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The Importance of Morphology and Palynology of Two Species of *Amaranthus* Found in Awka South Eastern Nigeria

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Abstract Study was carried out on the morphology of *Amaranthus hybridus and Amaranthus spinosus.* This is to find out the form and structure of the two species of *Amaranthus* under study and also to discover the areas of the walls of the two species where the walls are thinner or softer. The vegetative study was done in the field and in the laboratory by visual observation on the quantitative characters and analysed using a statistical test to compare the means of the two samples (T.test). *A. hybridus* has small fine hairs, robust, longitudinally rigid and greenish colour as it is commonly called, while *A. spinosus* has smooth stem, cylindrical and are mostly reddish in colour with spikes attached above the base of the petiole. The result of the pollen analysis of the two species showed no significant statistical difference in the sizes of the apertures in the pollen, though *A. hybridus* has a greater number than *A. spinosus*. The overall analysis showed there was no significant difference existing in the morphology of the two species. The slight difference existing in the morphology likewise palynology signifies their separation into different species while the similarities justify their placement under the genus *Amaranthus.*

Keywords Importance, Morphology, Palynology, *Amaranthus hybridus, Amaranthus spinosis*

1. Introduction

The genus *Amaranthus* L. belongs to the tropical family Amaranthaceae. According to [1] *Amaranthus* consists of about 60–70 species. 25 genera and 602 species are reported in Africa. Several species of *Amaranthus* exist in the tropics but the common grain *Amaranthus* types are*; A. hypochondriaus*, *A. cruentus* and *A. caudatus* [2]. The major weedy *Amaranthus* include *A. viridis*, *A. spinosus*, *A. retroflexus* and *A. hybridus*. *Amaranthus* species are herbs with four principal groups which are separated. These groups include; cultivated known as improved species or cultivars, wild and weedy referred to those growing in the field, racial grouped on the basis of geographic morphological patterns and landrace or accession on the basis of populations from specific locations [3]. Since ancient times, several species of *Amaranthus* have been cultivated in the old and new world as grain crops, pot herbs, ornamentals, and dye plants. The potential of grain and vegetable *amaranthus* as a food resource has been reviewed extensively [4]. Vegetable *amaranthus* species are used for food in different parts of the world. Grain amaranth can be utilized as seeds or flour to produce products such as cookies, cakes, pancakes, bread muffins, crackers, pasta and other products from bakery. In Nigeria, some *Amaranthus hybridus* leaves in addition to condiments are used to prepare soup. Their leaves are eaten as spinach or green vegetables in Congo [2]. *Amaranthus hybridus* L. and *Amaranthus spinosus* L. commonly found in south eastern Nigeria are the species of interest in this study. A system of classification of biological organisms which best reflects the totality of their similarities and differences is termed taxonomy [5]. Taxonomy is a science without data of its own and therefore utilizes the investigations of other branches of biology. Characters selected are those which are most easily observed and those which show promise as being reliable and discriminating in the taxon delimitation. Morphology, phylogeny, physiology, phytochemical, cytology, palynology and other taxonomic evidence are used by Taxonomists in the classification of plants to determine the similarities and differences used to group plants into various taxa. *Amaranthus hybirdus* L. and *Amaranthus spinosis* L. are grouped into genus based on their similarities and into different species based on the differences observed in them. [6] Study on the varieties of *capsicum* used macromorphological characters to reveal the intra and interspecific relationship among the varieties under the genus *Capsicum.* Plant morphology or phytomorphology is the study of the physical form and external structure of plants. This is useful in the visual identification of plants. It portrays a study of the development, form and structure of plants, and by implication, an attempt to interpret these on the basis of similarity of plan and origin. External morphological structures such as flowers, leaves, stems and fruits are used as the bases for plant classification. Phytomorphological data of plants are easily noticed and obtainable and thus are used frequently in plant identification [7]. The external morphological evidence provides the basic information for characterization of plants, identification, classification and relationships.[7], [8] showed morphological characters as vegetative, phenological floral characters, seeds and fruit morphology, thus morphological features of plants are those external diagnostic features of plant [9]. Palynology is "the study of pollen and spores"[10]. The first study of the Pollen of the Amaranthaceae was in 1826. Two pollen types were described by [11] based on the study of the twelve species representing these genera; the *Amaranthus* – type and the Gomphrena – type. The greatest progress in our learning of pollen within the Amaranthaceae was made by [12], who presented a large number of SEM micrographs of grains from taxa occurring in the new world. The taxonomic significance of morphology and palynology in the study of two species of *Draceana* which was stressed by [13] justifies their being under the same genus. Many taxonomic problems including the repositioning of several disputed taxa and interpretation of problems relating to the origin and evolution of different groups have been solved using Palynological characters.

2. Materials and Methods

Sources of Materials

The study was carried out at the Botanical garden of Nnamdi Azikiwe University Awka, Nigeria. The study started in February 2021 and ended in August 2021.

The leaves, stems, roots, flowers and seeds of *Amaranthus* species were collected from Mbakwu in Awka North Local Government Area Anambra State, Nigeria. The *Amaranthus* species was identified by assigning the plant to a particular taxonomic group and authenticated by a Plant Taxonomist in the Department of Botany Nnamdi Azikiwe University Awka, Anambra State Nigeria; the specimen voucher was also deposited in the herbarium of the above department.

Morphological Studies

The vegetative studies on the two *Amaranthus* species collected were done by visual observation and use of hand lens. Quantitative characters such as leaf morphology (leaf length and leaf width) stem morphology (stem colour and spine length), floral, fruit and seed morphology and qualitative characters such as leaf (leaf shape, leaf blade, leaf apex, leaf base, leaf surface, leaf colour and leaf arrangement), stem (stem size, stem shape and stem surface) were also analyzed accordingly.

Palynological Studies

Polleniferous material extracted by pollen extraction from the flowers of *A. hybridus* and *A. spinosis* was done with the aid of forceps and alcohol. The samples from each of the species were sieved through 200u –mesh copper wire gauze to filter off floral large particles. Samples were centrifuged at 1000rmp (revolution per minute) for five minutes. The supernatant was decanted and residue retained. To recover the polleniferous residue, the residue (ppt) was washed three times with water and centrifuged.

Acetolysis

The procedure employed in the preparation of palynomorphs for microscopic analysis is termed Acetolysis. The acetolysing agent is a mixture of concentrated tetraoxosulphate (VI) acid and acetic anhydride (1:9) 11. The following steps are involved in the treatment of the residue:

In a beaker, 2-3ml of acetic anhydride and concentrated H $_2SO_4$ was mixed as step one of the treatment. The preparation was done in a fume cupboard which serves as a device used to limit exposure to hazardous or toxic fumes. The acetolysis mixture was added to the samples in order to destroy all cellulosic materials for easy examination of pollen under the microscope. The centrifuge tube containing the pollen in suspension of an acetolysis mixture was placed in a water bath and heated at 70˚ C for 7 minutes and the content was stirred with a glass rod intermittently. The acetolysis mixture was carefully decanted without losing any residue. Distilled water was used in washing the pollen to acid contamination. Distilled water was added to the residue and was centrifuged again for 5 minutes at an average speed of 2500 rpm. The washing was repeated three times to ensure legitimate evacuation of acid. A few drops of glycerine (2ml) were added for storage and prevention of fungi contamination.

Microscopic Analysis of Temporary Slide

Two drops of prepared pollen were placed on 32x22mm sized slides, and a 22x22mm cover slip was gently placed on the slide. Nail polish was used to hold it firm on the slide. Pollen grain of *Amaranthus hybridus* and *Amaranthus spinosus* were studied and described based on their morphological features. The shape classes are based on the measurements of polar view (P) and Equatorial view (E) and the resulting P/E ratio. Pollen measurements were also taken using a Carl Zeiss eyepiece micrometer graticle, pollen characters were studied at magnifications of X400 and at x1000 respectively and photomicrographs taken.

Statistical Procedure

Data collected were analyzed using t-test and results presented were presented in mean ± SE, Equatorial views and the percentage of the pollen studied is 100%.

3. Results

Table 1. External morphology of Amaranthus *hybridus* and *Amaranthus spinosis*

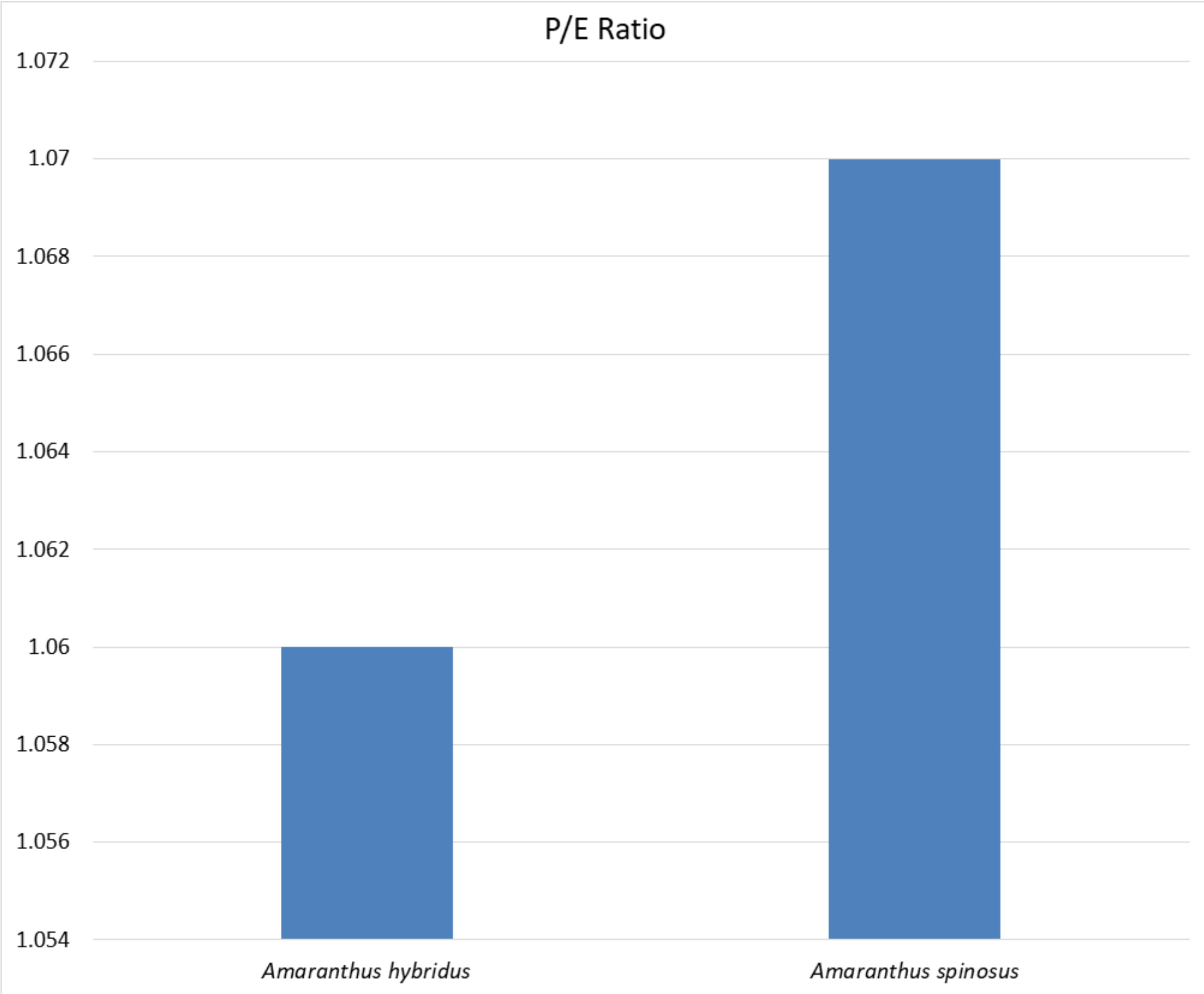
Morphological characteristics	*Amaranthus hybridus*	*Amaranthus spinosis*
Habit	Herbaceous	Herbaceous
Leaf type	Simple	Simple
Leaf arrangement	Alternate	Alternate
Leaf shape	Ovate to rhombic –ovate	Obovate to elliptical shape (oblanceolate
Leaf margin	Entire	Entire
Leaf apex	Retuse and apiculate	Mucronate
Leaf base	Cunneate or attenuate	Broadly lanceolate to ovate, tapering to a slender petiole and a pair of straight spines attached to the base of leaf petiole
Leaf mid rib	Conspicuous in adaxial and abaxial surfaces	Conspicuous veins with sparse hairs
Leaf colour	Green	Green
Leaf blade	Pubescent small fine hairs on the underside	Pubescent and fuzzy with hairs on the underside, leaf blade is long, petioled.
Stem	Green, thick and often ribbed or tingedwith red, with parallel lines	Smooth cylindrical, often reddish with small fine hairs
Root	Tap root	Tap root
Inflorescence arrangement	Arranged spirillay around the inflorescence axis. flowers occur singly or in several ranks	Arranged in a spiral around the inflorescence axis, occur singly or in several ranks
Flowers	Flowers occur singly or in several ranks, flowers are green radially symmetrical monoecious. Flowers do not have hypanthium.	Green in colour, radially symmetrical, monoecious, flowers lack hypanthium and in terminal spikes. aggrevated in dense terminal panicle.
Sepals or perianth	5 in number and separated	5 and separated
Calyx	Radially symmetrical	Radially symmetrical
Carpels	Fused to one another	Fused to one another.
Pistil	1	1
Ovary	Superior and lacks a stipe	Superior, lacks a stipe
Placentation	Basal	Basal
Androecuim	5 and lined up with the sepals	5 and lined up with the sepals

Table 1 Continued

Anther	Adnate	Adnate
Filament	Smooth with hairs or scales	Smooth with no hair or scales
Friut	pysix, an achene, dry dehiscent and single.	Pysix, an utricle achene and circular, dry indehiscent and single
Fruit locule	1	1
Fruit length	Ranges from1.5mm to 2.5mm	Ranges from 1.5mm to 2.0mm
Fruit shape	Obovoid (egg – shaped)	Ovoid (egg- shaped) or spherical
Seeds	Round, dark, brown, shiny, somewhat flattened and 1.0 to 1.5mm in diameter.	Flattened, round, deep brown, smooth and shiny measuring 1.omm in diameter.
Leaf length	9.32 ± 0.60cm	2.73cm ± 0.20cm
Leaf width	3.08cm ±0.21cm	1.23cm ± 0.21cm
Plant height	29.30 cm ± 1.95cm	26.70cm ± 2.20cm
Petiole length	4.67cm ± 0.44cm	2.11cm ± 0.23cm
Flower spike length	7.79cm ± 0.62cm	6.02cm ± 0.68 cm

Table 2. Description of the Pollen Morphology

Morphological characters of the pollen	***Amaranthus hybridus***	***Amaranthus spinosis***
Equatorial ratio	1.06µm	1.07µm
Length of polar axis	10.1µm -10.5µm	9.74µm -9.70µm
Length of equatorial axis	9.71µm -9.75µm	9.19µm -9.02µm
Distance between pores	0.84µm -1.67µm	0.4µm – 1.67µm
Number of apertures	Pantoporate, 13 -31 pori per grain	Pantoporate, 16 -34 pori per grain
Shape of pollen grains	Sphaeroidal to prolate – sphaeroidal to oblate	Sphaeroidal to prolate – sphaeroidal to oblate
Exine	Granulate, tectate	Granulate, tectate
Mesoporia	Flat with almost evenly distributed distance between pores	Flat with almost evenly distributed distance between pores
Pores shape	Varies between triangular, quadrangular and circular	Varies between triangular, quandrangular and circular
Pollen grain size	Ranges from very small to small	Ranges from very small to small.

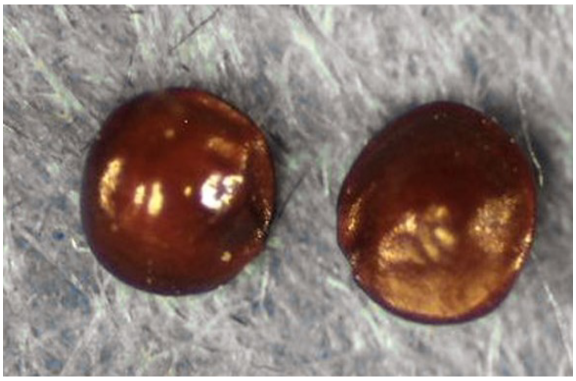
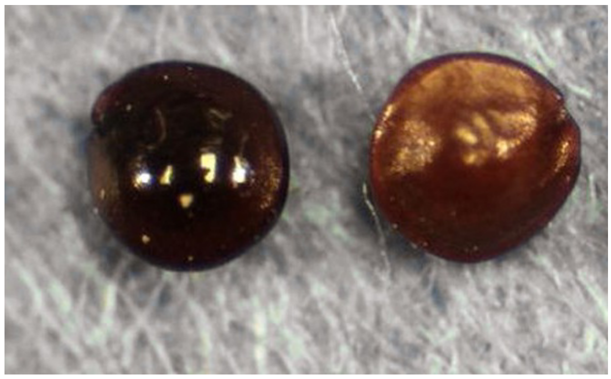


Note: unit of P/E ratio is micro-meter (um)

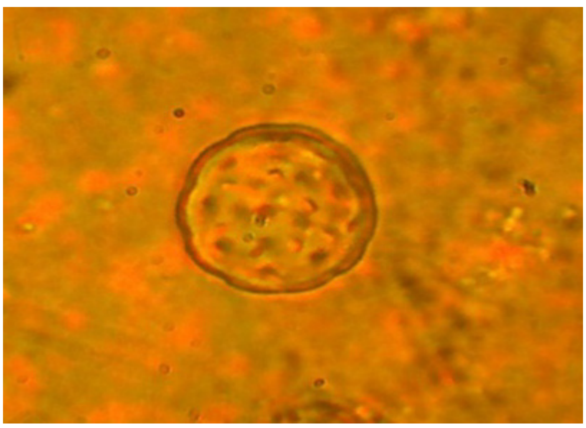
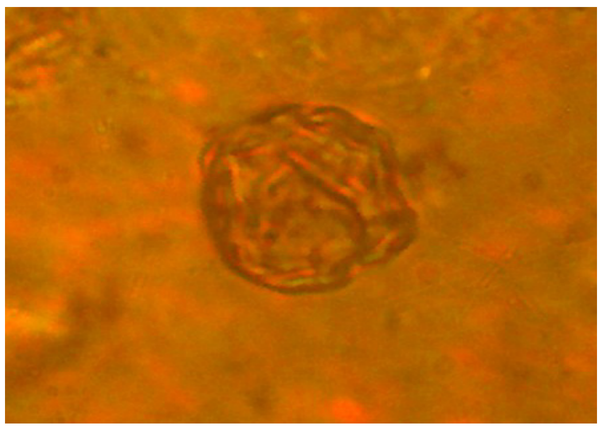
Figure 1. A graphical representation showing polar view (p) and equatorial view (E) and the resulting P/E ratio of the pollen grain studied. (vertical line represents X while the horizontal line represents Y

A B


C D

E F

G H

Figure 2. A-H SHOWING MORPHOLOGICAL PARTS OF THE TWO SPECIES OF *Amaranthus* (*A. hybridus, A. spinosis*, *A. hybridus* flower, *A. spinosis* flower *A. hybridus* seed, *A.spinosis* seed, *A. hybridus* pollen, and *A. spinosis* pollen)

4. Discussion

The research shows there are no real significant differences in the external morphology of the two species of *Amaranthus hybridus* and *Amaranthus spinosis* and both of them are herbs. However, the morphological features of the two species studied show some specific characteristics that can be used for taxonomic decision. However, the stem of *A. hybridus* has small fine hairs, robust, longitudinally rigid and greenish in colour while *A. spinosus* has a smooth stem, cylindrical and mostly reddish in colour with spikes attached above the base of the petioles (Table 1). The variation in the leaf shape, leaf apex, leaf base, leaf mid rib, stem, filament, fruit length and shape, seeds, leaf length and width, plant height, petiole length and flower spike of the species studied constitutes an important distinguishing factor that could be used for establishing intra and interspecific relationship (Table 1). [13] observed the same in the study of the morphology of two species of Dracaena revealing no significant difference. Significant statistical difference exists between the lengths and widths of the leaves as well as the flower spike length of the two species. *A. hybridus* had the longest leaf of 9.32cm± 0.60cm in length, broadest leaf of 3.08cm± 0.21cm and a flower spike length of 7.79cm± 0.62cm (Table 1). This supported the report of [14] that Amaranthus show a wide variety of morphological diversity among and even within certain species. Also [16] reported that morphological data of plants are easily observable and obtainable, and are "thus used most frequently in taxonomic studies". The inflorescence, flower and seed morphology of both plants are the same as shown by the result of the study (Fig 2). With the exception of the fruits of both species being achenes, the result of the inflorescence, flower, fruit and seed morphology of both species is in line with an earlier study of [14], in which the external morphology of *Amaranthus* was outlined as follows: habit and leaf form: shrubs, wild or cultivated; leaves alternate, herbaceous; leaves simple; lamina entire, ovate to rhombic ovate, net-veined. Inflorescence, floral, fruit and seed morphology: flower aggregated inflorescences, in spikes or in panicles. The terminal inflorescence unit is spike. Flower's minute, regular, 5 or 4 merous, cyclic or pentacyclic; perianth of sepals 5 and separated, whorled, isomerous petaloid, unisexual flowers present, plant hermaphrodite; androecium 5; androecial members adnate (not attached to the petals or sepals), free from one another, whorled; androecium exclusively of fertile stamens; stamens 5; anthers fused, dehiscing through longitudinal slit; gynoecium having a persistent style; styles 3; pistil 1 celled; gynoecium superior; placentation basal. This study also observed similar morphological characters in this study. These findings also were supported by [7]; the family Amaranthaceae is distinctive, the genus has distinguishing characters among the 75 species present across six continents. Fruit locule 1; fruit dries and splits open when ripe or does not; usually an achene; seeds round, dark and somewhat flattened. [14] reported that fruits of Amaranthaceae are in the form of capsules referred to as a unilocular pixdio that opens at maturity. The result of the pollen analysis of the two species showed that there was no significant statistical difference in the sizes of the pollen grains of species (Table 2). Pollen characteristics; equatorial ratio, length of polar axis, length of equatorial axis, distance between pores and number of apertures (fig 1) in the two species studied observed 1.06 P/E Ratio in *Amaranthus hybridus* and 1.07 P/E Ratio in *Amaranthus spinosis,* respectively (Fig 1). This constitutes an important factor in establishing the intra and interspecific relationship. Thus morphological data obtained in this study can be used in taxonomic study of Amaranthus species. The range of distance between pores differs in length (0.84μm - 1.67μm and 0.42μm - 1.67μm respectively) (Table 2), both of them are pantoporate. This was also supported by the study of [11] on the pollen types in Amaranthaceae morphology and evolutionary. The surface of their pollen grains is fine/smooth. Sizes of their aperture differ in number fig 2G, H; also this was supported by [11] in their study on pollen morphology of some species of Amaranthaceae. The number of apertures in the pollen of *A. hybridus* is greater than that of *A. spinosus*. The result is in line with an earlier study by [12] who reported that the pollen grains of *A. hybridus* and *A. spinosus* were spherical shaped with pantoporate or golf ball-like and aperture arrangement. Species in the genus *Amaranthus* are either monecious (*A. hybridus*), or dioecious (*Amaranthus palmeri*) [14]. The monoecious and dioecious species of *Amaranthus* species has differences observed between them. There was a greater number of apertures in the dioecious species on the visible surface (Table 2).

5. Conclusions

The overall result of the study showed that there is no significant difference in the morphology and palynology of *A. hybridus* and *A. spinosis*. In other words, the two species are closely related and this certifies their placement in the same genus *Amaranthus*. The slight differences existing between them (such as the spines found on *A. spinosus* but not found on *A. hybridus*) also justify their separation into different species.

REFERENCES

[1] Costea, M. and DeMason, D. (2001). Stem morphology and anatomy in *Amaranthus* L. (Amaranthaceae). Taxonomic significance. *Journal of the Torrey Botanical Society* 128(3): 254-281. https: // search.Proquest,com, doi/10.2307/3088717

[2] Dhellot, J.R., Matouba, E.,Maloumbi, M.G., Nzikou, J.M., Safou-Ngoma, D.G., Linder, M., Desobry, S and Parmentier, (2006). Extraction, chemical composition And nutritional characterization of vegetable oils: case of *Amaranthus hybridus* (var. 1 and 2) of Congo Brazzaville. *African Journal of Biotechnology* 5 (11): 1095-1101. htpp: /www.academicjournals.org/AJS ISSN 1684-5315.

[3] Christopher, B. (2008). *RHS A-Z encyclopedia of garden plants*. Dorling Kindersley Press. United Kingdom 1136pp. https:// www.scirp.org ISBN-13:978- 1405330978.

[4] Uchechi Ukam, N., Mgbekem, M., Edide, R. and Christopher Obizoba, I. (2016) Nutrient and Phytochemical Composition of Five Wild Green Leafy Vegetables Consumed in Erie-Biase Local Government Area of Cross River State. *Food and Nutrition Sciences*, 7,817-823. Doi: 10.4236/fns.2016.79082.

[5] Djirabaye Nadjiam (2021). Local Taxonomy and Diversity of Chadian Sorghum (Sorghum bicolor) L.) Moeoch) Accessions. *Agricultural Sciences* 12(5). DOI: 10.4236/as.2021.125033

[6] Aziagba, Bibian.O, Okeke, C.U, Ezeabara, Chinelo, A, Ilodibia, Chinyere, V, Uka Chiedozie, J. (2014). MacroMorphological Observations in *Capsicum* varieties Cultivated in Awka, Anambra State South Eastern Nigeria. *America Journal of Life Science Researches.* 3(1) 30-34. WWW. Worldfresearches.com https://www.diili.org

[7] Radford, A.E (2019) *Fundamentals of plant systematics*, Harper and Row New York, Pp. 302-322. ISBN- 10: 0060453052, ISBN-13: 978-0060453053. https://www.archive.org

[8] Sharma, O.P. (2013) *Plant Taxonomy*. Tata McGraw-Hill publishing Company Limited, New Delhi. 482pp.ISBN-9780070141599 https: //booksgoogle.com

[9] Gasser, C., Skinner, D. (2019) Development and evolution of the unique ovules of Flowering plants. *Biology* DOI: 10.1016/BS.CTDB.2018.10.007, Corpus ID: 58655075.

[10] Jennifer, M.K. O'Keefe, Fabienne Marret, Peter Osterloff, Mathew J. Pound and Lyudmila Shumilovskikh. (2021). *Geological Society*, *London.* Special Publications. 511, 1-11. https://doi.org/10.1144/SP511-2021-83.

[11] Angelini, P., Bricchi, D., Gigante, S., Poponessi, A., Spina, A. and Venanzoni, R. (2014) Pollen morphology of some species of Amaranthaceae s. Lat. common in Italy. *Flora Mediterranea* 24: 247-272 doi 10.7320/FIMedit24.247.

[12] Gretchen, D.J., and Vaughn M. B., (2007). *Grana.* A comparison of pollen counts: Light versus scanning electron microscopy. Vol.46 issue 1, 20-23pp https: //doi.org/10.1080/00173130601173897.

[13] Chinyere V. Ilodibia, C.U. Okeke, Maureen U. Chukwuma, Nneka, F. Okeke, Ebele, E. Akachukwu, Bibian, O. Aziagba (2015). Taxonomic significance of Morphology and palynology of two species of Dracaena found in south Eastern Nigeria. *International Journal of Biological Research* 3(1) 5-8. DOI:10.14419/ijbr.v3i1.3737

[14] Lavernee, S., Gueco, Teresita B.. Constacio De Guzman (2016). Diversity in the morphology of Amaranth (*Amaranthus* sp.) germplasm collection in the Phillipines. *Asian Journal of Agriculture and Food Sciences.* Vol.4 issue 1. ISSN: 2321-1571 ajouronline.com

[15] Aaron, S., Franssen, H., Daniel, Z., Skinner, D., Kassim, A., and Michael, J. (2009). Pollen morphological differences in *Amaranthus* species and interspecific Hybrids. *Journal of weed science society of America*. 49 (6): 732-737. DOI: 10. 1614/0043- 1745 (2001) 049[0732: PMDIAS] 2.0CO; 2

[16] Sellers, B. A., Smeda, J. R., Johnson, W. G., Kendig, J. A., and Ellersieck, M. R. (2003). Comparative growth of six *Amaranthus* species in Missouri. *Weed Science* 51: 329–333. DOI: https//doi.org /10.1614/0043-1745 (2003) 051(0329: [GOSAS] 2.0.CO; 2