Research in Plant Biology, 4(6): 17-23, 2014 ISSN : 2231-5101
www.resplantbiol.com

Regular Article

Pollen Grain Morphology of Some Selected Species of Asteraceae in South Western Nigeria

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Pollen morphology and diversity of twelve species of the Asteraceae were collected from different locations within Ile-Ife, South Western Nigeria and acetolysed. The parameters used for the characterization are: diameter of pollen grains, pollen wall thickness, number of pores, thickness between pores, length of spines and number of colpi. The pollen grains of the species studied are spheroidal in shape, in the group media and minuta, either echinate or spinate, yellow to yellowish brown, polypentaporate, tricolporate, acolpate and polycolporate, spines are short with thin walls and pores are densely situated and the measurements taken showed variations from species to species. The similarities in structure showed interspecies relationships and reasons for them to be in the same family while the interspecific variations in structures substantiate their existence as distinct species.

Keywords: pollen grain, diversity, Asteraceae, acetolysed, spheroidal, colpi, interspecific variations.

Asteraceae (Compositae) is a very large cosmopolitan family whose members are highly advanced and is the second largest family in the division Magnoliophyta with 1,100 genera and over 20,000 recognized species (Ming, 1999). The majority of Asteraceae species are herbaceous although an important component of the family consists of shrubs or even trees. Many plants in the family Asteraceae are economically important as weeds, ornamentals, medicinals and green vegetables (Olorode, 1984).

Pollen characteristics have been used considerably in the taxonomy of Angiosperms and they have been used in establishing phylogenetic relationships in the family Asteraceae, (Moore and Webb, 1978). Pollen grains of fossil and living plants have provided ample information on the floristic and climatic characters of geological formations (Erdtman, 1943). The first successful attempt at using pollen characters in the classification of plants was made by Prasad, (1963). Patel and Datta (1958) and Sowunmi (1973) are some of the workers who have also contributed to the understanding of the morphology of pollen grains and the significance of pollen architecture in tracing phylogeny. Pollen grains of some members of the family Asteraceae have been studied by Perveen (1999), Adedeji (2005) and Mbagwu *et al.,* (2009). All of them reported pollen grains of the Asteraceae to be spheroidal, echinate or spinate provided with circular

apertures. The aim of this study is to provide additional characters such as pollen morphology and diversity, structural details of the wall.

Materials and Methods

Pollen grains from fresh anthers of *Ageratum conyzoides* Linn., *Aspilia africana* (Pers) C.D Adams, *Bidens pilosa* Linn., *Chromolaena odorata* (Linn.) king & Robinson, *Crassocephalum crepidioides* Benth S. Moore, *Emilia praetermissa* Milne-Redhead, *Launaea taraxacifolia* Willd, *Synedrella nodiflora* Gaertn, *Tithonia diversifolia (*Hemsl) A. Gray, *Tridax procumbens*Linn., *Vernonia amygdalina* Del. Cent, and *Vernonia cinerea* Linn. were collected for the purpose of studying the morphology of the pollen grains. Pollen grains from 4-5 different flowers of the same species were collected. These were acetolysed by treatment with acetic anhydride and concentrated sulphuric acid following the procedure of Erdtman (1966). The acetolysed pollen grains were mounted in glycerine. In each case, measurements of largest equatorial diameter of 50 pollen grains were taken. Photographs of the pollen grains were taken with the camera attached to the Olympus microscope using x100 objective with oil immersion. The quantitative data was subjected to Single Linkage Cluster analysis.

Herbarium Survey: All plant species used in this work were collected by me, authenticated in the Ife Herbarium and voucher specimens deposited in the same herbarium.

Table 1: IFE Herbarium (Osun state, Nigeria) voucher specimen number for the twelve Asteraceae species studied

Species	Voucher specimen no.	Collectors
Ageratum conyzoides Linn.	IFE 16884	Akinnubi Olufunmilola
Aspilia africana (Pers) C.D. Adams.	IFE 16882	Akinnubi Olufunmilola
Bidens pilosa Linn.	IFE 16887	Akinnubi Olufunmilola
Chromolaena odorata (L.) Kings & Robinson	IFE 16881	Akinnubi Olufunmilola
Crassocephalum crepidioides (Benth) S. Moore	IFE 16880	Akinnubi Olufunmilola
Emilia praetermissa Milne Redhead.	IFE 16874	Akinnubi Olufunmilola
Launaea taraxacifolia Willd.	IFE 16877	Akinnubi Olufunmilola
Synedrella nodiflora Gaertn	IFE 16886	Akinnubi Olufunmilola
Tithonia diversifolia (Hemsl) A. Gray	IFE 16875	Akinnubi Olufunmilola
Tridax procumbens Linn.	IFE 16876	Akinnubi Olufunmilola
Vernonia amygdalina Del. Cent	IFE 16885	Akinnubi Olufunmilola
Vernoniacinerea Linn.	IFE 16883	Akinnubi Olufunmilola

Results

Generally, the pollen grains of species studied are spheroidal in shape, polypentaporate, acolpate, tricolporate and polycolporate. Pollen grains were either echinate or spinate, yellow to yellowish brown. Pollens are in the group media and minuta, Spines are short with thin walls and pores are densely situated. The size of pollen grains, length of spines, thickness of walls, number of pores, thickness between pores showed variations from species to species as shown in Table 1.

The qualitative result of the study of pollen grains for members of the family of Asteraceae studied is presented below;

Ageratum conyzoides Linn. (Plate 1A) : Pollens are polypantoporate, prolate-spheroidal and are small (minuta); spines are with sharp pointed ends are present while pores are densely situated.

Aspilia africana (Pers.) C.D. Adams (Plate 1B): Pollens are tricolporate, oblate-spheroidal and medium (media); spines with pointed ends while pores are densely situated.

Bidens pilosa Linn. (Plate 1C): Pollens are polypantoporate, oblate-spheroidal and medium (media);spines with blunt ends are present while pores are densely situated.

Chromolaena odorata (Linn.) King and Robinson. (Plate 1D): Pollens are polycolporate, oblate-spheroidal and are generally medium (media); spines are short with thin wall and pores are densely situated.

Crassocephalum crepidioides (Benth). S. Moore (Plate1E): Pollens are polycolporate, oblate-spheroidal and generally medium (media); pores are densely situated.

Emilia praetermissa Milne-Redhead (Plate 1F): Pollens are polycolporate, prolate-spheroidal and medium (media); pores are densely situated.

Launaea taraxacifolia Willd. (Plate 1G) : Pollens are acolpate, prolate-spheroidal and are medium (media); spines are small and short with sharp ends making the wall appear serrated; the wall is thin and pores are sparsely situated, usually between 3 in number.

Synedrella nodiflora Gaertn.(Plate 1H): Pollens are polycolporate, prolate-spheroidal and are medium (media); spines are short with pointed end with thin wall and pores are densely situated.

Tithonia diversifolia (Hemsl.) A. Gray(Plate 1I): Pollens are polypantoporate, prolate-spheroidal and are generally medium (media); pines are fairly long with pointed end with thin wall and the pores are densely situated.

Tridax procumbens Linn. (Plate 1J): Pollens are polypantoporate, prolate-spheroidal and are medium (media); spines are short with pointed ends with thin wall and pores are densely situated.

Vernonia amygdalina Del Cent. (Plate 1K): Pollens are polycolporate, prolate-spheroidal and are medium (media). Spines are short with thin wall and pores are densely situated.

Vernonia cinereaLinn. (Plate 1L): Pollens are polycolporate, prolate-spheroidal and are medium (media); spines are short with thin wall and pores are densely situated.

Table 2: Summary of the pollen grain morphological characters of species studied

Species	Exine pattern	Pollen shape	Apertural status
Ageratum conyzoides	Spinate	ProlateSpheroidal	Polypantoporate
Aspilia africana	Spinate	Oblate Spheroidal	Tricolporate
Bidens pilosa	Spinate	Oblate Spheroidal	Polypantoporate
Chromolaena odorata	Spinate	Oblate Spheroidal	Polycolporate
Crassocephalum crepidioides	Echinate	Oblate Spheroidal	Polycolporate
Emilia praetermissa	Echinate	Prolate Spheroidal	Polycolporate
Launaea taraxacifolia	Spinate	Prolate Spheroidal	Acolpate
Syndrella nodiflora	Spinate	Prolate Spheroidal	Polycolporate
Tithonia diversifolia	Spinate	Prolate Spheroidal	Polypantoporate
Tridax procumbens	Spinate	Prolate Spheroidal	Polypantoporate
Vernonia amygdalina	Echinate	Prolate Spheroidal	Polycolporate
Vernonia cinerea	Echinate	Prolate Spheroidal	Polycolporate

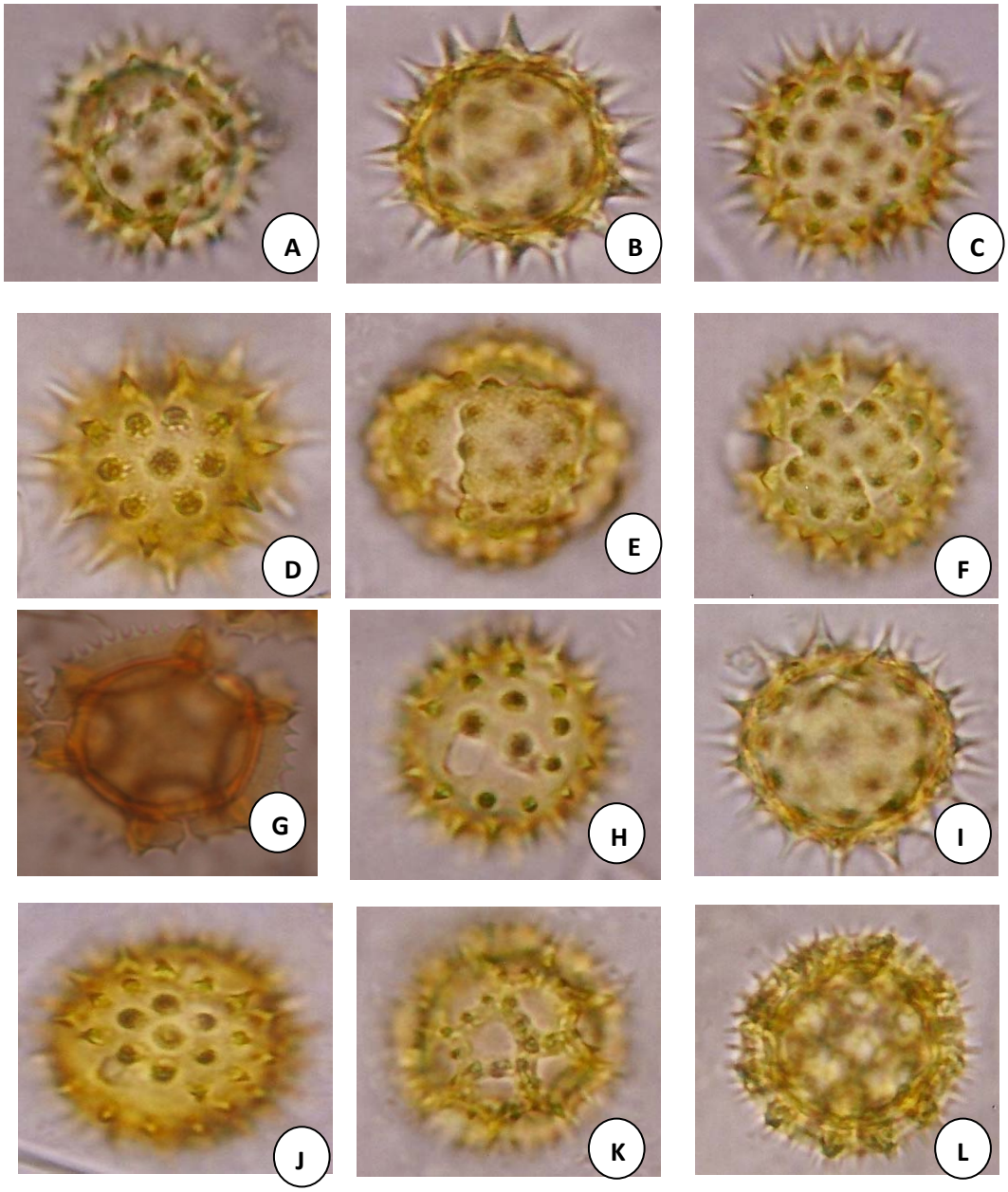

Plate 1: Pollen grains of the Asteraceae species studied. A. *Ageratum conyzoides* Linn., B. *Aspilia africana* (Pers) C.D Adams,C. *Bidens pilosa* Linn., D. *Chromoloaena odorata* (Linn.) king & Robinson, E. *Crassocephalum crepidioides* Benth S. Moore, F. *Emilia praetermissa* Milne-Redhead, G. *Launaea taraxacifolia* Willd, H. *Synedrella nodiflora* Gaertn, I.*Tithonia diversifolia* (Hemsl) A. Gray, J. *Tridax procumbens*Linn., K. *Vernonia amygdalina*Del. Cent, L. *Vernonia cinerea* Linn; Magnification X1300

Cluster Analysis

The taxa studied were subjected to Single Linkage Cluster Analysis using the pollen parameters. The result of the cluster analysis is presented in Fig 1. There are two major clusters. The first main cluster is delineated into two sub clusters; the first sub cluster comprises of four species namely; *A. conyzoides*, *E. praetermissa*, *S. nodiflora* and *B. pilosa* which were linked at similarity

coefficient level of 0.9966. *A. conyzoides* and *E. praetermissa* are closely related at similarity coefficient level of 0.9978. *S. nodiflora* and *B. pilosa* are closely related at the same similarity coefficient level. The second sub cluster comprises of four species which are; *V. cinerea*, *V. amygdalina*, *C. crepidiodes* and *C. odorata. V. cinerea* and *V. amygdalina* have the highest similarity coefficient level of 0.9998. *C. crepidioides* and *C. odorata* are linked together at coefficient level of 0.9982. In the second main cluster, *T. diversifolia* is distinctly separated from the other 3 species namely; *T. procumbens*, *A. africana* and *L. taraxacifolia.T. procumbens* and *A. africana* are at the same similarity coefficient level of 0.9990 and these two species are linked with *L. taraxacifolia* at similarity coefficient level of 0.9962.

Table3: **Measurements of diameter of pollen grains, Wall thickness, Number of pores, Thickness between pores and Length of spines**

Name of Species of pollen	Diameter of pollen grains (μm)	Pollen wall thickness (μm)	Number of pores (μm)	Thickness between pores (μm)	Length of spines (μm)
Ageratum conyzoides	21.12±0.36	2.9±2.00	11.0±2.00	2.93±0.15	2.9±2.00
Aspilia Africana	34.10±1.78	2.9±2.00	9.0±1.50	2.7±2.13	5.0±0.30
Bidens pilosa	38.27±0.70	2.9±2.00	16.0±2.00	3.93±0.18	6.9.±0.30
Chromolaena odorata	25.77±1.26	3.6±0.24	9.0±2.00	2.70±2.04	2.8±2.00
Crassocephalum crepidiodes	37.30±1.05	2.7±1.90	13.0±2.50	0.89±0.74	1.9±0.70
Emilia praetermissa	39.54±0.46	3.6±0.85	18.0±1.50	0.90±0.25	2.1±0.90
Launaea taraxacifolia	38.67±0.67	2.8±2.02	7.54±0.88	3.17±0.67	5.9±0.30
Synedrella nodiflora	33.75±0.52	2.9±2.04	15.0±1.35	3.60±0.19	4.6±0.30
Tithonia diversifolia	40.60±0.37	7.9±2.04	11.0±1.55	3.50±0.30	7.1±0.30
Tridax procumbens	40.06±0.43	3.4±0.34	9.0±1.55	2.9±2.02	4.8±0.40
Vernonia amygdalina	29.82±0.43	2.8±2.00	11.0±1.25	2.9±0.90	4.1±1.80
Vernonia cinerea	28.45±0.57	2.8±2.10	10.0±2.50	2.8±2.01	3.9±1.70

Discussion

Pollen architecture has great significance in the taxonomy of angiosperms and in revealing inter-relationship among them. (Moore and Webb, 1978). Palynology is not only useful in taxonomy but has proved to be useful in genetic and evolutionary studies, forensic science, allergy studies, tracing generational history of individual species and taxa, paleo-climatic studies and the study of the past human impact on vegetation. (Erdtman, 1943). In general appearance, the pollen grains of all species of Asteraceae studied were spheroidal, acolpate, colporate and polypantoporate with apertures. Wodehouse (1935) interpreted these apertures on the wall surface as furrows which have become so shortened that they coincided in extent with their enclosed pores. All the pollens were echinate or spinate, sticky and yellow to yellowish-brown colour. The exine pattern (Echinate) encountered in *C. crepidiodes* and *E. praetermissa* agreed to their placing in the same tribe (Senecioneae), also the exine pattern in V. amygdalina and V. cinerea (Echinate) also contribute to placing the species in the same tribe (Vernonieae). This agrees with the findings of Perveen (1999) about shape, colour and exine patterns.

Apart from the common generic features, there were also some differences in other pollen morphological features which characterized the species, In general, the pollen diameter of species in Asteraceae is small. Erdtman (1952) classified pollen grains according to size into groups: perminuta (diameter less than 10μm), minuta (diameter

10-25µm), media (diameter 25-50µm), magna (diameter 50-100µm), permagna (diameter 100-200µm), giganta (diameter greater than 200µm). According to this classification, the pollen grains of the species of Asteraceae studied belong to the groups minuta and media *A. conyzoides* is in the group minuta while the other species belong to the group media which connotes that the pollen grains diameter measured is classificatory for others and delimiting for *A. conyzoides.*

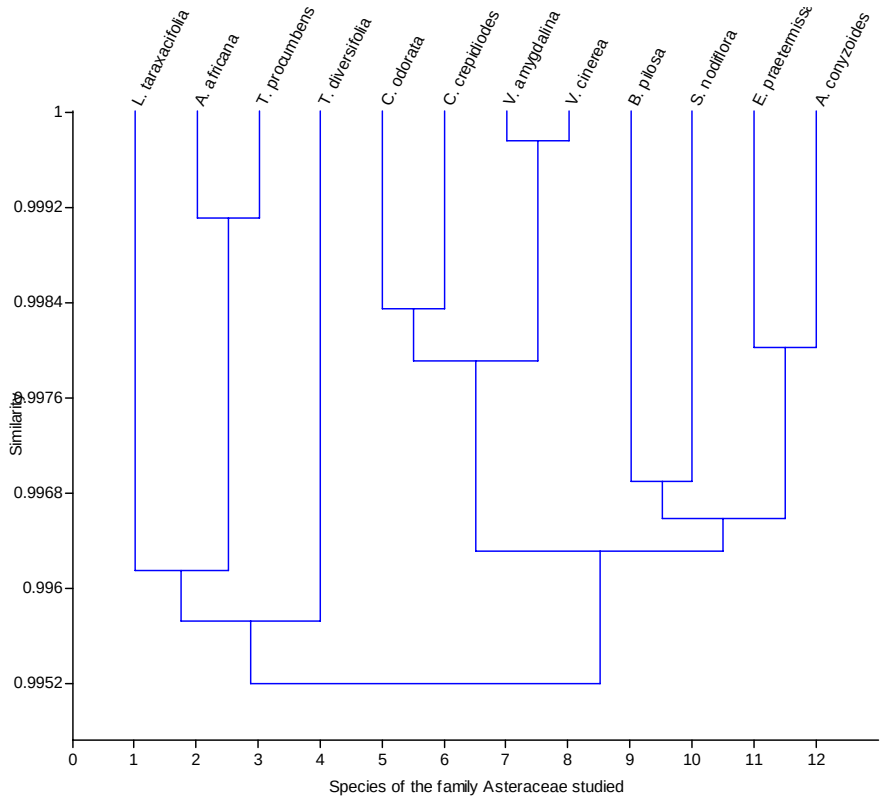

Fig. 1: Single Linkage Cluster Analysis of the Asteraceae species studied based on quantitative pollen grain parameters.

Spines were found in the pollen walls of all the species of the family studied. *S*hort spines occurred in *A. conyzoides, C. odorata, C. crepidioides, E. praetermissa, S. nodiflora, T. procumbens, V. amygdalina* and *V. cinerea* (Table 1) while long spines occurred in *A. africana, B. pilosa, L. taraxacifolia* and *T. diversifolia.* It is worthy of note that the pollen walls of all the species are thin. This has been previously reported by Perveen (1999) for the family Asteraceae. The pollen grains of *T. diversifolia* were distinct in having the longest spines and the thickest wall (Table 1) while that of *C. crepidioides* were distinct in having the shortest spines. They also had similar wall thickness with *E. praetermissa*. The pollen grain of *C. odorata* is distinct in having thick pollen wall while *E. praetermissa* have the largest number of pores but there is exception in *L. taraxacifolia* where the pores are very few in number. This separates *L. taraxacifolia* from the other species.The pattern of clustering does not agree with tribal classification except for *V. amygdalina* and *V. cinerea* that are clustered together at the highest similarity coefficient level which agrees to placing them in the same tribe (Vernonieae) (Fig. 1). Generally, the pollen pores are densely situated, therefore, thickness between pores are small except in *L.*

taraxacifolia where the pollen pores are sparsely situated leading to thickness between pores.

In the world of palynology, modern (and fossil) pollen of members of the Asteraceae are difficult to distinguish with the light microscope (LM) because they appear very similar. Most are polycolporate, have spines while a few have echinate (very small spines). However, from this study, some pollen grains have been differentiated based on some fairly distinct characters; *C. crepidiodes* have echina on the exine. The pollen of *E. praetermissa* appear somewhat similar to *C. crepidiodes* in the thickness between pores except that its grains are larger. *T. diversifolia* pollen grains are the largest, their spines have sharp edges. *A. conyzoides* pollen grains are the smallest of the grains in this group both in size and in the spines (Table 1). The pollen grains of *T. procumbens* are also similar to *T. diversifolia* in diameter of pollen grains measured (Table 1).

Number of colpi on pollen grains has been a useful tool in tracing evolutionary relationship among the species studied. The advanced dicotyledons have more colpi than the primitive ones, with either a colpus (monocolpate) or none at all (acolpate) (Walker, 1976 and Adedeji, 2005). The species studied have some common features which support their classification as members of a family and also some features which are useful tools in delimiting the different species as distinct species.

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