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Full Length Research Paper

Detection of fungi and aflatoxin in shelved bush mango seeds (*Irvingia* spp.) stored for sale in Uyo, Nigeria

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A survey of the fungi and aflatoxin contamination of bush mango seeds *(Irvingia* spp.) was conducted in Akwa Ibom State, Nigeria. Bush mango seeds sold at four major markets, located at Abak, Uyo, Ikot Ekpene and Itam in Akwa Ibom State were heavily contaminated with moulds. Eight different fungi were found associated with the bush mango seeds; *Aspergillus carbonarius, Aspergillus tereus, Aspergillus flavus, Aspergillus candidus, Penicillium expansum, Aspergillus niger, Candida tropicalis* and *Aspergillus glaucus. A. niger* have the highest rate of occurrence with high colony counts (1.0×10^3 – 4.3×10^3 colonies/g). The aflatoxins B_1 and G_1 concentrations ranged from 0.2 – 4.0 and 0.3 – 4.2 µg/kg, respectively. The result showed that bush mango seeds sold in Akwa Ibom markets require quality control and proper preservation.

Key words: Bush mango, aflatoxin, mycoflora, *Aspergillus,* Nigeria.

INTRODUCTION

The natural forest of West and Central Africa are rich in natural resources and have tremendous biodiversity (FAO, 1983), particularly in trees that provide food, fuel, fiber, medicine and various other products, including construction and building materials. *Irvingia gabonensis* (Engl) *and Irvingia wombolu vermoesen*, the eating and the cooking types of bush mango, respectively, have been identified by the International Centre for Research in Agro forestry as priority wild fruit tree species for domestication (Ladipo et al., 1995). *I. gabonensis* and *I. wombolu vermoesen* produce edible fruits and seeds. The sustainability of these natural resources has been the concern of various workers (National Research Council, 1991), particularly with continued clearing and selective exploitation of forest (Palmberg, 1984). Bush mango seed kernels are called “Ogbono” in Ibo (Okafor 1978) and “Apon” in Yoruba. Bush mango can provide sweet tasting fruit or thickener for soups and stew.

The dried seed of bush mango is widely accepted by consumer. The aim of this study was to investigate, detect and evaluate the presence of aflatoxin and aflatoxigenic fungi in bush mango seeds marketed in Akwa Ibom State, Nigeria, with special reference to its public health hazard.

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MATERIALS AND METHODS

Isolation of fungi

Bush mango seeds were collected from four different markets; Abak, Uyo, Ikot Epene and Itam town in Akwa Ibom State (Table 2). A total of twenty samples were collected (five samples from each market). Each sample was divided into two parts, one for extraction of aflatoxins and the other for isolation of fungi. The seed samples were sterilized by dipping them in 90% ethyl alcohol for 1 min and then rinsed in several changes of sterile distilled water. Small segments (1-2 cm) of moldy tissues from the seed were cut out with a sterile scalpel and placed in a triangle in previously prepared potato dextrose agar (PDA) in Petri dishes and incubated at 27 ± 2^{o}C. The cultures that appeared were primarily identified using cultural and morphological features (Banrnet and Hunter 1972) and

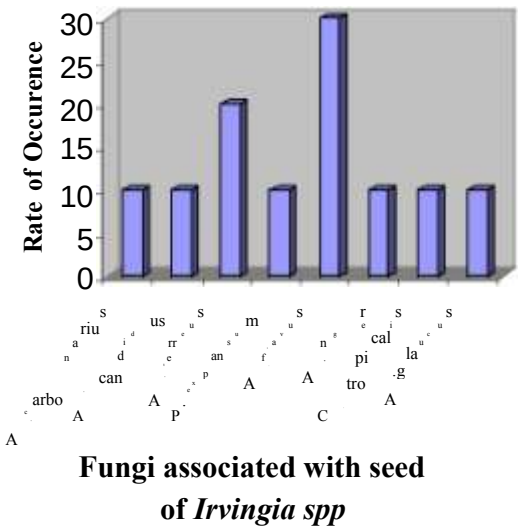

Figure 1 The rate of occurrence of fungi associated with marketed seed of *Irvingia* spp.

by comparison with already identified cultures, which were obtained from the plant pathology laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University Moor Plantation, Ibadan, Nigeria. The moisture content was determined by oven drying at 105°C for 4.5 h.

Detection of aflatoxins

Aflatoxins were extracted from the seed samples according to the method of Seitz and Mohr (1977). 10 g of the moldy bush mango seed samples obtained from each of the markets were extracted with chloroform and concentrated. Thin layer chromatography of aflatoxin and samples extracted were performed on silica gel DG 254. Of the extracted samples 5, 10 and 15 µl were spotted on three different points on a ruled base line of the TLC plates. Also 5, 10 and 15 µl of the aflatoxin standard were spotted on another three points near the previous sample extract spotted points. The plates were developed first with diethyl ether and then with chloroform : acetone (9:1, v:v). Aflatoxins were identified on the basis of co-migration with aflatoxin standards (Fluka) and by their characteristic fluorescent color under long ultra violet (UV) illumination were at 360 nm. The fluorescent spots of aflatoxins were scraped off the TLC and eluted by chloroform : methanol (9:1, v:v). The solvent was evaporated under nitrogen to dryness and the residue was dissolved in methanol. The concentration of aflatoxins (B_1 and G_1) in solution was determined by measuring its absorbance at 360 nm then calculated according to the method of Masri et al. (1969).

Confirmatory tests for aflatoxin were done by preparing three different derivatives of the isolated toxin or the aflatoxin standard with formic acid thionyl chloride, acetic acid-thionyl chloride and tri-fluoroacetic acid. The test was according to the method of Soloff and Friedman (1976)

RESULTS AND DISCUSSION

Results obtained in this study showed that *Aspergillus carbonarius, Aspergillus tereus, Aspergillus flavus, Aspergillus candidus, Penicillium expansum, Aspergillus niger Candida tropicalis* and *Aspergillus glaucus* were found to be associated with marketed bush mango seeds (Figure 1). Of the eight species, *A. flavus and A. tereus* were most dominant. The moulds probably infected the product during the process of cracking (shelled) to extract the cotyledons (kernels), drying, storing and transportation.

Fungal counts and the moisture content obtained from bush mango seeds are shown in Table 1. The moisture content ranged from 2.0-21.0%. In the open markets, bush mango seeds are displayed on trays, tables and in bowls for prospective consumers. These seeds are usually obtained from several farms pooled together and transported mostly via untarred farm roads causing injuries to the fruits. Some bush mango seeds were packed in contaminated containers. These seeds are usually kept in open markets until sold, thereby exposing them to microbial infection.

The extracted bush mango seeds and the standard aflatoxins produced bluish and greenish spots. This agreed with the work of Ciegler (1977) that aflatoxin occur in a variety of crops and animal products such as meat, milk and eggs. The production of aflatoxin is affected by several factors, which influence the mould growth such as moisture contents, relative humidity (RH), temperature, substrate composition and the presence of competing microorganisms (Ciegler, 1977). Sharma (1992) reported that the two major metabolites of *Aspergillus* sp. called aflatoxin were designated B_1 and G_1 because they fluoresce blue (B_1) and green (G_1) when expose to long-wave ultraviolet light. Fungal pathogens are known to produce secondary metabolites in plant tissues. These metabolites have been reported to be responsible for several ailments in animals including man. *A. flavus* is known to produce aflatoxins (Fennel et al., 1973) . Aflatoxins are highly carcinogenic causing hepatoma (cancer of the liver) and have also been associated with acute hepatitis in man, mostly in the developing world (Eaton and Groopman, 1994; Krogh, 1992; Prasad, 1992).

Aflatoxin was also detected from bush mango seeds in most of the markets surveyed, with the highest concentration was recorded in market sample from Ikot Epene (Table 2). The detection of aflatoxins in the marketed bush mango seeds in most of the samples indicates that *A. flavus* associated with the bush mango seed produces toxins. Aflatoxins have been reported detected in grapes and musts in France (Sage et al., 2002), edible nuts and nut products, milk and milk products (Prasad, 1992; Taveira and Midio 2001). Singh (1993) reported that out of 342 samples of different fruits and spices obtained from commercial centres, 95 of them were positive for aflatoxin.

The implication of this report is that most of the bush mango seeds presently on sale in our markets are partially acceptable for human consumption. Though in

Table 1. Moisture contents and mould colony counts of markerted *Irvinga* spp.

Locations	Market							
	Abak		Uyo		Ikot Ekpene		Itam	
	Moisture content %	Counts (Per 10^3)	Moisture content %	Counts (Per 10^3)	Moisture content %	Counts (Per 10^3)	Moisture content %	Counts (Per 10^3)
L1	19.0	4.3×10^3	10.0	3.3×10^3	17.0	3.1×10^3	9.0	3.2×10^3
L2	2.0	2.2×10^3	10.0	1.4×10^3	2.0	1.7×10^3	8.0	1.5×10^3
L3	13.0	3.2×10^3	6.0	1.6×10^3	8.0	1.0×10^3	9.0	1.3×10^3
L4	3.0	1.0×10^3	17.0	2.3×10^3	8.0	1.2×10^3	6.0	1.2×10^3
L5	9.0	2.0×10^3	20.0	1.2×10^3	5.0	1.0×10^3	6.0	1.5×10^3
L6	21.0	1.8×10^3	14.0	4.1×10^3	10.0	1.0×10^3	8.0	3.1×10^3
L7	6.0	3.4×10^3	8.0	3.0×10^3	3.0	1.2×10^3	2.0	2.0×10^3

Table 2. Aflatoxin content in bush mango seeds obtained from four markets in Akwa Ibom.
AFLATOXIN ANALYSIS (µg/kg)

LOCATIONS	ABAK		UYO		IKOT EKPENE		ITAM	
	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1
L1	0.20	0.40	0.03	0.30	3.0	2.80	0.40	3.0
L2	0.40	1.00	0.25	2.50	3.2	1.30	0.50	0.10
L3	1.00	0.80	3.00	3.00	4.0	1.20	1.00	0.10
L4	0.30	1.00	1.7	1.70	3.2	1.5	3.00	1.00
L5	1.40	2.40	2.00	2.00	1.5	0.40	0.50	0.10
L6	0.20	3.10	1.00	1.00	0.30	2.60	2.10	2.00
L7	2.2	4.20	2.00	2.00	3.5	0.30	3.00	2.00

Nigeria most foods are properly cooked before eating, once the raw materials are contaminated with aflatoxin, boiling will have no effect on the potency of the toxic materials. Aflatoxins have been found to be heat stable with a melting point of between 268 to 269^{O}C (Frazier and Westhoff, 1988). It is therefore important that both the bush mango growers and the marketers take necessary precautions in preventing contamination of the seeds to reduce possible contamination and hence reduce the risk of aflatoxin and other mycotoxins that are deleterious to human health. Since a number of toxigenic fungi are also major plant pathogens, there is considerable scope for reducing mycotoxin contamination of bush mango seeds through breeding cultures of plants resistance to diseases caused by these fungi.

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