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POLLEN FERTILITY AND KARYOTYPING STUDY OF *Terminalia catappa* And *Terminalia mantaly*

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ABSTRACT

The present study investigates pollen fertility, chromosomes number and karyomorphology of T. catappa and T. mantaly collected from Humid Forest Research Station, Umuahia Nigeria. The study was cytologically carried out using acto-orcein for pollen fertility percentage and somatic chromosome determination. The aim was to compare the pollen fertility and karyotypes of the two species. Pollen fertility was based on stainability test. The pollen fertility percentage mean obtained in T. catappa was 87.30 %, in T. mantaly, the pollen fertility percentage mean obtained was 73.80 %. Somatic chromosome number determined for the two species was 2n = 24, haploid chromosome number was n = 12. In karyotype analysis, chromosome length was between 3.18 to 3.26 µm. Two types of chromosome centromere were observed; metacentric (m) and sub-metacentric (sm) with the karyotype formula 5m+7sm in T. catappa and 9m+3sm in T. mantaly. This study has highlighted the differences in pollen fertility and karyomorpology of the two species investigated. Findings from this study can be applied in plant breeding and conservation programme.

Keywords: *Terminalia* species, pollen fertility, chromosome number, karyomorphology

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INTRODUCTION

The Genus *Terminalia* Linn. belongs to the family Combretaceae with 250 species. It is a predominantly tropical genus (Fan, 2015). About 25 species are present in West Africa (Hutchinson and Dalziel., 1954). *Terminalia* is a Latin word, and means 'leaves being borne in tufts.'' *Terminalia* have revealed a variety of chemical constituents such as tannins, flavoniods, terpenoids among others. Numerous biological activities have validated the use of this genus in treatment of various diseases in traditional medicine (Fahmy *et al*., 2015). According to Keay *et al*. (1964), Nigerian species of *Terminalia* include *T. superba, T. ivorensis, T. macroptera, T. laxiflora, T. mollis, T. avicennoides, T. schimperiana* (*glaucescens*) and *T. brownii*. The introduced species include *T. catappa, T. bellerica* and *T. mantaly*.

Pollen, which is a carrier of male gametes, includes three domains that are different in their chemical composition, morphology structure and their physiological and biological significance (Knox, 1984). These three domains of pollen-grain include exine, entine and nucleus. The complex exine structures of pollen are storage site for carbohydrates, glycoproteins, lipids, terpernoids and phenolics (Wiermam and Gubatx, 1992). The pollen nucleus is rich in chromatin materials and viable pollen stains pink to deep red with acetocarmine, while sterile (mostly shrived) pollen does not take any stain and thus remains almost white and transparent (Marutani *et al*., 1993). A viable or fertile pollen is one which after landing on the stigma of the same plant or other plants of the same variety or species, under normal conditions would start growing a pollen tube and finally discharges its

male gametes in embryo sac effecting fertilization (Ettore and Rudy, 2019; Yin-Long *et al*., 2012). Pollen fertility is the ability of the pollen to perform its functions of delivering male gametes to embryo sac. Pollen is a critical stage in the life cycle of the plants as fertile pollen is the crucial for sexual plant reproduction (Pushpa, 2021).

Pollen fertility, which can be determined using pollen viability tests in-*vitro* is very important in fruit and seed production in flowering plants. Therefore, the pollen fertility knowledge for any plant species is essential for plant breeders and commercial growers. Karyomorphological study provides evolutionary characteristics of karyotypes, as well as the cytological mechanisms. It is a fast and inexpensive approach to classify plant species by identifying the basic cytological parameters of a species, including chromosome number, ploidy level, karyotype asymmetry, and karyotype coefficient (Guerra, 2008). Chromosomes number and karyotype of a species are stable characteristics which can reflect its basic genetic information. The genus *Terminalia* become an interesting model for studying a plant karyotype evolution due to variation in their chromosome number. Ohri (1996) reported that *T. oliveri*, *T. myricarpa*, and *T. arjuna* are diploids (2n =24), *T. muelleri* shows triploid number (2n =36) and *T. bellirica* shows tetraploid (2n =48). The constancy and usefulness of the karyotype arises from the fact that, at a given stage of cell division and in a given tissue, each cell of an organism has a constant number of chromosomes of reasonably definite volume, length, and shape (Adedeji and Faluyi, 2003). Variation or constancy in the chromosome number, within taxa of different categories, proved to be important characters for taxonomic groupings. The pollen fertility status and karyomorphological studies on *Terminalia* species in Nigeria are scant. This present study was undertaken to study pollen fertility and karyotyping of *Terminalia catappa* and *T. mantaly* using aceto orcein staining.

MATERIALS AND METHODS

Collection of plant material (flower buds)

Terminalia species flower buds were collected from plants established in the field at Humid Forest Research Station, Umuahia, Nigeria. Flower buds were harvested at regular intervals (30 min) between 6 am -9 am and fixed directly in freshly prepared Carnoy's solution.

Fixation of flower buds

The fixative Carnoy's solution was prepared by mixing glacial acetic acid and ethyl alcohol in the ratio of 1: 3 V/V, the harvested flower buds were fixed for 24 hours and preserved in 95% alcohol in a refrigerator at about -4 °C.

Pollen fertility test and percentage pollen fertility

Some pollen grains from the mature anthers of *T. catappa* and *T. mantaly* flower buds were dusted on a slide and a drop of methyl blue stain was added to it. Then a cover slip was placed on the slide, and the excess stains were bottled out using a filter paper, then the edges of the slide was sealed with nail polish. The stained pollens were observed under the microscope using X4, X10 and X40 for better observation. Fertile or viable pollen grains were those that absorb the stains, that is, the stains got deeply into the cytoplasm, while other pollens were either partially stained or not stained at all. The number of the stained pollen were counted and also the number of the unstained or partially stained pollen were counted. The total number of pollens on the slide was also counted. The percentage pollen fertility/viability was calculated using the formula below.

$$PPV = (NSP/TNP) \times 100 \ldots\ldots (1)$$

Where:
PPV - Percentage pollen viability,
NSP - Number of stained pollens
TNP - Total number of pollens

Statistical Analysis

The data from the study of the two species from three replicates of the pollen fertility were subjected to statistical analysis using chi square mean method to generated the mean value of the average pollen fertility of the two species.

Karyotype analysis and study of *Terminalia catapa* and *T. mantaly*

Harvesting

Young healthy roots of the two *Terminalia* species (about 15 mm) were carefully collected at

two-hour interval from 7:00 and 9:00am after two of germination for the genotypes. Clean sterilized scapel was used to excise the growing root tips of the two *Terminalia* species.

Pretreatment
The harvested root tips of genotypes were rinsed twice in distilled water and pretreated in 0.002 ml solution of 8-hydroxylquinoline (0.058g dissolved in 100 ml of distilled water for four (4) hours.

Fixation
The pretreated root tips were rinsed twice in distilled water and fixed in 3:1 glacial acetic alcohol (3-part glacial acetic, to 1 parts ethanol). The fixative was freshly prepared for use and the root tips were fixed at room temperature for 24 hours, after which they were stored in 70 % ethanol for further use.

Hydrolysis
The fixed root tips were again rinsed twice in distilled water then hydrolyzed in water bath and controlled at 60 °C for six (6) minutes.

Storage
The root tips were stored in 70 % ethanol solution, and preserved in a refrigerator until it was needed for squashing.

Squashing and Staining
The hydrolyzed root tips were rinsed twice in distilled water, placed on a clean grease free slides (one root tip per slide) and excess fluid was removed using drying paper. The apical 1mm (whiter and denser) portion of the root tips were carefully cut off on the slide. One to two drops of 1 % aceto-orcein was added to the specimen and the material was macerated thoroughly. A thin cover slip was laid on top of the specimen and the slide was placed in a folded filter paper, and thumb pressure was applied to remove excess stain.

The cover slip was tapped gently with the blunt end of a biro. Tapping continued until the materials became well spread out and hardly visible. The corner slip was sealed with nail vanish and then viewed under the low and high power digital AMSCOPE 3000 camera microscope.

Viewing of the Slides
The slides were placed on the stage of the microscope and then adjusted for proper and clearer views at various magnifications; Photomicrographs of the cells at various stages of mitosis were taken at x4 x10, x40 and at x100 with oil immersion.

All measurements were recorded using the Image Pro Plus software and measurement options. Chromosomal morphology was described using nomenclatures proposed by Levan *et al*., 1994 while numerical characterization was done using other parameters.

Karyotyping analysis of *T. catappa* and *T. mantaly*.
Roots of newly germinated seeds (5-10 days) were collected and exposed to relevant cytological treatment. *Terminalia* species cells with well spread metaphase chromosomes revealing distinct morphology were carefully observed and selected for the karyotyping study. Measurement of long and short arm lengths were done in triplicates for each and from which the standard errors were computed. The selected chromosomes were further pretreated with saturated aqueous solution of monobromonapthalele. After soaking in Iron-alum mordanting solution and followed by hematoxyline treatment method to obtain a diploid number of 2n = 24 for the 2 species.

Statistical analysis of data
The data generated from the study on short arm length, long arm length, total length, arm ratio, R-value and centrometric index was analysis using the Image Pro- Plus software procedure and tested with the Minitab 17, 2018.

RESULTS
Pollen fertility results obtained are presented in Table 1 and Plate (1-2). Pollen fertility percentage obtained was between 75.56 to 97.60 % in *T. catappa* with average mean of 87.30%. (figure 1) In *T. mantaly*, the pollen fertility percentage obtained ranged from 67.30 to 85.71 % with average mean pollen fertility percentage of 73.80. The results showed that the pollen fertility percentage of the two species of *Terminalia* is above 50 %. (Figure 2).

Table 1: Viability of Pollen fertility percentage of *T. catappa* and *T. mantaly*.

Species	Pollen Fertility range	Mean Fertility Percentage
T. catappa	75.56 - 97.60	87.30±4.9
T. mantaly	67.30 - 85.71	73.80±3.9

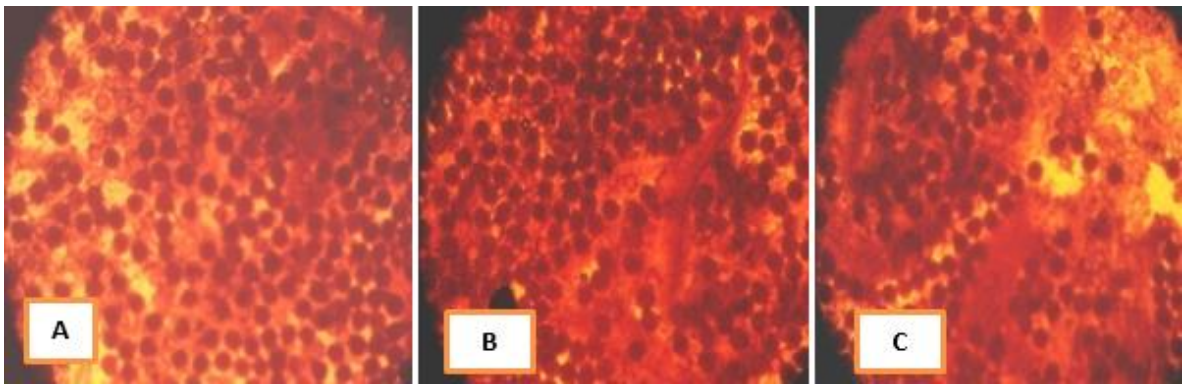

Plate1: Pollen mother cells for *T. catappa* in Triplicates A, B and C

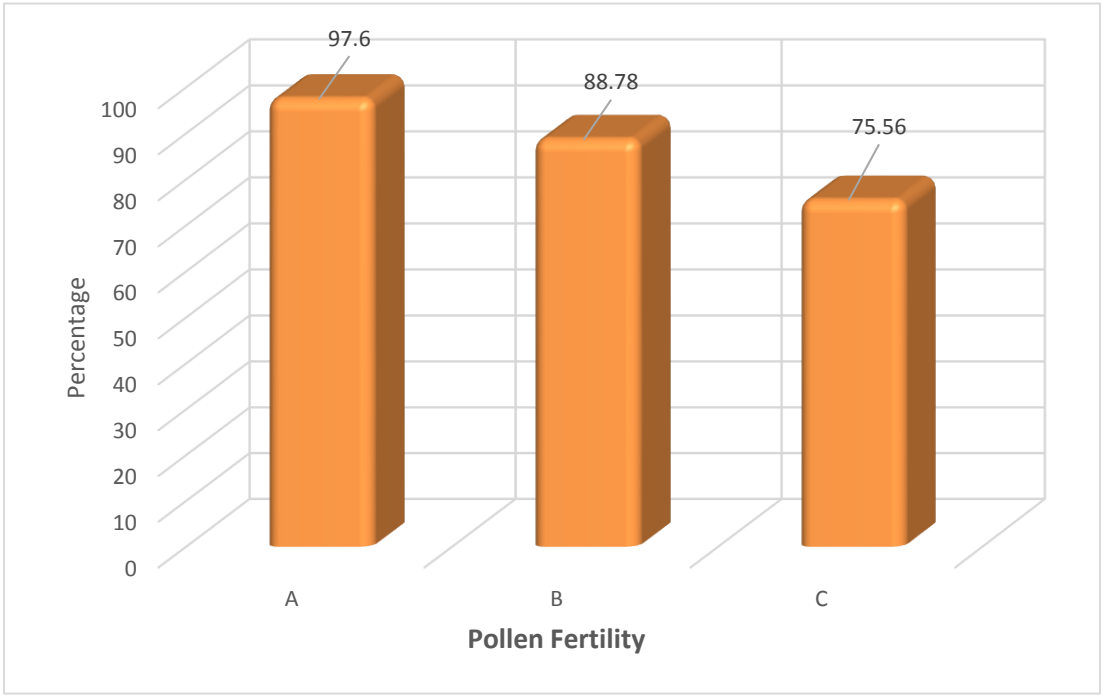

Figure 1: Percentage Pollen mother cells for *T. catappa* in Triplicates A, B and C

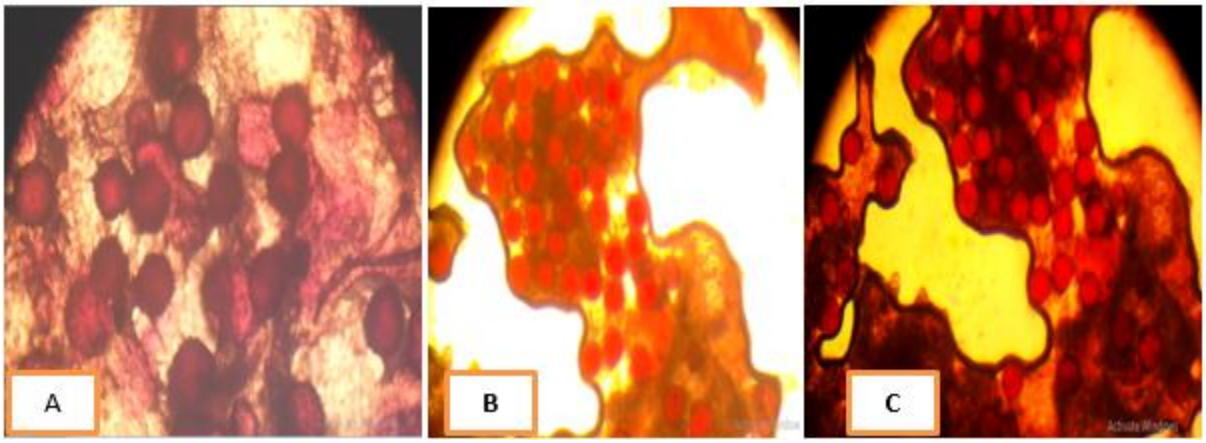

Plate 2: Pollen mother cells for *T. mantaly* in Triplicates A, B and C

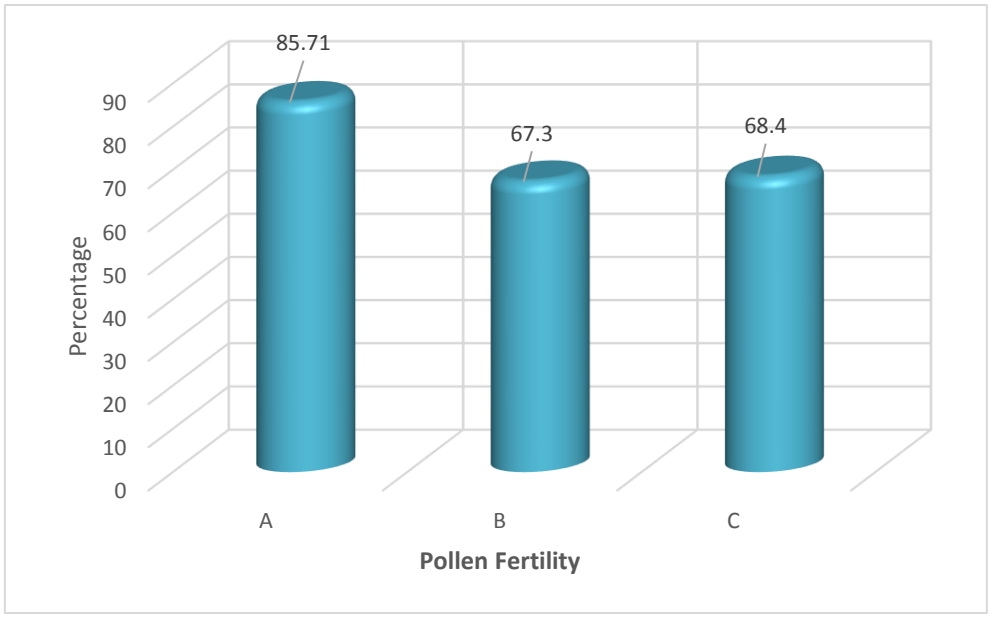

Figure 2: Percentage Pollen mother cells for *T. mantaly* in Triplicates A, B and C

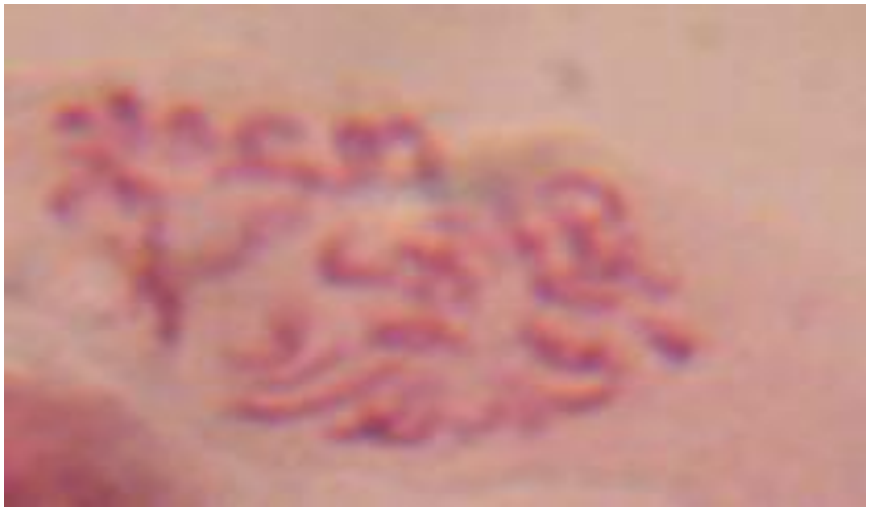

Plate 3: Chromosomes of *T. catappa* showing diploid chromosomes 2n = 24

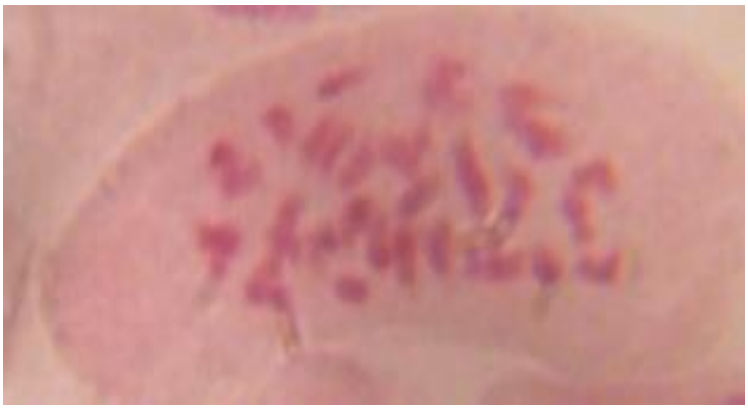

Plate 4: Chromosome of *T. mantaly* showing diploid chromosomes 2n = 24

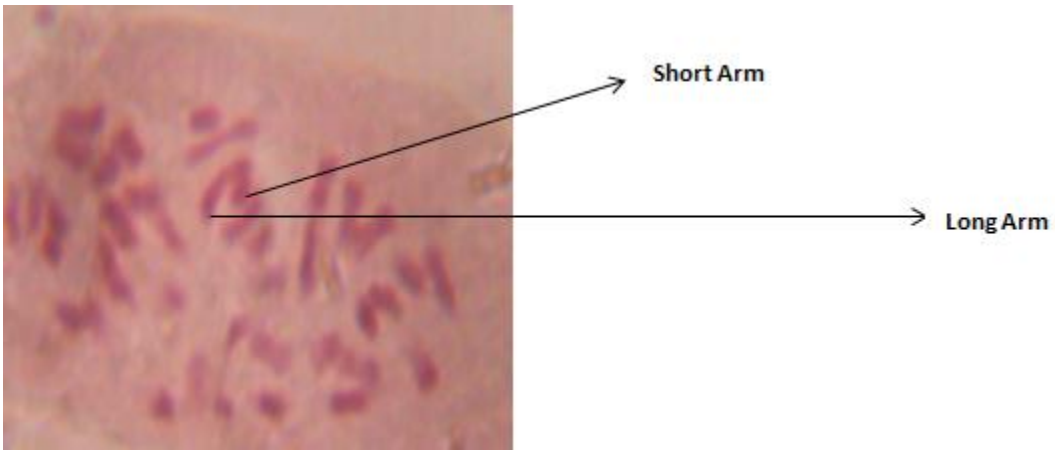

Plate 5: Chromosome with short and long arm.

Table 2: Chromosome parameter of two species of *Terminalia*

Terminalia Species	Short arm (S) µm	Long arm (L) µm	Total length (S+L) µm	Arm Ratio (L/S) µm	R- Value (S/L) µm	Centrometric index
T. catappa	1.30± 0.03	1.96±0.00	3.26± 0.03	1.51	0.66	1.96
T. mantaly	1.28± 0.01	1.90±0.03	3.18± 0.03	1.48	0.67	1.95

Karyotype analysis results

Plate 3-4 show the mitotic metaphase chromosomes of *T. catappa,* and *T. mantaly* respectively. The somatic chromosome number of the two species (*T. catappa and T. mantaly*) is 2n = 24. The karyotypes composition of the two species of *Terminalia* investigated were shown in Figure 1-2. The two of them had no secondary constriction. Table 2 depicts the basic chromosomes parameters. The chromosomes of the two species investigated were medium in size. The longest chromosome length was observed in *T. catappa* with a total length of 3.26µm while *T. mantaly* had a total length of 3.18µm. The chromosome length between the two species are comparable/similar. The chromosome arm length ratio (L/S µm) were between 1.48 to 1.51 µm. The highest arm length ratio was recorded in *T. catappa* while the shortest arm length ratio was observed in *T. mantaly* with the values of 1.51 and 1.48 µm respectively.

Based on the position of centromere, none of the species was found being diffused (holocentric or holokinetic). But they were localized (monokinetic). Two major position of centromere were observed among the pairs of the

chromosomes studied- metacentric and submetacentric chromosomes.

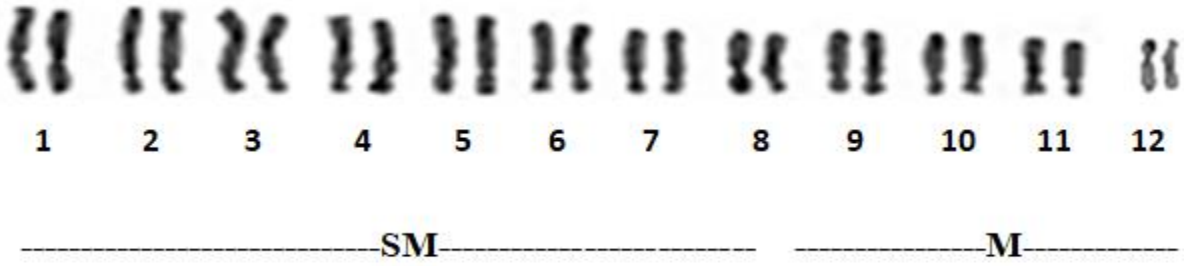

Figure 3: Chromosome composition of *T. cattapa.*

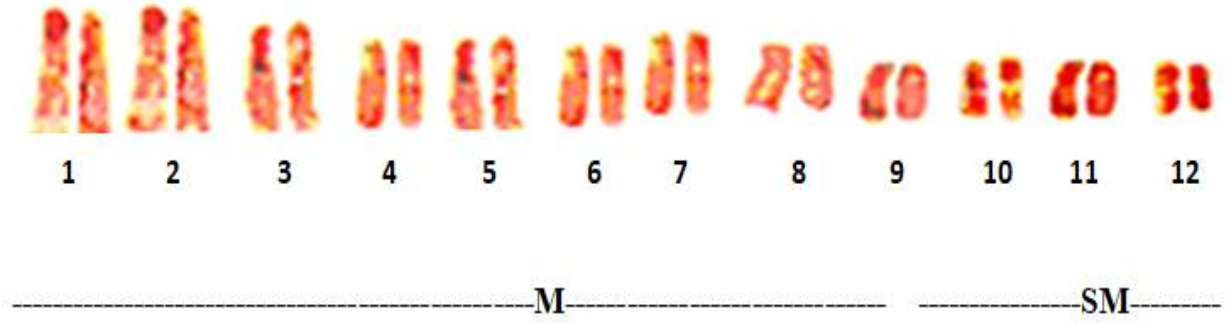

Figure 4: Chromosome composition of *T. mantaly.*

DISCUSSION

The results of pollen fertility study in the present study gave PPF mean of 87.30 % and 73. 80 % in *T. catappa* and *T. mantaly* respectively. Despite the fact that the two species are in the same genus, they were displayed different pollen fertility value although the pollen fertility percentage displayed by the two species was above 50 %. This result is in agreement with the findings of Reijieli and Anand (2002), 'that the species within a single genus showed different fertility percentage". The pollen fertility percentage obtained in *T. catappa* was higher (87.30 %) compared to 73.80 % which was obtained in *T. mantaly*. The implication of pollen fertility status in the two species is that, the high pollen fertility they had would make them to have enough sexual reproduction which would ensure the survival of the two species.

The karyotypes of several species have been established based on chromosome size and centromeric index in addition to the traditional process for karyotyping by adding a dye to metaphase chromosomes (Samira *et al*., 2020). Chromosome features and their count have been recorded in cytological characterization of germplasm (Sharma and Sharma, 2013). The genus *Terminalia* become an interesting model for studying a plant karyotype evolution due to variation in their chromosome number. Ohri (1996) reported that *T. oliveri*, *T. myricarpa*, and *T. arjuna* are diploids (2n =24), *T. bellirica* are tetraploid (2n =48) and *T. muelleri* showed triploid number (2n =36). Variation or constancy in the chromosome number, within taxa of different categories, proved to be important characters for taxonomic groupings. In this study, *T. catappa*, and *T. mantaly* also showed diploid number (2n =24) with basic number X=12. These findings are in line with the report made by Valkenburg and Waluye, (1991) and Rojas-Sandoval (2017) about *T. catappa*. The result is also similar to the report of Jephris (2013) who worked on the family combretaceae. He reported that the genus *Terminalia* has haploid (n) chromosome number between 12 to 16. The chromosome number (2n =24) obtained in *T. catappa* and *T. mantaly* from this study is a proof that *T. oliveri*, *T. myricarpa*, *T. arjuna*, which was

earlier studied by Ohri (1996) are in the same category of chromosome number (diploid number).

The chromosome morphology of the two species investigated was purely monokinetic. The position of the centromere has been taken as the criterion for designating chromosomes in different categories. Based on the relative position of the centromere, chromosomes are described as metacentric, submetacentric, acrocentric and telocentric (Dipak, 2009). On the basis of the number of centromeres, in this study the chromosomes of the two species were monocentric. In other words, none of the species investigated showed secondary constriction and this can be used as one of their similarities. Based on the position of centromere, *T. catappa* had four pairs of chromosomes which were metacentric (m) and eight pairs of chromosomes which were submetacentric (sm) but *T. mantaly* had nine pair of chromosomes which were metacenric (m) with three pairs of chromosomes which were submetacentric (sm). The two are monocentric but there are variation position of their centromere.

CONCLUSION

The investigation carried out on the pollen viability/fertility of *T. catappa* and *T. mantaly* showed that the pollen fertility percentage of the two species is above 50 %, but the pollen fertility percentage in *T. catappa* is higher than that of *T. mantaly*. The karyotyping analysis revealed that the two species of *Terminalia* investigated are diploid species with basic number x =12. The number of the centromere in the chromosomes of the two species which was one, made them to be monocentric species with the variations in the position of their centromere. Further work is therefore recommended to use large numbers of the species of *Terminalia* in order to gather more information about pollen fertility and karyotyping of the species of *Terminalia* particularly in Nigeria.

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