

Occurrence of Aflatoxin Producing Mould in *Irvingia Gabonensis* (Bush Mango) Seeds Sold Within Jos Metropolis

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Abstract: This study was conducted to determine the occurrence of aflatoxin producing mould in *Irvingia gabonensis* (Bush Mango) seeds sold within Jos metropolis. The total fungal load per sample was gotten from plate count and expressed as Colony Forming Units per gram of sample (cfu/g). The aflatoxigenicity of the fungal isolates was also evaluated on Coconut Extract Agar (CEA) by exposing the reversed side of plates to 365nm ultraviolet light. The result showed that mean fungal count of *I. gabonensis* ranges from 3.2×10^6 to 4.0×10^6 cfu / g. The fungi that were implicated for the contamination of the *I. gabonensis* include: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Aspergillus niger*, *Peneillium chrisogenu*, *Verticellium leceanii*, *Rhizopus oryzae*, *Fusarium sporotrichoides* and yeast. Strains of *V. leceanii*, yeast and *A. flavus* were the most predominant at 47.9%, 37.5% and 35.4% respectively. By a way of comparison, the result also showed that Bush Mango seeds from Terminus and Gadabiyu were the most contaminated, followed by Faringada and then Angwa Rukuba. Out of the 39 *Aspergillus* species isolated, only 4 strains exhibited aflatoxin producing potential. The presence of these aflatoxigenic mould on *I. gabonensis* seeds presents high health risk and calls for more regulations and periodical assessment of Bush Mango seeds sold in open markets.

Keywords: Aflatoxigenic, Fungi, *Irvingia Gabonensis*, Mycotoxin

1. Introduction

Fungi are ubiquitous which make them major spoilage agents of agricultural crops, foods and feedstuffs, that results in the reduction of postharvest quality of crops. Their occurrence in food pose potential health hazard to human beings and animals, due to their ability to produce poisonous secondary metabolites (Mycotoxin), that when ingested have enormous public health significant (Williams *et al.*, 2004; Miller *et al.*, 1996). Humans can ingest aflatoxins by the periodic consumption of contaminated food. Aflatoxin have been seen to occur in different kind of foodstuffs, such as

fruits, spices, cereals, oils, milks, meat, vegetables, etc. (Dor *et al.*, 2011).

Irvingia gabonensis (Bush mango), also known as wild mango, African mango is commonly called 'Ogbono' in Igbo and 'Apon' in Yoruba (Okafor, 1978). It produces edible fruits and seeds. The dried seeds of *Irvingia gabonensis* is widely consumed by humans in Nigeria as a good thickener for soup. Dried *Irvingia gabonensis* seeds has low water activity however, poor handling and storage, combination of environmental factors and continual exposure in the market

could predispose aflatoxigenic contaminants to it (Shaltout *et al.*, 1999).

One of the most important mycotoxins of health significance is aflatoxin. The toxins are primarily produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nominus*. Contamination of *I. gabonensis* seeds with these aflatoxigenic mould will pose a very serious health hazard because of its wide consumption by humans/ animals. (Leitao *et al.*, 1988; Dorner, 2002; Yiannikouris and Jouny, 2002). Aflatoxin are chemically and biologically active by-products produced by fungi growth naturally in wide range of plant products when conditions that support growth are present. The toxins are mutagenic, immunotoxic, carcinogenic, tetratoxigenic and nephrotoxic, capable of causing acute and chronic effects ranging from disorder of the central nervous system, cardiovascular/pulmonary system and death (Georggiet *et al.*, 2000; Hussein and Brasel, 2011; Bhat and Vasanthi, 2003). The health implication of mycotoxigenic fungal-contaminants is often very serious and apparently not known to consumers, since mycotoxins might have been produced but yet invisible to naked eyes. The mould might be killed by heat during processing but the mycotoxins can remain undestroyed in the final products thereby posing high health risk to the consumers of such products (Smith *et al.*, 1994).

It has been established by the United Nation Food and Agricultural Organisation that approximately 25% of the world's foods are contaminated with mycotoxins, especially aflatoxins (Smith *et al.*, 1994). About 4.5 billion people mostly in developing countries are at risk of chronic exposure to aflatoxins from contaminated food crops (Oyebaji *et al.*, 2013; Shuaib *et al.*, 2010). Due to these, the level of aflatoxins and similar toxic substances in foodstuffs have to be evaluated closely and to be continually kept under control. Else, related health risk like acute and chronic aflatoxications and even death will always be an issue. (Becer and Filazi, 2010). The analytical methods used in the developed countries are quite sensitive and reliable but very expensive to employ in developing countries like Nigeria. This have presented the need for aflatoxin detection methods that are reliable but inexpensive, so that it can be conducted with a level of technical skill acquirable in developing countries. If regularly and appropriately done, will reduce cases of intoxication and help set standards for agricultural products like *Irvingia gabonensis* seed which is widely consumed in Nigeria. This has raised major concerns among researchers and there is need for mycotoxin surveillance because of its wide occurrence in contaminated commodities (Negedu *et al.*, 2011).

2. Materials and Methods

2.1. Sample Collection

A total of 48 *I. gabonensis* seed samples were randomly purchased from hawkers and stationary sellers in the major markets within Jos metropolis. The markets include

Faringada, Angwa rukuba, Terminus and Satellite markets. Successively, 12 samples each were obtained in clean polythene bags at different sales points in the markets during the rainy season at four weeks interval, and transported to National veterinary Research Institute Microbiology laboratory Vom for appropriate analysis.

2.2. Mycological Analysis

The mycological analysis of the collected sample was carried out following standard method of Samson (2000). 10g of the pulverised dried *I. gabonensis* seeds was weighed and homogenously mixed in 90ml of sterile distilled water. It was then serially diluted to 5folds and 0.1ml of the resultant solution was spread plated on Sabaroud Dextrose Agar (SDA) incorporated with 40µg/ml chloramphenicol to prevent the growth of bacterial contaminants if present. Enumeration of colonies (cfu/g) was done after 7days of incubation at 27°C. Distinct colonies were then subcultured on fresh SDA media to obtain pure isolates for further examination.

The fungal isolates were identified using cultured, macroscopic and microscopic morphologies, and comparison with standard representative of species in relevant fungal atlas (Samson, 2000; Barnett *et al.*, 1998).

2.3. Screening for Aflatoxigenic Fungi

Screening for aflatoxin production potentials was carried on coconut extract agar (CEA) with 40µg/ml chloramphenicol to suppress bacterial growth, following standard methods (Davis *et al.*, 1987). All the pure fungal isolates were inoculated on CEA and incubated at 27°C for 5days. The aflatoxin producing potential of the isolates was determined by observing reversed side of plates under 365nm ultraviolet lamb. Using non-inoculated CEA medium as control, the emission of a characteristic blue fluorescence confirmed the presence of aflatoxin producing potentials of the isolates.

3. Results

The result showed fungal contamination of *Irvingia gabonensis* seeds which result in a significant total fungal load which ranges from 0.00 to 6.8×10^6 cfu/g. (Table 1). The result also showed that the *I. gabonensis* seed obtained from Terminus and Gadabiyu had the highest mean fungal load of 4.0×10^6 cfu/g, while samples from Angwa Rukuba had the lowest mean fungal load.

Nine fungal species were isolated from the *I. gabonensis* as presented in table 2. 33.8% of the fungal isolated were *Aspergillus* species which included *A. flavus*, *A. fumigatus*, *A. parasiticus* and *A. niger*. Other fungal genera isolated were *Penecillium chrisogenu*, *Verticelium leceanii*, *Rhizopus oryzae*, *Fusarium sporotrichoides* and yeast.

The result also showed that out of 39 *Aspergillus* species isolated, 17 were *A. flavus*, 9 were *A. fumigatus*, 5 were *A. parasiticus* and 8 were *A. niger* (Table 3). Only 2 *A. flavus* strains exhibited aflatoxin producing potential while one each

for *A. fumigatus* and *A. niger* respectively. None of the 5 *A. parasiticus* strains showed aflatoxin producing potential.

Table 1. Mean Fungal Load of *Irvingia gabonensis* (Bush Mango).

Sample No.	Terminus	Faringada	Angwa Rukuba	Gadabiyu
1	6.1×10 ⁶	1.6×10 ⁶	1.2×10 ⁶	1.5×10 ⁶
2	1.9×10 ⁶	3.0×10 ⁶	3.0×10 ⁶	5.3×10 ⁶
3	6.4×10 ⁶	6.0×10 ⁶	5.2×10 ⁶	5.3×10 ⁶
4	4.4×10 ⁶	1.0×10 ⁶	4.1×10 ⁶	4.2×10 ⁶
5	3.0×10 ⁶	3.4×10 ⁶	2.0×10 ⁶	2.2×10 ⁶
6	2.9×10 ⁶	4.6×10 ⁶	1.7×10 ⁶	1.5×10 ⁶
7	3.1×10 ⁶	3.8×10 ⁶	1.3×10 ⁶	3.6×10 ⁶
8	6.2×10 ⁶	6.8×10 ⁶	6.8×10 ⁶	6.7×10 ⁶
9	1.5×10 ⁶	4.7×10 ⁶	2.2×10 ⁶	6.8×10 ⁶
10	3.2×10 ⁶	2.8×10 ⁶	3.7×10 ⁶	1.5×10 ⁶
11	5.0×10 ⁶	5.6×10 ⁶	6.0×10 ⁶	2.7×10 ⁶
12	4.5×10 ⁶	2.3×10 ⁶	1.0×10 ⁶	6.7×10 ⁶
Mean	4.0×10 ⁶	3.8×10 ⁶	3.2×10 ⁶	4.0×10 ⁶

Table 2. Frequency of Occurrence of the Fungal Isolates.

Place of sample collection					
Fungal Isolate	Terminus	Faringada	Angwa Rukuba	Gada biyu	Total of specie
<i>A. flavus</i> (%)	3(25.0)	7(58.3)	5(41.7)	2(16.6)	17
<i>A. fumigatus</i> (%)	1(8.3)	2(16.7)	4(33.3)	2(16.6)	9
<i>A. parasiticus</i> (%)	-	2(16.7)	2(16.7)	1(8.3)	5
<i>A. niger</i> (%)	2(16.7)	1(8.3)	4(33.3)	1(8.3)	8
<i>P. chrisogenu</i> (%)	2(16.7)	4(33.3)	4(33.3)	1(8.3)	11
<i>V. leceanii</i> (%)	1(8.3)	5(41.7)	7(58.3)	10(83.3)	23
<i>R. oryzae</i> (%)	-	7(58.3)	1(8.3)	2(16.7)	17
<i>F. sporotrichoides</i> (%)	1(8.3)	1(8.3)	2(16.7)	-	4
Yeast (%)	6(50.0)	3(25.0)	1(8.3)	8(66.7)	18
Total	16	32	37	27	

Table 3. Screening for Aflatoxigenic Fungi.

Fungal Isolates	No. of Isolates	No. of + ve Samples (%)	No. of - ve Samples (%)
<i>A. flavus</i>	17	2(11.8)	15(88.2)
<i>A. fumigatus</i>	9	1(11.1)	8(88.9)
<i>A. parasiticus</i>	5	0(0.00)	5(100)
<i>A. niger</i>	8	1(12.5)	7(87.5)

4. Discussion

This study revealed that the Bush mango seed (*I. gabonensis*) sold in the market in Jos metropolis are contaminated with fungal species at significant total fungal load. This could be attributed to poor handling during harvest and transportation, poor storage, and exposure in the market as observed from the sellers (Shaltout *et al.*, 1999). Bosco F. and Mollea C. (2002) have also reported that raw materials, food and feeds are naturally susceptible to aflatoxigenic contaminants.

The study also showed contamination of Bush mango seeds sold with Jos metropolis with more than one genus of fungi. The genera implicated are *Aspergillus* species, *Rhizopus* species, *Penicillium* species, *Fusarium* species and Yeast. Most of these species have been reported to be pathogenic (Shanthini *et al.*, 2003). *Aspergillus* species such as *A. flavus*, *A. parasiticus*, *A. niger* and *A. fumigatus* which were also isolated in this research are the most dangerous of all the isolates. This is due to their high aflatoxin producing potentials and other mycotoxins of high health significance

(Adebajo, 1992; Olayemi *et al.*, 2012).

Fungal contaminants might be killed by heat during processing but the mycotoxins can remain undestroyed in the final product thereby posing health hazard to consumers (Smith *et al.*, 1994). The study confirmed the presence of 4 strain with aflatoxin producing potential. Subsequent exposure and as time goes on, more of the aflatoxigenic mould will proliferate on the *I. gabonensis* seeds and will cause more harm to consumers. Incidentally, bush mango seeds are highly consumed in plateau state. Therefore nonchalant attitude of government, non-governmental organizations and the masses toward handling practices of food and ingredients sold in open market is a pointer to severe outbreak of food diseases and poisoning in the future.

5. Conclusion

The *Irvingia gabonensis* (Bush Mango) seeds sold within Jos metropolis market were contaminated with varying levels of fungal loads from 1.0×10⁶cfu/g to 6.8×10⁶cfu/g which is alarming. *A. flavus*, *A. fumigatus* and *A. niger* isolated exhibited aflatoxin producing potentials. Other fungi isolated include *Penicillium chrisogenu*, *Verticellium leceanii*, *Rhizopus oryzae*, *Fusarium sporotrichoides* and yeast. This study therefore suggest that *I. gabonensis* (Bush Mango) seeds are susceptible to contamination by aflatoxigenic mould.

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