



ISSN NO. 2320-5407

Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/14836
DOI URL: <http://dx.doi.org/10.21474/IJAR01/14836>



INTERNATIONAL JOURNAL OF
ADVANCED RESEARCH (IJAR)
ISSN 2320-5407
Journal Homepage: <http://www.journalijar.com>
Journal DOI:10.21474/IJAR01

RESEARCH ARTICLE

PALYNOLOGY OF SOME SPECIES IN THE FAMILY CUCURBITACEAE FROM NIGERIA

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Manuscript Info

Manuscript History

Received: 31 March 2022

Final Accepted: 30 April 2022

Published: May 2022

Key words:-

Cucurbitaceae, Palynology, Aperture, Taxonomic, Magna

Abstract

Pollen morphology of ten (10) species representing nine (9) genera, two (2) varieties in the family Cucurbitaceae from Nigeria has been examined under light microscope. The pollen grains are largely monads. Exine wall ornamentation is most often reticulate in all the species of the family studied with the exception of pollens in *Cucurbita maxima* Duch. which are echinate, this is a diagnostic character for this species. Cell wall thickness is the least variable among all the characters studied. Almost all types of pollen shape classes have been observed within this family, namely spheroidal, subprolate, oblate-spheroidal, prolate-spheroidal, suboblate and prolate, with more than one shape type occurring in a species. It is worthy of note that pollen shape and aperture type can be used to delimit *Lagenaria siceraria* (spatulate fruit shape) from *Lagenaria siceraria* (oval fruit shape). However, the shapes are the same in *Citrullus lanatus* (black seeds water melon) and *Citrullus lanatus* (brown seeds water melon). Three (3) types of apertures, porate, colpate (very occasionally) and colpate are documented for the family in this study, while seven (7) pollen types have been distinguished based on the apertures: monoporate, monocolpate, bicolporate, triporate, tricolporate, tetraporate and tetracolporate. Tricolporate pollens were observed to occur more frequently than other apertural types. Pollen grains belong to the size categories Media and Magna and only very occasionally in the Minuta category. The implication of these size categories in the reproductive success of the plants in this family is highlighted.

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Introduction:-

The Cucurbitaceae family is also known as the cucurbit or gourd family. It is a fairly large plant family, consisting of about 125 genera and 965 species of mainly climbing plants (Ingle *et al.*, 2022). They have common features which are large leaves, creeping or climbing stems usually with simple or branched tendrils, fleshy fruits called pepo, with leathery exocarp, containing numerous seeds and a woody root stock.

Cucurbit species are among the economically most important vegetable crops worldwide and are grown in both temperate and tropical regions (Pitrat *et al.*, 1999; Paris, 2001 and Fapounda *et al.*, 2018). They are an important source of vegetables, fruits, edible seeds, seed oil, domestic utensils, medicines, water, animal fodder and fuel. According to Mukherjee *et al.* (2022), medicinal plants of the family Cucurbitaceae family consist of several edible fruits and vegetables consumed worldwide since ancient times and are also well known for their essential role in the

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ethnopharmacological as well as traditional medicinal systems globally. Ingle *et al.* (2022) also provided an insight into the chemical nature and medicinal potential of the chemical compounds found in the family Cucurbitaceae, most especially cucurbitacins which are structurally diverse triterpenes. The family is represented in Nigeria by 21 genera, many of which are of considerable economic importance.

Pruesapan and Van Der Ham (2005) examined the pollen morphology of the genus *Trichosanthes* of the family Cucurbitaceae. Moore and Webb (1978) examined pollen morphology of few species of the family Cucurbitaceae, Perveen and Qaiser (2008) examined the pollen flora of Pakistan while Akhtar *et al.* (2019) reported the palynological characteristics of some selected species of family Cucurbitaceae in Peshawar. There is an acute paucity of report on pollen morphology from Nigeria, hence this study.

Materials And Methods:-

Collection of Pollen Grains

A preliminary study of the species of family Cucurbitaceae was carried out in Obafemi Awolowo University, Ile-Ife, Osun State Nigeria, herbarium (IFE) and Forestry Research Institute of Nigeria Herbarium (FRIN) in Ibadan, Oyo State, Nigeria. Seeds of the cultivated plants were collected from the National Center for Genetic Resources and Biotechnology (NACGRAB) Moor Plantation Ibadan, Oyo State and Teaching and Research Farm, Obafemi Awolowo University (OAU) Ile – Ife, while seeds of the non-cultivated taxa were collected from the wild. They were planted until mature for pollen harvest. The ten (10) species with two (2) varieties in the family Cucurbitaceae studied are distributed within nine (9) genera, and are as listed below:

***Lagenaria siceraria* (Molina) Standl. (Oval Fruit Shape)**

***Lagenaria siceraria* (Molina) Standl. (Spatulate Fruit Shape)**

***Luffa aegyptiaca* Mill.**

***Telfairia occidentalis* Hook. f. (Staminate)**

***Cucumis melo* Linn.**

***Cucumis sativus* Linn.**

***Citrullus lanatus* (Thunb.) Matsum. & Nakai (Black seeds water melon)**

***Citrullus lanatus* (Thunb.) Matsum. & Nakai (Brown seeds water melon)**

***Cucurbita maxima* Duch.**

***Trichosanthes cucumerina* Linn.**

***Cucumeropsis mannii* Naudin. (Synonym *Cucumeropsis edulis* (Hook. f.) Cogn.)**

***Momordica charantia* Linn.**

Acetolysis

The pollen grains were acetolysed according to the standard methods of Erdtman (1952, 1960), with slight modification by Adedeji (2010) and mounted in unstained glycerin jelly.

Microscopy

Acetolysed pollen grains were examined under the light microscope. Quantitative data documented included diameter of pollen, polar length of pollen, exine wall thickness and ratio of length of polar axis to the equatorial diameter (P/E). Pollen shape, pollen aperture type and pollen size category were also documented. Quantitative data were taken from at least 50 pollen grains for each taxon with the aid of an ocular micrometer inserted in the eyepiece of the microscope. The measurements were later multiplied by the ocular constant with respect to the power under which they were taken. Photomicrographs of the acetolysed pollen grains were taken.

Data Analysis

The means of the quantitative data generated were calculated. Statistical analyses include Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) for significant differences among the taxa studied.

The terminology used is in accordance with Erdtman (1952, 1960), Kremp (1965), Faegri and Iversen (1964) and Walker and Doyle (1975).

Results:-

(Figures 1-2; Tables 1-2)

Lagenaria siceraria (Molina) Standl. (Oval Fruit Shape) Pollen grains are largely monads; Occasionally inaperturate, bicolporate and tricolporate when aperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grain ranges from 35.00 – 37.50 μm ; Pollen grain size category is media with an exine wall thickness of 2.5 μm . Shape of pollen grains ranges from Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate.

Lagenaria siceraria (Molina) Standl. (Spatulate Fruit Shape) Pollen grains are largely monads; Occasionally inaperturate, monoporate, bicolporate and tricolporate when aperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grains ranges from 27.50 – 37.50 μm ; Pollen grain size category is media with an exine wall thickness of 2.5 μm . Shape of pollen grains ranges from Oblate-spheroidal to Prolate-spheroidal to Subprolate.

Luffa aegyptiaca Mill. Pollen grains are monads: Inaperturate occasionally, monoporate and tricolporate when aperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grain ranges from 25.00 – 55.00 μm ; Pollen grain size category is media to magna with an exine wall thickness of 2.5 – 4.0 μm . Shape of pollen grains ranges from Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate.

Telfairia occidentalis Hook. f. (Staminate) Pollen grains are largely monads; Occasionally inaperturate and tricolporate when aperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grain ranges from 25.00 – 37.50 μm ; Pollen grain size category is media with an exine wall thickness of 2.5 μm . Shape of pollen grains ranges from Suboblate to Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate and Prolate.

Cucumis melo Linn. Pollen grains are largely monads; Occasionally inaperturate, largely monoporate, monocolpate (occasionally), triporate, bicolporate and tricolporate when aperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grain ranges from 30.00 – 37.50 μm ; Pollen grain size category is media with an exine wall thickness of 2.5 μm . The shape of pollen grains ranges from Suboblate to Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Prolate to Subprolate.

Cucumis sativus Linn. Pollen grains are monads; Monoporate and tricolporate when aperturate, though occasionally inaperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grain ranges from 32.50 – 37.50 μm ; Pollen grain size category is media with an exine wall thickness of 2.5 μm . The shape of pollen grains ranges from Spheroidal to Prolate-spheroidal to Subprolate and Prolate.

Citrullus lanatus (Thunb.) Matsum. & Nakai (Black seeds water melon) Pollen grains are largely monads; Occasionally inaperturate, monoporate and triporate when aperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grain ranges from 25.00 – 37.50 μm ; Pollen grain size category is minuta to media with an exine wall thickness of 2.5 μm ; Shape of pollen grains ranges from Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate.

Citrullus lanatus (Thunb.) Matsum. & Nakai (Brown seeds water melon) Pollen grains are monads; Monoporate, bicolporate and tricolporate when aperturate; Exine wall sculpturing is reticulate, equatorial diameter of pollen grain ranges from 25.00 – 37.50 μm ; Pollen grain size category is media with an exine wall thickness of 2.5 μm . The shape of pollen grains ranges from Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate.

Cucurbita maxima Duch. Pollen grains are largely monads, tetraporate when aperturate; Exine wall sculpturing is echinate or spinous, most often than not, the spines cover the pores (Fig. 2M); Number of spines ranges from 145 to 176; Length of spines ranges from 3.6 – 5.0 μm while distance between spines ranges from 6.20 – 9.10 μm ; Equatorial diameter of pollen grain ranges from 50.20 – 85.00 μm ; Pollen grain size category is media to magna with an exine wall thickness of 2.5 μm . Shape of pollen grains ranges from Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate and Prolate.

Trichosanthes cucumerina Linn. Pollen grains are monads, occasionally inaperturate, often tricolporate and tetracolporate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grains ranges from 32.50 – 45.00

μm ; Pollen grain size category is media with an exine wall thickness of $2.5 \mu\text{m}$. Shape of pollen grains ranges from Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate and Prolate.

***Cucumeropsis mannii* Naudin.** (Synonym *Cucumeropsis edulis* (Hook. f.) Cogn.) Pollen grains are largely monads; Monoporate, tricolporate and tetracolporate when aperturate, occasionally inaperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollens ranges from $22.50 - 37.50 \mu\text{m}$; Pollen grain size category is minuta to media with an exine wall thickness of $2.5 \mu\text{m}$. Shape of pollen grains ranges from Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate.

***Momordica charantia* Linn.** Pollen grains are monads; largely faintly tricolporate when aperturate; Exine wall sculpturing is reticulate; Equatorial diameter of pollen grain ranges from $45.00 - 57.50 \mu\text{m}$; Pollen grain size category is media to magna with an exine wall thickness of $2.5 \mu\text{m}$. Shape of pollen grains ranges from Suboblate to Oblate-spheroidal to Spheroidal to Prolate-spheroidal to Subprolate.

Figure 1:- Shapes of Pollens in the Species of Family Cucurbitaceae Studied: A – C: Spheroidal shape; D – F: Subprolate shape; G – I: Oblate-spheroidal shape. Magnification @ X400.

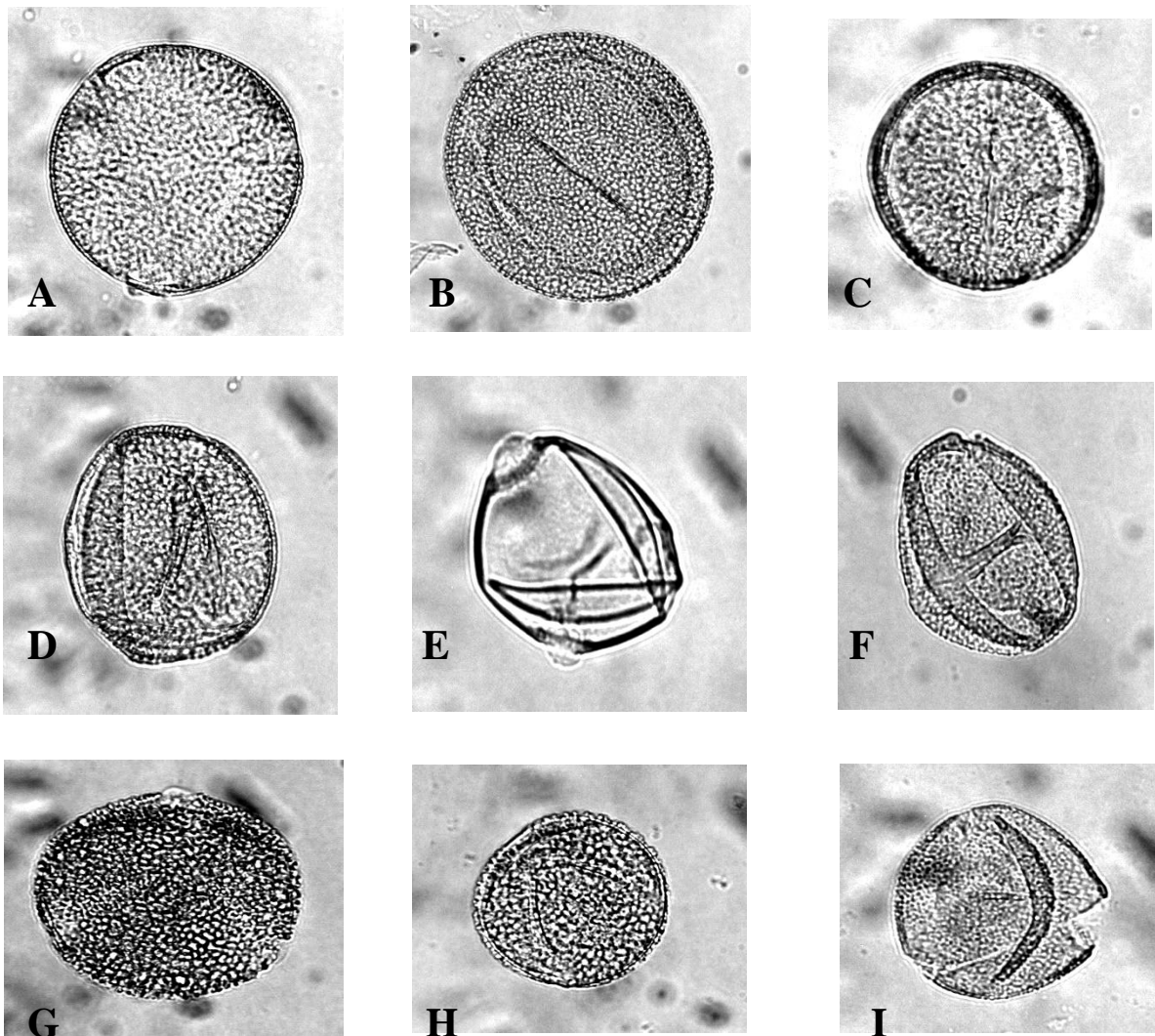


Figure 1 Continued: J – L: Prolate-spheroidal shape; M – O: Suboblate shape; P: Prolate Shape. Magnification @ X400

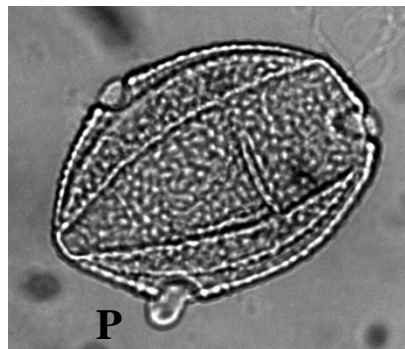
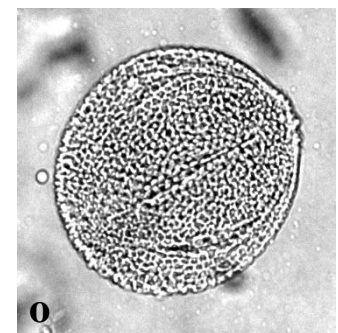
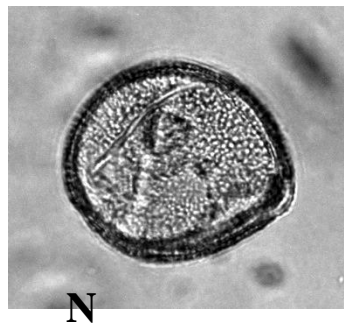
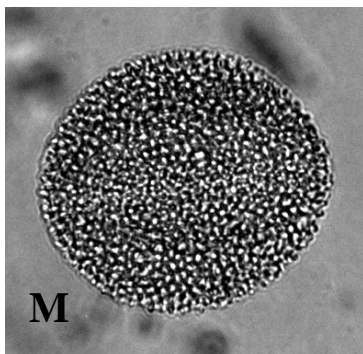
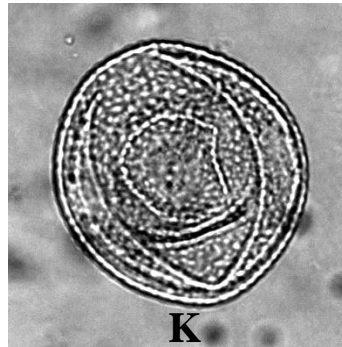


Figure 2:- Pollen Apertural Types in the Species of Family Cucurbitaceae Studied: A: Inaperturate; B-C: Monoporate; D: Monocolpate; E: Bicolpate; F – G: Triporate; H: Tricolpate; Arrows indicate positions of apertures. Magnification @ X400.

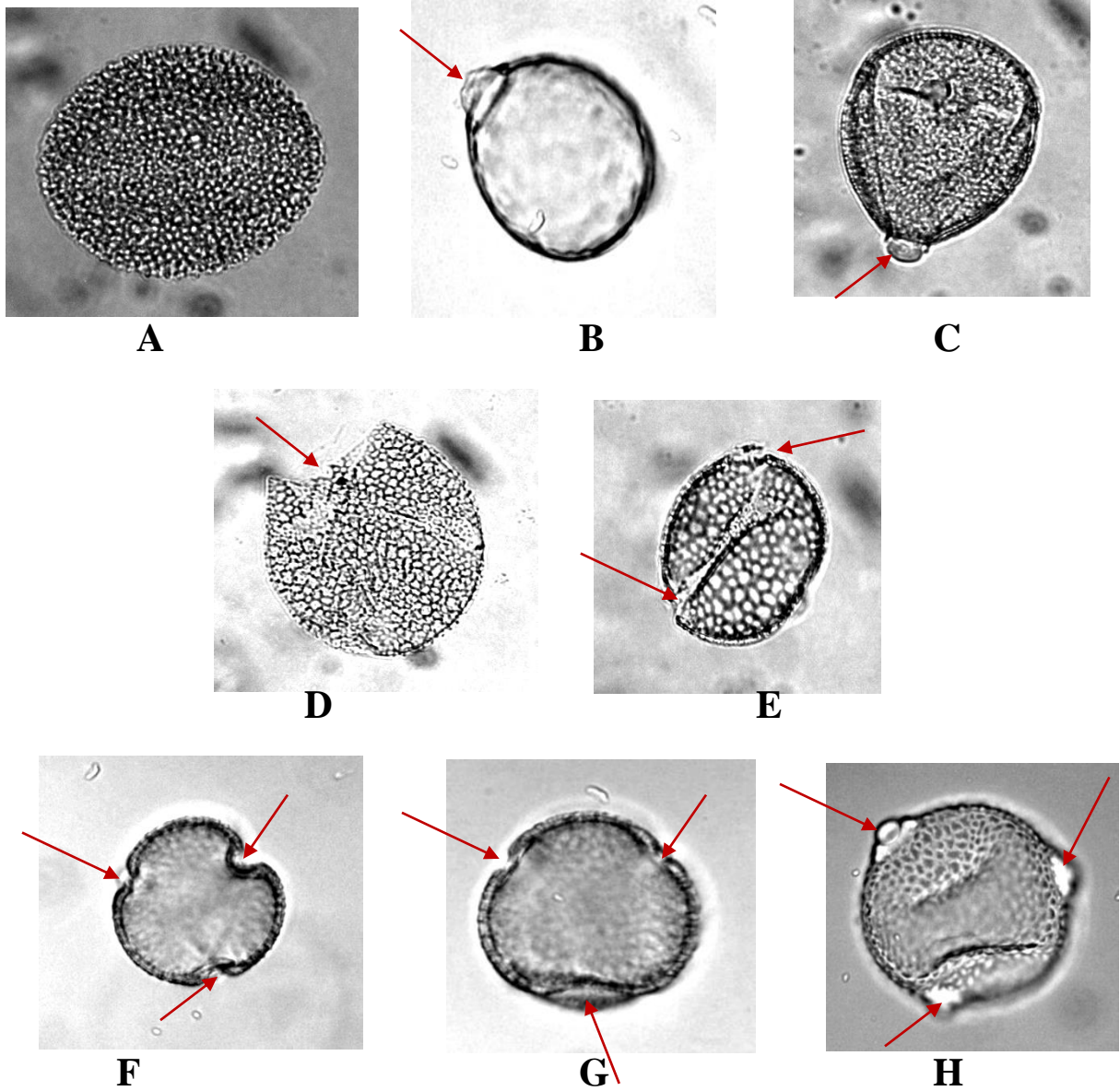


Figure 2 Continued: I – K (J with an open colpus): Tricolporate; L: Tetracolporate; M: Tetraporate (spines covering the pores). Arrows indicate positions of apertures. Magnification @ X400.

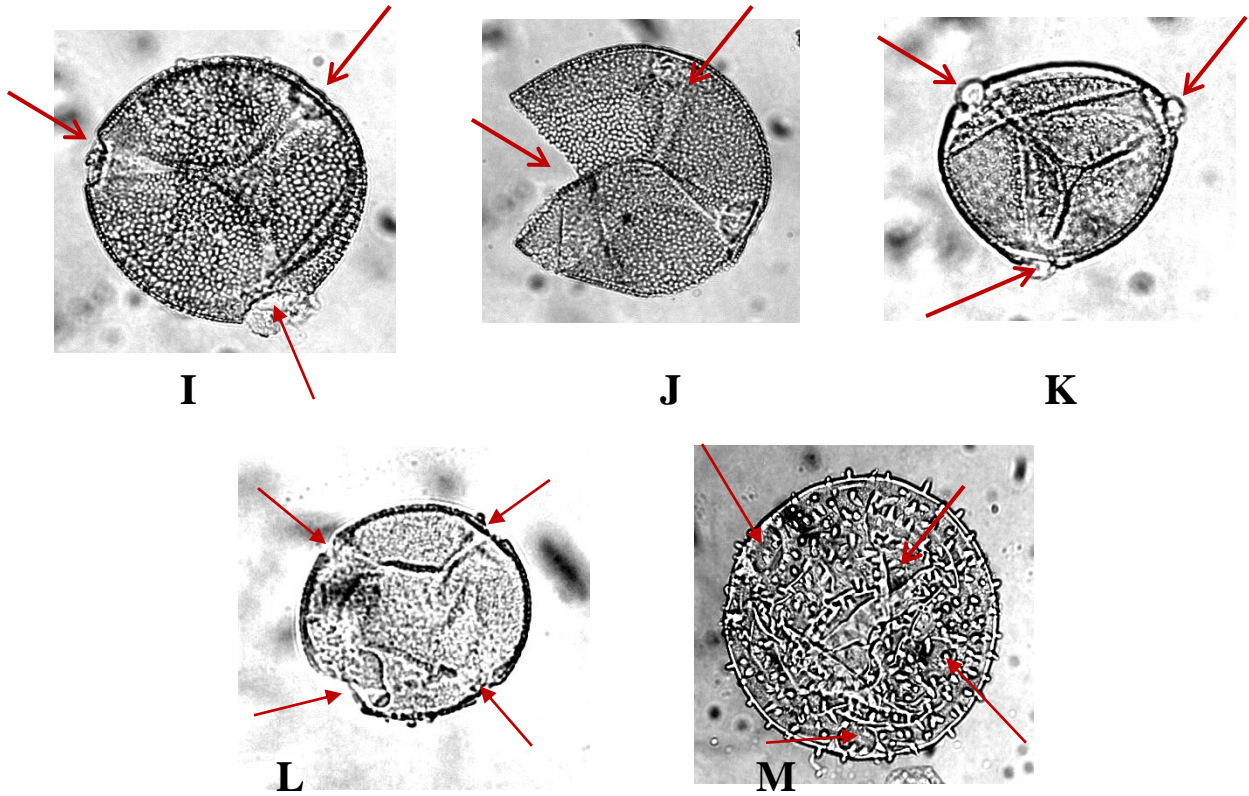


Table 1:- Summary of the Qualitative Characters of Pollen Grains in the Species of the Family Cucurbitaceae Studied.

Species	Shape	Aperture	Size Class
<i>Lagenaria siceraria</i> (Oval Fruit Shape)	Oblate-spheroidal, Spheroidal, Prolate-spheroidal, Subprolate	Bicolporate, Tricolporate	Media
<i>Lagenaria siceraria</i> (Spatulate Fruit Shape)	Oblate-spheroidal, Prolate- spheroidal, Subprolate	Monoporate, Bicolporate, Tricolporate	Media
<i>Luffa cylindrica</i>	Spheroidal, Oblate-spheroidal, Prolate-spheroidal, Subprolate	Monoporate, Tricolporate	Media to Magna
<i>Telfairia occidentalis</i>	Spheroidal, Prolate-spheroidal, Suboblate, Oblate-spheroidal, Subprolate, Prolate	Tricolporate	Media
<i>Cucumis melo</i>	Suboblate, Spheroidal, Oblate- spheroidal, Prolate-spheroidal, Prolate, Subprolate	Monoporate, Monocolpate, Triporate, Bicolporate, Tricolporate.	Media
<i>Cucumis sativus</i>	Subprolate, Prolate, Spheroidal to Prolate-spheroidal.	Monoporate, Tricolporate	Media
<i>Citrullus lanatus</i> (Black seeds water melon)	Prolate-spheroidal, Subprolate, Oblate-spheroidal, Spheroidal	Monoporate, Triporate	Minuta to Media
<i>Citrullus lanatus</i> (Brown seeds water melon)	Prolate-spheroidal to Subprolate, Spheroidal, Oblate-spheroidal	Monoporate, Bicolporate, Tricolporate	Media
<i>Cucurbita maxima</i>	Prolate-spheroidal to Subprolate, Oblate-spheroidal, Spheroidal, Prolate	Tetraporate	Media to Magna
<i>Trichosanthes cucumerina</i>	Prolate-spheroidal, Subprolate, Spheroidal, Prolate, Oblate- spheroidal	Tricolporate, Tetracolporate	Media
<i>Cucumeropsis mannii</i>	Subprolate, Oblate-spheroidal, Spheroidal, Prolate-spheroidal	Monoporate, Tricolporate, Tetracolporate	Minuta to Media
<i>Momordica charantia</i>	Oblate-spheroidal, Spheroidal, Suboblate, Prolate-spheroidal, Subprolate	Tricolporate	Media to Magna

Table 2:- Summary of Quantitative Pollen Characters in the Species of the Family Cucurbitaceae Studied, with Duncan Multiple Range Test Values (Means with the Same Alphabet along the Same Column are not Significantly Different).

Species	Polar Length (μm)	Equatorial Diameter (μm)	Exine Wall Thickness (μm)	P/E	NS	LS (μm)	DS (μm)
<i>Lagenaria siceraria</i> (Oval Fruit Shape)	34.60 \pm 0.55 ^{ef}	34.20 \pm 0.61 ^{ef}	2.50 \pm 0.00 ^b	1.02 \pm 0.02 ^c	-	-	-
<i>Lagenaria siceraria</i> (Spatulate Fruit Shape)	36.10 \pm 0.38 ^f	35.80 \pm 0.45 ^{fg}	2.50 \pm 0.00 ^b	1.01 \pm 0.02 ^c	-	-	-
<i>Luffa cylindrica</i>	50.50 \pm 1.18 ^h	45.10 \pm 1.45 ⁱ	2.70 \pm 0.14 ^b	1.14 \pm 0.04 ^{de}	-	-	-
<i>Telfairia occidentalis</i> (Staminate)	30.60 \pm 0.51 ^{bc}	30.50 \pm 0.71 ^{bcd}	2.50 \pm 0.00 ^b	1.01 \pm 0.03 ^c	-	-	-
<i>Cucumis melo</i>	32.20 \pm 0.30 ^{bcde}	31.70 \pm 0.40 ^{cde}	2.50 \pm 0.00 ^b	1.02 \pm 0.01 ^c	-	-	-
<i>Cucumis</i>	32.80 \pm 0.49 ^{cde}	35.10 \pm 0.42 ^f	2.50 \pm 0.00 ^b	1.17 \pm 0.02 ^e	-	-	-

<i>sativus</i>							
<i>Citrullus lanatus</i> (Black Seeds)	31.90±0.73 ^{bcd}	30.10±0.65 ^{bcd}	2.50±0.00 ^b	1.06±0.02 ^{cd}	-	-	-
<i>Citrullus lanatus</i> (Brown Seeds)	33.20±0.57 ^{de}	31.00±0.65 ^{cd}	2.50±0.00 ^b	1.08±0.02 ^{cd}	-	-	-
<i>Cucurbita maxima</i>	70.40±2.16 ⁱ	66.50±2.18 ^k	4.20±0.28 ^c	1.07±0.03 ^{cd}	156.4± 17.90 ^b	4.0± 0.41 ^b	8.75± 0.93 ^b
<i>Trichosanthes cucumerina</i>	41.60±0.56 ^g	37.10±0.64 ^g	2.50±0.00 ^b	1.13±0.02 ^{de}	-	-	-
<i>Cucumeropsis mannii</i>	29.90±0.65 ^b	29.30±0.82 ^{bc}	2.50±0.00 ^b	1.03±0.02 ^c	-	-	-
<i>Momordica charantia</i>	40.70±0.86 ^g	52.50±0.76 ⁱ	2.50±0.00 ^b	0.89±0.07 ^b	-	-	-

NS – Number of Spines; LS – Length of Spines; DS – Distance between Spines.

Discussion:-

Cucurbitaceae is a family of eurypalynous pollens, characterized by rather great variation in pollen morphology. However, at the level of tribes and sub – tribes, pollen morphology tends to be more consistent (Luis *et al.*, 2010). The pollen grains studied are largely monads. Exine wall ornamentation is largely reticulate in all the species studied with the exception of pollens in *Cucurbita maxima* where they are echinate, this is a diagnostic character for this species. Cell wall thickness is the least variable among the characters studied.

Almost all types of pollen shape classes have been observed within the family in this study, namely, spheroidal, subprolate, oblate-spheroidal, prolate-spheroidal, suboblate and prolate, with more than one shape type occurring in a species. Anjum and Quaser (2008) also reported that almost all types of shape classes were found within the species of the family Cucurbitaceae in Pakistan they studied. It is worthy of note that, paucity to absence of completely spheroidal pollen grain separates *Lagenaria siceraria* (spatulate fruit shape) from *Lagenaria siceraria* (oval fruit shape). However, the shapes are the same in *Citrullus lanatus* (black seeds water melon) and *Citrullus lanatus* (brown seeds water melon).

For most of the species, inaperturate pollens occur in very low frequency while most of the pollens are aperturate. Three types of apertures: porate, colpate and colporate have also been observed and documented in this study, while seven (7) pollen types can be distinguished based on the apertures: monoporate, monocolpate, bicolporate, triporate, tricolporate, tetraporate and tetracolporate. Garcia (2003) reported six (6) pollen apertural types from his study on 11 genera and 13 species of Cucurbitaceae from the state of Queretaro in Mexico.

Porate (pores only) and colporate (colpus and pores) pollen apertures have been observed in *Lagenaria siceraria* (Spatulate Fruit Shape), *Luffa cylindrica*, *Cucumis melo*, *Cucumis sativus*, *Citrullus lanatus* (brown seeds water melon) and *Cucumeropsis mannii*. They are largely porate only in *Citrullus lanatus* (black seeds water melon) and in *Cucurbita maxima*. They are colporate only in *Lagenaria siceraria* (oval fruit shape), *Trichosanthes cucumerina*, *Telfairia occidentalis* and *Momordica charantia* while colpate (monocolpate) was observed in *Cucumis melo* only.

Tricolporate pollens were observed to occur more frequently than other apertural types encountered in this work. This is in agreement with the work of Keraudren (1968) and Khunwasi (1998) who reported that tricolporate pollen type is common among the species in Cucurbitaceae.

The highest number of pores and colpi encountered in this work is four (4). Tetracolporate pollens were observed in *Trichosanthes cucumerina* and *Cucumeropsis mannii* while tetraporate were observed in *Cucurbita maxima*. Yue and Zhang (1986) and Pruesapan and Van Der Ham (2005) also reported that *Trichosanthes* pollen is usually 3-(col)porate, sometimes 4-(col)porate, rarely 5-(col)porate. The Duncan Multiple Range Test (DMRT) reveals the significant differences in the characters of the pollens in the family Cucurbitaceae studied.

Pollen grains have been classified into categories according to their diameter (Erdtman, 1952; Adedeji, 2005 and 2010). Erdtman (1952) classified pollens as Perminuta (diameter less than 10 µm), Minuta (diameter 10 - 25 µm), Media (diameter 25 - 50 µm), Magna (diameter 50 - 100 µm), Permagna (diameter 100 - 200 µm) and Giganta (diameter greater than 200 µm). Based on this classification, the pollen grains studied in this family belong to the Media and Magna categories and only very occasionally in the Minuta category. Polar length varied from 29.90±0.65 µm in *Cucumeropsis mannii* to 70.40±2.16 µm in *Cucurbita maxima*.

The permagna and giganta size categories, which are higher value size categories are not reported in this study. Hao *et al.* (2020) proposed that visits by pollen-foraging pollinators have selected against large pollen grains. An association between pollinator behaviour and pollen grain size was confirmed in their study by field studies of 80 flowering species in natural communities, showing that pollinators positively collected pollens from those species with relatively smaller pollen grains but rarely did so in species with larger ones. More specifically, large pollen would be favoured in the species whose pollen is little exploited by pollinators, whereas in species visited by pollen-collecting foragers, large numbers of smaller pollen grains may enhance reproductive success (Hao *et al.*, 2020). Hence, the Media and Magna pollen size categories, largely encountered in the species studied could have positive implication in the reproductive success of the plants.

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