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Identification of Three Medicinally Important Species of Family Asteraceae Based on Pollen Characters

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Abstract: Present work was conducted to examine pollen characters of three medicinal species of family Asteraceae i.e., *Helianthus annuus* L., *Sonchus arvensis* L. and *Tagetes erecta* L. Pollen morphology was examined under light microscope, on the basis of polar & equatorial diameter, exine & intine thickness, spines, colpi and endopore. Pollen fertility was also estimated. All species showed great variation among pollen characters. Prolate spheroidal and sub spheroidal pollens were detected in these species. On the basis of spines, echinated and psilated pollens were observed while uni, bi, tri and tetracolpate pollens were observed on the basis of colpi. It was concluded that palynological study is a valuable taxonomic tool due to the presence of a number of variety among pollens.

Keywords: Colpi, Echinated, Endopore, *Helianthus annuus*, Psilated, *Sonchus arvensis*, *Tagetes erecta*.

Introduction

Helianthus annuus L. (sunflower), *Sonchus arvensis* L. (perennial sow thistle) and *Tagetes erecta* L. (African marigold) belong to plant family Asteraceae. This family is one of the richest vascular plant families in the world comprising of over 1600 genera and 23000 species (Moreira-Munoz, 2011). Members of this family are found in almost every environment. *Helianthus annuus* L. is a native species of North America while *Sonchus arvensis* L. and *Tagetes erecta* L. are introduced species. These all prefer moist and humid environment but *Tagetes erecta* L. and *Helianthus annuus* L. can grow in sub humid and warm areas too (Jansen and Cardon, 2005). These species are mainly characterized by ray and disc florets, grouped in capitula and the fruit, cypsela, mostly with a pappus (Moreira-Munoz, 2011). Flowers of *Helianthus annuus* L. and *Sonchus arvensis* L. are yellow in color containing more xanthophyll pigments. While flowers of *Tagetes erecta* L. are found in two colors; yellow and orange. In these flowers, the only difference between yellow and orange is in the total amount of carotenoids (Valadon *et al*., 1967).

The above mentioned members of Asteraceae have great economic and medicinal importance. Sunflower is used for extraction of oil and biodiesel production (Demirbas, 2007). *Helianthus* and *Tagetes* are also cultivated for their ornamental purposes. Young leaves of perennial sow thistle can be used in salad. Its latex is oily and it may be a potential crop for production of oil and hydrocarbon (Lemna and Messersmith, 1990). Carotenoid pigments from *Tagetes* species are used for food coloring purpose (Vasudevan *et al*., 1997). *Sonchus arvensis* L. is a good source of penta cyclic triterpenes, which have importance in pharmaceutical industry (Hooper, 1984). Its roots are used in cough, bronchitis and asthma (Hussain *et al*., 2010). The ethanolic extract of leaves of *Tagetes erecta* L. has significant wound healing activity (Ghosh *et al*., 2004). This plant is also used as an analgesic, antiseptic, carminative, diuretic, expellant, stimulant and vermifuge (Neher, 1968). Due to all these medicinal properties, the study of these plant species has great importance.

Helianthus is considered as a diverse genus of Asteraceae family on the basis of morphological and genetic markers. Due to this reason its systematics is complex and has often reconsidered taxonomically (Heiser, 1954, 1961; Schilling, 2006). Similarly, *Tagetes* also exists in different species forms which show morphological similarities in many aspects like in some of the vegetative and reproductive parts. *Sonchus* is also morphologically and ecologically a diverse genus having different species with similar morphologies (Qureshi *et al*., 2002).

Many disciplines are associated with taxonomy which assist the taxonomists for proper identification, classification and systematic positioning of different taxa. Among these, palynology is an important taxonomic tool. It is usually used by modern taxonomists in order to identify and differentiate among closely related species (Zafar *et al*., 2007). Stix (1960) first studied the internal structure of pollen wall of Asteraceae. Qureshi *et al*., 2008 worked on pollen morphology of 3 genera of Asteraceae. Klimko *et al*., 2000 described pollen characteristics of three varieties of *Helianthus annuus* L. Thus palynology is useful both at and below the generic level and provides a basis for taxonomic studies. Beside identification and differentiation, palynology is also directly related with different other fields including agriculture, horticulture, plant breeding, biotechnology and forestry (Zafar *et al*., 2007).

Although many researches have been conducted on palynology of different Asteraceous

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species, but there is small amount of data, present on specifically *Tagetes erecta* and *Sonchus arvensis* species from their genera. Another reason for selecting these species was the presence of a large variety of pollen grains. Different authors have described different types of pollens in these plants. The main objective of the present work is plant profiling of three medicinal species belonging to family Asteraceae on the basis of their palynological characters and the basic purpose of the study is therefore to differentiate these species on the basis of palynological studies.

Materials and Methods

The experiment was designed to examine pollen morphology of three medicinal species of family Asteraceae (*Helianthus annuus* L.*, Sonchus arvensis* L. and *Tagetes erecta* L.)

To study different characters of pollen grains, fresh flowers of *Helianthus annuus* L.*, Tagetes erecta* L. and *Sonchus arvensis* L. were collected. Some florets from these flowers were separated from the capitula, kept on clean slide and opened with the help of dissecting needles. Anthers were separated from these and extra material was removed. A drop of acetic acid was put on the slide and the anthers were crushed in it. After crushing, the anther wall material was removed and the pollens were collected on the slide. Glycerin jelly was used to stain the pollen grains. It was prepared by mixing 1% safranine in glycerin jelly (Meo and Khan, 2005). A small amount of glycerin jelly was put on the pollens and heated to melt the jelly. After melting, a coverslip was placed on the material and pressed gently. The slide was labeled and the edges of coverslip were sealed with transparent nail varnish. Then it was examined under light microscope (model: Meiji techno MX - 4000). Different microscopic pollen characters were observed including polar and equatorial diameter, P/E ratio, exine and intine thickness, spine length and diameter, pollen fertility and size of colpi and endopore. For description of pollen characters, glossary of pollen and spore terminology was followed (Punt *et al*., 2007).

Micro photographs were taken by CCD digital camera (model: Canon Pc 1200 attached with MD lens MA 151/30/73 opter) fitted on light microscope. Identification of pollen characters was made by using power at high power plan i.e., 40×/0.65, ∞/0.17, F=200, WD=0.5 and at lower power plan i.e., 10×/0.25, ∞/0.17, F=200, WD=7.3. These micrographs were used for identification and characterization of different types of pollens on the basis of microscopic features. Data was evaluated by co-relation matrix and cluster analysis to determine interspecific relationship. Dendrogram was constructed on the basis of Unweight Pair Group Method with Arithmetic Average (UPGMA). The computer software SPSS v 11 was used for this purpose.

Results and Discussion

Examination of Pollen Morphology:

Following key was established for *Helianthus annuus* L., *Sonchus arvensis* L. and *Tagetes erecta* L. during the present study.

Identification Key to Species:

1a: Exine ornamentation is homobrocate *Sonchus arvensis* L.
1b: Exine ornamentation is not homobrocate 2
2a: Spine tip is obtuse *Helianthus annuus* L.
2b: Spine tip is not obtuse *Tagetes erecta* L.

***Helianthus annuus* L.:**

- **Pollen Class:** Prolate spheroidal, bicolpate and tricolpate.
- **Ornamentation:** Echinated and psilated.
- **Aperture:** Endoaperture is rounded to triprojectate and ectoaperture is absent.
- **Outline:** Polar view is rounded and equatorial view is elongated.
- **%Fertility:** 100%

***Sonchus arvensis* L.:**

- **Pollen Class:** Sub spheroidal, unicolpate to tetracolpate.
- **Ornamentation:** Echinated with obtuse tip and psilated.
- **Aperture:** Endoaperture is elongated.
- **Outline:** Polar view is rounded to triangular and equatorial view is rounded.
- **%Fertility:** 100%

***Tagetes erecta* L.:**

- **Pollen Class:** Prolate spheroidal and tricolpate.
- **Ornamentation:** Pentoporate and echinated.
- **Aperture:** Outline of endoaperture is rounded.
- **Outline:** Polar and equatorial view is rounded.
- **%Fertility:** 100%

Palynology is the science of pollen grains and spores. It is specifically associated with taxonomic study (Zafar *et al*., 2007). Pollen morphology is significant at specific level, due to remarkable differences in pollen characters among different plant species. Study of pollens is considered as a worthwhile taxonomic marker because species can easily be delimited on the basis of pollen characters (Perveen, 1999). Different researchers have extensively used pollen characters for taxonomic studies (Tomsovic, 1997; Huang, 1972). During present piece of work, pollen morphology of three different species of family Asteraceae was examined under light microscope. All the species (*Helianthus annuus* L., *Sonchus arvensis* L. and *Tagetes erecta* L.) showed great variations among their pollen shapes and sizes.

Different characters like polar and equatorial diameter, P/E ratio, exine and intine thickness, spine length and diameter, pollen fertility and size of colpi and endopore were considered in the present investigation (Table 1). In *Helianthus annuus* L. prolate spheroidal, bicolpate and tricolpate pollens were observed. Both echinated and psilated pollens were detected in this species (Plate 1). The pollens of *Tagetes*

erecta L. were prolate spheroidal, tricolpate, pentoporate and echinated (Plate 3). *Sonchus arvensis* L. showed more variety as compared to other two species. Sub spheroidal, unicolpate, bi-, tri- and tetracolpate pollens were observed in this plant species. Echinated pollens had obtuse tips (Plate 2). Perveen (1999) concluded that this plant family (Asteraceae) is a eurypalynous family. Diez *et al*. (1999) observed the pollen morphology of *Sonchus* and some other genera under light and electron microscope. They observed zoncolporate and echinolophate pollens among pollens of these species. Klimko *et al*. (2000) worked on pollen morphology of three varieties of *Helianthus annuus* L. they found no significant differences among pollens of these varieties. Noor *et al*. (2004) worked on palynology of *Tagetes petula* and different other cultivated species. They found a variety among pollen shapes including spherical, elliptical and oblong pollens.

In the present study, maximum ratio of polar to equatorial axis (P/E) was observed in pollens of *Helianthus annuus* and *Tagetes erecta* (i.e., 1.1) (Table 1). According to Klimko *et al*., 2000, values of P/E in three *Helianthus* varieties were found to be similar (1.0 – 1.04) as that of present results. In *Sonchus arvensis* this ratio was observed to be 1.0 (Qureshi *et al*., 2008) and 0.89 (Qureshi *et al*., 2002), differing from present results i.e., 0.96. In case of *Tagetes erecta* 0.85 P/E was observed, showing differences in results (Mazari *et al*., 2012). Pollens of *Helianthus annuus* L. had comparatively highest polar and equatorial diameters (230 µm and 205.6 µm respectively) while *Tagetes erecta* L. had lowest polar and equatorial diameters (178.2 µm and 160.7 µm respectively). Maximum exine thickness was observed in *Tagetes erecta* L. (13.1 µm) while intine was absent in this species. *Helianthus annuus* L. had maximum intine thickness (8 µm) and inter-membranal distance (21 µm). Smallest colpi was observed in *Tagetes erecta* L. (length= 19.6 µm, width= 37.9 µm). *Sonchus* species with trizonocolporate colpi character were observed by Qureshi *et al*., 2002. In contrast to this, Qureshi *et al*. (2008) and Klimko *et al*. (2000) did not detect the presence of colpi among *Sonchus* species and *Helianthus* varieties respectively. Spines of *Helianthus annuus* L. showed maximum length (24.9 µm) and diameter (21.1 µm). Largest endopore was observed in *Tagetes erecta* L. (length= 57.4 µm, width= 54 µm) while endopore lengths of other two species were nearly equal (Table 1). Qureshi *et al*. (2008), Klimko *et al*. (2000) and Mazari *et al*. (2012) did not detect the presence of endopore among *Sonchus* species, *Helianthus* varieties and *Tagetes erecta* respectively. Pollen fertility shows the capability of pollen grains to develop new generations through fertilization (Awan *et al*., 2001). The species, under present investigation, showed 100% fertility. 96-97% pollen viability was observed in *Helianthus* varieties (Klimko *et al*., 2000).

Pollen morphology is now considered as an important taxonomic tool because of the use of comparatively simple procedure for pollen studies and also due to presence of a great morphological diversity among pollen grains (Bashir and Khan, 2003). The present study showed great diversity among many characters including pollen shape, size, colpi number, spine length, endopore and wall thickness. The character of pollen spine has great significance in evolution and classification at both generic and specific levels (Zafar *et al*., 2007). The echinate pollen character is considered as a primitive feature as compared to that of psilate pollens (Wodehouse, 1935).

Table 1: Pollen Characters of *Helianthus annuus* L., *Sonchus arvensis* L. and *Tagetes erecta* L.

Species Name	Polar Diameter (µm)	Equatorial Diameter (µm)	Exine Thickness (µm)	Intine Thickness (µm)	Inter-membranal Distance (µm)	Colpi Length (µm)	Colpi Diameter (µm)	P/E	Spine Length (µm)	Spine Diameter (µm)	Inter-spinal Distance (µm)	Endopore Length (µm)	Endopore Width (µm)
Helianthus annuus L.	230	205.6	8.2	8.0	21.0	30.3	43.1	1.1	24.9	21.1	24.1	51.1	38.9
Sonchus arvensis L.	186.2	193.4	6.5	5.3	10.1	33.7	39.4	0.96	12.2	9.1	16.4	52.6	34.2
Tagetes erecta L.	178.2	160.7	13.1	0	0	19.6	37.9	1.1	10.8	11.6	9.8	57.4	54.0

Correlation analysis is considered as an excellent tool to understand the interrelationship of different species. The correlation among three species of family Asteraceae ranged from 0.98 to 0.99, where the maximum correlation index was found between *Sonchus arvensis* L. and *Helianthus annuus* L. as well as *Sonchus arvensis* L. and *Tagates erecta* L. i.e., 0.99 for both the comparisons (Table 2). While *Helianthus annuus* L. and *Tagetes erecta* L. showed comparatively less similarity i.e., 0.98. The results showed that all the three species of family Asteraceae, under study, are closely related to one another. The correlation among different pollen characters of three plant species ranged from 0.02 to 0.99 while negative correlation varied from -0.07 to -0.99 (Table 3). The maximum correlation (0.98) was observed among four pollen characters i.e., intine thickness related with inter-membranal distance and colpi width related with spine length while the minimum co-relation (0.54) was observed between colpi length and colpi width. Interspinal distance and endopore length showed more negative relation i.e., -0.94 (Table 3).

Cluster analysis on the basis of pollen characters showed that three species of family Asteraceae were divided into two groups at 100% phylogenetic distance. Group 1 included one species i.e., *Helianthus annuus* L. while group 2 included two species i.e., *Soncus arvensis* L. and *Tagetes erecta* L. (Figure 1).

Table.2: Correlation of Medicinal Species of Family Asteraceae on the Basis of Pollen Characters

Species Names	1	2	3
1	1		
2	0.99	1	
3	0.98	0.99	1

Key: 1. *Helianthus annuus* L., 2. *Soncus arvensis* L., 3. *Tagetes erecta* L.

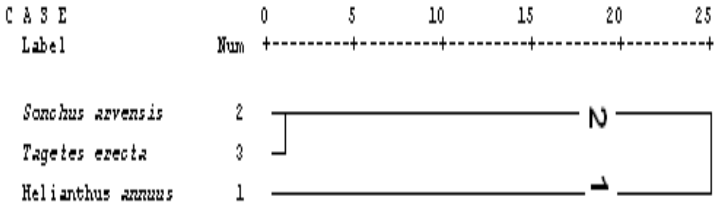


Figure.1: Cluster Analysis for Medicinal Species of Family Asteraceae on the Basis of Pollen Characters

Table.3: Correlaton of Different Pollen Charaters of Medicinal Species of Family Asteraceae

Pollen Characters	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1												
2	0.80	1											
3	0.37	-0.25	1										
4	-0.40	-0.87	0.70	1									
5	0.84	1.00	-0.18	-0.83	1								
6	0.94	0.96	0.02	-0.70	0.98	1							
7	0.42	0.88	-0.69	-1.00	0.84	0.71	1						
8	0.99	0.88	0.24	-0.53	0.91	0.98	0.54	1					
9	1.00	0.77	0.42	-0.36	0.81	0.92	0.37	0.98	1				
10	0.94	0.56	0.66	-0.07	0.62	0.76	0.09	0.89	0.96	1			
11	0.94	0.95	0.04	-0.68	0.97	1.00	0.70	0.98	0.93	0.78	1		
12	-0.78	-1.00	0.29	0.89	-0.99	-0.95	-0.89	-0.86	-0.75	-0.53	-0.94	1	
13	-0.42	-0.88	0.68	1.00	-0.84	-0.71	-1.00	-0.55	-0.38	-0.10	-0.70	0.90	1

Key: 1. Polar diameter, 2. Equitorial diameter, 3. P/E, 4. Exine thickness, 5. Intine thickness, 6. Inter-membranal distance, 7. Colpi length, 8. Colpi width, 9. Spine length, 10. Spine width, 11. Inter-spinal distance, 12. Endopore length, 13. Endopore width.

Conclusion

From the present work, it is concluded that the above mentioned species of family Asteraceae have a great diversity among their pollens due to which palynological study can be considered as a valuable taxonomic tool and it can be helpful while dealing with ambiguous taxonomic problems. It can also be considered that the nature of pollens in these species could be an evolutionary modification which might be inherited in plant species to determine their specific modes of pollination. Observed morphological similarities in pollens show their interspecific relationship and the reason for them to be in same family. On the other hand, observed differences in pollen morphology show the reasons for them to exist as distinct species.

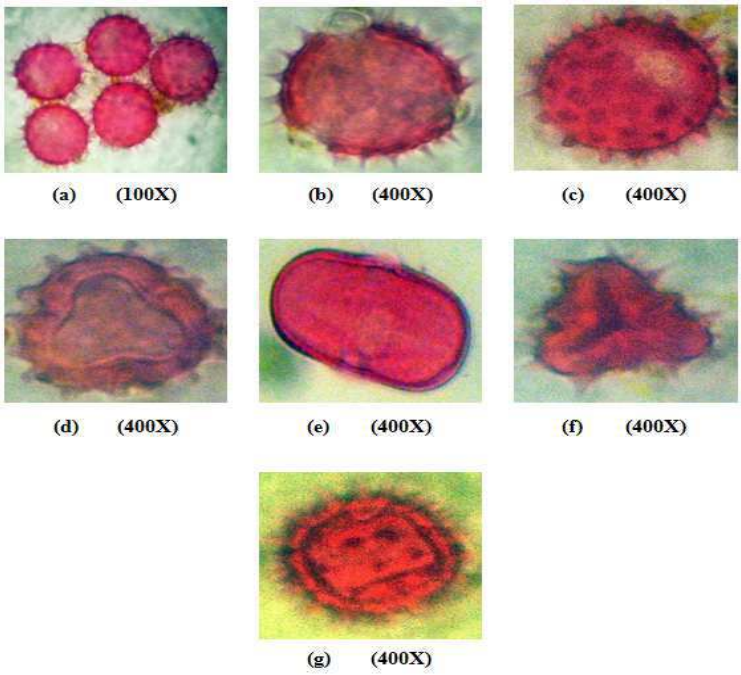


Figure.2: Pollen Grains of *Helianthus annuus* L. under Light Microscope. (a) Aggregate (b) Tricolpate (c-d) Polar view (e) Bicolpate (f) Triprojectate (g) Equatorial view

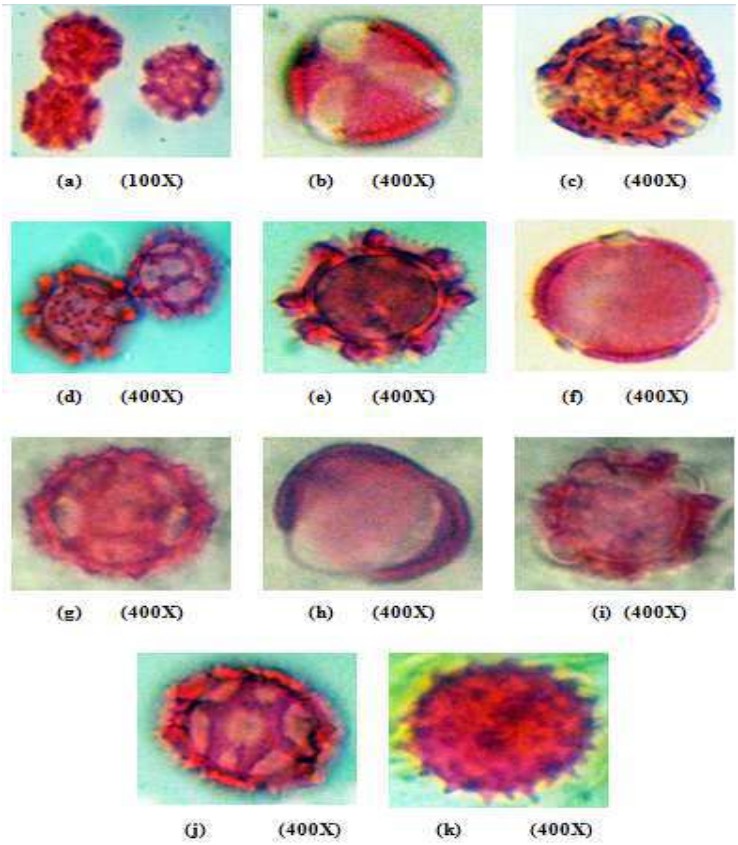
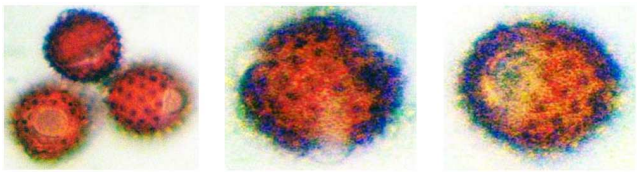


Figure.3: Pollen Grains of *Sonchus arvensis* L. Under Light Microscope: (a) Aggregate (b) Triangular (c) Tricolpate (d) Polar view (e) Tetracolpate (f) Psilated (g) Endoaperturate (h) Unicolpate (i) Bicolpate (j) Homobrocate (k) Rounded



(a) (100X) (b) (400X) (c) (400X)

Figure.4: Pollen Grains of *Tagetes erecta* L. under Light Microscope. (a) Aggregate (b) Tricolpate (c) Polar view

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