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POLLEN MORPHOLOGY OF SOME SELECTECTED VEGETABLES IN SOME AREAS IN THE SOUTHWEST NIGERIA

[1]Ajipe, J. O.* and [2]Edeghagba, B. O.
Department of Botany, University of Ibadan, Ibadan, Nigeria

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ABSTRACT

Pollen morphology of nine Nigerian vegetables cultivated in the south-west Nigeria was examined using light microscopy. The aim was to report on additional identification criteria for the plants especially when other means of identification are lacking. The nine plant species: *Murraya koenigii* L., *Amaranthus hybridus* L., *Ocimum gratissimum* L., *Talinum fruticosum* (L.) Juss., *Cochorus olitorious* L., *Telfaira aoccidentalis* (Hook.) F., *Celosia argentea* L., *Solanum macrocarpon* L., *Capsicum frutescens* L. and belonging to 7 families were collected from Lagos and Ibadan in the South-western Nigeria. These vegetables are highly consumed in Lagos because they were found in abundance in Lagos markets, although some were brought into those markets from neighbouring States. The flowers of these vegetables were collected, preserved in glacial acetic acid and transported to Palynology Laboratory for pollen analysis. Palynologyical studies were carried out through the process of acetolysis. The photomicrographs of the pollen were taken and various features of the pollen such as the shape of the pollen, the length and diameter of the apertures (which ranged from 0.20 µm to 18.00 µm), polar axis (which ranged from 7.00 µm to 19.00 µm), equatorial diameter (which ranged from 7.00 µm to 24.00 µm), and exine thickness (which ranged from 0.40 µm to 2.50 µm) were studied and documented. The results indicated that pollen grains exhibit considerable variation in their morphological characteristics. The results also confirmed that the pollen characteristics among species in the same family are similar to each other. The species that showed similarities in structure were said to exhibit inter-specific relationships, making them to be grouped in the same family while the species with differences in structures could be grouped in the same family. The study concludes that pollen characters may be significant in taxonomical studies and that if the pollen grains of these plants are found in an archeological context, inferences can be made about the people's diet and medicine.

Keywords: Pollen, Vegetables, Morphology, Archaeology, Palynology

INTRODUCTION

Vegetables are parts of plants or plant matter which include leaves, flowers, seeds, fruits, stems and roots that are widely consumed by humans as food. They play an important role in human nutrition, being part of daily diets in many households and can be eaten either raw or cooked. They are also rich in carbohydrates, proteins and contain chiefly cellulose, hemi-cellulose and pectin substances which account for their firmness and texture (Sobukola and Sairo, 2007). From antiquity, vegetables have been a major portion of human diet and medicine, and as such, from the archeological context, pollen morphology could be useful in making inferences on the types of vegetables which people fed on and used for medicinal purposes (Fisseha, 2002). Pollen morphology as an aspect of palynology, is concerned with the study of the features of microscopic structures known as pollen grains. It can be regarded as an expression of a certain part of the genome just like any character or feature which could be cryptic or macromorphological. In some groups or taxons, it may be useful for taxonomic studies, but in others pollen morphology may be less valuable (Ferguson, 1985).

*Author for correspondence
[2]Department of Biological Sciences, School of Science, Yaba College of Technology, Yaba-Lagos, Nigeria

For more than half a century, several taxonomic problems have been effectively solved through the application of pollen morphology. New families have emerged through the separation of some sub-families from other families; for instance, the separation of Bombacaceae from Malvaceae, Nelumbonaceae from Nymphaeceae, Paeoniaceae from Ranunculaceae, Fumariaceae from Papaveraceae and so on. Sowunmi (1973) conducted the first pollen morphological studies in Nigeria on about 150 Nigerian woody plants, and concluded that intra-specific variations observed among the morphological structures of the pollen grains can provide additional characters for use in plant taxonomy. The plants she worked on included *Alstonia booeni*, *Funtumia elastica* which belong to the family Apocynaceae; *Monodora tenuifolia*, *Annona senegalensis* of the family Annonaceae; *Lannea microcarpa*, *Mangifera indica* of the family Anacardiaceae; *Dracaena arborea* of the family Agaceae and so on. Adekanmbi and Ogundipe (2006) studied and explained the pollen morphology of twenty (20) cultivated plants in Nigeria. Mbagwu *et al*. (2009) conducted palynological studies on five species of the family Asteraceae in Imo State, Nigeria. The species were *Guternbergia nigritiana*, *Emilia praetemissa*, *Vernonia guineensis*, *Lagera pterodonta* and *Chromolena odorata*. They attributed natural variations of the pollen in these species to evolutionary modification which determine the mode of pollination and perpetuate a particular plant group. They concluded that the plants were grouped in the same families due to similarities in structure (interspecific relationships), and the differences in structures showed reasons for them to exist as distinct species (Mbagwu *et al*., 2009). Christensen (1986) studied the pollen grains of more than 120 species from Malvaceae with the aid of light and scanning electron microscopy in Denmark. The pollen grains were found to be spiny and spheroidal with an evolutionary trend from medium-sized to ancestral 3-zonocolporate pollen. In Pakistan, Ahmad *et al*. (2011) examined the pollen morphology of five species which belong to three genera of the tribe Chlorideae, in the family Poaceae with the aid of light microscopy (LM) and scanning electron microscopy. The three genera were *Tetrapogon*, *Chloris* and *Cynodon*. The results of their studies showed that all the species present in the tribe Chlorideae had circular pollen. Other variations including the equatorial view, polar and equatorial diameter, pore diameter and exine thickness of the pollen, were used in the identification and differentiation of the species. They concluded that identification, differentiation and delimiting of different taxa were helpful in determining the existence of variations in qualitative and quantitative characters of pollen grains in different species of the tribe and sculpturing pattern in the genus *Chloris* (Ahmad *et al*., 2011).

Cristiano *et al*. (2012) worked on the pollen morphology of 16 species of Rubiaceae family occurring in a dryland vegetation in Brazil. The results showed that the species were found to be distinguished palynologically. A comparative study and description of the pollen morphology of some members of Euphorbiaceae family was carried out by Paul *et al*. (2014). The results of their study showed that a large number of pollen of the Euphorbiaceae family were isopolar and radially symmetrical. There were also variations and similarities in pollen morphological characters within the genera and species which were enough to describe and classify them. Qaiser *et al*. (2015) described the pollen morphology of 28 species representing nine genera of the family Crassulaceae in Pakistan. Some of these genera were *Pseudosedium*, *Rhodiola*, *Rosularia*, *Tillaea* and *Orostachys*. The pollen grains of the species of these genera were found to be radially symmetrical, isopolar, sub-oblate, oblate-spheroidal, sub-prolate, prolate and tricolporate. They observed significant variations in shape and exine ornamentation of the studied species.

Consequently, pollen morphology is employed in plant systematics to classify plant groups with certain pollen features into different taxons (Ajipe and Adebayo, 2018). Although much pollen morphological studies have been carried out in Nigeria, there is still the need to conduct more studies on pollen morphology of different families (Sowunmi, 1973, 1995; Adekanmbi and Ogundipe, 2006). Therefore, this study was aimed at comparing pollen morphology of some selected vegetables (*Amaranthus hybridus*, *Celosia argentea*, *Cochorus olitorious*, *Solanum macrocarpon*, *Capsicum frutescens*, *Telfaira occidetalis*, *Ocimum gratissimum*, *Talinum triangulare* and *Murraya koenigii*) in some areas in the south-west Nigeria and providing the pollen morphological features of these vegetable plants. If the pollen grains of these plants are found in an archeological context, inferences can be made about the people's diet and medicine.

MATERIALS AND METHODS

The fresh flowers and buds of *Amaranthus hybridus* L., *Capsicum frutescens* L., *Celosia argentea* L., *Cochorus olitorious* L., *Murraya koenigii* L., *Ocimum gratissimum* L., *Solanum macrocarpon* L., *Talinum fruticosum* (L.) Juss. and *Telfaira aoccidentalis* (Hook.) F. were collected from Iyana-Oba, Lagos State and some gardens in University of Ibadan, Ibadan, on the 20th and 27th of September 2017. The flowers and buds were put inside different vial bottles containing glacial acetic acid and labelled accordingly. They were taken to the Palynology Unit, Department of Archaeology and Anthropology, University of Ibadan, Oyo State for pollen analysis.

In the Palynology Laboratory, the flowers and buds of each specimen were transferred into a plastic centrifuge tube and centrifuged at 5,000 revolutions per minute (rpm) for 10 minutes. The supernatant was decanted. The pollen grain was freed from the anther by crushing the sample with glass rods. The required acetolysis mixture (9:1) was prepared by adding 5 ml of concentrated sulphuric acid to 45 ml of acetic anhydride and poured into the tubes, mixed and heated for 10 min. in a water-bath. The samples were left to cool and thereafter centrifuged. The supernatant was then decanted (Edtman, 1960). Distilled water was added to the samples in the tube, mixed, centrifuged and the supernatant was decanted. This process was repeated twice to wash off the acetolysis mixture. The samples were treated with 50% glycerol, mixed, centrifuged and decanted. Drops of 100% glycerol were added to the samples, mixed thoroughly and transferred to the vials from where the slides were mounted. When mounting the slides preclear slides were used. They were appropriately labelled. A drop of the residue in the vial was dropped on each slide and covered with coverslip. A coverslip of 22x22 mm was placed on the slides and a little pressure was applied to enable even spread. The sides of the coverslip were sealed twice with colourless nail polish in order to prevent dehydration. The slides were then examined with Olympus microscope at X400 magnification for counting and Leica microscope at X1000 magnification for the morphological features of each pollen to be studied. The parameters examined include the aperture, sculpturing pattern, polar axis, equatorial diameter, length of the os or pore, exine thickness, the colpi length and width. From a slide, about ten (10) pollen grains were examined in order to obtain precise values and information to describe and identify the pollen studied.

RESULTS AND DISCUSSIONS

Pollen descriptions

Amaranthus hybridus

The pollen grains are spheroidal in shape with perforate exine pattern as shown in Plate 3. The apertures are referred to as pores, with length ranging from 0.20 µm to 0.60 µm and width ranging from 0.40 µm to 0.50 µm. The polar axis and the equatorial diameter ranged from 7.00 µm to 9.00 µm. The exine thickness ranged from 0.60 µm to 1.00 µm.

Celosia argentea

Here, the pollen grains are imperfect spheroidal in shape with perforate exine pattern. They also have pores as their apertures as shown in Plate 7; with width ranging from 0.60 µm to 0.80 µm. The polar axis ranged from 8.00 µm to 10.00 µm. The equatorial diameter ranged from 7.00 µm to 9.00 µm and the exine thickness ranged from 1.50 µm to 1.80 µm.

Cochorus olitorious

The pollen grains are spheroidal in shape with perforate exine pattern. Plate 6 shows the equatorial view of the colporate grain (compound aperture, colpi and ora) with the polar axis ranging from 14.00 µm to18.00 µm. The pores or the ora had length ranging from 3.00 µm to 4.00 µm and width ranging from 1.80 µm to 2.20 µm.

length of the colpi ranged from 11.00 µm to 14.00 µm and the width ranged from 0.50 µm to 0.80 µm. The exine thickness ranged from 0.80 µm to 1.20 µm; the equatorial diameter ranged from 8.00 µm to 10.00 µm.

Solanum macrocarpon

The pollen grains are prolate in shape. Plate 8 shows the equatorial view of the colporate grain (compound aperture, colpi and ora). The pores or the ora have the length ranging from 2.00 µm to 3.00 µm and width ranging from 0.60 µm to 1.00 µm. The length of the colpi ranged from 7.50 µm to 8.50 µm and width ranging from 0.50 µm to 0.80 µm. The polar axis ranged from 8.50 µm to 10.00 µm. The equatorial diameter ranged from 7.50 µm to 9.00 µm; the exine thickness ranged from 0.60 µm to 1.20 µm.

Capsicum frutescens

The pollen grains are spheroidal prolate in shape having colporate aperture (compound aperture, colpi and ora) as shown in Plate 9. The polar axis ranged from 7.00 µm to 9.00 µm and the exine thickness ranged from 0.40 µm to 0.60 µm. The length of the pores or ora ranged from 1.60 µm to 2.20 µm and the width ranged from 0.80 µm to 1.00 µm. Colpi length ranged from 6.00 µm to 7.00 µm and the width ranged from 0.60 µm to 1.00 µm. The equatorial diameter ranged from 7.00 µm to 8.50 µm.

Telfaira occidetalis

The pollen grains are oblate-spheroidal in shape with reticulate exine pattern. Plate 2 shows the polar view of the tricolporate grain. The ora have length ranging from 1.60 µm to 2.00 µm and width ranging from 0.80 µm to 1.20 µm. The length of the colpi ranged from 15.00 µm to 18.00 µm and width ranging from 1.00 µm to 1.60 µm. The polar axis ranged from 19.00 µm to 25.00 µm. The equatorial diameter ranged from 14.00 µm to 19.00 µm and the exine thickness ranged from 0.80 µm to 1.40 µm.

Ocimum gratissimum

The pollen grains are oblate-spheroidal in shape with reticulate exine pattern as shown in Plate 4. They are colpate grains with polar axis ranging from 8.00 µm to 12.00 µm. The length of the colpi ranged from 6.50 µm to 9.0 0 µm and the width ranged from 1.60 µm to 2.00 µm. The exine thickness ranged from 1.00 µm to 1.40 µm and the equatorial diameter ranged from 9.00 µm to 17.00 µm.

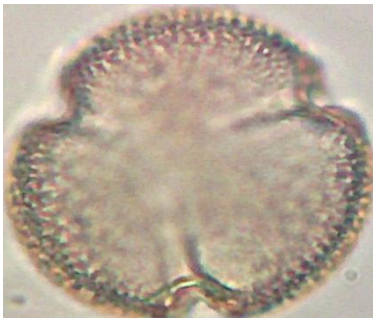
Talinum fruticosum

The pollen have perforate exine pattern and are spheroidal in shape as shown in Plate 5. They have pores with the width ranging from 2.00 µm to 3.00 µm. The polar axis ranged from 16.00 µm to 24.00 µm. The equatorial diameter ranged from 16.00 µm to 24.00 µm and the exine thickness ranged from 1.00 µm to 1.40 µm.

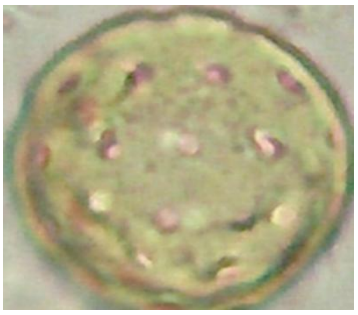
Murraya koenigii

Plate 1 shows the polar view of one of the pollen grains of *Murraya koenigii*. The pollen grains are oblate in shape with reticulate exine pattern. They are colpate grains with polar axis ranging from 16.00 µm to 19.00 µm. The length of the colpi ranged from 10.00 µm to 13.00 µm and the width ranged from 0.20 µm to 0.40 µm. The exine thickness ranged from 2.00 µm to 2.50 µm and the equatorial diameter ranged from 20.00 µm to 23.00 µm.

(1) *Murraya koenigii* (PV)



(2) *Telfaira occidentalis* (PV)

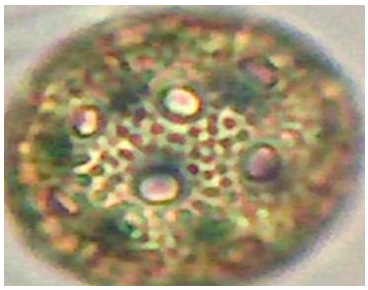


(3) *Amaranthus hybridus*

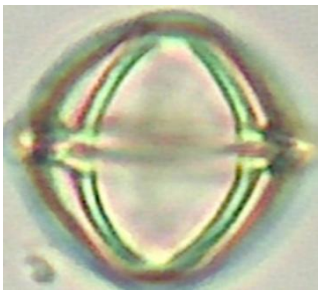
(4) *Ocimum gratissimum*

(5) *Talinum fruticosum*

(6) *Cochorus olitorious* (EV)



(7) *Celosia argentea*



(8) *Solanum macrocarpon*

(9) *Capsicum frutescens* (EV)

Plates 1-9: Photomicrographs of pollen grain morphology of vegetable species studied
Magnification: X 1,000

TABLE 1: Quantitative characteristics of the pollen grains of some vegetables in southwest Nigeria (n=10).

Specimen	Polar axis (P) (µm)	Equatorial diameter (E) (µm)	Length of colpus (µm)	Width of colpus (µm)	Length of os/pore (µm)	Width of os/pore (µm)	Exine thickness (µm)
Murraya koenigii	17.70±1.25^{b}	21.80±0.92	11.70±1.34	0.31±0.10	0.44±0.18	Nil	2.17±0.24
Amaranthus hybridus	8.25±0.89^{a}	8.25±0.89^{a}	Nil	Nil	Nil	0.45±0.07	0.83±0.16^{a}
Ocimum gratissimum	9.80±1.60^{a}	14.10±2.38	7.65±0.88	1.83±0.16	Nil	Nil	1.10±0.17^{b}
Talinum fruticosum	19.80±2.78^{bc}	18.90±2.47^{b}	Nil	Nil	3.40±0.52	2.45±0.50	1.10±0.14^{b}
Cochorus olitorious	15.30±1.34	9.10±0.88^{a}	12.30±0.95	0.63±0.12	1.78±0.18	1.94±0.13	1.02±0.11^{ab}
Telfaira occidetalis	20.75±1.84^{c}	17.60±2.01^{b}	16.50±1.08	1.18±0.22	Nil	0.96±0.13	1.01±0.20^{ab}
Celosia argentea	8.95±1.01^{a}	8.90±1.31^{a}	Nil	Nil	2.40±0.46	0.69±0.09	1.62±0.12
Solanum macrocarpon	9.00±0.58^{a}	8.08±0.40^{a}	7.65±0.53	0.61±0.12	1.87±0.20	0.82±0.18	0.88±0.21^{ab}
Capsicum frutescens	8.20±0.63^{a}	7.40±0.57^{a}	6.55±0.50	0.81±0.14	1.98±1.02	0.92±0.10	0.51±0.09

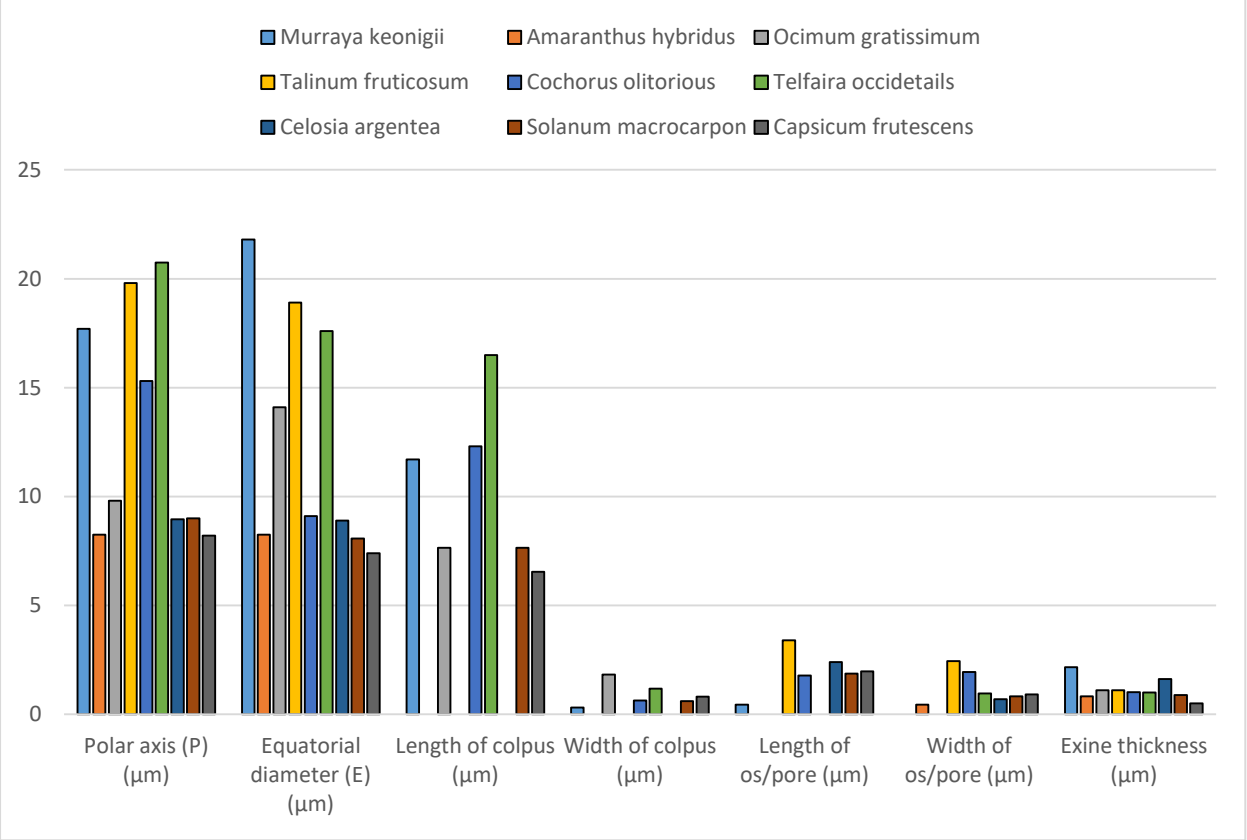


Fig. 1: The graph (bar chart) showing the mean of various features of the pollen grains of all the vegetable samples.

Results of this study have shown that the pollen morphology among species belonging to the same family had features similar to one another. For example, as shown in Plates 3 and 7, the pollen types of *Amaranthus hybridus* and *Celosia argentea* have pores as their aperture. They are both spherodial in shape and have perforate exine pattern. From Table 1, the values of polar axis and equatorial diameter of *A. hybridus* and *C. argentea* have the same superscript, meaning they are not significantly different from each other. From Figure 1 the polar axis and equatorial diameter of *A. hybridus* ranged from 7.00 µm to 9.00 µm, which is similar to those of *C. argentea* with polar axis which ranged from 7.00 µm to 10.00 µm and the equatorial diameter ranging from 8.00 µm to 11.00 µm. From Table 1, the mean with the standard deviation of polar axis and equatorial diameter of *A. hybridus* is 8.25 µm±0.89 µm, similar to those of *C. argentea* with the mean and standard deviation of the polar axis as 8.95 µm±1.01 µm and the equatorial diameter as 8.90 µm±1.31 µm. Also, from Figure 1 and Table 1, the length of the ora of *A. hybridus* ranged from 0.20 µm to 0.80 µm and their width ranged from 0.40 µm to 0.50 µm, with their mean and standard deviation as 0.45 µm±0.07 µm, and for *C. argentea*, the diameter of the ora ranged from 0.60 µm to 0.80 µm with the mean and standard deviation of 0.69 µm±0.9 µm. Therefore, the pollen of *A. hybridus* and *C. argentea* are referred to as porate grains, and their characteristic justifies their being grouped together under the family of Amaranthaceae. Likewise, as shown in Plates 8 and 9, *Solanum macrocarpon* and *Capsicum frutescens* belong to the same family *Solanaceae*. Both pollen grains of *S. macrocarpon* and *C. frutescens have* compound aperture: ora and colpi; thus, they are referred to as colporate grains. They are both prolate in shape. The polar axis and equatorial diameter of *S. macrocarpon* and *C. frutescens* were not significantly different from each other. Figure 1 shows that polar axis of *S. macrocarpon* ranged from 8.50 µm to 10.00 µm with equatorial diameter ranging from 7.50 µm to 9.00 µm; these were similar to those of *C. frutescens* with polar axis ranging from 7.00 µm to 9.00 µm and the equatorial diameter ranging from 7.00 µm to 8.50 µm. The mean with standard deviation of polar axis of *S. macrocarpon* was 9.00 µm±0.58 µm and that of the equatorial diameter was 8.08 µm±0.40 µm; values were

similar to those of *C. frutescens*, with the mean and standard deviation for polar axis as 8.20 µm±0.63 µm and the equatorial diameter as 7.4 µm±0.57 µm. In the same vein, Figure 1 shows that the length of the colpi of *S. macrocarpon* ranged from 7.50 µm to 8.50 µm and the width of the colpi ranged from 0.50 µm to 0.80 µm; the length of the ora ranged from 2.00 µm to 3.00 µm and the width of the ora ranged from 0.60 µm to 1.00 µm. The values were similar for *C. frutescens*, with the length of the colpi ranging from 6.00 µm to 7.00 µm and the width of the colpi, which ranged from 0.70 µm to 1.00 µm; the length of the ora ranged from 1.60 µm to 2.00 µm and the width of the ora ranged from 0.80 µm to 1.00 µm. Table 1 shows that the mean and standard deviation of the length of the colpi for *S. macrocarpon* was 7.65 µm±0.53 µm, and the width was 0.61 µm±0.12 µm. The length of the ora was 1.87 µm±0.20 µm while the width was 0.82 µm±0.18 µm. These values were similar to those of *C. frutescens*, with the mean and standard deviation of colpi as 6.55 µm±0.50 µm for the length and 0.81 µm±0.14 µm for the width; the standard deviation of the ora was 1.98 µm±1.02 µm for the length and 0.92 µm±0.10 µm for the width. Figure 1, shows that the exine thickness of *S. macrocarpon* ranged from 0.60 µm to 1.20 µm, while that of *C. frutescens* ranged 0.40 µm to 0.60 µm. The other species could not be grouped together in the same family because of their structures.

There were some similarities between some pollen grains of plants that did not belong to the same family like *Murraya koenigii, Telifaira occidentalis* and *Ocimum gratissimum* as shown in Plates 1, 2 and 4. They had the same exine pattern (reticulated) and the same aperture (colpus). Table 1 shows the results of the mean and standard deviation of various features of pollen grains of the 9 species considered. The polar axis of *A. hybridus, O. gratissimum, S. macrocarpon, C. frutescens* and *C. argentea* did not differ significantly. The polar axis of *Talinum fruticosum*, *M. koenigii*, and *T. occidentalis* differed . The equatorial diameter of *A. hybridus, C. olitorious, C. argentea, S. macrocarpon* and *C. frutescens* was similar. *T. fruticosum* and *T. occidentalis* also had the same equatorial diameter. The exine thickness of *O. gratissimum* and *T. fruticosum* was similar. *C. olitorious, T. occidentalis* and *S. macrocarpon* had the same thickness. *M. koenigii, C. Argentea* and *C. frutescens* also had the same exine thickness.

In this study, the different outlines were related to the number of apertures, type of sculpturing, shape class and size. Comparing the description of the studied species with the previous descriptions, some of the variations could have resulted from methodologies employed in pollen preparation, such as the chemical treatment which maight have affected the size and shape of pollen. Some of the differences could have resulted from the type of microscope used. While the present study used the light microscope, El Ghazali (1989) and Bonnefile and Riollet (1980) used the scanning electron microscope (SEM).

CONCLUSION

This study has revealed the morphological structures of the pollen grains of some vegetable plants consumed in Lagos. Pollen morphological characters such as length of exine, the length and diameter of the apertures, the shape of the pollen, polar axis, equatorial diameter and exine thickness varied amongst the species studied. Similarities were also observed amongst some parameters studied. These similarities and differences formed the basis of grouping the vegetable plants into the same or different groups.

The distinctive morphological features of the pollen grains observed in these plants could be used in archaeological studies to determine the types of food and medicine used by the people in the past.

RECOMMENDATION

This study recommends that pollen morphology should be used as a tool for floral identification in taxonomic studies.

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