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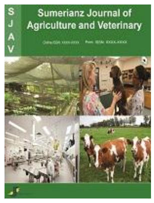

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Effects of the Presence of the African Mistletoe *Tapinanthus Bangwensis* [Engl. and K. Krause] Danser on the Mineral Nutrient Composition Leaf Chlorophyll and Relative Water Content of *Citrus Sinensis* and *Irvingia Gabonensis* Host Plants

Edagbo D. E.*
Plant Genetic Resources Unit, National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria
Email: dedagbo@yahoo.com

Oyetunji O. J.
Department of Botany, University of Ibadan, Ibadan, Nigeria

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Abstract

Background: Information on the African Mistletoes with the focus on their interactions with host plants in view of the physiological and biochemical processes underlying the relationship is scanty. It is therefore necessary to engage in further study of the process for in-depth knowledge. Experiment: The stems and leaves of the mistletoe on *Citrus* and *Irvingia* plants and their hosts' stems and leaves were collected, cleaned and dried in the sun. The dried materials were ground into powder and subjected to mineral analysis test. Tests were also conducted on fresh leaf samples for chlorophyll and relative water contents. Results: Mineral nutrients such as Na, K, Mg, N, Zn, Mn were significantly higher in the uninfested hosts when compared with infested in both *Citrus sinensis* and *Irvingia gabonensis* but the mineral nutrients contained in the parasite were in preponderance to that available in the infested. Leaf chlorophyll (chl) study revealed the possession of higher chlorophyll a and b content in the uninfested (chl a 41.11, b 37.29 mg/ml *Cs;* chl a 49.70, b 45.81 mg/ml Ig) when compared with infested (chl a 37.09, b 33.18 mg/ml *Cs;* chl a 46.65, b 41.99 mg/ml Ig) hosts; however comparative chlorophyll contents of the mistletoe to both hosts varied. Estimation of percentage Relative Water Content (RWC) of leaves during the season of dry (ds) and rainy (rs) conditions were higher for the infested (ds 83.07 rs 90.61 *Cs;* ds 89.25 rs 94.83 Ig) compared to uninfested (ds 81.81 rs 87.65 *Cs;* ds 75.09 rs 92.89 Ig) hosts while the mistletoe (ds 90.23 rs 95.08) exhibited comparative higher RWC to the hosts. Conclusion: The physiological relationship between the mistletoe and its hosts often led to diminished nutrients and chlorophyll content with acquisition of increased RWC in the infested hosts. The increased RWC implied a physiological process for water use efficiencies in *Citrus sinensis* and *Irvingia gabonensis*.

Keywords: Parasitism; Mineral elements; Physiological relationship; Water uptake.

1. Introduction

Mistletoes are a polyphyletic group of shrubby hemi-parasites of aerial stems in the order Santalales. They comprise species occurring in five families namely, the Loranthaceae, Viscaceae, Eremolepidaceae, Misodendraceae and Santalaceae [1-4]. Taxonomically, the mistletoes constitute a highly specialized and diverse group of angiosperms. They are obligate stem parasites whose dependency ranges from holoparasitic to hemiparasitic, and they are characterized by the development of a haustorium, an absorptive organ that serves as a sort of root, attaching to the host and penetrating its conductive tissues in order to pass nutrients to the parasite. They affect host viability by withdrawing essential resources and thus compete with their host for water, inorganic nutrients and organic compounds [5].

Hemiparasitic mistletoes lack specific mechanisms or endodermis-like structure (barriers) in their haustoria for discrimination and active uptake of mineral nutrients beyond that of simple diversion of host xylem sap [6, 7]. Generally for mistletoes, the flow of nutrients is predominantly one-way, from host to parasite. Chemical analyses of mistletoes from a large sample of host species provide evidence that these parasites can cope well with many different nutrient sources. Some elements may vary by one or two orders of magnitude in samples from the same mistletoe species on different host trees and species. It has been observed that in many instances the best correlation for predicting the concentrations of elements in mistletoe is the concentration of elements in the host [6].

Mistletoes affect hosts viability by withdrawing nutrients and other essential resources necessary for their survival. The parasite competes with its host for water, inorganic nutrients and organic compounds and this contributes to the debilitation of this host to the extent of its resource sink. Mistletoes affect hosts in many ways, including reduced growth, diminished vigour, premature mortality, impaired quality and quantity of wood, reduced fruit set, and heightened susceptibility to attack by other agents such as insects or fungi [5, 8, 9]. When one part of the host is intensively attacked by mistletoe, the reproductive and photosynthetic potential of the part distal to the infestation declines leading to death of the part. But the extent of damage caused to the host depends on size of the

*Corresponding Author

parasite, the growth rate and metabolic activity of the parasite, the degree of dependency on the host for resources, and the stage of development of the host [5].

Much information have emanated from studies of the mistletoes in Europe and other continents, on several aspects of the interactions and relationships of these group of parasitic plants with their hosts. In contrast, information on the African Mistletoes with the central focus on their mechanism of interaction with the host plants, the physiology and biochemistry still remain scanty. Work on the comparative ecophysiological study of the African Mistletoe, *Tapinathus bangwensis* on two host plants was conducted by Oyetunji and Edagbo [10]; nonetheless, this research seeks to revalidate and establish the plausible effects of the presence of the African Mistletoe, *Tapinathus bangwensis* on the mineral nutrient composition, leaf chlorophyll and relative water contents of *Citrus sinensis* and *Irvingia gabonensis* as host plants while adopting modified techniques and methodologies of the above referenced study. Majorly, the introduction of comparative assessment of the properties of the infested host plants with the uninfested vis-à-vis the properties of the mistletoes were some of the distinct features of this study.

2. Materials and Methods

2.1. Site of the Study

The stems and leaves of the mistletoe on *Citrus* and *Irvingia* plants and their hosts' stems and leaves were collected, cleaned and dried in the sun. The dried materials ground into powder and kept in air tight containers for further analysis. These plant samples were collected from the *Citrus* orchard of the National Cereals Research Institute and *Irvingia* plantation of the National Centre for Genetic Resources and Biotechnology (NACGRAB), both at Moor Plantation Apata, Ibadan, South-Western, Nigeria (located at latitude, $07^0$3872' - $07^0$3860'N; longitude, $003^0$8420' - 003^0 8415'E; and at an altitude of 3 m). The laboratory works were conducted at NACGRAB and the Institute of Agricultural Research and Training (IAR&T), Moor Plantation Apata, Ibadan. Sample materials were randomly collected from the selected and marked plants in both rainy and dry seasons. The annual rainfall ranged from 750 to 1557 mm and temperature range was $23/34^0$C (minimum/maximum). Relative humidity was between 45 and 89% throughout the year.

3. Determination of Mineral Elements

3.1. Potassium Calcium and Sodium

Ash was obtained from the powdered samples of mistletoe and the infested and uninfested *Citrus* and *Irvingia* hosts. To the ash placed in a crucible was added 5 ml 2M HCl for it to be digested while the heating mantle was used to heat it to dryness. After a further addition of 5 ml 2M HCl and heating to boil; filtration was then carried out on a 100 ml volumetric flask using a Whatman No.1 filter paper. Distilled water was used to mark up the filtrate obtained and this was stoppered, made ready for reading of the concentrations of K, Ca and Na on PFP7 Model of the Jenway Digital Flame Photometer using each mineral element corresponding filter. Each element concentration was calculated with this expression:

$$\%K \text{ or } \%Ca \text{ or } \%Na = \frac{\text{Meter Reading (MR) X Slope X Dilution factor}}{10,000}$$

3.2. Iron and Magnesium

These two elements were determined using the Atomic Absorption Spectrophotometer (AAS). Prepared Serum of Iron and Magnesium (obtained from the parasite and hosts) were filtered and dilutions were made. It was a 1/10 dilution for Iron and 1/25 for Magnesium. Each of them was standardized using their respective standards.

$$\textbf{Calculation: } \frac{\text{Test Reading}}{\text{Reading of Standard}} \text{ X Concentration of Standard}$$

$$= \text{Concentration of Test (mg/100 ml)}$$

3.3. Phosphorus

The ash obtained from each of the ground sample of the mistletoe and hosts was treated with 2M HCl solution similar to what was done for Calcium. A 50 ml standard flask was collected into which ten (10) ml filtrate solution was run using a pipette. Another 10 ml solution containing vanadate yellow was added into the flask, marked up with distilled water and this was stoppered then allowed to stand for 10 minutes to attain full yellow development. Absorbance or Optical Density (OD) of the solution was read at a wavelength of 470 nm on Spectronic 20 spectrophotometer to obtain concentration of phosphorus. Calculation for the percentage phosphorus was obtained by the expression below:

$$\% \text{ Phosphorus} = \frac{\text{Absorbance X Slope X Dilution factor}}{10,000}$$

3.4. Nitrogen-Free Extract (NFE) Test

Determination of the amount of Nitrogen Free Extract (NFE) was achieved through a calculation of the difference upon analysis of all the other items in proximate analysis from samples of the parasitic mistletoe, infested and uninfested *Citrus* and *Irvingia* hosts. All the nutrients that could not be considered arising from the previous methods of proximate analysis are now captured. These consisted majorly of vitamins, digestible carbohydrates and other non-nitrogen soluble organic compounds. This was achieved with the application of the subtraction of SUM of (moisture % + % crude protein + % Ether Extract + % Crude Fibre + % Ash) from 100.

NFE =(100 – [% M + % Cp + % EE + % CF + % Ash]).

3.5. Determination of Zinc, Manganese Lead and Cadmium using Buck 200 AAS (Atomic Absorption Spectrophotometer)

Ash from each of ground samples of the mistletoe and hosts (infested and unifested *Citrus* and *Irvingia*) was digested as per the process with calcium and potassium. With 100 ml volumetric flask in place, distilled water was used to wash the digested ash and marked up. Using a suction tube, the diluents were sucked into the Buck 200 Atomic Absorption Spectrophotometer (AAS). Each trace element was read at its respective wavelength in line with the corresponding hollow cathode lamp using the appropriate fuel and oxidant combination. For example with Zn: appropriate usage for fuel and Oxidant: Air-Acetylene; Wavelength: 213.9; Sensitivity (ug/ml): 0.03.

Meter reading of the element was used to calculate the concentration with the formula below:

Ppm or mg/kg (any of the elements) = Meter reading X Slope or Gradient X dilution factor.

% (any of the elements) = ppm or mg/kg divided by 10, 000

3.6. Chlorophyll Determination

The leaf chlorophyll determination of the mistletoe and host plants was conducted using spectrophotometric method as described by Bojović and Stojanović [11]. The leaf samples used for the assessment were obtained from ten infested *Citrus* and *Irvingia* hosts with associated mistletoe parasite including leaves from similar uninfested hosts. Fresh plant leaves of 0.5g with added 10 ml of 80 % acetone was taken and homogenized in tissue homogenizer. Extract obtained was allowed to centrifuge at 2500 rpm for 5 min then the supernatant obtained was diluted with 9 ml of 80% acetone per ml of extract. Extract produced was read on spectrophotometer at 645, 663 and 750 nm wavelengths. Often before the calculation of chlorophyll concentrations, correction for any turbidity in the solution was achieved through the subtraction of absorbance at 750 nm from absorbance at the other two wavelengths.

3.7. Calculations

Calculations to determine chlorophyll concentrations of the plant samples include the following:

Chlorophyll *a* [milligrammes/millilitre (mg/ml)] = 12.7 A663 - 2.69 A645

Chlorophyll *b* (mg/ml) = 22.9 A645 - 4.68 A663

Where:

A645 = measurement of absorbance at 645 nm wavelength;

A663 = measurement of absorbance at 663 nm wavelength.

Total Chlorophyll (mg/ml) = Chlorophyll *a* + Chlorophyll *b*

3.8. Estimation of Leaf Relative Water Content (RWC)

Mistletoe and host species were selected (ten infested *Citrus* and *Irvingia* hosts with associated parasite as well as similar host species free of infestation) from which fresh leaves were collected. Each leaf sample was cut into small disc using a sharp cork borer. The leaf samples were subsequently weighed and recorded as fresh weight sample (W). These leaves in distilled water (15g leaves: 75cl distilled water) were then allowed to hydrate to full turgidity for 4 hours at normal room temperature (25^0C) and light (dim light). At end of 4 h, the samples were removed from water and quickly dried of any surface moisture with filter paper. The samples wiped off of moisture were weighed immediately to obtain fully turgid weight (TW). The next process after noting the turgid weight was to oven dry the samples at 80^0C for 36 h. After the time lapse for the drying process, leaf samples were cooled in desiccators and weighed for the determination of dry weight (DW). The mathematical expression for eliciting the relative water content of each sample was determined as shown below:

$$RWC (\%) = \frac{W - DW}{TW - DW} \times 100$$

Thus W = fresh weight of sample; TW = turgid weight of sample; DW = dry weight of sample.

It should be noted that the Leaf Relative Water Content Estimation experiment was carried out for two years across the dry and rainy seasons.

4. Statistical Analysis

Data obtained were analysed statistically using the SPSS 21 Statistics Program. Statistical analysis was done using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT).

5. Results

5.1. Mineral Element Composition of the Stems and Leaves of Tapinanthus Bangwensis with the Infested and Uninfested Citrus and Irvingia Hosts in the Dry Season

Assessment of the mineral nutrients contents of the stems and leaves of *Tapinanthus bangwensis* with the infested and unifested *Citrus* and *Irvingia* hosts was carried out and the result was shown in Table 1. *Tapinanthus* on *Citrus* had similar Na content (0.15%) with the infested host while the uninfested had higher content (0.20%) which was significantly different ($\alpha = 0.05$). However, the parasite on *Irvingia* had the highest Na content (0.27%) which was significantly higher than the contents in both the infested and uninfested hosts. The K in the mistletoe-*Citrus* association revealed that the parasite had K content (0.83%) which was significantly higher than the infested host but the uninfested host had the highest K (1.16%) uptake which was statistically different from both. *Tapinanthus* on *Irvingia* was noted to have the highest K content (2.20%) significantly higher (α = 0.05) than that of the infested host but not statistically different from the uninfested. The P content in the mistletoe-host with the infested and unifested *Citrus* and *Irvingia* hosts revealed similar amount of P uptake in the mistletoe and the hosts which were not statistically different. The Ca contents of the parasite and the two hosts (infested and unifested *Citrus* and *Irvingia*) were similar. The Mg contents of the uninfested hosts (0.64%, *Citrus* and 0.69% *Irvingia*) were higher than that of the infested hosts and the parasite. The N content in the *Tapinanthus* on *Citrus* was statistically similar with the uninfested host while the infested had the least amount of N. Similar N contents were recorded in the *Irvinga*-mistletoe association.

The Fe content (0.71 %) of *T. bangwensis* on *Citrus* was significantly higher (α = 0.05) than that of the infested and uninfested *Citrus* while the quantities of Fe observed in the uninfested *Citrus* was the next higher amount (0.66 %). The highest content of Fe was observed in the uninfested *Irvingia* (0.73 %) which was significantly higher (α = 0.05) than that of the mistletoe and the infested host while the least Fe content was in the infested host (0.53 %). The mistletoe on *Citrus* had uptake of Zn (0.65 %) that was significantly higher than the infested and uninfested *Citrus* which both had similar Zn content (0.59 %). The Zn in *T. bangwensis* (0.74 %) on *Irvingia* was significantly higher (α = 0.05) than the infested host but lower than the amount in the uninfested host (0.75 %). The Mn uptake of the mistletoe on *Citrus* (0.03 %) and *Irvingia* (0.04 %) were significantly higher than that in the hosts. The Pb content (0.02 %) of the parasite on *Citrus* was significantly higher than that in the infested but similar with that of the uninfested *Citrus*. Also in the mistletoe - *Irvingia* association, the parasite had higher Pb content (0.02 %) than the infested but statistically similar with the uninfested. The Cd content of the parasite on *Citrus* was similar with the infested and uninfested hosts while the Cd content in the parasite on *Irvingia* was significantly higher (α = 0.05) than the infested but similar to that of the uninfested.

5.2. Mineral Element Composition of the Stems and Leaves of Tapinanthus Bangwensis with the Infested and Uninfested Citrus and Irvingia Hosts in the Rainy Season

Shown in Table 2 is the result of the mineral elements composition of the stems and leaves of *Tapinanthus bangwensis* with the infested and uninfested *Citrus* and *Irvingia* hosts in the rainy season. The highest amount of Na was found in the uninfested *Citrus* (2.52%) while *Tapinathus* possessed Na (1.57%) that was significantly higher (α = 0.05) than that in the infested *Citrus* host. Similarly Na in the unifested *Irvingia* (2.45%) had the highest content and the infested (0.29%) had the least. The K content it was observed to be significantly higher in the uninfested hosts (5.08% *Citrus* and 5.34% *Irvingia*) when compared to the parasite and the infested hosts. The P uptake in the mistletoe on both *Citrus* and *Irvingia* were statistically similar with the uninfested hosts but higher than those found in the infested hosts (0.57% *Citrus* and 0.51% *Irvingia*). The infested hosts (0.28% *Citrus* and 0.28% *Irvingia*) were found to have the least amount of Ca while the uninfested hosts (0.64% *Citrus* and 0.68% *Irvingia*) had the highest. The contents of the Mg and N were in similar pattern with that observed in Ca whereby the infested hosts had the least amount of the mineral elements while the uninfested had the highest.

The Fe content in the uninfested *Citrus* (0.72 %) was significantly higher (α = 0.05) than that in the mistletoe (0.51 %) and the infested host (0.40 %). The Fe content in the *Irvingia* – mistletoe association displayed similar trend observed in the *Citrus* – mistletoe association with the Fe in uninfested *Irvingia* at 0.82 %, mistletoe, 0.46 % and the infested *Irvingia*, 0.39 %. Zn uptake in the *Citrus* hosts and its parasite showed that the least amount of Zn was with the infested *Citrus* (0.35 %) while the uninfested had the highest (0.64 %). In the case of *Irvingia*, the uninfested *Irvingia* possessed Zn (0.74 %) that was significantly higher than that in its infested counterpart (0.34 %) and the parasite (0.36 %). Mn in the uninfested *Citrus* (0.04 %.) was the highest which was significantly higher than that of the infested *Citrus* (0.02 %) and the mistletoe (0.03 %). The mistletoe on *Irvingia* and the uninfested *Irvingia* possessed similar amount of Mn at 0.03 % which was higher than that in the infested *Irvingia* host (0.02 %). The Pb content in the parasite on *Citrus* and its infested host was similar at 0.02 % while the infested *Irvingia* host (0.03 %) had higher content than its parasite (0.01 %). Similar amount of Cd were found in mistletoe (0.03 %) and its *Citrus* host (0.03 %) in as well as the parasite (0.02 %) and its *Irvingia* host (0.02 %).

5.3. Leaf Chlorophyll Content of Tapinanthus Bangwensis and its Two Hosts in the Dry Season

Table 3 shows the leaf chlorophyll contents of the Mistletoe and its two host plants for test conducted in the dry season. The chlorophyll *a* content of *T. bangwensis* (53.47 mg/ml) on *Citrus sinensis* was significantly higher (α = 0.05) than the amount in the infested and uninfested hosts but the least pigment was found in the infested (37.09 mg/ml). Evaluation of chlorophyll *a* content in the *Irvingia* –mistletoe association showed that the uninfested host had significantly higher amount (49.70 mg/ml) of the pigment than the parasite and the infested host. In the chlorophyll *b* content test for the *Citrus* –mistletoe association, the parasite had significantly higher (α = 0.05) amount (43.96 mg/ml) than the infested and uninfested hosts while the infested had the least chlorophyll *b* content (33.18 mg/ml). The chlorophyll *b* content for *Irvingia* and its parasite showed that the uninfested had significantly higher content (45.81 mg/ml) than the infested host and the mistletoe. The total chlorophyll content of the parasite and its hosts revealed for the *Citrus* –mistletoe association that the parasite had significantly higher (α = 0.05) content (97.43 mg/ml) than both the infested and uninfested hosts while the uninfested also displayed higher content than the infested. In the *Irvingia* –mistletoe association, the uninfested host had significantly higher content than the infested and the parasite with the parasite (80.07 mg/ml) observed to have the least total chlorophyll content. The ratio of chlorophyll *a* to chlorophyll *b* was statistically similar for the infested and uninfested *Citrus* though higher for the parasite. However, the chlorophyll *a* to *b* ratio in the *Irvingia*-mistletoe association was similar for the parasite and the hosts.

5.4. Leaf Chlorophyll Content of Tapinanthus Bangwensis and its Two Hosts in the Rainy Season

Result of the assessment of the leaf chlorophyll contents of *Tapinanthus bangwensis* and its two hosts in the rainy season is outlined in Table 4. The chlorophyll *a* contents of the leaf of *Tapinanthus* on *Citrus* (53.73 mg/ml) and *Irvingia* (44.18 mg/ml) were significantly higher than those of the infested hosts (*Citrus* 34.70 mg/ml and *Irvingia* 33.98 mg/ml). The highest chlorophyll *a* contents were obtained in the uninfested hosts (*Citrus* 55.63 mg/ml and *Irvingia* 55.59 mg/ml). The chlorophyll *b* content of the leaf of the parasite on *Citrus* was statistically similar to that in the infested and uninfested hosts while the chlorophyll *b* leaf content in the *Tapinanthus* (40.38 mg/ml) was higher than that in the infested *Irvingia* host (31.37 mg/ml) but lower to that of the uninfested (52.14 mg/ml). Total leaf chlorophyll content of *Tapinanthus* on *Citrus* (104.22 mg/ml) and *Irvingia* (84.56 mg/ml) were significantly higher than those of the infested hosts (*Citrus* 66.86 mg/ml and *Irvingia* 65.27 mg/ml) but the ratio of chlorophyll *a* to *b* was similar for the parasite and the infested hosts.

5.5. Relative Water Content (%) Estimation of Leaves of Tapinanthus Bangwensis and its Two Hosts in the Dry Season

Table 5 shows the Relative Water Content (RWC) estimation of leaves obtained from the mistletoe and the host plants carried out in the Dry Season (DS). In the conduct of the first Dry Season (DS1) evaluation exercise, *T. bangwensis* (85.21%) on *Citrus* had significantly higher ($\alpha = 0.05$) water content than the infested and the uninfested *Citrus*. The results of the RWC of the mistletoe (83.78%) on *Irvingia* also showed that the parasite had significant higher RWC than *Irvingia*. However, the infested *Citrus* had significantly higher water content than the uninfested while the infested and uninfested *Irvingia* had similar RWC values. In the second experiment (DS2), the infested *Citrus* (75.06%) had significantly higher RWC than the parasite and uninfested plant while the infested *Irvingia* (85.43%) had similar RWC with the parasite which was higher than than that of the uninfested *Irvingia* (79.88%). Assessment in DS3 revealed that the mistletoe and infested *Citrus* had higher RWC which were statistically similar but different from the uninfested (75.82%) which was the least. RWC observed in the *Irvingia*-mistletoe association in DS3 showed also that the parasite and infested host had statistically similar RWC which were higher than the uninfested. In DS4, the infested *Citrus* (90.71%) had RWC value that was significantly higher than what was recorded for *T. bangwensis* and the uninfested host. The infested *Irvingia* had the highest RWC (84.07%) which was significantly different from that in the parasite and the uninfested host while the parasite had the least RWC (77.42%). The result in the DS5 evaluation showed that the mistletoe and its *Citrus* hosts had RWC values which were similar and in close range (89.73-90.69%). The infested *Irvingia* host (88.46%) had higher RWC uptake than the parasite and the uninfested host.

5.6. Relative Water Contents (%) Estimations of Leaves of Tapinanthus Bangwensis and its Two Hosts in the Rainy Season

The result of the Relative Water Contents (RWC) estimations of the leaves of the mistletoe and the host plants performed in the Rainy Season (RS) is presented in Table 6. The initial RWC test in the rainy Season (RS1) showed that *Tapinanthus bangwensis* had significantly higher ($\alpha = 0.05$) RWC (88.58%) than the infested and uninfested *Citrus* hosts with the least RWC obtained in the uninfested (78.19%). In the *Irvingia*–mistletoe association, similar RWC pattern was observed, as the mistletoe had the highest (92.77%) with the uninfested the least RWC. In the Second (RS2) test, *Tapinanthus* (92.15%) had significantly higher RWC than the infested and uninfested *Citrus* hosts and for the *Irvingia*-mistletoe interaction, a similar trend was obtained. The RS3 assessment showed that *Tapinanthus* on *Citrus* still had significantly higher ($\alpha = 0.05$) RWC than the infested and uninfested hosts. During the same period (RS3), the parasite on *Irvingia* (95.33%) also had significantly higher RWC than the infested and uninfested hosts. In RS4, *Tapinanthus* on the *Citrus* and *Irvingia* hosts had the predominant higher RWC than the infested and uninfested hosts while the uninfested had the least RWC in the two hosts. The RWC of the mistletoe and host plants as observed in RS5 followed similar trend as noted in previous experiments. The parasite exhibited higher RWC than the infested and uninfested in the *Citrus* and *Irvingia* hosts while the infested hosts in the two host instances had the least RWC.

6. Discussion

A comparison of the mineral elements obtained from mistletoe and host plants showed reduced nutrients contents in the infested *Citrus sinensis* and *Irvingia gabonensis* hosts whether in the dry or rainy season. But an assessment of the nutrients composition in the parasite and the uninfested hosts showed a few similarities though with some variations. There were higher uptakes of macro-nutrients like Na, K, Mg, N, in the uninfested *Citrus* host and micro-nutrient like Fe in *Irvingia* across the seasons when compared with that of parasite while N, Pb, Cd in the uninfested *Citrus*, P, N, Pb, Cd uptakes in the uninfested *Irvingia* were comparatively similar with that of the Mistletoe. Hence, this work agreed with the findings of Devkota [5], Glatzel and Geils [6], Ogunmefun, *et al.* [12] and Oyetunji and Edagbo [10], on differential constituent mineral nutrients of mistletoes in relation to their host source. Furthermore, it was observed that the macronutrients and micronutrients obtained in the mistletoe on *Irvingia* host plant were comparatively higher than the same nutrient elements got from the mistletoe on *Citrus*. Basically this higher differential accumulation of the nutrients in favour of the parasite on *Irvingia* might be due to the higher nutrient uptake by the host, which served as enriched source for its dependent parasite. However, the infested hosts suffered a diminished nutrient status when compared with the uninfested in their constituent nutrient elements. The macronutrients were depleted in the infested host plants by the parasite. Many of the micronutrients contents were also very low in these hosts. However, the parasite possessed relatively similar nutrients contents with the uninfested host plants. This suggests that the parasite is a burden on the host plants and these host plants need to absorb more nutrients to offset the nutrient requirement of the parasites. The lower nutrient status of the infested hosts so obtained from the comparative assessment of the accumulated mineral nutrients in the host plants supported other research findings among which included the works by Karunaichamy*, et al.* [13] and Hosseini*, et al.* [14] which implicated mistletoes as nutrient sink that often withdrew mineral nutrients from their hosts and invariably disrupt their nutrient balance. Indications from the evaluation of the mineral nutrients in the mistletoe-host association showed that in general the parasite possessed a comparative higher mineral nutrient content than their infested host (this is in corroboration of the assertion by Karunaichamy, *et al.* [13]. Equally noteworthy is the varied constituent elements of the major cations exhibited by *Tapinanthus bangwensis* on the two host plants similar to some other African mistletoes (e.g. *Phragmanthera incana* - [12]) which are non-host specific showed the adaptability of its internal metabolic constituents. The adaptive and receptive nature of the cellular metabolic structure of the African mistletoes such as *Tapinanthus bangwensis* afforded them the advantage of infesting wide range of several economic and agronomic trees and thus annexing them as host plants.

The assessment of the leaf chlorophyll contents of infested and uninfested *Citrus* and *Irvingia* host plants showed that concentrations of chlorophyll *a* and *b* were higher for the uninfested group but the ratios of chlorophyll *a* to *b* in the leaves of the host plants were comparatively similar. Results of evaluation of the leaf chlorophyll content of sampled plants were in conformity with observations by Abubacker*, et al.* [15] and Murugan*, et al.* [16] which affirmed reduction in the leaf chlorophyll content of host plants as a result of parasite infestation. This reduced leaf chlorophyll content may arise because of low availability of nutrients (competitive use by host-parasite) such as magnesium, phosphorus and even water culminating to inadequate chlorophyll pigmentation as well as reduced photosynthetic efficiency. The host-parasite interactions which have been acknowledged to be one of the major sources of reduction of the chlorophyll content of the hosts usually result in decline in their photosynthetic capacity [6, 17, 18]. Hence the presence of the parasite on the host plants could be described as a militating force which usually hampered the photosynthetic mechanism of the host (often leading to less carbon assimilation) but did not necessarily implied an impaired metabolic process of a dysfunctional photosynthetic apparatus. This may be why the host-parasite plants are able to co-exist for some considerable length of time without obvious damage to the host (as the minimum photosynthate necessary for their sustenance was catered for).

Estimation of Relative Water Contents (RWC) for the host-parasite plants showed patterns congruent with some past studies but a few peculiarities were also observed. *T. bangwensis* exhibited a predominantly higher RWC over the *Citrus* and *Irvingia* host plants in rainy season but its relationship with both hosts in the dry season is best described as indeterminate with the series of fluctuations displayed. In few instances, the host plants had comparatively higher RWC, at some points it was vice versa while at some other times there were similarities. Considering the pattern of the RWC so exhibited in the host-parasite relationship it is apparent that the assertion by Oyetunji and Edagbo [10] which stated that the rate of mistletoe's adjustment and adaptation to water flux and drought conditions is dependent to a larger extent on the host source, remained valid. Meanwhile, the infested host plants possess RWC capacities which majorly were significantly higher than the uninfested in both the dry and rainy seasons and this was consistent with the findings by Murugan*, et al.* [16]. The higher RWC of the infested hosts reflected the dissipation of extra energy in the metabolic process within these hosts caused by pressure (drain) on their transpiration stream to cater for both the host plants as well as the parasite's solute needs. The parasite through its haustorial attachment to the hosts' xylem is able to exert the suction pressure whereby its water and mineral nutrients demands are the compulsory priority of initial settlement. Mistletoes have been severally acknowledged to possess low water use efficiencies and must as well maintain more negative water potential than their hosts so as to ensure constant inflow of water with consistently opened stomatal aperture for higher transpiration process [17, 19]. Hence the physiological processes for meeting up with the biological water sustaining demands of the host often lead to the increased RWC exhibited by the infested host plants. The higher RWC status of *T. bangwensis* relative to the host plants in the rainy season earlier mentioned could be said to result from high relative humidity of the atmosphere and high water table of the surrounding soil often leading to low evapo-transpiration rate. This connotes a steady state of water availability and therefore little stress for water use demands. The RWC relationship of the host-parasite association in the dry season was said to be indeterminate because of the fluctuations observed. This was so because changes in the season brings about a gradual shift in the relative humidity, level of the water table in the soil and wind speed which all have their effects on the RWC of the sampled plants. The early dry season will often be accompanied by minimal variation up to the latter dry season of marked differences in outcomes and output of the sampled plant materials. Rigling*, et al.* [18], in their work asserted that mistletoe infection increases drought stress on their host. Thus, under dry environment of deprivation, mistletoes activities could lead to the death of host plants.

7. Conclusion

The presence of the mistletoe, *Tapinanthus bangwensis* on host plants exerts nutritional and water use pressure on the susceptible hosts. This therefore affects the mineral nutrients distribution, chlorophyll contents and water uptake of the hosts. Hence, the metabolic activities and survival of *Tapinanthus bangwensis* on hosts especially its dense population results in significant impact on available mineral nutrients, chlorophyll and water contents of the host plants. The solutes and photosynthates diverted from host plants by the mistletoe during dry season often tend towards a lesser productivity in host plants when compared with similar status in rainy season.

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Table-1. Mineral Elements Composition of *Tapinanthus bangwensis* and its Two Hosts in the dry season

Sample	Na %	K %	P %	Ca %	Mg %	N %	Fe %	Zn %	Mn %	Pb %	Cd%
T. bangwensis on Citrus	0.15^{d}	0.83^{c}	0.22^{c}	0.49^{a}	0.59^{b}	0.54^{c}	0.71^{b}	0.65^{c}	0.03^{b}	0.02^{a}	0.01^{b}
Infested Citrus	0.15^{d}	0.63^{d}	0.24^{bc}	0.36^{a}	0.49^{c}	0.48^{d}	0.59^{e}	0.59^{d}	0.02^{c}	0.01^{b}	0.01^{b}
Uninfested Citrus	0.20^{c}	1.16^{b}	0.27^{ab}	0.36^{a}	0.64^{a}	0.58^{bc}	0.66^{d}	0.59^{d}	0.02^{c}	0.02^{a}	0.01^{b}
T. bangwensis on Irvingia	0.27^{a}	2.20^{ab}	0.27^{ab}	0.56^{a}	0.68^{a}	0.69^{a}	0.69^{c}	0.74^{b}	0.04^{a}	0.02^{a}	0.02^{a}
Infested Irvingia	0.14^{d}	0.59^{d}	0.24^{bc}	0.45^{a}	0.39^{d}	0.63^{b}	0.53^{f}	0.53^{e}	0.01^{d}	0.01^{b}	0.01^{b}
Uninfested Irvingia	0.23^{b}	1.24^{b}	0.30^{a}	0.49^{a}	0.69^{a}	0.71^{a}	0.73^{a}	0.75^{a}	0.03^{b}	0.02^{a}	0.02^{a}

Means with same letter were not significantly different in each column by DMRT at $\alpha = 0.05$

Table-2. Mineral Elements Composition of *Tapinanthus bangwensis* and its Two Hosts in the rainy season

Sample	Na %	K %	P %	Ca %	Mg %	N %	Fe %	Zn %	Mn %	Pb %	Cd %
T. bangwensis on Citrus	1.57^{b}	1.88^{b}	1.81^{a}	0.50^{b}	0.47^{c}	1.32^{b}	0.51^{c}	0.45^{c}	0.03^{b}	0.02^{b}	0.03^{a}
Infested Citrus	0.34^{d}	0.62^{c}	0.57^{b}	0.28^{d}	0.38^{d}	0.31^{b}	0.40^{d}	0.35^{d}	0.02^{b}	0.02^{b}	0.03^{a}
Uninfested Citrus	2.52^{a}	5.08^{a}	1.55^{a}	0.64^{b}	0.68^{b}	1.39^{a}	0.72^{b}	0.64^{a}	0.04^{a}	0.01^{c}	0.02^{ab}
T. bangwensis on Irvingia	0.77^{c}	1.50^{b}	1.79^{a}	0.40^{c}	0.41^{d}	1.28^{c}	0.46^{cd}	0.36^{d}	0.03^{ab}	0.01^{c}	0.02^{ab}
Infested Irvingia	0.29^{d}	0.49^{c}	0.51^{b}	0.28^{d}	0.36^{d}	0.30^{b}	0.39^{d}	0.34^{d}	0.02^{b}	0.03^{a}	0.02^{ab}
Uninfested Irvingia	2.45^{a}	5.34^{a}	1.85^{a}	0.68^{a}	0.76^{a}	1.45^{a}	0.82^{a}	0.74^{a}	0.03^{b}	0.01^{c}	0.01^{b}

Means with same letter were not significantly different in each column by DMRT at $\alpha = 0.05$

Table-3. Leaf chlorophyll Content of *Tapinanthus bangwensis* and its two hosts in the dry season

Sample	Chlorophyll *a* (mg/ml)	Chlorophyll *b* (mg/ml)	Total Chlorophyll (mg/ml)	Chlorophyll *a* / Chlorophyll *b*
Leaves of *T. bangwensis on Citrus sinensis*	53.47^{a}	43.96^{a}	97.43^{a}	1.22^{a}
Leaves of *infested Citrus sinensis*	37.09^{c}	33.18^{b}	70.28^{d}	1.12^{b}
Leaves of uninfested *Citrus sinensis*	41.11^{bc}	37.29^{ab}	78.41^{cd}	1.11^{b}
Leaves of *T. bangwensis on Irvingia gabonensis*	42.29^{bc}	37.78^{ab}	80.07^{bcd}	1.12^{b}
Leaves of infested *Irvingia gabonensis*	46.65^{ab}	41.99^{ab}	88.64^{abc}	1.12^{b}
Leaves of uninfested *Irvingia gabonensis*	49.70^{a}	45.81^{a}	95.51^{ab}	1.09^{b}

Means with same letter were not significantly different in each column by DMRT at $\alpha = 0.05$

Table-4. Leaf chlorophyll content of *Tapinanthus bangwensis* and its two hosts in the rainy season

Sample	Chlorophyll *a* (mg/ml)	Chlorophyll *b* (mg/ml)	Total Chlorophyll (mg/ml)	Chlorophyll *a* / Chlorophyll *b*
Leaves of *T. bangwensis on Citrus sinensis*	53.73^{a}	50.49^{a}	104.22^{a}	1.07^{a}
Leaves of *infested Citrus sinensis*	34.70^{c}	52.15^{a}	66.86^{c}	1.08^{a}
Leaves of uninfested *Citrus sinensis*	55.63^{a}	50.48^{a}	104.12^{a}	1.07^{a}
Leaves of *T. bangwensis on Irvingia gabonensis*	44.18^{b}	40.38^{b}	84.56^{b}	1.00^{a}
Leaves of infested *Irvingia gabonensis*	33.98^{c}	31.37^{c}	65.27^{c}	1.08^{a}
Leaves of uninfested *Irvingia gabonensis*	55.59^{a}	52.14^{a}	107.74^{a}	1.07^{a}

Means with same letter were not significantly different in each column by DMRT at $\alpha = 0.05$

Table-5. Relative Water Content (%) estimation of leaves of *Tapinanthus bangwensis* and its two hosts

SAMPLE	DRY SEASON				
	09/02/15 DS1	17/02/15 DS2	02/03/15 DS3	16/02/16 DS4	02/03/16 DS5
T. bangwensis on Citrus sinensis	85.21^{a}	72.23^{e}	80.00^{d}	88.66^{c}	89.73^{b}
Infested Citrus sinensis	83.07^{c}	75.06^{c}	80.71^{c}	90.71^{a}	90.06^{b}
Uninfested Citrus sinensis	81.81^{d}	73.55^{d}	75.82^{e}	89.72^{b}	90.69^{a}
T. bangwensis on Irvingia gabonensis	83.78^{b}	85.43^{a}	90.23^{a}	77.42^{f}	87.37^{d}
Infested Irvingia gabonensis	78.26^{e}	85.26^{a}	89.25^{b}	84.07^{d}	88.46^{c}
Uninfested Irvingia gabonensis	78.44^{e}	79.88^{b}	75.09^{f}	79.04^{e}	81.22^{e}

Means with same letter were not significantly different in each column by DMRT at $\alpha = 0.05$
DS1 – DS5: Dry season, 1st – 5th data estimation

Table-6. Relative Water Contents (%) estimations of leaves of *Tapinanthus bangwensis* and its two hosts

SAMPLE	RAINY SEASON				
	29/06/15 RS1	**02/07/16 RS2**	**08/09/15 RS3**	**22/04/16 RS4**	**18/05/16 RS5**
T. bangwensis on Citrus sinensis	88.58^{c}	92.15^{b}	90.33^{b}	94.12^{b}	85.22^{b}
Infested Citrus sinensis	80.84^{e}	82.29^{d}	79.86^{d}	90.61^{d}	82.82^{d}
Uninfested Citrus sinensis	78.19^{f}	80.20^{e}	76.16^{e}	87.65^{e}	84.04^{c}
T. bangwensis on Irvingia gabonensis	92.77^{a}	93.92^{a}	95.33^{a}	95.08^{a}	90.16^{a}
Infested Irvingia gabonensis	89.39^{b}	91.88^{b}	90.45^{b}	94.83^{a}	75.17^{f}
Uninfested Irvingia gabonensis	83.25^{d}	90.45^{c}	88.65^{c}	92.89^{c}	77.80^{e}

Means with same letter were not significantly different in each column by DMRT at $\alpha = 0.05$

RS1 – RS5: Rainy season, 1st – 5th data estimation