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# Host Preference, Salt Balance and Molecular Characteristics of African Mistletoes in Selected Areas of Sokoto State, Nigeria

**Tenufa, M. H. [a], Maishanu, H. M. [a], Anka, S. A. [a*], Muhammad, A. S. [b] and Gumi, A. M. [a]**

[a] *Department of Plant Science, Faculty of Chemical & Life Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.*
[b] *Department of Crop Science, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Mistletoes are hemi parasitic plants that are widely distributed and parasitize host trees globally. The aim of this study was to identify African mistletoes using DNA markers, determine their host preferences and salt balance in selected communities of Usmanu Danfodiyo University, Sokoto, Nigeria. A total of 8communities were surveyed and the number of mistletoes on the host trees were counted and recorded. In addition, host characteristics such as canopy spread, tree height, water holding capacity and mistletoe-host nutrients ($N^+$, $K^+$, $Ca^{2+}$ and $P^+$ concentrations) equilibrium was determined. Molecular identification of collected mistletoe samples was done using DNA Barcoding with Rbcl targeted specific primers and sequence analysis using DNA Subway database. The results revealed that *Acacia nilotica* tree was highly infested by the mistletoe. Host canopy spread, basal area and height had no influence over mistletoe infestation. The

**Corresponding author: E-mail: suleaanka95@gmail.com;*

concentration of $N^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ and $P^+$ were found to be higher in the mistletoes compared to the host trees. Molecular identification of the collected mistletoes revealed close relationship with *Moquiniella rubra,* belonging to the family Loranthaceae. It can be concluded from the result that the mistletoes in the study areas are *Moquiniella rubra* and depend solely on the host trees for their nutrients requirement and survival.

*Keywords: Mistletoes; host preference; salt balance; African Mistletoes.*

## 1. INTRODUCTION

The word mistletoe is derived from two German words; *mist*- dung and *tang*- twig or stick [1]. Generally, mistletoes are hemi-parasitic flowering plants bearing ever green leaves that photosynthesize and depend on their host tree for water and mineral nutrients [2]. They are widely distributed in all major biomes and climate types, except in the extremely cold regions [3]. Mistletoes consist of more than 1400 species around the world belonging to the order Santalales [4], and evolved five times independently and are not monophyletic [5]. Recent molecular phylogenetic work by Mellado et al*.* [6] has greatly clarified our concepts of which members of Santalales are mistletoes and how they are related to one another. In Nigeria, six genera of Loranthaceae have been identified viz: *Tapinanthus*, *Agelanthus*, *Loranthus*, *Globemetulla*, *Phragmanthera* and *Englerina* [7].

Despite the large host range of the majority of parasitic plants, many also show high levels of host preference. Mistletoes host choice can be considerably influenced by relatively host abundance, host characteristics such as branch size, age, height and the duration of association between the host and the parasite (Didier et al., 2009). For centuries, mistletoes have been considered as pests that kill trees and devalue natural habitats on a particular region of the world. Researchers have revealed that Mistletoes are now all over the world causing damage to their host plants, posing serious threats to plantation by parasitizing cultivated plants, affecting host physiology leading to reduced growth, survival and reproduction of their host trees. Ibrahim and Ayodele [8] revealed that heavy infestation of African mistletoes on their host plant can kill the host plant completely. In West Africa, mistletoes are found on many trees of economic importance, such as Sheabutter tree (*Vitellaria paradoxa*), Neem tree (*Azadirachta indica* L.), Sweet orange (*Citrus sinensis* L.), Grape (*Citrus paradisi* L.), Cocoa (*Theobroma cacao* L.) and Rubber (*Hevea brasiliensis*) [9].

Many parasitic plants can simultaneously parasitize many host species as different host species may supply a parasite with different resources, a mixture of host species may be superior to a single host alone. Boussim et al*.* [10] reported that mistletoe (*Tapinanthus globiferus*) parasitizes 126 host species and believed that it is less specific compared to other mistletoe species. Despite the large host range of the majority of parasitic plants, many also show high levels of host preference. Mistletoes host choice can be considerably influenced with relatively host abundance, host characteristics such as branch size, height and the duration of association between the host and the parasite. African mistletoes make requisite mineral nutrients at the expense of their host tree by absorbing mineral salts directly from the vascular tissue of the host tree, resulting in salt imbalance and death of the distal branch of the host tree. As little is known about the host preference and molecular identification of African mistletoes in Nigeria, it is therefore imperative to identify the mistletoes in Sokoto state and reveal certain host characteristics which could influence mistletoes appearance and infection on their host tree. Though, mistletoes classification and nomenclature have become thorny issues in Nigeria, as most tribes generally refer to all mistletoes by a common local name, regardless of which family they belong to, little has been done on taxonomy and distribution of mistletoes in Nigeria [11]; (Ibrahim and Ayodele, 2011). This research aims to identify African mistletoes using DNA markers and determine the host preference and salt balance in selected communities of Usmanu Danfodiyo University, Sokoto-Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Description of the Study Area

The Study Area is situated in Sokoto State on latitudes $11^0$ 30 to $14^0$ 00'N, longitudes $4^0$ 00 to $6^0$ 40'E and altitude 351.0m above sea level, in the Sudan savanna ecological zone of Nigeria [12]. Soil of the area is sandy to sandy loam in texture. It is friable and easily cultivated with poor water holding capacity. The soil in the University area is young, lacking horizon development and is classified as entisol [13].

Climate of the area is semi-arid characterized by erratic and scanty rainfall during the usually short wet seasons (June to October). The area also experiences long dry seasons that consist of a period of dry winds and dusty harmattan (November to February) and a hot dry period (March to May). Precipitation data ranged between 458.19mm and 755.03 mm with mean annual precipitation of 585.15 mm [12]. Monthly temperature ranged between 17.02$^0$ C in January to 41.45$^0$ C in April. Monthly average temperature of the area ranged from 22.13$^0$C to 35.92$^0$C and annual average temperature ranged from 22.90 to 35.98$^0$C [12]. Relative humidity of the area ranged from 21 to 49% in dry seasons and from 51 to 79% in wet seasons [14]. The prevailing regional winds that usually bring rainfall to the area are the tropical maritime air masses coming usually coming from the south. The tropical continental air masses come from Sahara in the North; they are sometimes called hungry winds. The mean highest wind speed is usually in May with up to 182.25 m/s while the mean lowest wind speed occurs in October with 59 m/s [15]. The sparse distribution of trees and relative aridity of the region being closer to the Sahara Desert forms part of the primary motives for this study. In addition to that, the widespread distribution of mistletoes infestation on the fewer trees in the area is becoming worrisome. Hence the need for this study, so as to conserve the scarce forest reserve of the area particularly the native tree species, which are already threatened due to over exploitation.

### 2.2 Area Identification, Sites Selection and Area Demarcation

The study was conducted in selected communities in the main campus of Usmanu Danfodiyo University Sokoto. A total of 8 communities viz; Kwalkwalawa, Sayya-GidanGada, GidanYero, Shellan-Makera, VC's Quarters, Gidan Asarkei, Danrini and Gudan-Gebe communities were surveyed for presence of the mistletoes. Cursory survey was conducted to identify affected areas within the University communities where mistletoe infestation on tree species was prevalent. Purposive sampling technique was applied to identify the suitable locations where the infestation of mistletoe was occurring. The sampling was done according to the method adopted by Suria [16]. In each of the eight communities above, where this study was conducted, three study plots measuring 50m$^2$ were allocated. In an attempt to reduce encroachment and reduce variability between the study plots, a distance of 100m was maintained between the plots.

### 2.3 Sample Collection

At each study plot (50m$^2$), mistletoes were collected by random sampling technique. The collection was done by using a clean/sterilized sharp cutting knife, to avoid infection on the host trees. The shoots, fruits and suckers of the African mistletoes as well as the leaves and shoots of both parasitized and non-parasitized host plant were randomly handpicked and collected separately in a dark polythene bag. The samples were taken to the herbarium for identification and subsequent Laboratory analysis. The herbarium technique used followed the procedure adopted by Blenco [17]. Data collected from field were utilized for evaluation of mistletoe-host preference, mistletoe-host salt balance and molecular characteristics study of mistletoes identified.

### 2.4 Evaluation of Mistletoe-Host Preference

The level of infestation of the African mistletoes on host trees was determined by the percentage of infestation using the equation (i) below as described by Tizhe et al. [18].

(i) IP $= \left\{\frac{\mathbf{Q}}{\mathbf{M}}\right\} \times \mathbf{100}$ Where, Q = Number of infested plants; M = Total number of host plants observed; 100 = Constant value.

The canopy spread (S) of the host trees were computed using Axis method, by taking two measurements of the longest (E1) and shortest (E2) branches [19].

(ii) $S = \left(\frac{\mathbf{E1+E2}}{\mathbf{2}}\right)$

The heights of all the trees were measured using Haga Altimeter [20].

The Diameter at Breast Height (DBH) of each of the tree was measured at 4.5ft from the ground level using a measuring tape round the tree stem so as to get the circumference (C). After which the Basal Area (BA) of each tree was calculated using the formula below as described by Bob et al. [19].

(iii) **TBA =** $\frac{\Pi \times D^2}{4}$ Where Π = Pie, D = Diameter, 4 = Constant value, Hence the Circumference is given as $C = \Pi \times Diameter$. The water holding capacity (WHC) for each tree was calculated using the formula WHC = WM – DM [21]. Where WHC = water holding capacity, WM = wet mass and DM = dry mass.

## 2.5 Determination of Salt Balance

The concentration of sodium and potassium in the plant tissues were analyzed using flame photometer. Calcium and Magnesium were determined using EDTA and Phosphorus was determined using spectrophotometer following the method described in Taffouo et al. [22] and Dibong et al. [23].

## 2.6 Molecular Identification

The leaves and shoot of the African mistletoes were collected from various locations of the study area for identification using DNA bar cording techniques.

## 2.7 DNA Isolation

The fresh green leaves of the collected samples were weighed (~100 mg) and immediately used for DNA isolation using CTAB mini prep protocols. Leaf tissues were grinded to fine powder after freezing with liquid nitrogen in a pre-chilled mortar. The fine powdered tissues were then transferred to a tube of Pre-warmed CTAB buffer (2.0% CTAB (w/v); 0.1 M Tris Cl, pH 8; 0.02M of EDTA, pH 8; 1.4 M NaCl) and the mixture was incubated at 65°C for 20 min. Supernatant was collected after centrifugation and equal volume of Chloroform: Isoamyl alcohol (24:1) was mixed. After centrifugation, aqueous phase was collected, mixed with equal volume of isopropanol and incubated for 20 min at -20°C. Centrifugation was done to pellet down DNA. Pellet was washed with 70% (v/v) ethanol, air dried and dissolved in nuclease free water. The sample was treated with RNase enzyme at 37°C and subsequently purified by phenol-chloroform method (Sambrook and Russell, 2001). The concentration and quality of purified DNA were checked in Nano drop spectrophotometer (Thermo Scientific, USA) employing 260/280 and 260/230 ratio and also by 1% (w/v) agarose gel electrophoresis.

## 2.8 DNA Fragment Amplification Using RbcL Primers

The isolated DNA was used to amplify RbcL fragments of each sample using RbcL plant specific primers in Veriti 96 well Thermal cycler. The primers are:

rbcLaF-5′ATGTCACCACAAACAGAGACTAAAGC3′ and rbcLaR- 5′GTAAAATCAAGTCCACCRCG3′ [24,25].

The PCR conditions were 95°C for 1 minute, followed by 35 cycles of 95°C for 30second, 51°C for 30second and 68°C for 1 minute, followed by a final elongation step at 68°C for 5 minute.

## 2.9 DNA Amplification Using *Rbcl* Primers

The isolated DNA sequences were generated and analyzed using AB13730XL automated machine sequencer. The generated sequences were searched against reference sequences in the Barcode of Life Database (BOLD) using BLASTN approach and default parameters in order to reveal the ancestral relationship among mistletoes themselves and between mistletoes and their host.

# 3. RESULTS

The results showed that the percentage of infestation of the African mistletoes varies among the host trees in the study area with *Acacia nilotica* L. having the highest level of infestation of 15% while *Albizialebbeck* (L.) Benth, *Terminalia mantaly* L. and *Moringa oleifera* L. had the lowest level of infestation of 0.9% each (Table 1). In determining the mean canopy spread, it was observed that *Eucalyptus camaldulensis* had the highest canopy spread of 15.0m wide, followed by *Magnifera indica* with a canopy spread of 12.6m wide while *Adansonia digitata* had lowest canopy spread of 2.0m wide. The result of the plant height revealed that *Eucalyptus camaldulensis* had greater height of 15.1m followed by *Ceiba pentandra* with a height of 14.3m whereas *Acacia senegal* had the shortest height of 5.6m (Table 2).

The basal area of *Adansonia digitata* tree was the largest with 0.52 $\mathrm{m}^2$ while tree with the smallest basal area was *Acacia Senegal* with 0.002 $m^2$. The result of the stem bark Water Holding Capacity (WHC) revealed that *Acacia nilotica* shoot had the highest water holding capacity weighing 13.0g while *Magnifera indica* had the lowest WHC weight of 0.8g. The result of salt concentration revealed that $Na^+$, $K^+$, $Ca^+$, $Mg^+$ and $P^+$ varied in salt concentration between the hosts and the parasite (Table 2).

The concentration of $Na^+$, $Mg^+$, $Ca^+$ and $K^+$, were found to be high in the parasites compare to the host trees except the parasites of *Terminalia mantaly* and *Ceibapentandra* which had low concentration of $Ca^+$. The concentration of $Mg^+$ was also found to be low in the parasites compare to their host except the parasite of *Albizia lebbeck, Piliostigma reticulatum* and *Ficus gnaphalocarpa* that had high concentration of $Mg^+$. The concentration of $P^+$ was found to be low in all the species compared to the host trees (Table 3). Alignment of the samples' sequences in the Barcode of Life Data Base (Fig. 6) revealed close relationship with the sequence of *Moquinielarubra* chloroplast partial rbcL gene for ribulose bisphosphate carboxylase large subunit specimen with voucher RBGK 5742. The phylogenetic tree was built using the molecular sequences generated with MEGA (Fig. 5).

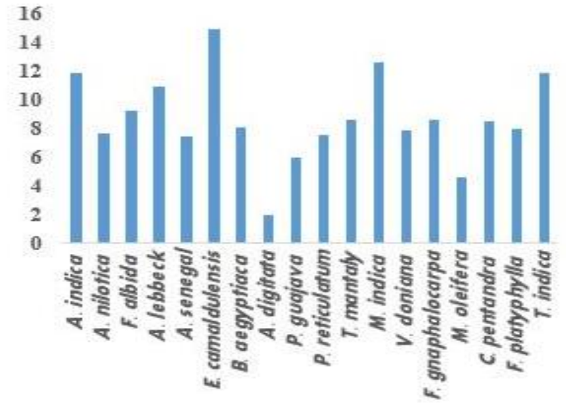


**Fig. 1. Mean canopy spread (m) of the selected trees in the study area**

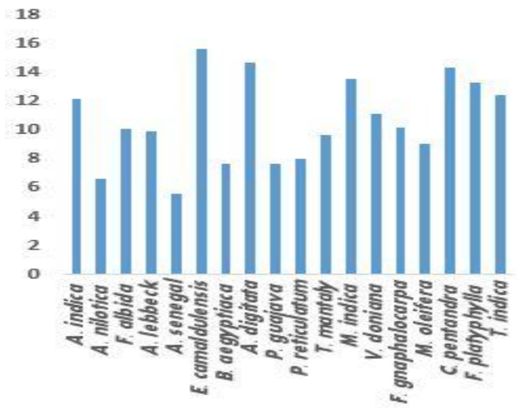


**Fig. 2. Mean Tree Height (m) of the selected trees in the study area**

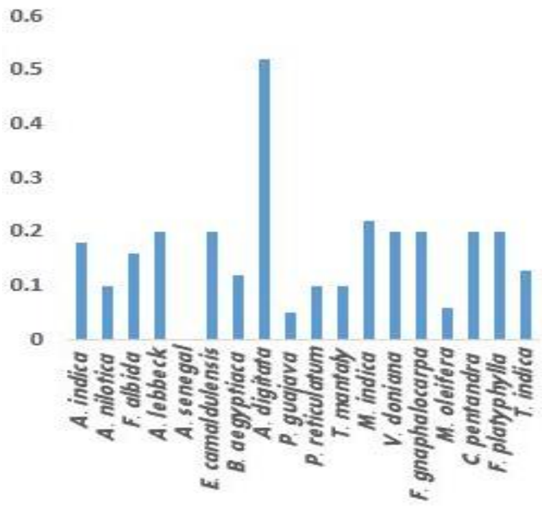


**Fig. 3. Mean Basal Area ($m^2$) of the selected trees in the study area**

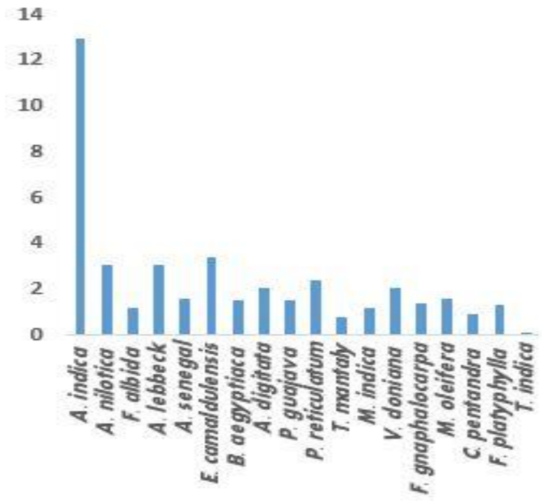


**Fig. 4. Water holding capacity (g) of each tree species in the study area**

**Table 1. Degree of infestation of African mistletoe on host trees**

| Trees surveyed | Degree of infestation | | | | |
|---|---|---|---|---|---|
| | **No of trees Surveyed** | **Number Of trees Infested** | **Percentage Of trees Infested (%)** | **No of trees Not infested** | **Percentage of trees Not infested (%)** |
| *Azadirachta indica* A. Juss. | 73 | - | - | 73 | 23.0 |
| *Acacia nilotica* L. | 67 | 48 | 15.0 | 19 | 5.9 |
| *Faidherbia albida* (Del.) | 22 | 16 | 5.0 | 6 | 1.9 |
| *Albizia lebbeck* (L.) Benth. | 4 | 3 | 0.9 | 1 | 0.3 |
| *Acacia senegal* (L.) Willd. | 2 | - | - | 2 | 0.6 |
| *Eucalyptus camaldulensis* Dehnh. | 10 | - | - | 10 | 3.1 |
| *Balanite aegyptiaca* L. | 35 | 19 | 5.9 | 16 | 5.0 |
| *Adansonia digitata* L. | 19 | - | - | 19 | 6.0 |
| *Psidium guajava* L. | 9 | 5 | 1.6 | 4 | 1.3 |
| *Piliostigma reticulatum* (DC.) Hochst. | 6 | 4 | 1.3 | 2 | 0.6 |
| *Terminalia mantaly* L. | 3 | 3 | 0.9 | - | - |
| *Magnifera indica* L. | 46 | - | - | 46 | 14.4 |
| *Vitex doniana* Sweet. | 3 | - | - | 3 | 0.9 |
| *Ficus gnaphalocarpa* L. | 6 | 4 | 1.3 | 2 | 0.6 |
| *Moringa oleifera*Lam. | 11 | 3 | 0.9 | 8 | 2.5 |
| *Ceiba pentandra* (L.) Gaertn. | 2 | 1 | 0.3 | 1 | 0.3 |
| *Ficus platyphylla* Delile. | 1 | - | - | 1 | 0.3 |
| *Tamarindus indica* L. | 1 | - | - | 1 | 0.3 |
| **Total** | **320** | **106** | **33.1** | **214** | **67.0** |

**Table 2. Host characteristics that influence mistletoes occurrence and abundance**

| Hosts | Host characteristics | | | |
|---|---|---|---|---|
| | MCS (m) | MTH (m) | MTBA ($m^2$) | WHC (g) |
| *Azadirachta indica* A. Juss. | 11.9 | 12.2 | 0.18 | 1.7 |
| *Acacia nilotica* L. | 7.7 | 6.6 | 0.10 | 13.0 |
| *Faidherbia albida* (Del.) | 9.3 | 10.1 | 0.16 | 3.1 |
| *Albizia lebbeck* (L.) Benth. | 10.9 | 9.9 | 0.2 | 1.2 |
| *Acacia Senegal* (L.) Willd. | 7.5 | 5.6 | 0.002 | 3.1 |
| *Eucalyptus camaldulensis* Dehnh. | 15.0 | 15.6 | 0.2 | 1.6 |
| *Balanites aegyptiaca* L. | 8.1 | 7.7 | 0.12 | 3.4 |
| *Adansonia digitata* L. | 2.0 | 14.7 | 0.52 | 1.5 |
| *Psidium guajava* L. | 6.0 | 7.7 | 0.05 | 2.1 |
| *Piliostigma reticulatum* (DC.) Hochst. | 7.6 | 8.0 | 0.1 | 1.5 |
| *Terminalia mantaly* L. | 8.6 | 9.7 | 0.1 | 2.4 |
| *Magnifera indica* L. | 12.6 | 13.6 | 0.22 | 0.8 |
| *Vitex doniana* Sweet. | 7.9 | 11.1 | 0.2 | 1.2 |
| *Ficus gnaphalocarpa* L. | 8.6 | 10.2 | 0.2 | 2.1 |
| *Moringa oleifera* Lam. | 4.6 | 9.1 | 0.06 | 1.4 |
| *Ceiba pentandra* (L.) Gaertn. | 8.5 | 14.3 | 0.2 | 1.6 |
| *Ficus platyphylla* Delile. | 8.0 | 13.3 | 0.2 | 0.9 |
| *Tamarindus indica* L. | 11.9 | 12.4 | 0.13 | 1.3 |

**Table 3. Concentrations of sodium, potassium, calcium, magnesium and phosphorus of african mistletoes in relation to their host trees**

| Host/parasites | Elements (mg/kg) | | | | |
|---|---|---|---|---|---|
| | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $P^+$ |
| *Azadirachta indica* A. Juss | 77.5 | 2000 | 145 | 90.3 | 6.00 |
| Parasite | -- | -- | -- | -- | -- |
| *Acacia nilotica* L. | 82.5 | 2150 | 70 | 90 | 9.42 |
| Parasite | 130.0 | 2750 | 92 | 87.6 | 8.74 |
| *Faidherbia albida* (Del.) | 75.0 | 1250 | 224 | 160.8 | 7.45 |
| Parasite | 142.5 | 3250 | 144 | 109.2 | 7.77 |
| *Albizia lebbeck* (L.) Benth | 105.0 | 2150 | 128 | 98.4 | 8.79 |
| Parasite | 200.0 | 4500 | 162 | 123.6 | 6.97 |
| *Acacia Senegal* (L.) Willd. | 107.0 | 2150 | 135 | 95.5 | 6.85 |
| Parasite | -- | -- | -- | -- | -- |
| *Eucalyptus camaldulensis* | 75.5 | 1900 | 131 | 92.5 | 8.25 |
| Parasite | -- | -- | -- | -- | -- |
| *Balanites aegyptiaca* L. | 85.0 | 2050 | 65 | 85 | 8.59 |
| Parasite | 125.0 | 2340 | 81 | 72.3 | 7.12 |
| *Adansonia digitata* L. | 95.0 | 2000 | 153 | 98.3 | 6.70 |
| Parasite | -- | -- | -- | -- | -- |
| *Psidium guajava* L. | 175.0 | 1900 | 132 | 94.8 | 6.86 |
| Parasite | 245.0 | 4900 | 130 | 94.8 | 6.86 |
| *Piliostigma reticulatum* DC | 106.0 | 2000 | 147 | 100.5 | 8.25 |
| Parasite | 128.0 | 2150 | 172 | 102.2 | 6.85 |
| *Terminalia mantaly* L. | 70.0 | 750 | 190 | 130.8 | 6.06 |
| Parasite | 150.0 | 1000 | 116 | 85.2 | 6.82 |
| *Magnifera indica* L. | 87.5 | 2000 | 188 | 122.4 | 6.86 |
| Parasite | -- | -- | -- | -- | -- |
| *Vitex doniana* Sweet | 182.0 | 1750 | 125 | 92.8 | 8.89 |
| Paraite | -- | -- | -- | -- | -- |
| *Ficus gnaphalocarpa* L. | 120.0 | 1750 | 144 | 100.8 | 9.50 |
| Parasite | 145.0 | 2250 | 146 | 103.2 | 10.46 |
| *Moringa oleifera* Lam. | 182.5 | 1750 | 128 | 97.2 | 5.86 |
| Parasite | 125.0 | 4500 | 108 | 84.0 | 8.57 |
| *Ceiba pentandra* L. Gaertn | 80.0 | 750 | 238 | 163.2 | 6.23 |
| Parasite | 157.5 | 750 | 142 | 105.6 | 7.29 |
| *Ficus platyphylla* Del. | 80.8 | 1900 | 120 | 82.5 | 8.42 |
| Parasite | -- | -- | -- | -- | -- |
| *Tamarindus indica* L. | 75.0 | 1000 | 104 | 84.5 | 8.00 |
| Parasite | -- | -- | -- | -- | -- |

*Key: Na=Sodium; K= potassium; Ca=Calcium; M=Magnesium; P= Phosphorus, (--) No parasite*

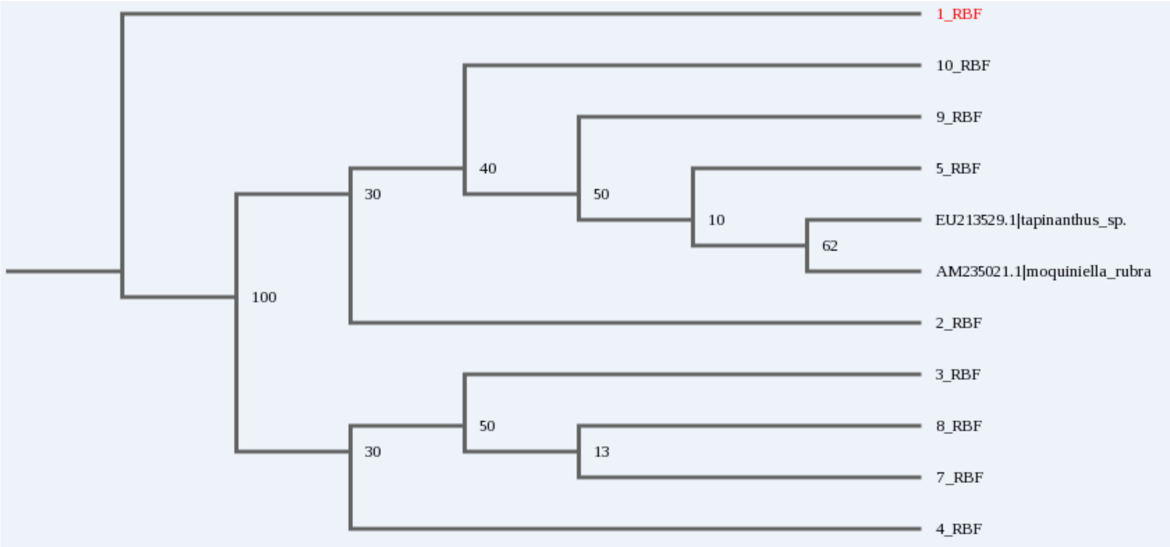


**Fig. 5. Phylogenetic tree of collected samples**


**Fig. 6. Snapshot of the sample after alignment**


**Fig. 7. Snapshot of trimmed sequences of the mistletoe samples using sequence trimmer**

## 4. DISCUSSION

The Result of the degree and severity of infestation of the African mistletoes in the study area clearly indicated that trees of economic importance were highly infested by the mistletoes. This is similar to the findings of Oluwole et al. [26] who reported high-level infestation of African mistletoes in the Samaru area of Zaria, Nigeria. The infestation on these trees could be due to widespread dispersal of seeds of the African mistletoe (*Moquinielarubra*) in the study area by a broad array of birds that depend on these mistletoes for food, as they consume the seed of the mistletoes, thereby enhancing the transfer of the sticky seed from one tree to another.

Many mistletoe types show a high preference for some hosts compared to others. The Result in Table 2 shows clearly that *Azadirachta indica, Terminalia mantaly* and *Eucalyptus camaldulensis* with wide canopy spread and greater heights were not affected by the mistletoes while those host trees with narrow canopy spread and small heights were highly affected. This is because sparse canopy tree cover is a suitable habitat for the mistletoe seed disseminators (birds), as these birds are able to fly through these trees more easily. Contrary to the findings of Boussim et al. [10], which revealed that host tree with large basal area has an influence on mistletoes prevalence and infestation, in the present findings, host trees with small basal area were highly infested with mistletoes while those with large basal area were not infested. This finding corroborates that of Aukema et al. [5], who reported a weak relationship between larger host size and infestation severity of African mistletoes.

Host tree species that have shown high bark water holding capacity such as *Acacia nilotica, Faidherbia albida* and *Balanites aegyptiaca* were found to have abundant mistletoes parasitizing their branches compared to other host trees with low bark water holding capacity as shown in Table 2. This is because mistletoes require water for growth and photosynthesis in some parts of their life cycle. This finding corroborates that of Callaway et al. [21] who revealed that tree with high water-holding capacity support mistletoes and other epiphytes, which may parasitize on them. The salt content analysis of African mistletoes revealed that the concentration of $Na^+$, $K^+$, $Ca^{2+}$,$Mg^{2+}$, and $P^+$ varied significantly among the host tree in the study area as indicated in Table 3.

It was observed that the concentrations of $Na^+$, $K^+$ and $Mg^{2+}$ was high in the parasite compared to some host trees, this is similar to the report of Desire [27] and Taffouo et al. [22]. The high concentration of these mineral salts in the parasite was probably because mistletoes main target is to constantly derive mineral salt necessary for their chemical processes, chief among which are $Na^+$ and $K^+$. This finding is similar to that sampling and Dibong et al. [23]. In the present study, the concentration of $P^+$ was observed to be higher in the leaves of the host trees compared to that of the parasite. The reason behind this phenomenon is not clear, although, the high concentration of $P^+$ may not be needed in the parasite for the host trees to be successful in retaining its nutrients. The concentration of $P^+$ was observed to be high in the parasite compared to *Faidherbia albida* and *Ficus gnaphalocarpa.* This finding agrees with the earlier report of Bannister et al. [28]. At the mistletoes leaves have more concentration of Potassium compared to their host trees. This could be because both host and parasite leaves draw nutrients from the same xylem sap, whereas some mistletoe have additional contact with their host, which permits the nutrient gain from the phloem sap as well as the xylem sap and the flow of this nutrient is one way from the host to the parasite [29-31].

All the gene sequences generated were trimmed using DNA SUBWAY sequence trimmer (a fast track to gene annotation and genome analysis) (Fig. 7), after which the trimmed sequences were aligned to ascertain the similarities or differences that exist among them (Fig. 6). Alignment of all the sample sequences generated using DNA SUBWAY revealed very close similarity among the samples. Search against a reference sequence in the Barcode of Life Database, using the Basic Local Alignment Tool (BLAST) revealed that all the sequences were 98.15% similar to the sequence of *Moquiniella rubra* with assertion number AM 235021.1:76-570 and voucher RBGK 5742 in the NCBI (National Data for Biotechnology Information) database.

## 5. CONCLUSION

Analysis of Water Holding Capacity (WHC) of the host trees has shown that mistletoes in the study area parasitized the host with high water content (*Acacia nilotica, Faidherbia albida*, and *Balanites* *aegyptiaca*) more than the hosts with low water content.

Trees such as *Azadirachta indica, Terminalia mantaly* and *Eucalyptus camaldulensis* with wide canopy spread and greater heights were not affected by the mistletoes while those host trees with narrow canopy spread and small heights were highly affected. This is because sparse canopy tree cover is a suitable habitat for the mistletoe seed disseminators (birds), as these birds are able to fly through these trees more easily.

Analysis of the ions such as Sodium ($Na^+$), Potassium ($K^+$), Calcium ($Ca^{2+}$), Magnesium ($Mg^{2+}$) and Phosphorus ($P^+$) in the mistletoe leaves in relation to the host tree revealed a significant ($p< 0.05$) difference in their concentration; this was because the parasite had more of these mineral salts than the host trees.

The DNA analysis of mistletoes samples collected from the study area revealed a strong relationship with *Moquiniella rubra* as obtained during the search against sequences in the reference database of NCBI. Thus, it can be strongly concluded that the majority, if not all of the mistletoes encountered in the present study are *Moquiniela rubra* belonging to the family Loranthaceae.

In the present study, trees of economic importance such as *Acacia nilotica, Faidherbia albida and Balanites aegyptiaca* were found to be highly infested while *Moringa oleifera, Psidium guajava, Albizia lebbeck, Ceiba pentandra* and *Piliostigma reticulatum* were mildly infested.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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