Original Research Article

HOST PREFERENCE, SALT BALANCE AND MOLECULAR CHARACTERISTICS OF AFRICAN MISTLETOES IN SELECTED AREAS OF SOKOTO STATE, NIGERIA

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 ofUsmanuDanfodiyo 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. The results revealed that *Acacia nilotica* tree washighly infested by themistletoe.Host canopy spread, basal area and height had no influence over mistletoe infestation. The 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 *Moquiniellarubra*,belonging tothe family Loranthaceae. It can be concluded from the result that the mistletoes in the study areas are *Moquiniellarubra* and depend solely on the host trees fortheir nutrientsrequirement and survival.

INTRODUCTION

The word mistletoe is derived from twoGerman words; *mist*- dung and *tang*- twig or stick (Vidal-Russell and Nickrent, 2008). Generally, mistletoes are hemi-parasitic flowering plants bearing ever green leaves that photosynthesize and depend on their host tree for water and mineral nutrients (Milius, 2003). They are widely distributed in all major biomes and climate types, except in the extremely cold regions (Okubamicheail*et al*., 2011). Mistletoes consist of more than 1400 species around the world belonging to the order Santalales (Judd *et al*., 2002), and evolved five times independently and are not monophyletic (Aukema, 2004). Recent molecular phylogenetic work by Mellado*et al.* (2016) 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* (Watson, 2001).

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 (2011) 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 (*Vitellariaparadoxa*), Neem tree (*Azadirachtaindica* L.), Sweet orange (*Citrus sinensis* L.), Grape (*Citrus paradisi* L.), Cocoa (*Theobroma cacao* L.) and Rubber (*Heveabrasiliensis*) (Beghoet *al.*, 2007).

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.* (2004) reported that mistletoe (*Tapinanthusglobiferus*) 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 (Didier *et al*., 2009).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 (Sonke*et al.,* 2000). 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 (Bako *et al.,* 2003; Ibrahim and Ayodele, 2011). This researchwork studiedhost preference and molecular characteristicsof theAfrican Mistletoes in selected communities of UsmanuDanfodiyo University, Sokoto.

MATERIALS AND METHODS

The study was conducted in selected communities in the main campus ofUsmanuDanfodiyo University Sokoto. A total of 8 communities viz;Kwalkwalawa, Sayya-GidanGada, GidanYero, Shellan-Makera, VC's Quarters, GidanAsarkei, Danrini and Gudan-Gebecommunities were surveyed for presence of themistletoes.

Sample Collection

The shoots, fruits and suckers of the African mistletoes as well asthe leaves and shoots of bothparasitized and non-parasitized host plant were randomlyhandpickedbyusinga sharp knife andcollected separately in a dark polythene bag.The samples were taken to the herbarium for identification andsubsequentLaboratory analysis.

Evaluation of Mistletoe-Host Preference

The level of infestation of the African mistletoes onhost trees was determined by thepercentage of infestation using the equation: IP = $\left\{\frac{\mathbf{Q}}{\mathbf{M}}\right\} \times \mathbf{100}$ (Tizhe*et al*., 2015).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, thus:S = $\left(\frac{\mathbf{E1+E2}}{\mathbf{2}}\right)$ by taking two measurements of the longest (E1) and shortest (E2) branches (Bob *et al*., 2015).The heights of all the trees was measured using Haga Altimeter (NFI 2005). 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 treestem so as to get the circumference (C). After which the Basal Area (BA) of each tree was calculated using the formula **TBA** = $\frac{\mathbf{\Pi \times D^2}}{\mathbf{4}}$ (Bob *et al.*2015). Where Π = Pie, D = Diameter,4 = Constant value, Hence the Circumference is given as $C = \Pi \times Diameter$. Thewater holding capacity (WHC) for each tree was calculated using the formula WHC = WM – DM (Callaway *et al.,* 2002). Where WHC = water holding capacity,WM = wet mass and DM = dry mass.

Determination of Salt Balance

The concentration of sodium and potassiumin 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 inTaffouo*et al*. (2008) and Dibong*et al*. (2010).

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.

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.

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′ (Levin *et al.*, 2004; Kress and Erickson, 2007).

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.

DNA Amplification using *Rbcl* primers

The isolated DNAsequences 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.

RESULTS

The results showed that the percentage of infestation of the African mistletoes varies among the host trees in the study area with *Acacianiloticа*L. having the highest level of infestation of 15% while *Albizialebbeck* (L.) Benth, *Terminalia mantaly*L. and *Moringaoleifera*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 *Magniferaindica* with a canopy spread of 12.6m wide while *Adansoniadigitata*had lowest canopy spread of 2.0m wide. The result of the plantheight revealedthat *Eucalyptus camaldulensis* had greaterheight of 15.1m followed by *Ceibapentandra*with a height of 14.3m whereas*Acacia senegal*had the shortest height of 5.6m (Table 2).

The basal area of *Adansoniadigitata*tree was the largestwith 0.52m^2 while tree with the smallest basal area was *Acacia Senegal* with 0.002m^2. The result of the stem bark Water Holding Capacity (WHC) revealed that *Acacia niloticа*shoothad the highest water holding capacity weighing 13.0g while *Magniferaindica*had the lowest

WHCweightof0.8g. The result of salt concentration revealed that Na^+, K^+, Ca^+, Mg^+ and P^+ variedin 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 *Albizialebbeck, Piliostigmareticulatum* and*Ficusgnaphalocarpa*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 (Figure 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 (Figure 5).

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 (%)
*Azadirachtaindica*A. Juss.	73	-	-	73	23.0
*Acacia nilotica*L.	67	48	15.0	19	5.9
Faidherbiaalbida (Del.)	22	16	5.0	6	1.9
Albizialebbeck (L.) Benth.	4	3	0.9	1	0.3
Acacia senegal(L.) Willd.	2	-	-	2	0.6
*Eucalyptus camaldulensis*Dehnh.	10	-	-	10	3.1
*Balaniteaegyptiaca*L.	35	19	5.9	16	5.0
*Adansoniadigitata*L.	19	-	-	19	6.0
Psidiumguajava L.	9	5	1.6	4	1.3
Piliostigmareticulatum(DC.) Hochst.	6	4	1.3	2	0.6
*Terminalia mantaly*L.	3	3	0.9	-	-
*Magniferaindica*L.	46	-	-	46	14.4
*Vitexdoniana*Sweet.	3	-	-	3	0.9
*Ficusgnaphalocarpa*L.	6	4	1.3	2	0.6
*Moringaoleifera*Lam.	11	3	0.9	8	2.5
Ceibapentandra(L.) Gaertn.	2	1	0.3	1	0.3
*Ficusplatyphylla*Delile.	1	-	-	1	0.3
*Tamarindusindica*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)
*Azadirachtaindica*A. Juss.	11.9	12.2	0.18	1.7
*Acacia nilotica*L.	7.7	6.6	0.10	13.0

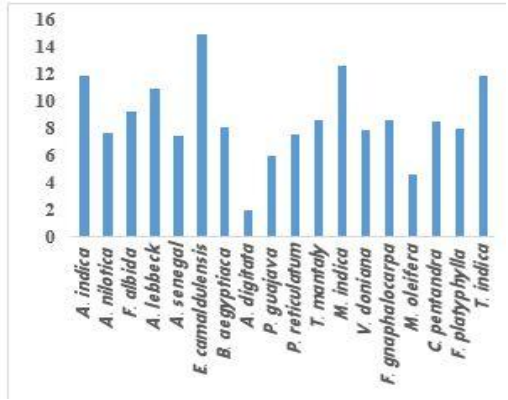
Faidherbiaalbida (Del.)	9.3	10.1	0.16	3.1
Albizialebbeck (L.) Benth.	10.9	9.9	0.2	1.2
Acacia Senegal (L.) Willd.	7.5	5.6	0.002	3.1
*Eucalyptus camaldulensis*Dehnh.	15.0	15.6	0.2	1.6
*Balanitesaegyptiaca*L.	8.1	7.7	0.12	3.4
*Adansoniadigitata*L.	2.0	14.7	0.52	1.5
Psidiumguajava L.	6.0	7.7	0.05	2.1
Piliostigmareticulatum(DC.) Hochst.	7.6	8.0	0.1	1.5
*Terminalia mantaly*L.	8.6	9.7	0.1	2.4
*Magniferaindica*L.	12.6	13.6	0.22	0.8
*Vitexdoniana*Sweet.	7.9	11.1	0.2	1.2
*Ficusgnaphalocarpa*L.	8.6	10.2	0.2	2.1
*Moringaoleifera*Lam.	4.6	9.1	0.06	1.4
Ceibapentandra(L.) Gaertn.	8.5	14.3	0.2	1.6
*Ficusplatyphylla*Delile.	8.0	13.3	0.2	0.9
*Tamarindusindica*L.	11.9	12.4	0.13	1.3

KEY: MCS=Mean Canopy Spread**; MTH=**Mean Tree Height**; MTBA=**Mean Tree Basal Area**; WHC=**Water Holding Capacity

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^+
*Azadirachtaindica*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
Faidherbiaalbida(Del.)	75.0	1250	224	160.8	7.45
Parasite	142.5	3250	144	109.2	7.77
Albizialebbeck(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	--	--	--	--	--
*Balanitesaegyptiaca*L.	85.0	2050	65	85	8.59
Parasite	125.0	2340	81	72.3	7.12
*Adansoniadigitata*L.	95.0	2000	153	98.3	6.70
Parasite	--	--	--	--	--
*Psidiumguajava*L.	175.0	1900	132	94.8	6.86
Parasite	245.0	4900	130	94.8	6.86
*Piliostigmareticulatum*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
*Magniferaindica*L.	87.5	2000	188	122.4	6.86
Parasite	--	--	--	--	--
*Vitexdoniana*Sweet	182.0	1750	125	92.8	8.89
Paraite	--	--	--	--	--
*Ficusgnaphalocarpa*L.	120.0	1750	144	100.8	9.50
Parasite	145.0	2250	146	103.2	10.46
*Moringaoleifera*Lam.	182.5	1750	128	97.2	5.86
Parasite	125.0	4500	108	84.0	8.57
*Ceibapentandra*L. Gaertn	80.0	750	238	163.2	6.23
Parasite	157.5	750	142	105.6	7.29
*Ficusplatyphylla*Del.	80.8	1900	120	82.5	8.42
Parasite	--	--	--	--	--
*Tamarindusindica*L.	75.0	1000	104	84.5	8.00
Parasite	--	--	--	--	--

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



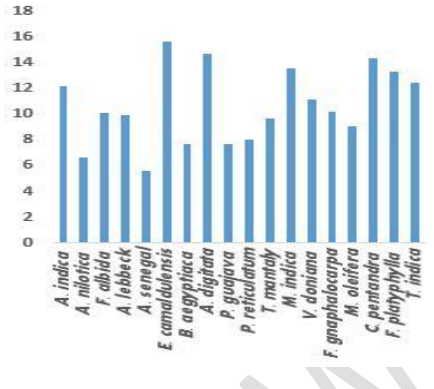
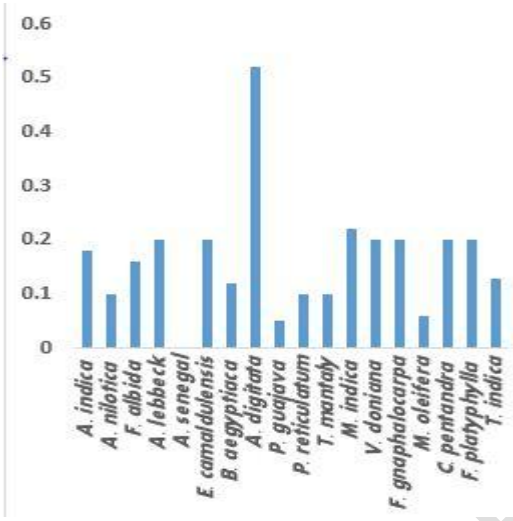


Figure 1: Mean canopy spread (m) of the selected trees in the study area; Figure 2: Mean Tree Height (m) of the selected trees in the study area



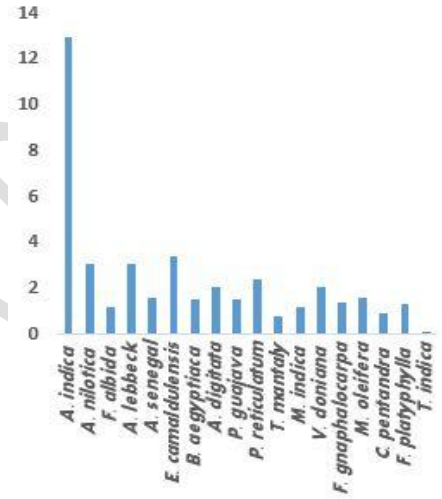


Figure 3: Mean Basal Area (m^2) of the selected trees in the study area;Figure 4: Water holding capacity (g) of each tree species in the study area

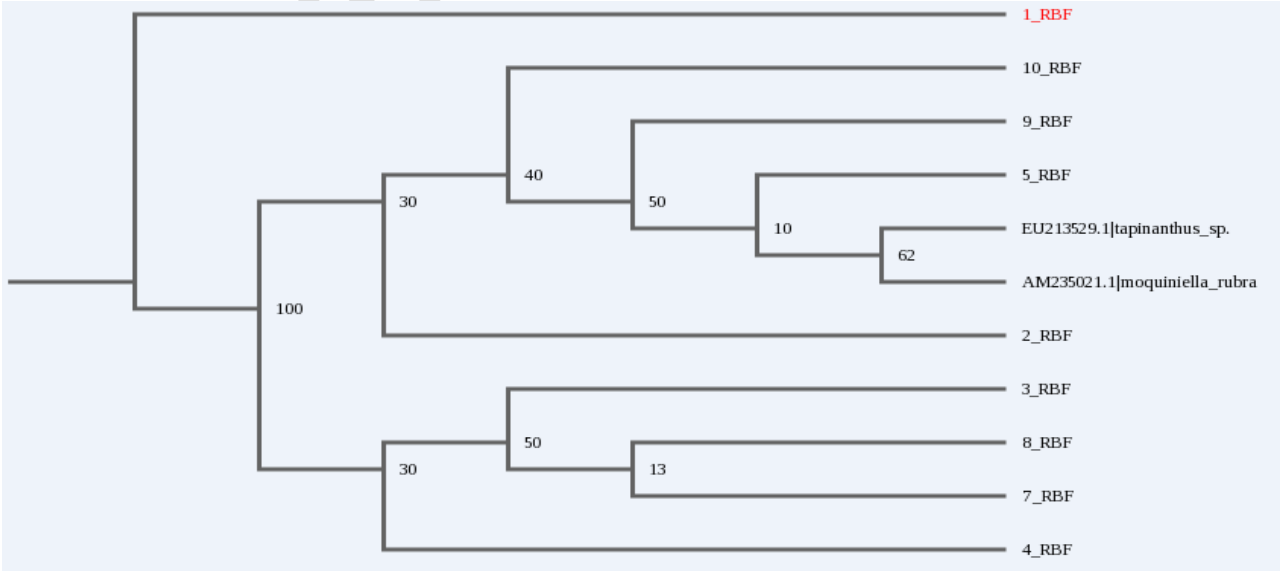


Figure 5: Phylogenetic tree of collected samples

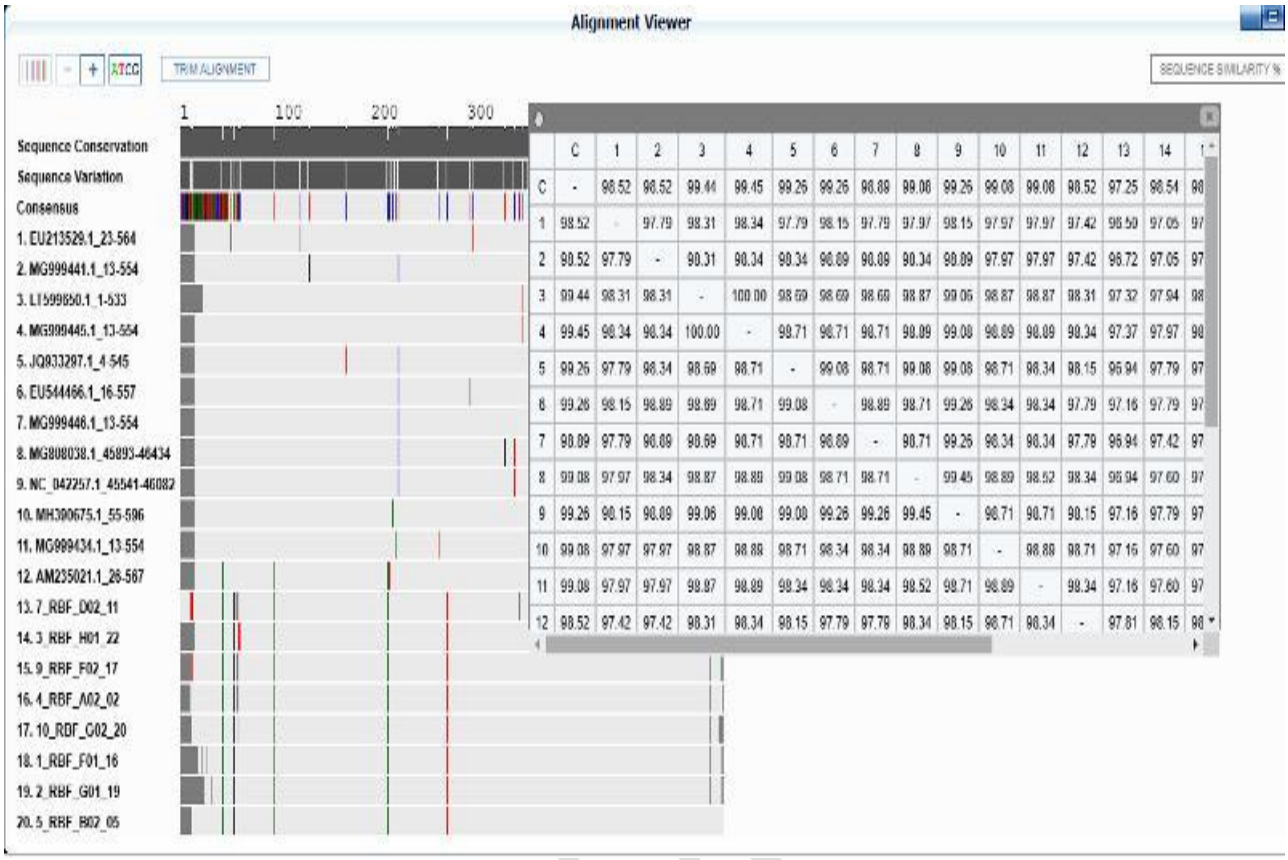


Figure 6: Snapshot of the sample after alignment

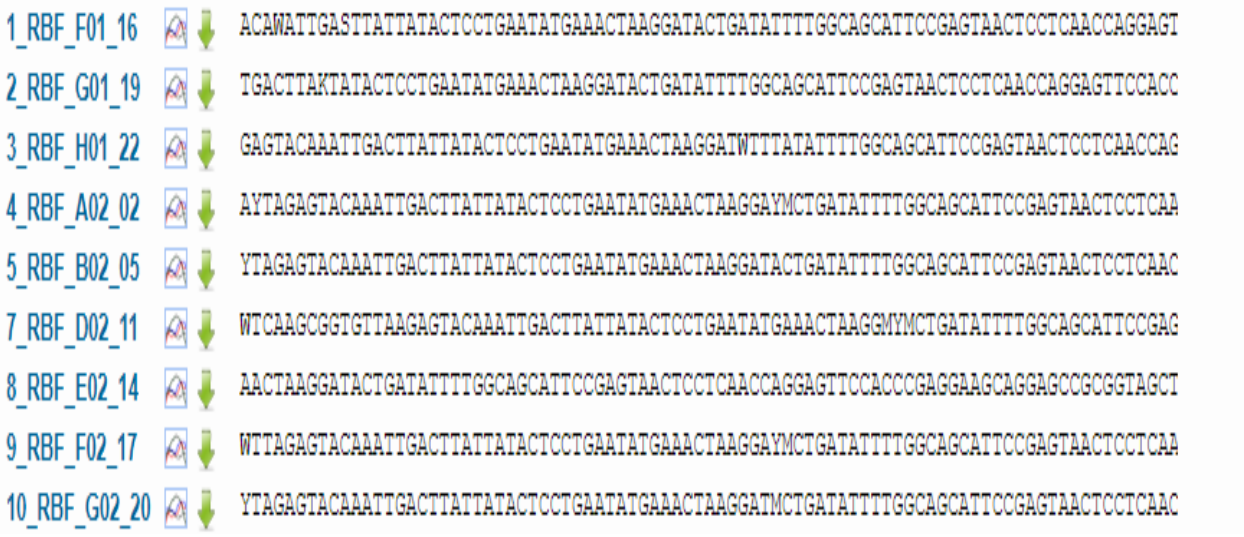


Figure 7: Snapshot of trimmed sequences of the mistletoe samples using sequence trimmer

DISCUSSION

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.* (2004) who reported high level infestation of African mistletoes in Samaru area of Zaria, Nigeria. 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 *Piliostigmareticulatum*were mildly infested The infestation on these trees could be due to widespreaddispersal of seeds of the African mistletoe (*Moquinielarubra*) in the study area by 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 (Van Ommeren *et al*., 2002).

Many mistletoe types show high preference to some host compared to others. 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 contrary to the finding of Didier *at al*. (2009), who reported that mistletoes host preference was due to wide canopy spread and height of the host trees. This is because sparse canopy trees cover is a suitable habitat for the mistletoe seed disseminators (birds), as these birds are able to fly through these trees more easily (Ward, 2005).The resultsof Boussim*et al*. (2004) which revealed that host tree with large basal area has influence on mistletoes prevalence and infestation was contrary to thepresent findings,in whichhost trees with small basal area were highly infested with mistletoes while those with large basal area were not infested. This finding corroboratesthat of Aukema*et al*. (2004) who reported weak relationship between larger host size and infestation severity of African mistletoes.

Host tree species that have shown high bark water holding capacitysuch as *Acacia nilotica, Faidherbiaalbida*and*Balanitesaegyptiaca*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 part of their life cycle. This finding corroboratesthatof Callaway *et al.* (2002) which 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 concentration of Na^+, K^+, Mg^{2+} were high in the parasite compare to some host tree, this is similar to the report of Desire (2005) and Taffouo*et al.* (2008). 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 is Na^+ and K^+. This finding is similar to that of Bannister *et al.* (2002) and Dibong*et al.* (2010). 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;this is contrary to the report of Bowie *et al.* (2004) on nutrient status of mistletoes and their host. The reasonbehindthisphenomenon is not clear, although, thehigh concentration of P^+maynot beneeded inthe 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 *Faidherbiaalbida*and*Ficusgnaphalocarpa.* This finding agrees with the earlier report of Bannister *et al*. (2002) that themistletoes leaves have more concentration of Potassium compared to their host trees. This is 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 nutrients 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 (Kuijit, 2005).

All thegene sequences generated were trimmed using DNA SUBWAY sequence trimmer (a fast track to gene annotation and genome analysis) (Figure 7), after which the trimmed sequences were aligned to ascertain the similarity or differences that exist among them(Figure 6). Alignment of all the samples sequences generated using DNA SUBWAY revealed very close similarity among the samples.Search against reference sequence in the in Barcode of life Database, using Basic Local Alignment Tool (BLAST) as suggested by Levin *et al*. (2004) revealed that all the sequences were 98.15% similar to the sequence of *Moquiniellarubra* with assertion number AM 235021.1:76-570 and voucher RBGK 5742 in the NCBI (National Data for Biotechnology Information) database.

CONCLUSION

From the findings of in this study, it could be concluded that the host characteristics investigated had no influence on mistletoes-host interaction. Other factors such as compatibility, interaction with birds and seedling establishment may be more important in determining mistletoes-host interaction. Analysis of Water Holding Capacity (WHC) of the host trees has shown that mistletoes in the study area parasitized host with high water content (*Acacia nilotica, Faidherbiaalbida*, and *Balanitesaegyptiaca*) compared to host with low water content. Analysis of ions content 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 strong relationship with *Moquiniellarubra*as obtained during search against sequences in the reference database of NCBI. Thus, it can be strongly concluded that majority,if not allof the mistletoesencountered in presentstudy in the province of UsmanuDanfodiyo University Sokotoare *Moquinielarubra*belonging to the family Loranthaceae.

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