May 2013- July 2013, Vol. 3, No. 3, 1933-1941. E- ISSN: 2249 –1929

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Biological Science

CODEN (USA): JCBPAT Research article

Comparative Ecophysiological Study of *Tapinanthus Bangwensis*, [Engl. And R. Krause] Danser (African Mistletoe) On Two Host Plants

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Received: 19 June 2013; **Revised:** 14 July 2013; **Accepted:** 16 July 2013

Abstract: Some ecophysiological aspects of African mistletoe, *Tapinanthus bangwensis*, [Engl. and R. Krause] Danser on two hosts were investigated. The leaf mineral nutrient analysis, total reducing sugar content and chlorophyll content of the mistletoe and the hosts were estimated. The characteristic feature observed was that the Na concentration was similar in the mistletoe and its *Citrus* species host while it was significantly higher in *Irvingia* species. The Ca, Mg, P, N and chlorophyll were more in the hosts relative to the mistletoe. Also, the relative water content estimation carried out showed a high percentage level in which the hosts had slightly higher rates than the mistletoe at the period of rainy season but a contrary result was obtained in the dry season when the mistletoe maintained higher water content than the hosts. Based on the results achieved in this study, it can be asserted that mistletoe thrives on its hosts relative to the available nutrients, water content and to a slight extent on the host photosynthate; while the extent to which mistletoe can affect the host is dependent on how much of the resource is diverted by the parasite and also the overall supply available to the host.

Key words: Mistletoe, Host, Mineral nutrient, Sugars, Chlorophyll, Relative water content

INTRODUCTION

Mistletoes belong to a large family of about 75 genera and approximately 1000 species[1] . The family originated in the Southern hemisphere and dispersed, apparently early, between fragments of Gondwana. It is now widely distributed on land surfaces of the former super continent. The family has three terrestrial, root parasitic genera and 72 genera of aerial branch parasites[1] . The Loranthacean mistletoes are tropical and occur as parasites on both angiosperms and gymnosperms. Six major genera are found in Nigeria namely: *Tapinanthus* [Blume] Reichb., *Agelanthus* Tieghem, *Loranthus* L., *Globimetula* Tieghem, *Phragmanthera* Tieghem *and Englerina* Tieghem. *Tapinanthus* is far more widespread in the Nigerian Savanna. The taxa infest many wild and domesticated tree and shrub species of ethnobotanical and economic value, causing various degrees of structural and economic damage[2].

Mistletoes are very important in curative medicine. They are known to be highly potent in curing circulatory problems and also as anticancer agents[2]. Mistletoe extracts are widely used in complementary and alternative cancer therapy in Europe. The extracts possess cytotoxic as well as immunostimulatory effect. The activity principle of the mistletoe (*Viscum album* L.) phytotherapeutics could be considered as combined cytotoxic and 'biological response modifying' activities (increasing host defense against cancer) that result from the activities of the plant lectins and the other biologically relevant substances. In Nigeria, several herbal preparations from leaves and twigs of mistletoes such as *T. bangwensis* have become popular for the treatment of variety of diseases, such as diabetes and hypertension, which have been reported to be on the increase in the country[2].

Mistletoes, as perennial flowering plants and aerial parasites of trees, face several interesting physiological challenges. Mistletoe seeds must firmly attach to a host branch and the seedlings must overcome host defenses and secure access to organic and inorganic resources of the host. To grow and reproduce, mistletoes must successfully compete for a share of the host's water, avoid mineral deficiencies, tolerate differences in host xylem sap chemistry and, over time, flower and seed within the host canopy. They are a diverse group of plants that meet these challenges in various environments and with a variety of physiological mechanisms[3] . Despite the ecological and medicinal importance of the African mistletoes, the physiological processes responsible for their biological activities and the extent and degree of their interactions with their hosts are yet to be fully understood; especially in the tropics.

Many aspects of African Mistletoes biology still poorly known and therefore provide extensive opportunities for further research. In view of the scanty knowledge on host-parasite relationship which is pervasive in most economic trees in the country; it has therefore becomes imperative to research into the phyto-physiological studies of these group of plants.

This study therefore carried out to elicit the comparative physiological processes, which involved water relations, mineral nutrient accumulation, sugar production, and leaf chlorophyll synthesis in *Tapinanthus bangwensis* and its two susceptible hosts, *Citrus sinensis* and *Irvingia gabonensis*.

MATERIALS AND METHODS

Site of the study: *Tapinanthus bangwensis* (African mistletoe parasitizing on two host plants; *Citrus sinensis* and *Irvingia gabonensis*) and these hosts studied for their eco-physiological behavioural patterns in respect of parasitic relationship.

Samples of these three plants were collected from some plantation fields at Moor plantation (a research centre) Apata, Ibadan, South-western, Nigeria (located between latitude, 07°38718' - 07°38599'N ; longitude, 003°84198' - 003° 84153' E; and at an altitude of 3 m) with laboratory work and analysis carried out at the Plant Genetic Resources laboratories, National Centre for Genetic Resources and Biotechnology (NACGRAB), Plant and Soil fertilizer laboratory, Institute of Agricultural Research and Training (IAR & T) and at the Plant Physiology laboratory, Department of Botany, University of Ibadan (UI). Samplings randomly collected from the selected and marked plants in both raining 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.

DETERMINATION OF MINERAL ELEMENTS

Calcium, Potassium & Sodium: The plant sample obtained digested by adding 5 mL of 2M HCl to the ash in a crucible and heat to dryness on a heating mantle. Five milliliter of 2 M HCl added again, heat to boil, and filtered through a Whatman No.1 filter paper into a 100ml volumetric flask. The filtrate made up to mark with distilled water, and used as stock for reading of concentration of Calcium, Potassium and Sodium using Jenway Digital Flame Photometer (PFP7 Model). The concentration of each of the element calculated using the formula:

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

Phosphorus: The ash of each sample obtained treated with 2M HCl solution as described for Calcium above. Ten (10) mL of the filtrate solution was pipette into 50 mL standard flask and 10mL of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow colour development. The concentration of phosphorus obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic 20 spectrophotometer or colorimeter at a wavelength of 470nm. The percentage phosphorus calculated using the formula:

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

Determination of Magnesium using Buck 200 AAS (Atomic Absorption Spectrophotometer): The digest of the ash of each sample above as obtained in calcium and potassium washed into 100 mL volumetric flask with demonized and made up to mark. This diluent aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination. For Mg fuel and Oxidant: Air-Acetylene; Wavelength: 285.2; Sensitivity (ug/L):15.

The meter reading for the element used to calculate the concentration using the formula:

Ppm or mg/kg (Mg) = Meter reading X Slope or Gradient X dilution factor.

% Magnesium = ppm or mg/kg divided by 10, 000

Nitrogen-Free Extract (NFE) Determination: Nitrogen Free Extract (NFE) calculated by difference after analysis of all the other items method in the proximate analysis. This includes all the nutrients

not assessed by the prior methods of proximate analysis. These are composed mainly of digestible carbohydrates, vitamins and other non-nitrogen soluble organic compounds. This was done by subtracting sum of (moisture % + % crude protein + % Ether Extract + % Crude fiber + % Ash) from 100.

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

Determination of Total Reducing Sugar: The phenol-Sulphuric Acid Method of Dubois *et al*[4]., (1956) used. Two gram of sample dissolved in 250 mL of distilled water and centrifuged to get the supernatant for the analysis. 1mLof the diluted solution were pipetted into test-tubes and 1mL of 52% phenol was added to each test-tube, 5ml of 96% H_2SO_4 was also added drop by drop. The test tubes allowed standing for 10 minutes before their contents transferred into clean, grease-free cuvettes and read with a Spectrophotometer at a wavelength of 490 nM. A blank was also prepared as above but distilled water took the place of sample being analyzed. The blank used to set the equipment to the zero mark. Glucose and fructose used as standard.

Leaf relative water content (RWC) estimation: Fresh leaves collected from each plant. A sharp cork borer used to cut the leaves samples into small discs. These were weighed and recorded as the sample's fresh weight (W), after which the samples were hydrated to full turgidity in distilled water for 4h under normal room light (dim light) and temperature (25^0C). After 4h, the samples taken out of water, dried of any surface moisture quickly and lightly with filter paper, and immediately weighed to obtain fully turgid weight (TW). Samples were then oven dried at 80^0C for 36 h and after being cooled down in desiccators, weighed to determine the dry weight (DW). Relative water content calculated using the mathematical expression below:

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

Where:

W = sample fresh weight;

TW = sample turgid weight;

and DW = sample dry weight

Determination of leaf chlorophyll content: The chlorophyll content of the hosts and mistletoe leaves were estimated according to the method of Hipkins and Baker[5]. Two grams of the leaves were collected in a polythene bag. These were ground with pestle and mortal in 80% v/v aqueous acetone in the dim light and filtered with No. 1 Whatman filter paper. Some 10ml of the filtrate (extract) taken into flat bottom volumetric flask and made to 50 mL with 80% v/v aqueous acetone. Absorbance read in a spectrophotometer at 645, 653 and 663 nm wavelengths. The measurement replicated thrice for each plant. The measurements carried out in a dim room to avoid photo-oxidation of the chlorophyll pigments. The chlorophyll content (mg/L) in each of the samples calculated using the following simultaneous equation:

Chlorophyll (chla) = $12.7A_{663} – 2.69_{645}$; Chlorophyll (chlb) = $22.9A_{645} – 4.68A_{663}$

Total chlorophyll (Tchl) = $20.2A_{645} + 8.02A_{663}$; Where A is the absorbance.

Statistical analysis: Statistical analysis of the study done using the analysis of variance (ANOVA) where applicable and the different means of treatments compared using Student-Newman-Keuls Multiple Comparisons Test with the statistical package, Graph Pad Instat.

RESULTS

The result of the mineral nutrient contents of the mistletoe and its hosts shown in **Table 1.** The Sodium content of the mistletoe and its *Citrus* host was not significantly different but was significantly higher in *Irvingia* than its parasite. The value of Potassium in both the mistletoe and hosts showed that the host plants had significantly higher Potassium content. The Calcium, Magnesium, Phosphorus and Nitrogen content of the mistletoe and host plants exhibited a trend similar to that observed in Potassium wherein the hosts had significantly ($P < 0.001$) higher content of these mineral nutrients. The result also showed that the mineral nutrients uptake of the host affected the level of accumulation of these elements in the parasite as well. This relationship is highly significant ($P < 0.001$).

Table -1: Nutrient concentrations of the mistletoe and hosts

S/N	Sample	% Na	% K	% Ca	% Mg	% P	% N
1	*T. bangwensis on Citrus*	0.44ns	0.77***	1.05***	0.88***	0.12***	0.57***
2	*Citrus*	0.45ns	0.83***	1.12***	0.95***	0.20***	0.74***
3	*T. bangwensis on Irvingia*	0.45***	0.74***	1.10***	0.92***	0.14***	0.60***
4	*Irvingia*	0.65***	0.94***	1.25***	0.99***	0.28***	0.79***

The values are means of three replicates. * = significant at p<0.001; ns = not significant**

Table-2 shows the total reducing sugars contents as contained in the mistletoe and hosts. The data revealed that the reducing sugar content (fructose and glucose) of the hosts were significantly higher ($P < 0.001$). Meanwhile a closer assessment of the fructose and glucose contents in the mistletoe and hosts showed that fructose was significantly higher in both the parasite and the hosts. The quantity of the fructose and glucose in the mistletoe that was parasitic on *Irvingia* were higher than that on *Citrus*. However, the host plants had more of these reducing sugars than the parasite.

Table-2: Reducing sugar content of the mistletoe and hosts

S/N	**Sample**	**% Fructose**	**% Glucose**
1	*T. bangwensis on Citrus*	1.42 ***	0.50***
2	*Citrus*	1.87 ***	1.06***
3	*T. bangwensis on Irvingia*	1.66***	0.76***
4	*Irvingia*	2.13***	1.43***

The values are means of three replicates. * = significant at p<0.001**

The relative water content (RWC) of the mistletoe and the hosts **(Table-3)** during the rainy and dry seasons were high in both plants with little difference between them. Seasonal variation of the parameter was observed in both plants. In the rainy season, the mistletoe had between 80 and 85% RWC and *Citrus* had between 88 and 92%, while *Irvingia* had between 88 and 93%, its mistletoe had between 86 and 90%.

Thus in the rainy season, the hosts had higher ($P < 0.001$) RWC than the mistletoe. There was a departure in RWC trend during the dry season from those of the rainy season. The RWC of the mistletoe and the hosts revealed values which were higher ($P < 0.001$) in the parasite. This was such that the RWC range obtained for *Citrus* was 75-86% and it was between 77 and 86% in the mistletoe while *Irvingia* had between 71 and 80%, its mistletoe had 88-91%.

It was observed that the RWC obtained in the mistletoe that was parasitic on *Irvingia* was higher than that on *Citrus* throughout both seasons. Also noted in the host plants, was the higher rate of RWC in *Irvingia* compared to *Citrus* throughout the period of the rainy season when the test was conducted; whereas the reverse was the outcome in the dry season, as *Irvingia* had the lower RWC for a major part of this period.

Table-3: Relative water content (%) estimation of the mistletoe and hosts

S/N	**Sample**	**Rainy Season**				**Dry Season**		
		13/07/10	20/07/10	28/07/10		22/02/11	24/02/11	01/03/11
1	*T. bangwensis on Citrus*	80.59***	83.54***	85.41***		77.93**	86.34ns	80.39ns
2	*Citrus*	89.37***	92.78***	88.92***		75.74**	86.20ns	78.42ns
3	*T. bangwensis on Irvingia*	90.85***	87.11***	86.16*		88.30***	91.74***	89.44***
4	*Irvingia*	93.01***	90.01***	88.96*		71.92***	79.39***	80.16***

The values are means of three replicates. * = significant at $p < 0.001$, ** = significant at $P < 0.01$, * = significant at $P < 0.05$.**

The value of chlorophyll a **(Table-4)** in the mistletoe was significantly lower than in its hosts. The chlorophyll b content in the mistletoe and hosts revealed divergent results. In the mistletoe-*Citrus* association, the values were not significantly different but with the mistletoe-*Irvingia* relationship, the chlorophyll b content in the parasite was significantly higher than what obtained in the host. Overall, the total chlorophyll content of the mistletoe and hosts exhibited values in which the total chlorophyll of the *Citrus* was significantly higher while the total chlorophyll content for the mistletoe-*Irvingia* relationship was not statistically different. The ratio of chlorophyll a/b was statistically different between the mistletoe and hosts; with the ratio of Chlorophyll a to Chlorophyll b in the parasite been much less than in hosts.

Table-4: Leaf chlorophyll content of the mistletoe and hosts

S/N	Sample	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a / Chlorophyll b
1	*T. bangwensis on Citrus*	1.4641***	5.0444ns	6.2758***	0.29***
2	*Citrus*	2.6499***	5.1769ns	7.5348***	0.51***
3	*T. bangwensis on Irvingia*	1.9442***	3.826***	5.7681ns	0.51***
4	*Irvingia*	3.6367***	2.6952***	5.8442ns	1.35***

The values are means of three replicates. * = significant at p<0.001; ns = not significant; CHL a = chlorophyll a; CHL b = chlorophyll b; T CHL = Total chlorophyll**

DISCUSSION

The mineral elements in the mistletoe and that of its host plants showed differential accumulation of the analyzed nutrients in both plants. The proportion of Na in *Citrus* and its parasite was similar but the concentration of Na in *Irvingia* was significantly higher than in the mistletoe. This observation is in tandem with the theory put forth by Glatzel and Geils[3] that some elements may vary by one or two orders of magnitude in samples from the same mistletoe species on different host trees and species. The tissue concentrations of the other mineral elements which included K, Ca, Mg, P and N in the hosts and mistletoe showed that the hosts accumulated significantly higher proportion of these elements contrary to those reported by other workers in temperate mistletoes[6] . These results bore some similarities and dissimilarities to other assessed mistletoe species in their nutrient uptakes. Since, mistletoes are generally known to show variations in their physiological capability and adaptation, it will be logical therefore not to expect a uniform level of concentration of the various mineral elements contained in them. In this study, the concentrations of elements in the mistletoe supports the observation by Glatzel and Geils[3] , in which it was reported that the best correlation for predicting the concentrations of elements in mistletoe is often (but not always) the concentration of elements in the host. The nutrient uptake by the mistletoe and hosts showed that both displayed similar order of nutrient accumulation which at its initial stage might be of no obvious detriment to the hosts; but the extent to which the hosts could be affected depend not only on how much of the resource is diverted by the parasite, but also on the overall supply available to the hosts [7]. The nutrient uptake by the mistletoe evidently showed correlated fluctuations relative to hosts' source, such that for example, when the percentage Calcium in *Citrus* was 1.12, mistletoe's uptake was 1.05 and in *Irvingia* when it was 1.25, mistletoe had 1.10. Phosphorus and Sodium were the lesser nutrient elements contained in the mistletoe and hosts while Calcium and Magnesium were the more abundant in both. This is to say therefore, that, in a case of heavy infestation by mistletoe, such a host plant is prone to nutrient deficit, stunted growth, severe damage and ultimately death, if no control measure taken.

The values of the reducing sugars (i.e. glucose and fructose) of the mistletoe and hosts revealed a higher proportion in the hosts relative to the parasite. The sugars are major photosynthates and mistletoes have been known to carry out limited photosynthesis while they also derive some proportion of their photosynthates from the hosts; insomuch that the hosts maintain their optimum sugar content with little or no manifest reduction caused by their contact with mistletoe. This study have also further shown that a larger proportion of the reducing sugar content of the mistletoe and hosts is fructose and it can therefore be thus ascribed as the main source of energy in both plants. The

quantity of reducing sugar obtainable in the mistletoe is relative to the quantity available in the hosts; a trend similar to what obtained in the assessment of concentrations of mineral elements in mistletoe[3]. In attestation of the postulation put forth above, it was noted that the fructose and glucose proportion in the mistletoe that was parasitic on *Irvingia* were higher than that on *Citrus* and this was in view of the fact that *Irvingia* had more reducing sugars when compared to *Citrus*. This further suggests that the parasite depends on the host for little sugar supply from its host to complement its weak photosynthetic activities. This could cause a stress on the host particularly when there is an unfavourable weather condition.

Mistletoe and the host plants were observed to possess high level of relative water contents. The hosts had higher relative water content than the mistletoe in the rainy season. The reverse was the scenario in the dry season whereby the relative water content of the mistletoe became higher than that of the hosts. This was so because the parasite was able to maintain its optimum water requirement while the hosts generally exhibited a slight decline in their water content. This observation thus lends credence to an earlier study on water relations in mistletoe by KirkPatrick[8] as noted by Hawksworth *et al.*[9], *(1996*); whereby he posited that *Pinus contorta* infected by *Arceuthobium americanum* under optimal moisture conditions displayed conductance usually less than that of the host while during summer drought condition, however, conductance in the parasite was typically from 2 to 5 times that of the host. Based on the observation from this study, it can be averred that the rate of mistletoe adjustment and adaptation to water flux and drought conditions is dependent to a larger extent on the host source. It was noted that while mistletoe was able to obtain maximally its water requirement from *Irvingia* in both rainy and dry season; despite this host's higher level of water depletion, the case is not the same with *Citrus*. In *Citrus*, it was observed that the mistletoe was able to access water relative to the available quantity. This confirmed why African mistletoes usually maintain some leafy condition in the dry season relative to its host. This situation could be very dangerous to the hosts in case of severe or prolong drought. The high relative water content of the mistletoe may improve the mineral nutrition of the parasite[10] particularly during dry period.

Chlorophyll contents of the African mistletoe leaves were lower and/or equal to those of hosts on a fresh weight basis. This corroborates the observation by researchers like Graham *et al.* [11] , who worked on mistletoe and hosts chlorophyll apparatus; also in support was a similar effort by Johnson and Choinski[12] who showed *Tapinanthus vittatus* parasitizing *Diplorhynchus condylocarpon* had lower total chlorophyll content on a fresh weight basis. In addition, the value of the ratio of chl a to chl b in the parasite was much less than in hosts signifying more difference between chl a and chl b in the mistletoe. The high Chl b to a proportion in this study indicated that the mistletoe, *Tapinanthus bangwensis* had a relatively small proportion of Chla. Meanwhile, mistletoes have been shown to carry out photosynthesis at low rates and reported to possess chloroplasts with large deficiencies in photosystem activities[13]; it would therefore not be inappropriate to link this consistently low proportion of chlorophyll a in the mistletoe to these observable traits since variations in the proportion of the other components (chlorophyll b & total chlorophyll) of the photosynthetic apparatus have been observed to be of inconsequential effect. The low Chl a/b in the mistletoe might be a mechanism to combat the effects of shading by the host plant on its photosynthesis. This means the mistletoe could effectively utilize diffused (low) light. Some research had indicated that dwarf mistletoes usually possessed less than 25% of the chlorophyll level of their hosts' foliage. This result showed a far high percentage of the parasite chlorophyll level, which signifies higher photosynthetic activities in the African mistletoe. This assertion is supported by the level of the reducing sugars in the mistletoe obtained in this study. Physical observation of African mistletoes shows that they are usually very

greenish just as their hosts. This could the reason why they do not usually kill their hosts in the tropics. Marshall *et al.* [14] also noted that the differences in photosynthetic rates of mistletoes and their hosts were not statistically significant, despite the low photosynthetic rates in mistletoes.

CONCLUSION

The study of the host- mistletoe ecophysiology of *Tapinanthus bangwensis* on the two hosts, *Citrus sinensis* and *Irvingia gabonensis* shows that mistletoe thrives on its hosts on the strength of the available water, mineral nutrients, sugars (photosynthates) and the effective leaf chlorophyll content. Often, though may not always be the case, a rise or decline in these nutritive parameters is accompanied by a correlated change in the mistletoe [3]. Mistletoe as observed in this study possessed a consistently low proportion of chlorophyll a while the ratio of chlorophyll a to b in the parasite is much less than in the hosts. The value of chlorophyll b and total chlorophyll content varies between the mistletoe and hosts. The major source of energy in both mistletoe and the host plants is Fructose. In the event of nutrient shortfall and/or water stress, the host plant is more liable to suffer the immediate impact.

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