Open access Journal International Journal of Emerging Trends in Science and Technology
Impact Factor: 2.838 DOI: http://dx.doi.org/10.18535/ijetst/v3i03.12

Mycoflora Associated with Baobab (*Adansonia digitata*) and their Effect on the Nutritional Composition of the Leaf and Fruit

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ABSTRACT
Mycoflora associated with leaf and fruit of Baobab (Adansonia digitata) and their effect on the nutritional composition were investigated. Three different mycoflora were isolated from the leaf they include Aspergilus flavus, Lasiodiplodia theobromae and Cladosporium spp while six mycoflora were isolated from the fruit Aspergillus flavus, A. niger, Lasiodiplodia, theobromae, Cladosporium spp, mucur mucedo and pseudofussicoccum. It was observed that there was a relative decrease in the fat, crude fibre and crude protein content of the infected leaves and fruits while the Ash contents of the infected leaves and fruits were relatively higher. So also there was a decrease in the moisture content of healthy fruit while the moisture content of the healthy leaves was relatively higher compared with the fungal infected ones. A correlation test carried out shows that there was no significant difference P (>0.005) between the moisture, fat and crude protein of infected leaves and the fat and crude fibre of infected fruits and apparently healthy ones, while the ash and crude fibre of leaves and the ash, moisture and crude protein of the fruits indicates a significant difference P (<0.005).
Keyword: *Mycoflora, Nutritional Composition, Baobab, Leaf, Fruit.*

1. INTRODUCTION

Baobab (*Adansonia digitata* L.) is a large iconic tree indigenous to Africa where it is found in many countries. It is an emblematic, culturally important and physically majestic sub-tropical tree. The baobab has been referred to as "arbre a palabre", meaning the place in the village where the elders meet to resolve problems. In the past decade, it has attracted the interest of several pharmaceutical companies and researchers due to its various traditional uses (medicinal, nutritional and cosmetic).

There have been various reports of baobab *(Adansonia digitata*) trees covered with black mould on their branches and stems and in some cases it has been suggested that this might be linked to death of these iconic trees [7]. This condition has commonly been referred to as ''sooty mould'' in the literature (Piearce et al. 1994). The infection appears to begin as orange brown spots, mostly on the undersides of branches.

The spots subsequently turn black and can coalesce to form larger patches [14]. Fallen twigs covered with the black fungus have commonly been found on the ground below severely affected trees in Zimbabwe [14], and were presumed to have died due to the black mould infection. The fungus collected between 1989 and 1991 from the black stems of trees in Zimbabwe was identified as an *Antennulariella sp*. [14] and it was concluded E. M. Cruywagen (&) _ P. W. Crous _ J. Roux that this condition was mainly due to long-term environmental stresses and that the sooty mould was a secondary infection.

Sharp (1993) reported instances of ''sooty mould'' in Malawi, South Africa, Zambia and Zimbabwe.

These reports, including observations made over an approximately 10-year-period, were closely associated with more than a decade of drought [15].

In 1991, an article in New Scientist [1] reported that an unknown black fungus was colonising the branches and trunks of apparently healthy baobab trees in South Africa and Zimbabwe. It was speculated that the fungi started growing on the trees after rain had ended the drought of the previous decade.

The term ''sooty mould'' refers to fungi that grow on the exudates of insects living on plants, but are also able to grow without these exudates [3,8].

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Fungal Mycoflora

All samples (bark, leaves and fruit pulp) were separated and were surface sterilized with 1% sodium hypochlorite solution [9]. Isolations were conducted by plating sections of material on to isolation media. Potato dextrose agar (PDA) was prepared as instructed by the manufacturer. All isolations were incubated at 25°C until the growth of fungi were observed and the microscopies of the isolates were determined as described by Kora *et al.* (2002) method. The fungal isolates were sub-cultured, the pure cultures were obtained by plating the isolates on PDA and incubated at 25°C for 5days. The pure cultures we prepared on slides and Identification was done with reference to Barnett and Hunter (1998).

2.2 Analysis of fungal effects on the nutritional composition of leaves and fruit pulp of baobab trees.

Samples used for the analysis were collected from fresh healthy leaves and fruit pulps and fungal infected ones. The samples were weighed, cut into pieces and dried in an oven at 100^0C for 2hours. The dried pieces of the samples were grounded into powder and analyzed for ash, moisture, Crude Protein, Crude Fibre, and fat following the official methods of analysis by Association of official Analytical chemist 15th edition (1990). Samples were collected from five plants and each sample was replicated 3 times. The analysis was carried out in the department of animal science Faculty of Agriculture Bayero University, Kano.

Procedures involved are described below:

a. Determination of Moisture

2gm of grounded samples were weighed into container (crucible), the crucibles were shaken gently to uniformly distribute the samples and expose them for maximum drying.

The samples were placed into an oven that has been pre heated to 100^0Cfor 3hrs. The samples were left uncovered in an oven for 24hrs at 100^0C. The samples were then moved to the desiccator and covers were placed on each container as it is transferred. The desiccator were sealed and allowed to cool for 2hrs. The container with cover and dried samples were weighed and the weights were recorded to the nearest 0.1mg.

$$\% \text{ Total Moisture} = \frac{W3-W2}{W2-W1} X\ 100$$

W1 = tare weight of container (with cover) in grams

W2 = weight of sample and container (with cover) in grams

W3 = dry weight of sample and container (with cover) in grams

b. Ash Determination

The crucible was removed from the oven to the desiccator, the crucible were allowed to cool and weighed to the nearest 0.1mg (W1). 2g of samples were weighed accurately into crucible, the weight of the crucible and the sample were recorded to the nearest 0.1mg (W2). Ash in furnace at 600^0c for 2hrs until the furnace reaches the temperature. The crucibles were allowed to cool in furnace to less than 200^0c. The crucibles were then placed in desiccator with vented top. The crucible and the ash were allowed to cool and weighed to the nearest 0.1mg (W3)

$$\%\text{ASH} = \frac{W3-W1}{W2-W1}\ X\ 100$$

W1 = tare weight of crucible in grams

W2 = weight of crucible and sample in grams

W3 = weight of crucible and ash in grams

c. Crude Fat Ether Extract.

A filter paper was folded into a thimble shape and weighed (W1)

2g of samples were weighed and placed into the thimble (W2).

The thimble was slipped into a thimble holder.
250ml of pet (ether/ diethyl) was added using a glass funnel from the top of the condenser.
The sox let apparatus was assembled, extraction was done for about 4hrs on a Hi setting (condensation rate of 5 to 6 drops per second).
After extraction the ether was allowed to drain out from thimble for about 30minutes.
The thimble was removed from the holder and allowed to drain and dry at 700c for 30 minutes
It was then transferred to a desiccators and allowed to cool. It was then weighed to the nearest 0.1mg (W3)

%Crude Fat = $\frac{(W1-W2) - W3}{W2} X\ 100$

W1 = initial thimble weight in grams
W2 = sample weight in grams
W3 = weight of sample and thimble in grams

d. Crude Protein

0.2gm of samples was weighed into a digestion tube, 15ml of conc. Sulfuric acid was added, and the tube was swirl gently until the sample and acid were thoroughly mixed. 5g of catalyst mixture was added. The mixture was heated cautiously until the solution was clear. The temperature was then raised and the solution was boiled for 2hrs, the solution was allowed to cool and transferred into the content of the tube into 100ml volumetric flask and diluted to volume with distilled water and mixed thoroughly.

I. Distillation

10ml of 2% boric acid and 2 drops of mixed indicator was measured into a 100ml Erlenmeyer flask10ml of aliquot of the digest into a distillation apparatus. 15ml of 40% Naott was added into the mixture, and then Nitrogen was distilled into the boric acid/indicator flask for about 10-15 minutes.
Thereafter the condenser tip was rinsed with distilled water.

II. Titration

The distillate was titrated with standard 0.025N sulfuric acid to a pink end point and the burette reading was taken.

III. Calculation

%N=

$$\frac{0.014\ \left(\frac{MeN}{100g}\right) X\ Titer\ Value\ (TV) X\ Volume\ of\ digest\ (100ml)\ X\ Normality\ of\ acid\ (0.025)}{Weight\ of\ (0.2g) x\ volume\ of\ aliquot\ used\ (10ml)}$$

% Crude Protein = %N X 6.25

e. Crude Fibre

2g of samples were weighed (W1) and transferred into a 9cm hard filter paper supported on a filter cone on a 60º funnel. The samples were extracted with three 25ml portions of ether and vacuum was applied until the samples were dry.
The extracted samples were transferred by brushing into a 600ml beaker of the fibre digestion apparatus. 200ml of 1.25% of sulfuric acid solution was added. The beaker was then placed on the digestion apparatus with pre adjusted heater and boiled for exactly 30 minutes. The beaker and the filter content were removed through California Buchner funnel, the beaker was rinsed with 75ml of boiling water and washed through the funnel. The process was repeated with 50ml portions of water and suck dry. The residue was returned to the beaker by blowing back through funnel.
200ml of boiling 1.25 sodium hydroxide solutions was added; it was then returned to the heater and boiled for 30minutes. The beaker and the heater were then removed and washed with 25ml of boiling 1.25%sulfuric acid solution, three 50ml portions of water and 25ml of alcohol. The fibre mat and residue were dried at 130± 2°C for 2hrs. It was then transferred into a desiccator and weighed (W2).The samples were then allowed to cool in the desiccator and weighed (W3)

Crude Fibre = $\frac{W2-W3}{W1} X\ 100$

Data analysis

Paired sample test was adopted to statistical compared fungal infected fruit and leaf and apparently healthy ones.

3. RESULT AND DISCUSSION

Aspergillus flavus, Lasiodophodia theobromae and cladosporium spp, where isolated from the fruits and leaf of Baobab plants where as additional fungi *A. niger*, *cladosporium* spp and *mucor mucedo* were isolated from the fruit. Tsao 1993 observed that these mycoflora were also isolated from rubber plants and were pathogenic to the plant.

The proximate composition of the fungal infected and apparently healthy leaves of *Adansonia digitata* was shown in Table 1, while the proximate composition of the fungal infected and apparently healthy fruits was shown in Table 2.

Result of the proximate composition of healthy and fungal infected leaves and fruits indicates that there was an increase in the ash and moisture content of the infected fruits of *Adansonia digitata* also the ash content of the fungal infected leaves was higher. This correlated with the findings of [12]. However, there was an increase in the crude protein crude fat, crude fiber of the healthy fruits and leaves relatively to the apparently fungal infected samples. It could therefore be deduced that the increase in moisture and ash in the fruits, and the increase in ash of the leaves may be caused by degradation due to mycoflora. Similarly these degradative activities might result to relative reduction in the protein, crude fat and crude fibre [12].

Wehmeyer (1971) Reported that baobab contain significant amount of essential amino acids that animal are incapable of synthesizing within their own bodies and are essential for human growth, maintenance and reproduction. This suggests that these pathogens might have denied man these essential nutrients upon consumption through their degradative activities thereby causing some great damaging to human health [12].

Table 1: Proximate Composition of Healthy and Unhealthy Leaves of Baobab (*Adansonia digitata*)

Nutrients	HLAD Concentration (%) Mean + Std. Error	F ILAD concentration (%) Mean + Std. Error	Significant difference
Ash	1.27 ± 0.11<	1.60 ± 0.12	S
Moisture	17.55 ± 0.63 >	15.20 ± 0.50	NS
Fat	1.80 ± 0.41>	1.49 ± 0.68	NS
Crude Fibre	12.45 ± 0.28>	11.62 ± 0.30	S
Crude Protein	13.95 ± 0.33>	12.83 ± 0.30	NS

HLAD = Healthy leaves of *Adanosni adigitata*, **FILAD** = Fungal infected leaves of *Adansonia digitata*
NS= No significant difference, **S**= there is Significant difference.

Table 2: Proximate Composition of Fungal Infected and Healthy Fruit Pulp of (*Adansonia digitata*)

Nutrients	HFAD Concentration (%) Mean + Std. Error	FILAD Concentration (%) Mean + Std. Error	Significant difference
Ash	3.97 ± 0.18<	4.28 ± 0.15	S
Moisture	14.34 ± 0.58<	15.35 ± 0.54	S
Fat	1.10 ± 0.08 >	0.78 ± 0.07	NS
Crude Fibre	5.73 ± 0.14 >	5.13 ± 0.15	NS
Crude Protein	5.73 ± 0.14 >	2.67 ± 0.19	S

HFAD = Healthy fruits of *Adansonia digitata*, **FIFAD =** Fungal infected fruits of *Adansonia digitata*
NS= No significant difference, **S**= there is Significant difference.

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