded in *M. aurea*. The number of perforation ranges between 8–15 (*A. sieberi*, *A. fragrantissima* and *T. santolinoides*) and 10–18 (*P. abyssinica*). It should be noted that the pollen grains of the seven studied plant species were echinate (Table 4) and the ornamentation of inter-spinal area is generally of Rugulate-perforate nature. Mesfin *et al.* (1995) claimed that the ornamentations between spines are a significant characteristic

for Asteraceae. The pollen grains of the species in the current study are mostly, sticky and yellow to yellowish-brown in colour. The pollen walls are thin, which in agreement to that recorded by Perveen (1999) for the members of Asteraceae.

Conclusion

Pollen morphology is a useful taxonomic trait in higher plants. The ornamentations, pollen shape, the numbers of perforations, diversity of spine characteristics as well as exine sculpture are considered useful diagnostic characters for the differentiation of closely related species. Aperture type is found to be consistent within Asteraceae.

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