ISSN:
Print - 2277 - 0593
Online - 2315 - 7461

Journal of Natural Sciences, Engineering and Technology

POLLEN STUDIES ON FOUR SPECIES OF *CRASSOCEPHALUM* (MOENCH.) S. MOORE (ASTERACEAE) IN SOUTHWESTERN NIGERIA

***A.S. OYELAKIN AND M.S. AYODELE**

Department of Biological Sciences, Federal University of Agriculture, Abeokuta, P.M.B. 2240, Abeokuta, Ogun State, Nigeria.
***Corresponding author:** princehabbey@yahoo.com **Tel:** +2348035216157

ABSTRACT

Twenty-three accessions of four species of the genus *Crassocephalum* were investigated on their pollen size, pollen morphological structure and fertility. Pollen sizes were obtained from polar and equatorial diameter measurements. Pollen fertility was accessed through the staining property in cotton blue in lactophenol reagent. The structural form of pollen grains was examined by means of a light microscope (X100 magnification). There was no significant difference in pollen size, structure and fertility among species investigated. *C. crepidioides* had relatively the largest size of pollen grains (28.10±1.67 µm) (media), while *C. rubens* pollen were relatively most fertile (98.7%). However, pollen grains of the species were tricolporate with three germ pores except in *C. crepidioides* which had up to six germ pores (multiporate). All pollens in the species were fenestrate type with high fertility. The study suggests that number of germ pores in addition to pollen morphology be used in species separation.

Key words: *Crassocephalum*, pollen grains, Pollen morphological structure and fertility

INTRODUCTION

The genus *Crassocephalum* (Moench.) S. Moore comprised of green leafy vegetables popular and widespread in many tropical and subtropical regions but more prominent in Tropical Africa (Fowomola and Akindahunsi, 2005). *Crassocephalum* species constitute special delicacies in Southwestern Nigeria and feature prominently among numerous edible plants common to the Yoruba culture (Bankole *et al.*, 2003). The edible fleshy mucilaginous leaves and stems are eaten as vegetable and as medicine for several different ailments (Grubben and Denton, 2004; Fowomola and Akindahunsi, 2005). The leaf sap of *C. crepidioides* is used to treat stomach upset and fresh wounds (Tindall, 1983). A decoction of the leaves of *Crassocephalum* species is used as lotion for headache and as a mild stomachic. Leaf extract of *C. crepidioides* showed moderate antimutagenic activity in *Salmonella typhinurium* and produces dihydroisocoumarins that has shown antimalarial activities against *Plasmodium falciparum* (Schippers, 2000).

Several studies and reviews have been carried out on the size, structure, and fertility of pollen grains of species in the family Asteraceae (Jones, 1979; Hodalova and Martonfi, 1995; Ayodele, 1999; Otieno and Mesfin 1984; Nair and Lawrence, 1985; Moore and Webb, 1978). The usefulness of pollen size, shape, and structure as specific characteristics in the

family Asteraceae has been the subject of these several studies. Clausen (1962) citing Eneroth (1951) demonstrated that it is possible to distinguish species in the family Asteraceae on the basis of pollen size in spite of some overlap of the species in this respect. Similarly, Oschurkova (1959) who studied 16 Russian species reported that many of them could be separated by the same criterion. Leopold (1956) reported that it was difficult to distinguish the 9 birch species native of New England on pollen size alone, but concluded that differences in pollen diameter could also be used to separate the shrubby birch species. Kapp (1969) warned that size alone could not be used as a parameter to separate pollen grains. Jones (1981) suggested that other pollen characters in addition to pollen size be used in species separation. Scientific reports on the pollen grains of the Nigerian species of the *Crassocephalum* are not available. The pollen size, structure and fertility in the genus *Crassocephalum* therefore, require some attention.

The objective of this study is to provide detailed description of the pollen grains of some four Nigerian species of *Crassocephalum* with reference to their pollen size, structure and fertility. The data obtained would be used to draw conclusions on the pollen of the *Crassocephalum rubens* (Juss.ex.Jacq) S. Moore, *C. crepidioides* (Benth.) S. Moore, *C. biafrae* (Oliv. & Hiern) S. Moore *and C. togoense* C.D. Adams.

MATERIALS AND METHODS

Achenes were collected from field populations of *Crassocephalum* during series of field trips covering different ecological locations in southwestern Nigeria (Table 1) between January 2006 and December 2007. 23 accessions collected from different populations were labelled with different accession numbers. Specimen from these populations had their identities confirmed using Herbarium specimens at the Forestry Research Institute of Nigeria (FRIN) and Obafemi Awolowo University Ile-Ife, Nigeria. Ten to twenty achenes of each accession were planted in sterilized loamy soil contained in 10-litre plastic buckets-five buckets per accession. They were arranged 1m apart in four rows at the Department of Biological Sciences experimental garden, University of Agriculture, Abeokuta, Ogun State, Nigeria. The germinated seedlings were nurtured till flowering. Slides for pollen studies were prepared by dusting pollen grains from the opened flowers in a drop of cotton blue in Lactophenol on a clean glass slide and covered with a cover slip. Five slides from five different flowers collected from different plants of the same accession were prepared for each accession. Pollen fertility was estimated by counting pollen grains from ten fields on each of the five slides prepared for each species at X100 magnification. Pollen grains with cytoplasmic content stained deep blue were considered fertile while those that were not stained or only partially stained or with collapsed outline were considered sterile.

Table 1: Location & collection data of field populations of *C. crepidioides*, *C. rubens*, *C. biafrae* & *C. togoense*

Name & accession number of species	Location of collection	Description of location
C. crepidioides (Benth.) S. Moore AS/010,012,015,018,& 022	Routes: Osiele village, along Ibadan-Abeokuta road, No. 21, Gbada Salami Street, Idimu Lagos, Adodo village, Igbeti/Ogbomoso, Aguo village Ibadan-Oyo road, University of Ado-Ekiti campus.	Guinea & Derived savannah in yam/Maize farmland, vegetable garden, waste place, roadside
C. rubens (Juss. Ex Jacq.) S. Moore (purple floret) AS/001, 004, 005, 009, 014, & 017	Routes: Guguru village along Igbeti/Ogbomoso Adodo village, near Ogbomoso, Obantoko/ Adatan,Asero, Abeokuta, Okuku, along Ikirun/Ilorin road. Guguru village, near Ogbomoso/Igbeti, roadside, besides local government secretariat, Epe.	Derived & Guinea savannah in yam/maize farm, road side, yam/ pepper farmland, cleared land
C. rubens (Juss. Ex Jacq.) S. Moore (light purple floret) AS/002, 003, 006, 007 & 016	Routes: Telemu village along Iwo-Osogbo road, Gurugu village, along Ogbomoso/Igbeti, Aramoko -Ado-Ekiti road. Joju village, Sango-Otta	Guinea & Derived savannah in yam farmland, waste place by the roadside, abandoned plot of land, uncultivated burnt forest
C. rubens (Juss. Ex. Jacq.) S. Moore (white floret) AS/013, 019 & 020	Routes: Igbeti township, Oru/ Ijebu Igbo road. Ibadan/ Abeokuta; Bakatari Township	Guinea & Derived savannah in Yam farmland ,waste place road-side in dilapidated building
C. biafrae (Olive. Hiern) S. Moore AS/008 & 011	Routes: Fiditi along Oyo-Ibadan road. , Moniya	Derived Savannah with loamy soil; Cocoa plantation.
C. togoense C.D. Adams AS/021 & 023	Routes: Apomu Ikire; Eleekara, via Idi-Igba, Ilora, Ibadan -Oyo road.	Derived Savannah with sandy loamy soil; abandoned farmland and weedy roadsides.

Pollen fertility percentages for each species were calculated by expressing the number of fertile pollen grains as a percentage of the total pollen grains counted.

$$\text{i.e. \% pollen fertility} = \frac{\text{Stained pollens}}{\text{Total pollens counted}} \times 100$$

Pollen size was determined by measuring the polar and equatorial diameter of fifty full and deeply stained randomly selected pollen grains from ten field of view on the five slides prepared for each accession at X400 magnification using ocular micrometer and variations were noted (Brookes and Thomas, 1967). The ocular measurements were later converted to microns using stage micrometer. Mean and standard deviations were calculated for the measurements. Statistically analysis of the data was done, employing analysis of variance and coefficient of variation. The coefficient of variation (CV) for the pollen sizes was also computed to compare the variation in pollen sizes within each of the species in the genus. Photomicrographs of pollen grains stained with cotton blue in Lactophenol were taken at X 400 magnification to show the fertile and sterile pollen grains and the pollen structure.

RESULTS AND DISCUSSION

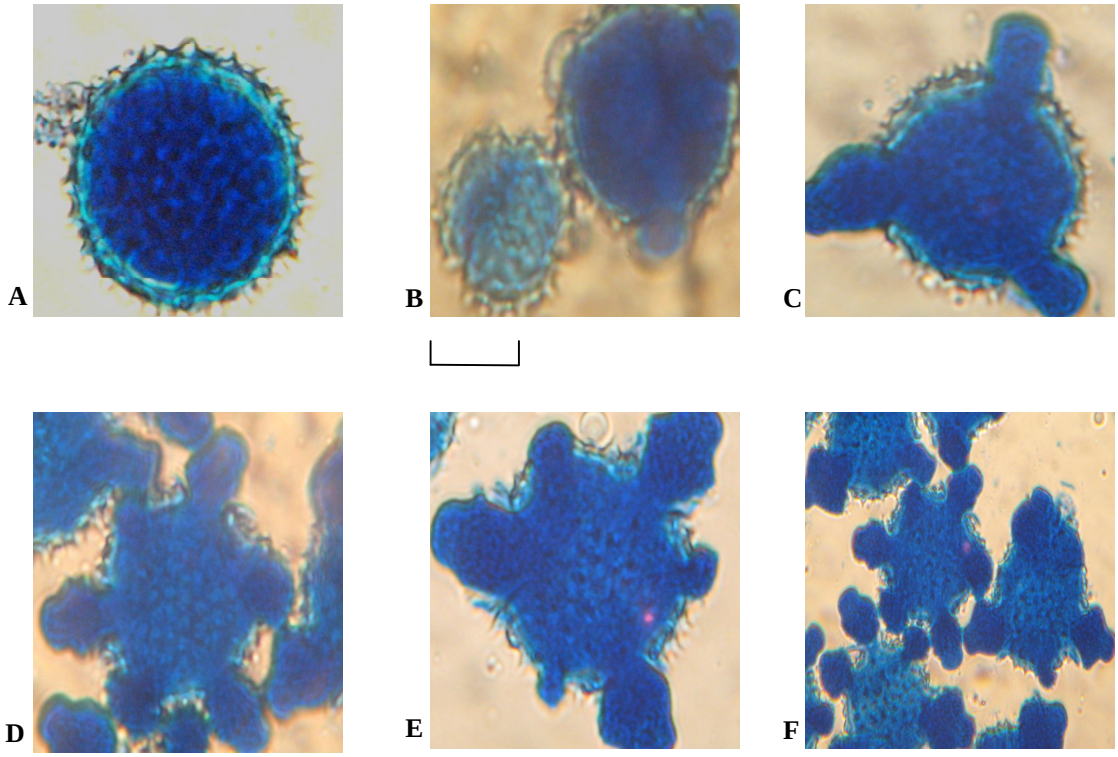
Little or no variations in size were observed in the pollen grains of four species investigated (Table 2).

Photomicrographs of pollen grains for the species studied were recorded (Fig. 1). All the species have fenestrate pollen grains as described by Moore and Webb (1978). These were single grains with a coarse network of high echinate ridges (pointed projecting spinous elements) separated by large spaces (lacunae) in a fixed geometrical pattern. This type of pollen grains is characteristic of the family Asteraceae (Moore and Webb, 1978). The species studied have asteroid fundamental pattern with caveate exine carrying spines with a bulbous base and aperturate form of pollen grains similar to most pollen grains in the family Asteraceae (Mesfin, 1984; Nair and Lawrence, 1985; Otieno and Mesfin, 1992). Pollen grains in *C. rubens, C. biafrae* and *C. togoense* were tricolporate with three germ pores. This is similar to the report of Hodalova and Martonfi, (1995) on *Senecio nemorensis* with the presence of three germ pores also in Asteraceae family. *C. crepidioides* was multicolporate with up to six germ pores (Fig. 1).

Table 2: Data on Pollen Characters of *C. crepidioides*, *C. rubens*, *C. biafrae* & *C. togoense*

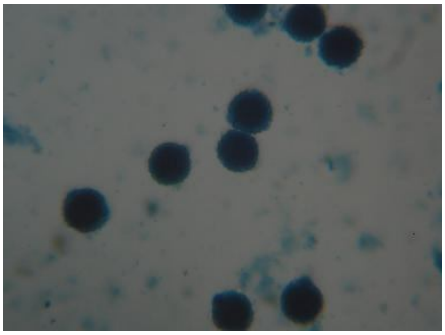
Species	Collection Number	*Number of pollen counted	Pollen fertility (%)	Pollen size Mean ± S.D (μm)	Coefficient of variation	Number of germ pores
C. rubens (white florets)	AS/013	719	97.36	23.85±1.56	6.54	3
	AS/019	625	98.78	25.10±1.75	6.97	3
	AS/020	588	88.97	25.47±1.81	7.11	3
C. rubens (purple florets)	AS/001	528	95.08	24.74±1.52	6.14	3
	AS/004	669	95.81	24.76±1.58	6.38	3
	AS/005	586	95.90	25.20±1.68	6.67	3
	AS/009	708	96.47	25.10±1.61	6.41	3
	AS/014	477	98.01	25.33±1.73	6.83	3
	AS/017	907	97.13	25.05±1.58	6.31	3
C. rubens (light purple fl.)	AS/002	822	97.81	24.87±1.66	6.67	3
	AS/003	536	97.20	25.02±1.53	6.11	3
	AS/006	576	96.35	24.78±1.47	5.93	3
	AS/007	209	85.17	20.36±1.60	7.86	3
	AS/016	726	98.07	26.69±1.28	6.80	3
C. crepidioides	AS/010	1112	87.50	24.12±1.61	6.67	3,4
	AS/012	1030	96.99	25.18±1.74	6.91	3,4,5,6
	AS/015	957	84.43	27.01±1.86	6.89	3,4
	AS/018	792	96.72	24.88±1.70	6.83	3,4
	AS/022	924	97.51	28.10±1.67	5.94	3,4
C. togoense	AS/021	887	97.18	24.70±1.64	6.64	3
	AS/023	709	96.33	26.40±1.71	6.48	3
C. biafrae	AS/008	589	87.50	22.53±1.56	6.93	3
	AS/011	895	92.80	20.50±1.68	7.20	3

*=Number on which fertility estimate was based.
Mean and standard deviation values were based on 50 pollen diameter measurements



Scale line represents 25μm

Figure 1: Fertile and Sterile Pollen Grains of *Crassocephalum* Species Studied. Fertile Pollen Grains Stained Deep Blue
A Pollen structure in *C. togoense* showing caveate exine with spines
B. Fertile and sterile pollen grains in C. *biafrae*
C. 3 germ pores in *C. rubens*
D. E, and F. up to 6 germ pores in *C. crepidioides*



G. Some Fertile Pollen Grains in *C. rubens*

Figure 1: Fertile and Sterile pollen grains of *Crassocephalum* species studied. Fertile pollen grains stained deep blue

Pollen size in *C. rubens* varied between 20.36 -26.69μm (media) while it was between 24.70- 26.40μm (media) in *C. togoense*. *C. biafrae* recorded the lowest pollen size (20.00 -22.53 μm) (minuta) (Erdtman, 1952). Pollen fertility was generally high in all the species, highest recorded (98.78%) in *C. rubens*. This seems associable with their potentials for self fertility. Variations observed in pollen fertility and size among the species collected either within the same area or those collected from different areas of southwestern Nigeria were not statistically significant (Table 2) ($P > 0.05$).

These observations correspond with the earlier report on the pollen size of the genus *Vernonia* (Ayodele, 1999) in the family Asteraceae.

The occurrence of up to six germ pores as observed in *C. crepidioides* in this study is significant, since such observation has not been reported in the genus *Crassocephalum*. Polyploidy may be a reasonable suggestion for the presence of six germ pores. The speculations on the polyploidy status of *C. crepidioides* had earlier been reported through chromosome counts of 2n=40 (n=20), while 2n=20 (n=10) for diploids by Olorode (1974) and Olorode and Okoli (1978). *C. crepidioides* with multiporate germ pores probably belongs to a race of higher ploidy than the other diploid species with tricolporate germ pores.

From the observations made, the following conclusions can be drawn: no taxonomically significant differences ($P< 0.05$) between the species studied for size and fertility of pollen grains were found except in *C. crepidioides* with up to six germ pores. This study has however, shown that pollen size alone cannot be used to differentiate the diploid species from polyloid species. It may of course be possible to separate the four species on the basis of the number of germ pores on their pollen wall apart from the size variation, as Jones (1981) has suggested that other pollen characters in addition to pollen size be used in species separation. The study suggests that the number of germ pores on the pollen of the species be considered as a useful tool in plant speciation and in cytotaxonomic grouping of plants either at generic or specific level.

ACKNOWLEDGEMENT

Professor D.A. Agboola is appreciated for finacial support rendered during the course of this study and Dr. A.E. Adegbite for taken me to different ecological locations in Nigeria. Mr Bamiduro's role of watering the plants anytime I was away on field trip is well apprecitaed.

REFERENCES

Ayodele, M.S. 1999. Size variations in pollen of *Vernonia* Schreb. (Asteraceae) of different plant growth (Habits) forms. *Comp. Newsletter,* 26: 4-11.

Bankole, M.O., Ayodele, M.S., Adejumo, O.T. 2003. The antimicrobial effects of some Asteraceae commonly eaten as vegetables in Southwest Nigeria on some enteric pathogens.*Comp. Newsletter,* 40: 56-61.

Brookes, D., Thomas, K.W. 1967. The distribution of pollen grains on microscope. Slides part 1.The non randomness of the distribution. *Pollen et spores,* 9: 621-629.

Clausen, K.E. 1962. Size variations in pollen of three taxa of *Betula* (1). *Pollen et spores*, 4: 169-174.

Erdtman, G. 1952. *Pollen morphology and plant taxonomy*. Chronical Botanica Co., Walthman, Mass, U.S.A.

Fowomola, M.A., Akindahunsi, A.A. 2005. Protein quality indices of sand box (*Hura crepitans*) seed. *J. Fd. Agri. Environ.* 3: 16-19.

Grubben, G.J.H., Denton, O.A. 2004. Vegetables. In: Plant Resources of Tropical Africa: 8-25 PROTA, Foundation Leiden, CTA, Wageningen.

Hodalova, I., Martonfi, P. 1995. Pollen morphology in the *Senecio nemorensis* group (Compositae) from the Carpathians. *Comp. Newsletter,* 26: 61-70.

Jones, S.B. 1981. Synoptic classification and pollen morphology of *Vernonia* (Compositae: Vernonieae). *Rhodora,* 83: 59-75.

Kapp, R.O. 1969. Pollen and Spores. 249 Wm.C. Brown Company Publishers, Dubuque, Iowa.

Leopold, B.E. 1956. Pollen size frequency in New England species of the genus *Betula*. *Grana Palynologica* 1: 140-147

Mesfin, J. 1984. The genus *Bedens* (Compositae) in Tropical Africa. *Symb. Bot. Upsal*., 24(1): 1-38.

Moore, P.D., Webb, J.A. 1978. An Illustrated Guide to Pollen Analysis: 50-51. Hodder and Stoughton, London.

Nair, P.K.K., Lawrence, R. 1985. Advances in pollen-spore Research: 145-235. Scholar publishing House, B-707 Sector-C.Mahanagar, Lucknow, India.

Olorode, O. 1974. Chromosome numbers in Nigerian Compositae. *Bot.J. Linn. Soc.,* 68: 329-335.

Olorode, O., Okoli, B.B. 1978. Cytogenetic studies in some Nigerian species of *Crassocephalum.* (Senecioneae- Compositae). *Niger. J. Genet*., 2: 81-90.

Oschurkova, M.V. 1959. The description of pollen of different species of *Betula* occurring in the USSR. (In Russia). *Problemy Botanik*a., 4: 68-91.

Otieno, D.F., Mesfin, T. 1992. Pollen morphological studies in *Senecio* (Compositae-Senecioneae) from Ethiopia. *Comp. Newsletter*, 20/21: 22-28.

Schippers, R.R. 2000. *African Indigenous Vegetables. An Overview of the Cultivated Species*. Natural Resources Institute, Chatham, U.K., NRI, ACP-EU Technical Centre for Agricultural and Rural Corporation 214Pp.

Tindall, H.D. 1983. *Vegetables in the Tropics*. Macmillan Education Ltd Houndmills, Basingstoke, Hampshire RG 212xs.Printed in Hong Kong. Pp 84-86.

(Manuscript received: 25th May, 2011; accepted: 27th June, 2012).