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Pollen Morphological Study of Some Selected Flowering Plants within Adekunle Ajasin University Main Campus, Akungba-Akoko, Ondo State, Nigeria

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ABSTRACT

On the Adekunle Ajasin University Main campus, Akungba-Akoko, Ondo State, Nigeria, the pollen morphological study of seven (7) flowering plant species representing three (3) angiospermic plant Families (Asteraceae, Euphorbiaceae, and Fabaceae sub-family Caesalpinoideae) was examined, analyzed, and studied palynologically to determine the morphological features and attributes as an aid in the identification and classification of its members within each family. Pollen grains from fresh polleniferous materials were extracted using acetolysis methods. Detailed morphological evaluations were performed at x100 magnification. Results showed similarities and differences between species in the same genera, as well as differences between species in other families. Pollen study of these species revealed several similarities between species within the same family. Aperture type, forms, pollen grain size, sculpturing pattern, pollen dimension, and exine pattern were all studied for differences and similarities. *Euphorbia hirta* L and *Euphorbia heterophylla* L, both Euphorbiaceae species, have tricolpate apertures, are prolate in shape, reticulate, and contain small to medium pollen sizes. Asteraceae has tricolporate apertures, prolate spheroidal shapes, spinate, short and long spines, and pollen sizes ranging from microscopic to enormous (*Ageratum conyzoides* L, *Tithonia diversifolia*, and *Tridax procumbens*). Pollen from the family Fabaceae subfamily Caesalpinioideae (which includes *Caesalpinia pulcherrima* (L.) Swartz. and *Senna alata* (Linn.) Roxb) were found to be subprolate, coarsely reticulate, tricolporate, and with pollen sizes ranging from medium to large. Aperture measures such as number and diameter, pollen size, and spine height were suggestive in all of the species studied. Because all of the species studied exhibited comparable form and symmetry, they were grouped together and assigned to the same family.

Keywords: Asteraceae, Caesalpinioideae, Euphorbiaceae, Pollen morphology.

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I. Introduction

Pollen grains are known to be the male microgametophytes of seed plants, which produces male gametes. They have been dispersed by insects (entomophilous) and wind (anemophilous) respectively. When pollen grains are been dispersed, they are carried from the stamens to the pistil of flowering plants or from the male cone to the female cone of coniferous plants, for pollination to takes place (Shivanna, 2005). Palynology is a Science that studies pollen grains and spore morphology and this has found its practical applications in plant systematics, medicine, criminology, biogeography, agriculture, aeropalynology and stratigraphy for correlation of soil bearing rocks strata, etc. (Ige, 2009; Essien, 2020; Ige & Essien, 2021).

Pollen analysis has been widely used to trace the history of cultivated and uncultivated plants, according to Meo and Khan (2004). The process of cross-pollination involves the transfer of haploid male genetic material from one flower's anther to the stigma of another (Shivanna & Tandon, 2020). Occurrence of self-pollination on the other hand happens when the anther of the same flower pollinates its stigma.

Analysis of fossil pollen grains is the most important approach to reconstruction of past flora, vegetation and environment (Sowunmi, 1987; Sowunmi, 1981a; 1981b; Traverse, 1988; Faegri & Iversen, 1989; Ige & Essien, 2019). Pollen identification: the basis of palynology, is based exclusively on pollen morphology (Shubharani *et al*., 2013; Paul *et al*., 2014; Essien & Ige, 2019). Understanding the functional features of pollen, such as pollination biology and pollen-pistil interaction, requires knowledge of pollen morphology (Shivanna & Johri, 1989; Shivanna, 2005). Pollen morphology is particularly significant in taxonomy, phylogeny, palaeobotany, aeropalynology, and pollen allergy (Sowunmi, 1987; Essien, 2019). Pollen grain from various plants can serve as a source of nectar and apiary development success is tied to pollen meal for honeybees (Kalpana & Ramanujam, 1997).

In this study, samples of fresh polleniferous materials were gotten from three (3) different angiospermic plant families

which include those of the Families Asteraceae, Euphorbiaceae and Fabaceae sub-family Caesalpinoideae.

Within the order Asterales, there are around 32,000 recognized species of flowering plants divided into over 1900 genera (Barreda *et al*., 2015). The composite or sunflower family is a frequent name for this family. Asteraceae includes shrubs, vines, and trees, as well as annual, biennial, and perennial herbaceous plants (The Plant List, 2016). The family covers a wide range of environments, ranging from sub-polar to tropical (MBG, 2013). The presence of hundreds of small individual florets bound together by protective involucres in flower heads, or more properly, capitulla, is the most prevalent feature of this family. The Asteraceae family is important economically since it produces basic foods, garden plants, and herbal medicines (Judd *et al*., 2007). *Ageratum conyzoides*, *Tridax procumbens*, and *Tithonia diversifolia* are the plants researched in this family.

Euphorbia is a flowering plant genus with a wide range of species. Euphorbia is a genus in the Euphorbiaceae family, also known as the spurge family. Euphorbias can range in size from tiny annuals to enormous, long-lived trees. It is one of the largest flowering plant genera, with over 2,000 species (Stebbins & Hoogland, 1976). The plants all have a deadly, milky, white latex-like sap as well as distinctive and distinct floral structures. The genus may be defined by the shape and structure of its flower heads, or by the qualities of its members' gene sequences. The head of its flower seems to be a single blossom (a pseudanthium) when viewed in its entirety (PBI, 2019). *Euphorbia hirta* and *Euphorbia heterophylla* are the species researched in this family.

The Fabaceae family of flowering plants is a big and commercially important one. Trees, shrubs, and perennial or annual herbaceous plants with easily identifiable fruit (legume) and compound, stipulate leaves are included (Stevens, 2006). With over 765 genera and nearly 20,000 recognized species, the family is the third biggest land plant family in terms of number of species, after Orchidaceae and Asteraceae (Watson & Dallwitz, 2007). The Fabaceae family includes anything from enormous trees (like *Koompassia excelsa*) to little annual herbs, with the bulk of them being herbaceous perennials. Inflorescences of this family are ambiguous and sometimes reduced to a single bloom. Following fertilization, the blooms produce legume-like fruits with a short hypanthium and a solitary carpel with a short gynophore (Watson & Dallwitz, 2007). *Ceasalpinia pulcherrima* and *Delonix regia*, both belonging to the Caesalpinioideae subfamily, were the plants researched.

II. Materials and Methods

Ageratum conyzoides, Euphorbia hirta, Euphorbia heterophylla, Tridax procumbens, Tithonia diversifolia, Caesalpinia pucherrima, and *Senna alata* anthers were gathered from fresh flowers on the Adekunle Ajasin University Main campus in Akungba-Akoko. At the Adekunle Ajasin University Herbarium of the Department of Plant Science and Biotechnology, the collections were identified and authenticated. Acetic anhydride, ethanol, glacial acetic acid, sulphuric acid, microscope, oil immersion, cover slips, and glass slides were all used. With a piercing needle and a pair of forceps, anthers from both flower buds and fully opened flowers were carefully removed and crushed in a beaker containing ethanol. Before being decanted, these were sieved and centrifuged for 5 minutes at 2,000 revolutions per minute (RPM). The pollen sediments were treated with glacial acetic acid to eliminate water before acetolysis. The acetolysis mixture was made from acetic anhydride and concentrated sulphuric acid (H_2SO_4) in a 9:1 ratio. The sediments were boiled in a water bath according to Erdtman's (1969) procedures, which were adapted by Agwu and Akanbi (1985), Paul *et al*. (2014), and Essien and Ige (2019). The mixture was placed in a water bath at 100 degrees Celsius for 5 minutes, agitated, centrifuged for 5 minutes, and the supernatant decanted. After being rinsed with glacial acetic acid, the precipitates were washed twice with distilled water, centrifuged, and decanted. The recovered precipitates were kept in glycerin and ethanol solution in plastic vials (2:1). On a microscope slide, one drop of properly agitated precipitates suspension was mounted and covered with an 18×18mm cover slip. To keep the precipitates from drying out, the mount was coated with colorless nail varnish (cortex). Microscopically, the prepared slide was examined with an Olympus microscope at x40 magnification for counting and a Leica microscope at x100 magnification for comprehensive morphological examinations. With the use of reference descriptions and photomicrographs, pollen identification, morphological investigation, and analysis were completed.

III. Results and Discussions

Two plant species each in the Family Euphorbiaceae and Fabaceae sub-family Caesalpinoideae namely: *Euphorbia hirta, Euphorbia heterophylla*, *Caesalpinia pulcherrima and Senna alata* and three plant species in the Family Asteraceae: *Ageratum conyzoides Tridax procumbens* and *Tithonia diversifolia* respectively were studied and analysed palynologically. For each pollen type studied, the morphological features that were investigated include but not limited to aperture type, exine pattern, exine thickness, exine sculpturing, polarity, symmetry and shape, and pollen size. An average of ten (10) pollen grains was utilized to obtain the dimensions and other pollen morphological features, which aided in the easy description and identification of the pollen investigated.

A. Description of Pollen Morphological Features

Euphorbia hirta

Aperture:	Tricolpate (aperture with three colpi), colpi is clearly distinct.
Exine pattern:	Sexine finely reticulated, nexine thinner than sexine.
Exine thickness:	Medium
Sculpturing:	Fine reticulation
Polarity:	Isopolar
Symmetry:	Radially symmetrical
Pollen shape:	Subprolate to prolate
Pollen size:	Small size
Dimension:	P= 19.05 µm (16.7-21.4) µm E= 16.55 µm (13.6-19.5) µm P/E = 1.15 Longest axis: 21.4 µm

Euphorbia heterophylla

Aperture: Tricolpate (aperture with three colpi), colpi not clearly distinct.
Exine pattern: Sexine is thick; Gammate
Exine thickness: Medium
Sculpturing: Coarsely reticulated
Polarity: Isopolar
Symmetry: Radially symmetrical
Pollen shape: Prolate spheroidal
Pollen size: Medium size
Dimension: P= 25.35 µm (22.6-28.1) µm
E= 25.1 µm (20.5-29.7) µm
P/E = 1.01
Longest axis: 29.7 µm

Ageratum conyzoides

Aperture: Tricolporate; 3 aperture with colpi and pore, pores are densely situated
Exine pattern: Spinate
Exine thickness: Pollen wall is thin
Sculpturing: Spinules, Echinate; having very short/small spines, thin walls.
Polarity: Isopolar
Symmetry: Radially symmetrical
Pollen shape: Prolate-spheroidal, circular.
Pollen size: Small size
Dimension: P= 20.05 µm (17.8-22.3) µm
E= 18.35 µm (21.7-15.0) µm
P/E = 1.11
Longest axis: 22.3 µm

Tridax procumbens

Aperture: Tricolporate; 3 aperture with colpi and pore, pores are densely situated
Exine pattern: Spinate
Exine thickness: Pollen wall is thin
Sculpturing: Spinate; having spines, thin walls.
Polarity: Isopolar
Symmetry: Radially symmetrical
Pollen shape: Prolate-spheroidal
Pollen size: Similar to *Tithonia diversifolia* in pollen size
Dimension: P= 38.2 µm (30.7-45.7) µm
E= 35.7 µm (29.6-41.8) µm
P/E = 1.07
Longest axis: 45.7 µm

Tithonia diversifolia

Aperture: Polycolporate; apertures more than 6 colpi and pores
Exine pattern: Spinate
Exine thickness: Pollen wall is thick
Sculpturing: Spinate (Having longer and sharper spines), thick walls.
Polarity: Isopolar
Symmetry: Radially symmetrical
Pollen shape: Prolate-spheroidal
Pollen size: Large in size
Dimension: P= 40.95 µm (31.2-50.7) µm
E= 37.35 µm (31.7-43.0) µm
P/E = 1.09
Longest axis: 50.7 µm

Caesalpinia pulcherrima

Aperture: Tricolporate; 3 apertures with colpi and pore
Exine pattern: Sexine thicker than nexine
Exine thickness: Pollen wall is thick
Sculpturing: Coarsely reticulate
Polarity: Isopolar
Symmetry: Radially symmetrical
Pollen shape: Prolate-spheroidal
Pollen size: Large in size
Dimension: P= 45.6 µm (38.7-52.5) µm
E= 40.55 µm (34.3-46.8) µm
P/E = 1.12
Longest axis: 52.5 µm

Senna alata

Aperture: Tricolporate; 3 aperture with colpi and pore
Exine pattern: Nexine is thinner, granulate
Exine thickness: Pollen wall is thick
Sculpturing: Coarse scabrate
Polarity: Isopolar
Symmetry: Radially symmetrical
Pollen shape: Sub-prolate
Pollen size: Medium in size
Dimension: P= 32.25 µm (28.3-36.4) µm
E= 30.55 µm (26.6-34.5) µm
P/E = 1.05
Longest axis: 36.4 µm

Pollen morphology has great significance in the taxonomy of angiosperms and in revealing inter- relationship among them (Moore & Webb, 1978). The species studied have some features which support their classification as members of a family. Some of these features are useful tools in delimiting the different species as distinct species. In this study, variations within species of the same genera and/or family were reduced. These variations were observed in their aperture type, exine pattern, shapes, sizes, sculpturing pattern and their polar and equatorial dimensions (Table I). Members of the same genera and/or family had most of their morphological features in common. For example, in the Family Euphorbiaceae, *Euphorbia hirta* and *Euphorbia heterophylla* were reported to have reticulate sculpturing pattern and tricolpate aperture respectively. Variation between *Euphorbia hirta* and *Euphorbia heterophylla* in terms of pollen sizes is not much prominent as it ranges from small size in *Euphorbia hirta* to medium size in *Euphorbia heterophylla*. The difference could have been much more pronounced had it been one is small and the other large.

Pollen grains from the Asteraceae family are spheroidal and have colporate pores. These openings on the wall surface were interpreted by Wodehouse (1935) as furrows that had shortened to the point where they matched the size of the enclosed pores. The pollen of *Ageratum conyzoides*, *Tridax procumbens* and *Tithonia diversifolia* were reported to be spinate; that is, they possess spines. In terms of pollen size, *Tridax procumbens* is similar to *Tithonia diversifolia* whereas *Ageratum conyzoides* are small in size and also echinate (having small spines). The pollen walls of *Ageratum conyzoides* and *Tridax procumbens* are thin but that of *Tithonia diversifolia* is thicker than the other species within

TABLE I: SUMMARY OF POLLEN MORPHOLOGICAL CHARACTERISTICS OF THE SPECIES STUDIED

Pollen Morphological features	*Euphorbia hirta*	*Euphorbia heterophylla*	*Ageratum conyzoides*	*Tridax procumbens*	*Tithonia diversifolia*	*Caesalpinia pulcherrima*	*Senna alata*
Polar distance	19.05	25.35	20.05	38.2	40.95	45.6	32.25
Mean and Range (μm)	(16.7- 21.4)	(22.6- 28.1)	(17.8- 22.3)	(30.7-45.7)	(31.2- 50.7)	(38.7- 52.5)	(28.3- 36.4)
Equatorial distance	16.35	25.1	18.35	35.7	37.35	40.55	30.25
Mean and Range (μm)	(13.6- 19.5)	(20.5- 29.7)	(21.7- 15.0)	(29.6- 41.8)	(31.7- 43.0)	(34.3- 46.8)	(26.6- 34.5)
P/E	1.15	1.01	1.11	1.07	1.09	1.12	1.05
Pollen shape	Sub-prolate to prolate	Prolate spheroidal	Prolate spheroidal circular	Prolate spheroidal	Prolate spheroidal	Prolate spheroidal	Sub-prolate
Aperture	Tricolpate	Tricolpate	Tricolporate	Tricolporate	Polycolporate	Tricolporate	Tricolporate
Exine pattern	Nexine thinner than sexine	Rather thick sexine pattern, Gammate.	Spinate	Spinate	Spinate	Sexine thicker than nexine	Granulate, nexine is thinner
Sculpturing	Fine reticulation	Coarsely reticulation	Echinate/spinules	Long spines	Longer spines	Coarsely reticulate	Coarsely scabrate
Pollen size	Small	Medium	Small	Large	Large	Large	Medium

the same Family. In the family Fabaceae sub-family Caesalpinioideae, the pollen grains of the species studied were all reported to be generally radially symmetrical, isopolar and tricolporate. The tectum is coarse, either coarsely reticulate or coarsely scabrate. *Caesalpinia pulcherrima* had coarsely reticulate tectum with distinct colpi while *Senna alata* is coarsely scabrate. The shape is prolate-spheroidal, prolate or sub-prolate. The size of the pollen of *Caesalpinia pulcherrima* is large; the polar and equatorial dimension ranges from 45.6 μm (38.7-52.5 μm) polar dimension to 40.55 μm (34.3-46.8 μm) equatorial dimension respectively while that of *Senna alata* is medium in size ranging from 32.25 μm (28.3-36.4 μm) polar dimension to 30.55 μm (26.6-34.5 μm) equatorial dimension (Table I).

Generally, comparing the pollen morphology of all the species studied from each Family, their distinct morphological characters create the difference amidst taxon. The aperture type is normally consistent within a family. The Family Euphorbiaceae is majorly tricolpate, reticulate, prolate and mostly not enlarged in size. The Family Asteraceae is completely distinct with their colporate aperture type, consisting of spines; prolate-spheroidal in shape and have all ranges of size whereas the family Fabaceae sub-family Caesalpinioideae are mostly tricolporate in aperture type, coarse reticulation in terms of exine pattern and unlike species from other families, their pollen grains were not small in size.

IV. CONCLUSION

The use of these quantitative and qualitative features to analyze pollen morphology of some of these selected plant species from the Family Euphorbiaceae, Asteraceae, Fabaceae sub-family Caesalpinoideae provides justification for the existing classification of the individual plant into each plant family. This research discovered that there are some commonalities among species within the same family, as well as distinct variations among species within other families. Aperture types and numbers, exine pattern, pollen form, sculpturing pattern, size, polar and equatorial dimensions, and other morphological features are used to express these differences and similarities. The findings of this study are expected to aid in the detection, identification and classification of its members within each family. Because of the similarities and differences in pollen morphology among the species studied, pollen features could be beneficial in determining affinity and taxon categorization. This research is expected to be crucial for identifying these floras and enhancing the conservation status of these economically significant plants, as well as serving as a reference tool for grouping plant species into their respective families.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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