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Biochemical Changes Induced By the Effect of Six Pathogenic Fungi on Dialium *Guineense:* Black Velvet Edible Fruit

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Abstract: *The following fungi (Aspergillus niger, Aspergillus flavus, Botryodiplodia theobromae, Fusarium oxysporum, Penicillium chrysogenum and Rhizopus stolonifer,) were isolated from diseased fruits. Changes in the Proximate content in D. quineense edible fruit inoculated with the test organisms were carried out. The effect of the test organisms on the mineral composition and vitamin content was carried out using the methods recommended by Association of Official Analytical Chemist (AOAC). There was a significant increase (P<0.05) by several degrees caused by the test fungi in moisture, protein and ash, while there was a significant decrease in dry matter, crude lipid, carbohydrate and crude fiber when compared to the uninoculated control. There was significant decrease (P<0.05) in all the mineral compositions (Ca, Cu, Fe, Mg, P, K and Na) caused by the fungi inoculated fruits when compared to the uninoculated fruit control. The vitamin content (Vit. A, B_2, B_3, and C) of the fungi inoculated fruits decreased significantly when compared with the control. The vitamin C content of D. guineense is known to be very high (49.50%), but was reduced drastically by Botryodiplodia theobromae (20.50%). The inoculated fungi caused an increase in anti-nutrient (oxalate, phytate, tannin, saponins and trypsin inhibitors) content of the edible pulp.*

Keywords*: Biochemical compositions, D. guineense, Nutrients, Pathogenic fungi*

I. Introduction

Black Velvet tamarind (*Dialium guineense*) is a woody plant that occurs in the rain forest region of West Africa. It grows up to 15m high with dark green glossy leaves each measuring 6cm to 8cm long and 2.5cm wide at the widest part of the leaf (Okegbile and Taiwo, 1990). The young leaves are sometimes chewed for its tangy taste. A well-established velvet tamarind plant bears one set of fruit per year and yields up to 200kg of fruits annually. The ripe fruits are available from January till May but the peak period for harvest is between March and April, (Orhue et. al., 2007). The velvet tamarind pulp is eaten in Southeastern Nigeria (where it is known as *"Icheku" or Nchichi"*) because of its refreshing properties and pleasant scorching taste. The mature dry circular pods are cracked open (manually) to release the semi-dry edible fruit pulp, which also embeds the seed. Black velvet Tamarind has been found to contain high about of vitamin C (ascorbic acid) and other nutrients (Dike, 2010). These nutrients are being affected by pathogenic fungi in diverse degrees. (Ikechi-Nwogu and Nwaukwu, 2012) identified six pathogenic fungi that are found growing on the edible fruit of Tamarind. These pathogenic fungi are; *Aspergillus niger*, *Aspergillus flavus, Botryodiplodia theobromae, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus stolonifer*, which have mycotoxins that lowers the nutritional value of the fruit.

This study is carried out to find out the effect of the above fungi on the biochemical composition (Proximate, Vitamin C, Mineral content and Anti-nutritional factors) of the *Dialium guineense* fruit.

II. Materials and Methods

2.1. Isolation and identification of fungi.

The fungi used in this study (*Aspergillus niger*, *Aspergillus flavus, Botryodiplodia theobromae, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus stolonifer,)* were isolated from diseased friuts using the standard Blotter method recommended by the international Seed Testing Association (ISTA, 1976 and Agar method (Klement and Voros, 1974). The fungi were identified under a Stereobinocular microscope based on their habit characteristics. Slides were made to confirm identification following descriptions by international Mycological Institude (IMI fungi Descriptions). Pure, single spore cultures of each fungi were obtained by growing them on potato dextrose agar (PDA). The cultures were grown in complete darkness in an incubator for 7days at 21 ± 2^0C they were used as inoculum.

2.2. Inoculation of Seeds with Fungi

One hundred grams of healthy *Dialium guineense* fruits were weighed out and cleaned by removing the pulp aseptically. The cleaned edible fruit was then put into 25ml conical flasks, plugged with non –absorbent cotton wool and covered with foil and then autoclaved at 121^{o}C for 15 minutes at 15PSI to eliminate any internal and external seed borne micro- organism (Ward and Diener, 1961). After autoclaving the flasks were allowed to cool and 100ml of sterile distilled water was added to each flask and shaken gently to wet all the seeds and to create a humid and conducive environment for the fungi to be inoculated to have an even distribution. Each flask containing seeds was inoculated with a disc of 7 day old mycelium spores of each fungus obtained from the pure culture of isolated fungi from infected seeds. This was done with a 1.5cm diameter sterile cork borer.

The flasks were gently shaken for about 15minutes to obtain uniform distribution of the mycelium among the seeds. The control flask, received the same treatment, but there was no fungi added to it. The entire flasks which include the fungi inoculated and uninoculated seeds were incubated at room temperature in complete darkness for 14 days.

A total of 18 flasks were used, 3 flasks replicate for each set of fungi inoculated seeds and uninoculated seeds. They were incubated for 14 days in a dark incubated area. At the end of the incubated period, the flasks of each fungal treatment and flasks for control were harvested for biochemical analysis. The seeds in each flask were transferred into a pre-weighed watch glass, dried at 45^{o}C for 24 hours and the spores and mycelia of the fungi removed by sieving (Ward and Diener, 1961). Biochemical analysis of various nutrient component (Dry Matter, Moisture, Crude Lipid, Crude Protein, Crude Fiber, Ash content and Carbohydrates in both fungus-inoculated and uninoculated seeds at the incubation period of 14 days were determined, also the effect of the fungi on the Vitamin C content and the anti-nutrient content of the fruit was carried out following procedures recommended by the Association of Official Analytical Chemists (AOAC, 1995). The results of each component were subjected to statistical analysis using the Analysis of Variance (ANOVA).

III. Results and Discussions

The effects of the fungi on the biochemical component of the fruits were carried. The moisture content of the fungi – inoculated seeds increased (p=0.05) when compared with the uninoculated control (6.6). This is presented in (Table 1). *Botryodiplodia theobromae* (15.5) caused the highest increase in moisture followed by *Rhizopus stolonifer* (12.24) The increase caused by the fungi is due to their utilization of the component of the seeds as food nutrient thereby producing water in the process. Similar results were recorded by Ataga and Akueshi (1986) in sunflower seeds inoculated with fungi. Ataga and Umechuruba (1997) also reported increase in moisture content of African Yam Bean inoculated with storage fungi. The reverse is the case for Dry Matter. There was a significant decrease (P=0.05) from the control seeds when compared to inoculated seeds with Fungi. This fungus produces extracellular cellulolytic and pectic enzyme and secondary metabolites which may be responsible for the drastic depletion of dry matter (Okonkwo et al., 1990).

There was also a decrease in carbohydrate of fungi inoculated seeds as when compared with the uninoculated control. The decrease in the inoculated seeds could be due to the utilization of storage starch and sugar as a carbon source by the microorganisms during respiration and also a source of energy for microbial growth (Monday, 2005).

The protein content of the fungi inoculated seeds, increased significantly when compared to the uninocualted control. The increase caused could be due to the presence of proteinaceous mycelium in the fungi. Cherry and Beuchat, (1975) obtained a similar result in their study of protein changes in groundnut seeds infected with *Neurospora sitophila and Rhizopus oligosporu* which he said resulted from slight protein synthesis by the proliferation of the fungal hyphae and the synthesis of enzyme protein or other constituents.

There was a significant decrease of lipid of the uninoculated control when compared to the fungi inoculated seeds. This agrees with the findings of Ogundero, (1992) who explained that the decrease in oil content could be due to the hydrolysis of oil to free fatty acid (FFA). This occurred at different rates for the individual fungi pathogens.

There was a drastic and significant decrease in fiber of all the seeds inoculated with the individual fungi when compared to the control. This experiment agrees with the report of (Onifade et al, 2004), about a decrease in crude fiber content of sweet potato flour enriched with *A. niger*. He also explained that the crude fiber tends to decrease during fermentation. This he concluded was a result of being utilized by the fermentation microbes.

There was significant increase in ash of the individual fungi when compared to the uninoculated control. For the fungi, Ataga and Umechuruba (1997) resolved that the increase could be attributed to the presence of minerals like potassium and phosphorous in the mycelia of the fungi.

The mineral content of the *D. guineens*e fruit infected by the different test fungi showed that there was a significant decrease in the mineral composition of the seeds when compared to the uninoculated control. This

is a shown in (Table 2). This shows that when infected fruits are eaten, they lack adequate basic mineral nutrients.

Black velvet Timarid has a high content of Vitamin C and Vitamin A as shown in (Table 3). However, the text fungi caused significant decrease in the vitamin content of the edible fruit.

The text organisms caused a significant increase in the antioxidant content of the edible fruit. This could have resulted as a result of the introduction of mycotoxins by the fungi. This causes food poison. This is shown in (Table 4).

TABLE 1: Changes in the Proximate Content in D. quineense Edible Fruit inoculated with *Aspergillus niger, Aspergillus flavus, Botryodiplodia theobromae, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus stolonifer*

Test Fungi	Proximate Composition (%W/W)/100g						
	Dry Matter	Moisture	Crude Lipid	Crude Protien	Carbohydrate	Crude Fibre	Ash
(Uninoculated) Control	93.3±1.48a	6.6±0.10c	4.25±0.03a	5.71±0.10b	84.1±1.32a	0.5±0.01a	3.01±0.01d
Aspergillus niger	89.65+1.43b	10.35±0.12c	3.19±0.23a	6.34±0.31a	75.53±0.07b	0.23±0.03c	4.59±0.09c
Aspergillus flavus	91.28±1.02a	8.72±1.47c	2.10±0.55b	6.27±0.01a	73.32±0.02c	0.4±0.03b	3.98±0.06d
Botryodiplodia theobromae	84.5±1.67c	15.5±1.02a	1.25±0.02c	6.89±0.16a	60.2±1.75e	0.3±0.01c	6.50±0.02a
Fusarium oxysporum	86.25±0.15c	9.75±0.23c	2.95±0.12b	6.02±0.01b	68.5±0.14d	0.15±0.01c	5.02±0.03b
Penicillium chrysogenum	92.08±0.11a	7.92±0.01c	2.56±0.03b	6.56±0.01a	70.26±0.01d	0.2±0.04c	5.29±0.01b
Rhizopus Stolonifer	85.0±0.15c	12.24±1.21b	3.90±0.02a	6.75±1.21a	69.4±1.25d	0.25±0.04c	4.62±0.06c
L.S.D(0.05)	1.54	0.4	0.8	0.6	1.27	0.1	0.3

Mean of two determinations with three replications ± Standard Error. L.S.D- Least Significant Difference
Values with the same alphabet along column are not significantly different at (P<0.05)

TABLE 2: Changes in the Mineral Content in *D. quineense* Edible Fruit inoculated with *Aspergillus niger, Aspergillus flavus, Botryodiplodia theobromae, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus stolonifer*

Test Fungi	Mineral Composition (%W/W)mg/100g						
	Calcium (Ca)	Copper (Cu)	Iron (Fe)	Magnesium (Mg)	Phosphorus (P)	Potassium (K)	Sodium (Na)
(Uninoculated) Control	47.0±0.40a	1.0±0.04a	4.1±0.40a	300±14.12a	42.9±0.40a	260±14.14a	390±15.42a
Aspergillus niger	45.5±1.02a	0.9±0.01a	3.9±0.12b	250±10.19d	40±0.05b	190±10.50e	250±16.12d
Aspergillus flavus	46.4±0.12a	0.7±0.38a	3.7±0.35c	235±10.25e	39.25±1.23c	230±11.70b	200±11.12f
Botryodiplodia theobromae	35.5±1.32d	0.65±0.04a	3.6±0.25c	275±10.35c	35.62±1.23c	220±10.50c	213±11.02e
Fusarium oxysporum	30.2±0.12e	0.35±0.01b	3.25±0.42d	150±15.2g	32.2±2.25d	105±13.2f	150±13.2g

Penicillium chrysogenum	43.0±21.24b	0.80±0.01a	3.4±0.12c	200±10.2f	37.5±2.35c	200±2.50d	300±12.5c
Rhizopus Stolonifer	39.5±1.21c	0.5+0.72a	3.50±0.28a	280±7.25b	36.3±1.27c	190±11.5e	350±15.3b
L.S.D(0.05)	0.9	0.1	0.1	1.53	1.50	1.54	1.55

Mean of two determinations with three replications ± Standard Error. L.S.D- Least Significant Difference
Values with the same alphabet along column are not significantly different at (P<0.05)

TABLE 3: Changes in Vitamin Content in *D. quineense* Edible Fruit inoculated with *Aspergillus niger, Aspergillus flavus, Botryodiplodia theobromae, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus stolonifer*

Vitamin Composition (%W/W)mg/100g	**Test Fungi**							
	Control	*Aspergillus niger*	*Aspergillus flavus*	*Botryodiplodia theobromae*	*Fusarium oxysporum*	*Penicillium chrysogenum*	*Rhizopus stolonifer*	LSD
Vitamin A	3.00a	1.70b	1.90b	1.50 b	1.05b	2.5b	2.90a	0.5
Vitamin B2	0.07a	0.05a	0.05a	0.025b	0.06a	0.055a	0.04b	0.02
Vitamin B3	0.52a	0.35c	0.45b	0.20e	0.25d	0.37c	0.36c	0.03
Vitamin C	49.50a	40.5b	39.5b	20.50e	35.5d	37.5c	36.5c	1.56

Mean of two determinations with three replications. L.S.D- Least Significant Difference
Values with the same alphabet along row are not significantly different at (P<0.05)

TABLE 4: Changes in Anti-nutrient Content in *D. quineense* Edible Fruit inoculated with *Aspergillus niger, Aspergillus flavus, Botryodiplodia theobromae, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus stolonifer*

Anti-nutrient Composition (%W/W)mg/100g	**Test Fungi**							
	Control	*Aspergillus niger*	*Aspergillus flavus*	*Botryodiplodia theobromae*	*Fusarium oxysporum*	*Penicillium chrysogenum*	*Rhizopus stolonifer*	LSD
Oxalate	0.90c	1.25a	1.90a	1.50 a	2.50a	1.00b	1.15ab	0.6
Phytate	1.30c	1.70b	1.80b	1.45c	2.03a	2.00b	1.9b	0. 2
Tannin	0.60c	0.80b	0.60c	0.75b	1.50a	1.00b	0.90b	0.1
Saponins	0.20d	0.80b	0.90b	0.65c	1.80a	0.05c	0.60c	0.1
Trypsin Inhibitors	15.40d	17.9c	20.4b	19.5b	25.3a	20.5b	17.5c	1.56

Mean of two determinations with three replications. L.S.D- Least Significant Difference
Values with the same alphabet along row are not significantly different at (P<0.05)

IV. Conclusion

The research emphasized the role pathogenic fungi affect the nutritional composition of the edible fruit of *D. guineenses*, a fruit mostly eaten in Africa. It is advised that infected fruits should not be eaten because it causes food poison.

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