



Food Condiment Series 1: Fungal Contamination of Two Food Condiments (*Brachystegia eurycoma* and *Detarium macrocarpum*) Sold in Some Local Markets in Rivers State

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Authors' contributions

This work was carried out in collaboration between both authors. Author INCG designed the study, performed the statistical analysis, wrote the protocol and wrote the manuscript. Author CH managed the analyses of the study. Author INCG managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This work is aimed at identifying these organisms in order to reduce the contamination of food condiments. A survey of seed-borne fungi associated with the seeds of *Brachystegia eurycoma* and *Detarium macrocarpum* (Whole and Ground) obtained from two different local markets in Rivers State namely; Etche and Bori was conducted. Fungi were isolated using Standard Blotter Method and pure cultures of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus spp*, *Botrydipodia theobromae*, *Fusarium solani*, *Rhizopus stolonifer*, *Penicillium spp*, *Penicillium notatum* and *Fusarium moniliforme*, were obtained on Potatoes Dextrose Agar (PDA). Comparing the fungal contamination of the food condiments *Detarium macrocarpum* and *Brachystegia eurycoma* from 2 different markets in Rivers State, shows that the fungi obtained from Etche market has the highest percentage incidence of 181.83% as compared to Bori market (79.16%). It shows that these

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legumes are good substrate, for the growth of pathogenic fungi that produce toxins that are detrimental to human health because of the diseases they cause. Fungi produce toxins which cause harm to us thus, consumers are advised to buy the whole seeds of (*Brachystegia eurycoma* and *Detarium macrocarpum*) and use immediately. Preservation of food condiments should be a major concern in order to improve food security and safe guard the health of the consumers.

Keywords: Fungal contamination; mycotoxins; *Detarium macrocarpum*; *Brachystegia eurycoma*.

1. INTRODUCTION

Brachystegia eurycoma belongs to fabaceae family. In Nigeria, they have different names: In Edo State, it is known as okuen, Cross Rivers State call it okung and in the Igbo speaking state like Abia State, it is known as Achi. It is a less important legumes, used to lessen the problem of protein malnutrition in developing countries [1]. It is an economic tree crop with large twisted branches that grows up to 35 m tall [2]. It is used as shade for ornamental tree, especially in the dry season. *B. eurycoma* is a woody plant found in the rain forest zone. The seeds are rich in protein and carbohydrate and it flowers in from April-May and matures from September-January [2]. The plant has flat brown pods shiny brown edible seeds that are disk-shaped, 2 cm in diameter. When mature, their seeds are thrown out their pods explosively [2].



Plate 1. Dehulled *Brachystegia eurycoma* seeds

It occurs in southern Nigeria, Gabon and western Cameroon. It is used in construction, cabinet making, furniture works, flooring, carpentry and interior joinery. The wood can also be used to make veneer and plywood. The seeds are spicy and consumed as condiment. They are used to increase the viscosity of soups in Nigeria. It is also used to make food wrappers and gums. According to researchers, it helps in maintaining heat within the body when consumed, in other words, it is a good source of nutrient and helps in the control of the body temperature [3]. These seeds help to fight against colon and rectal

cancer when eaten as they help in softening hard stools [4].

The tree is used as a food and medicine. Exudates from the stem are useful antibacterial for the treatment of wounds and infections. Then the seed tincture has useful anti-inflammatory compound.

Detarium microcarpum is a member of the sub family Caesalpinaceae and Fabaceae family [5] that thrives in both the Rainforest and Savanna zones in Nigeria. It is known by various names as "Ofor" (Ibo), "Taura" (Hausa), and "Ogbogbo" (Yoruba) in Nigeria.

The plant is a tree of up to 10 m high with twisted bole and spreading crooked branches, the wood is hard and dark brown in colour. It is a large tree with a twisted trunk and widely spread crooked branches. The fruits are drupe like, circular or disc-shaped with a tangled network of fibers. The seeds occur singly and are within the hard disc shaped, brownish shell.



Plate 2. Boiled *Detarium microcarpum* seeds

It flowers during the rainy season and bears fruit from September-January/May and in November, it sheds its leaves and produces new ones in March [6].

The fruit are eaten raw or cooked. It is used to emulsify, flavour and as thickening agent used to thicken soup, bake cakes and bread, added when producing baby food and used to prepare

local beer [6]. The leaves are used as a condiment or vegetables, as are its flowers [6].

Tuberculosis, meningitis and diarrhea are treated using the roots, stems, bark, leaves and fruits. They are also used to treat farm animals [6]. Decoctions made from the barks, leaves and roots are used to treat venereal diseases, rheumatism, urogenital infections, hemorrhoids, stomach-ache, intestinal worms and dysentery, headache, sore throat, back pain, convulsions, constipation, measles, hypertension, itch and painful menstruation. They are also used to treat against malaria, impotence and leprosy. The fresh bark or leaves are applied to wounds, to prevent infections and cure infections. The fresh leaves and bark can be squeezed and given to fainted patient to revive them. The leaves pounded and mixed with milk is very efficient for the treatment of snakebites. In Chad the heated roots are also used to repel mosquito. They enrich the soil with nitrogen through the bacterial root nodules as well as serving as "nutrient pumps," bringing up nutrients that have been leached to soil horizons deeper than the topsoil, and eventually releasing them in the form of leaf litter and decaying organic plant residues [6]. They are used for carpentry, piles, fences and joinery.

When the seeds are sun-dried and stored in the same container with other spices and stimulants or exposed in the market, it is liable to harbour propagules of many common fungi associated with post-harvest deterioration and toxin production since humid tropical climate favour fungi.

Some fungi could be mere surface contaminants while others could be deep seated, surviving long enough to cause considerable damage both to the seed quality and the consumer. Many of these surface contaminants include fungi which have been isolated from seeds, fruits, vegetables and stored products in the tropics particularly in Niger Delta and are reported to produce mycotoxins in high concentrations. These have been associated with fatal disorders in consumers and in the production of biochemical changes which reduced the seed and fruits quality [7,8]. *Aflatoxins*, *Ochratoxins*, *Petulin* and other Mycotoxins secreted by the *Aspergillus spp*, *Penicillium spp* and *Fusarium spp*, have been associated with such *mycotoxicoses* as liver damage, carcinoma, weakness and reduction in growth [9].

This study is aimed at investigating fungal species that contaminate the seed of *D. macrocarpum* with the view of authenticating its consumption and ethno medicinal uses.

2. MATERIALS AND METHODS

Mature seeds of *D. macrocarpum* and *B. eurycoma* were bought from two (2) different markets in Rivers State; Bori market in Khana Local Government Area and Umuanyagwu market in Etche in Local Government Area respectively.

2.1 Isolation

Fungi were isolated from the seeds of *D. macrocarpum* and *B. eurycoma* using Standard Blotter Method recommended by International Rules for Seed Testing [10]. Sterilized Petri dishes were lined with 3 layers of sterilized 9cm filter paper. Sterilized distilled water was used to wet them and excess water poured out. The seeds were sorted to remove visible diseased ones then surface sterilized by dipping into 70% ethanol for 2 minutes and rinsed twice in sterilized distilled water; then placed in Petri dishes equidistantly and incubated at 25°C in the laboratory for 7 days. The following fungi such as; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus spp*, *Botrydiodia theobromae*, *Fusarium solani*, *Rhizopus stolonifer*, *Penicillium spp*, *Penicillium chrysogenum* and *Fusarium moniliforme* were found growing on the seeds of *D. macrocarpum* and *B. eurycoma* then sub-cultured on Potato Dextrose Agar medium from which pure cultures were made solidify [11].

2.2 Inoculation/ Preparation of Pure Culture

The work benches are first surface sterilized using 70% and cotton wool. An inoculating needle was flamed until red hot then dip in alcohol to cool. With the heat-sterilized needle a small portion of the fungi colony were picked and transferred into a sterilized plate containing the solidified Potato Dextrose Agar and the needle flamed again until red hot, to kill all adhering spores and hyphae. After which, the culture were allowed to grow in an incubator [11].

For the control seeds, each were placed in a Petri dish without surface sterilization. After the incubation period, the seeds were examined

using a binocular stereomicroscope (25-50x) magnification, for incidence of fungi on them. If present (+) and if absent (-). The number of seeds with the same colour of fungi was counted and recorded. The total number of seeds with fungal growth was calculated. Fungi were identified and finally the results were analyzed using Analysis of variance (Anova).

Mean % incidence or occurrence of fungi from each market was calculated using the formula;

Mean % =

$$\frac{\text{Total no. of occurrence of a particular fungi} \times 100}{\text{Total no. of plated sample}} \quad 1$$

3. RESULTS/ DISCUSSION

From the results obtained, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus spp*, *Botryodiplodia theobromae*, *Fusarium solani*, *Rhizopus stolonifer*, *Penicillium spp*, *Penicillium notatum* and *Fusarium moniliforme*, were found growing on Potato Dextrose Agar (PDA). The percentage incidences gotten are as follows: *Aspergillus flavus* (47.13%), *Aspergillus niger* (63.19%), *Aspergillus specie* (0.13%), *Botryodiplodia theobromae* (0.25%), *Fusarium solani* (10.56%), *Rhizopus stolonifer* (17.19%), *Penicillium spp* (26.10), *Penicillium notatum* (12.50%), and *Fusarium moniliforme* (111.81%). *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium moniliforme* were consistently higher than *Aspergillus spp*, *Botryodiplodia theobromae*, *Fusarium solani*, *Rhizopus stolonifer*, *Penicillium spp* and *Penicillium notatum* in percentage incidences. Comparing the fungal contamination of food condiments *Detarium macrocarpum* and *Brachystegia eurycoma* gotten from the 2 different markets in Rivers State, it shows that

the fungi obtained from Etche market has the highest percentage incidences of fungi as compared to the Bori (Table 1).

Aspergillus niger, has the highest occurrence followed by *Aspergillus flavus*, *Penicillium spp*, *Rhizopus stolonifer*, *Penicillium chrysogenum*, *Fusarium moniliforme*, *Fusarium solani*, *Botryodiplodia theobromae* and *Aspergillus spp*.

Fusarium moniliforme was dominant in the two markets, while *Botryodiplodia theobromae* was found only in Etche market.

Comparing whole seeds from the two markets, the Etche market has the highest number of *Fusarium moniliforme*, followed by *Aspergillus flavus*, *Aspergillus niger*. While *Fusarium solani* occurred only in Etche. For the grinded seeds, *Aspergillus flavus* is higher in Etche market followed by *Fusarium moniliforme*, *Penicillium spp*, *Penicillium chrysogenum*, *Aspergillus niger*, in Bori market is higher.

The ground specimen was found to have a higher level of percentage incidences of fungi due to the fact that they are exposed to fungi activity than the whole forms of the seed. Thus, the ground form is more prone to fungal attack.

The Climatic conditions prevalent in Etche market had been reported to favour the survival of some fungi also isolated from other fruits, vegetables, food spices and stimulants [12,13].

However, [9] had recommended grinding and packaging in tins or polythene bags of foods and condiments as a means of conserving the light sensitive vitamin and riboflavin and preventing it from microbial contamination.

Table 1. Mean% incidence of fungi isolated from seeds (ground and whole) of *Detarium macrocarpum* obtained from two different markets in Rivers State

Fungi	Etche market		Bori market	
	Whole	Ground	Whole	Ground
<i>Aspergillus niger</i>	30	8	2	13
<i>Aspergillus flavus</i>	80	63	43.5	4
<i>Fusarium moniliforme</i>	84	43	15	15
<i>Rhizopus stolonifer</i>	3	-	2.15	5
<i>Penicillium spp.</i>	-	12	4	0.5
<i>Penicillium chrysogenum</i>	-	8.25	0.5	6
<i>Aspergillus spp.</i>	-	2	-	-
<i>Fusarium solani</i>	40	-	-	-
<i>Botryodiplodia theobromae.</i>	-	2	-	-

Table 2. Total and Mean% incidence of Seed borne fungi isolated from seeds (Whole and Ground) sample of *Brachystegia eurycoma* and *Detarium macrocarpum* obtained from Etche market

Seed-borne fungi	Etche market			
	<i>B. eurycoma</i>		<i>D. macrocarpum</i>	
	Whole	Ground	Whole	Ground
<i>Aspergillus niger</i>	10	86	30	8
<i>Aspergillus flavus</i>	—	90	80	63
<i>Fusarium moniliforme</i>	1.65	92	84	43
<i>Rhizopus stolonifer</i>	—	—	3	—
<i>Penicillium spp.</i>	—	55.20	—	12
<i>Penicillium notatum</i>	2	15	—	8.25
<i>Aspergillus spp.</i>	—	—	—	2
<i>Fusarium solani</i>	—	0.20	40	—
<i>Botryodiplodia theobromae</i>	—	—	—	2
Total	13.65	338.4	237	138.25
Mean	1.5	37.6	26.3	15.4

Table 3. Total and Mean% incidence of Seed borne fungi isolated from seeds (Whole and Ground) sample of *Brachystegia eurycoma* and *Detarium macrocarpum* obtained from Bori market

Seed-borne fungi	Bori market			
	<i>Brachystegia eurycoma</i>		<i>D. macrocarpum</i>	
	Whole	Ground	Whole	Ground
<i>Aspergillus niger</i>	4	68.5	2	13
<i>Aspergillus flavus</i>	40	2.5	43.5	4
<i>Fusarium moniliforme</i>	10.5	6.5	15	15
<i>Rhizopus stolonifer</i>	—	50.5	2.15	5
<i>Penicillium spp.</i>	5	8.5	4	0.5
<i>Penicillium notatum</i>	4	6	0.5	6
<i>Aspergillus spp.</i>	—	—	—	—
<i>Fusarium solani</i>	—	—	—	—
<i>Botryodiplodia theobromae</i>	—	—	—	—
Total	63.5	142.5	67.15	43.5
Mean	7.1	15.83	7.46	4.83

From our results, grinding would be unacceptable as a means of preventing or eliminating microbial contamination of *D. macrocarpum* and *B. eurycoma*. Grinding appeared to have activated spores and mycelia fragments of some apparently dormant fungi within the fruit as more fungal species were isolated [13].

The samples that got rotten completely at the end of the incubation period after analysis showed the presence of gram positive bacteria alongside with *Fusarium moniliforme*.

Consumers should be aware that micro-organisms are present in these food condiments that we consume every day and various ways should be employed in other to make these food

condiments free from microbes. Right from the initial state of production to the time the produce reaches the consumers, the farmer has to combat many unfavorable circumstances. Among these are pests, micro-organisms which ingest the farmland foreign matter which may be dangerous or otherwise poisonous substances or impurities which get into the produces from materials used in processing micro-organisms and dirt introduced into the produce through unhygienic practice off the people who handled the produce as well as the loss of quality that results in short comings in storage practices.

Pathogenic organisms which get into food stuff cause diseases to the consumer. The excreta of rodents and birds contain millions of microbes. These can cause deadly diseases as well as

poisoning due to toxic materials secreted by fungi also have been proved to cause cancer, and food condiments processed in dirty and unhygienic conditions are unfit for human consumption.

Premises used to dry and store these food condiments should be clean and hygiene and free from dust, cobwebs, dirt etc. Food condiments should be kept separately from other fruits, vegetables etc to avoid transfer of fungal contaminants.

The differences between the fungi found in the whole and ground samples as shown in the result might be due to the method of processing which makes the ground seeds prone to fungi attack.

4. CONCLUSION

In conclusion, this study has shown that *D. macrocarpum* and *B. eurycoma* are good substrate for the growth of pathogenic fungi which are infectious and detrimental to human because of the diseases they cause such as: damage and weakening of the immune system, to tumors in the human urinary tract, liver cancer, fever, cough, chest pain and breathlessness [14]. So foods should be properly washed, stored in a protected environment and measures should be taken to prevent fungal contamination of food during preparation of meal. There are needs to improve the handling, storage and environments where food items are. In this case, food condiments should be a major concern in order to improve food security and safe guard the health of the consumers. Improved safety of foods can be achieved through awareness raising programs involving several partners such as local authorities, the food vendors, government departments, consumer organizations, standard setting bodies and some non-governmental organizations.

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Food legislations have tried to address the new challenges brought about by market food vending but have met with a lot of resistance. A number of initiatives aimed at developing innovative ways of improving the safety of market

foods and improving the livelihood of vendors have been introduced. They include the following:

- Development of information materials and training of public health and food safety officials.
- Training to help street food vendors comply with regulations and implement safer food handling practices.
- Improvements to equipment and materials used by vendors to prepare store or serve street food.
- Education campaigns to increase consumer awareness about nutrition and safety of street foods.
- Updating codes of hygienic practice and other food safety recommendations.
- Workshops at state and local level for exchanging information and experiences on market foods. (Whitney, 1968).

Comparing the fungal contamination of food condiments *Detarium macrocarpum* gotten from the 2 different local markets in Rivers State, study reveals that the fungi obtained from Etche market has the highest percentage incidences of fungi as compared to the Bori market.

REFERENCES

1. Ikegwu JO, Okechukwu PE, Ekumankana EO. Physio-chemical and pasting characteristics of flour and starch from Achi (*Brachystegia eurycoma*) seeds. Journal of Food Technology. Published by Medwell Journals. 2010;8(2):58-66.
2. Oyen LPA. *Brachystegia eurycoma*. Records from plant resources of tropical Africa, (PROTA) Wageninngen, Netherlands; 2012.
3. Onimawo A, Egbekun MK. Comprehensive Food Science and Nutrition. Revised Edition, Ambik Publishers, Benin City; 1998.
4. Ndukwu MC. Determination of selected physical properties of *Brachystegia eurycoma* seeds. Res. Agric. Eng. 2009;55(4):165-169.
5. Abdalbasit AM, Mohammad ES, Ahmad B, Abdelwahab SI. *Detarium macrocarpum* fruit proximate chemical analysis and sensory characteristics of concentrated juice and jam. African Journal of Biotechnology. 2009;8(17):4217-4221.
6. Kouyaté AM, Van DP. Medicinal Plant; 2006.

- Available:http://database.prota.org/PROTAhtml/Detarium_microcarpum
7. Ogundana SK, Ajulo E. Fungal flora of pulped pepper (*Capsicum frutescens*) stored under various conditions. International. Biodeterioration Bulletin. 1980;16:67-71.
 8. Ekundayo CA. Biochemical changes caused by Mycoflora of yam slices during sun drying. Microbes letters 1986;32:13-18.
 9. Ekundayo CA. Mycoflora and vitamins content of sun dried food condiments in Nigeria. Plants Foods for Human Nutrition. 1987;14:16-24.
 10. ISTA. International seed testing association. International rules for seed testing. Seed Science and Technology Neergaad 1979;4:51-77. Seed Pathology Vols.1 & 2, Macmillan Press Ltd. London. 1976;268.
 11. Ataga AE, Elenwo EN, Nwachukwu EO. Laboratory exercises and series in mycology. ACOTEC Technology, P.H., Nigeria. 2010;(13-21):97-183.
 12. Onuegbu BA. Studies on post- harvest microorganisms of okra (*Abelmoschus esculentus*) L, Monch. M.Sc. Disertation, University of Port Harcourt. 1987;66.
 13. Elenwo EN. Seed borne fungi consumed with alligator pepper (*Aframomum melegueta*) in southern Nigeria. Niger Delta Biologia; 1996;1(2):67-71.
 14. Ikechi-Nwogu C, Nwaukwu IA. Fungi pathogens associated with forest fruit *Dialium quineense* (Icheke) in Port Harcourt Metropolis. Scientia Africana. 2012;11(No.1):107-113.

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