

## Fungi and Aflatoxin Occurrence in Fresh and Dried Vegetables Marketed in Minna, Niger State, Nigeria

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### Abstract

Mycoflora and aflatoxin contamination was determined in 200 samples of fresh and dried vegetable, which are widely used because of their nutritional and medicinal principles. Fifty (50) fresh pumpkin (*Curcubita spp*), fresh spinach (*Spinacia oleracea*), bitter leaf (*Vernonia amygdalina*) comprising of (25 dried and 25 fresh) and 50 samples of tomato (*Solanum lycopersicum*) (25 dried and 25 fresh) were collected from local vegetable vendors in Minna metropolis, Nigeria for investigation. Identification of isolates was carried out based on their morphological and microscopic characteristics. A total of 165 isolates made up of 4 genera (*Aspergillus*, *Penicillium*, *Fusarium* and *Mucor*) were identified. Aflatoxins (AF) were determined using High Performance Liquid Chromatography technique with UV detection. The dried bitter leaf samples was more ( $P < 0.05$ ) susceptible to aflatoxin contamination than fresh bitter leaf and pumpkin leaf samples. Fifty two percent (52%) of all aflatoxins contamination was by AFG1, no significant ( $P > 0.05$ ) difference was observed between AFB1 (40%) and AFB2 (44%) in the dried bitter leaf samples. Aflatoxins were detected in tomato (fresh and dried) and spinach samples at lower concentrations (0.00-9.12  $\mu\text{g}/\text{kg}$ ). This report calls for a need for enlightenment on proper agricultural, storage and hygienic practices alongside the hazards related with aflatoxin-contaminated foods.

**Keywords:** Fungi; Aflatoxin; HPLC; Vegetables; Minna

### Introduction

A preliminary survey of fungi and mycotoxin contamination of fresh and dried vegetable plants stored for sale in Nigerian markets revealed that they are suitable substrates for various fungi growth and aflatoxins contamination [1]. The fungi family have continuously raised global food safety concerns due to their ability to colonize food items and either cause physical damage or release secondary metabolites which may be toxic [2]. Fungi of the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Mucor* are they known toxigenic strains [3], which produce toxic metabolites [4]. These vegetables are cheap and provide valuable nutritional data, as they are good sources of proteins, vitamins, minerals and essential amino acids as well as antioxidants that are frequently used for both dietary and medicinal purposes. Vegetables are readily used either in fresh or dried forms. Drying is practised in Nigeria to make the products more durable and the bulk of it preserved so they may be available all year round. The dried products are susceptible to infestation with fungi and other contaminants either already present on the primary product, or during the drying process as a result of fungi promoting conditions and dust. Further spoilage can occur during storage, handling and transportation as well as market [5]. Vegetable products that are susceptible to fungi growth can also be contaminated with aflatoxins [6].

The four major aflatoxins (AF), AFB1, AFB2, AFG1 and AFG2 are the most important mycotoxins in foods and feeds because of their high prevalence in nature and toxicity [7]. Aflatoxins especially AFB1 has been established to be a powerful carcinogen [8], cytotoxic [9], mutagenic and teratogenic [10]. Aflatoxins have repeatedly caused death in Kenya [11], India and Malaysia. However, natural occurrence of mycotoxins in vegetables therefore has been demonstrated in many countries [12]. However, reports on mycotoxin contaminations of vegetables in the West African region are rare, still standing out as a cutting-edge reference material is the comprehensive work of Hell et al. [13] who studied fungi and aflatoxins incidence in dried vegetable samples from Benin republic, Togo and Mali.

This study is therefore intended to bridge data gap and present some worth comprehensive report on the fungi isolated from (fresh and dried) bitter leaf, tomato, (fresh) pumpkin and spinach marketed within town Minna, Nigeria as well as their susceptibility to aflatoxin using HPLC technique for detection.

### Materials and Methods

#### Sample collection

Fresh and dried samples of the four different vegetables were collected from twenty-five vegetable vendors from four markets in Minna metropolis, Niger state, Nigeria during the months of June and July. A total of 200 samples; fresh Bitter leaf (25), sun-dried bitter leaf (25), fresh Tomato (25), sun-dried Tomato (25), fresh Spinach (50) and fresh Pumpkin (50) were collected, labelled, packaged in sample collection bags, and taken to the laboratory. In the laboratory, samples were divided into two halves. One half was grounded and stored at  $-20^{\circ}\text{C}$  in the deep freezer until used for mycotoxins (aflatoxins) analysis. The other half was used immediately for fungal growth and isolation studies.

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## Fungi isolation and identification

The method employed was used with some modifications in isolations and identification of fungi. For each plant collections, 10 g were rinsed with sterile distilled water, inoculated into petri dishes containing potato dextrose agar (PDA) and allowed to solidify before incubating at room temperature for 48 to 74 hours. The colonies, which developed in the plates were counted using the colony counter (Model 6399/Stuart Scientific Co. Ltd., Great Britain) and expressed as colony forming units per millilitre (CFU/ml) of samples. Series of sub-culturing were made for all the counts to obtain pure culture isolates. Each isolate was grown on a PDA slant and stored for further use.

Fungal isolates were stained with lactophenol cotton blue and observed under the microscope using  $\times 40$  and  $\times 10$  objectives lens.

Identification of fungi depends largely on morphological characteristics such as the type and arrangement of spores produced, as well as the mycelial type. The isolates were assigned to tentative identity using Taxonomic description.

## Aflatoxins extraction

Aflatoxins were extracted according to Hell et al. [13], without modification. Each fresh and dried ground vegetable sample (10 g) was mixed with 50 ml of methanol (BDH Chemicals Ltd Poole, England)/water (85:15 v/v), blended for 3 mins and filtered with whatman filter paper. The filtrate, which was about 40 ml, was mixed with an equal volume 40 ml of 10% NaCl (BDH Chemicals Ltd Poole, England). The mixture was poured into a separator funnel and defatted with 25 ml of n-hexane (BDH Chemicals Ltd Poole, England). The hexane layer was discarded and the aqueous layer partitioned twice with 25 mL of chloroform (May and Baker Ltd Dagenham, England). The chloroform layers were pooled, decanted over anhydrous sodium sulphate (Cambrian Chemicals Beddington, England) for dehydration and then evaporated to near dryness using a regulated water bath at 50°C. The residue was transferred to an amber vial and kept in a refrigerator at a temperature of -200°C until analyzed.

## Quantitative analysis of aflatoxins

Aflatoxins residues were reconstituted in 1 ml of chloroform for high-pressure liquid chromatography (HPLC). Aflatoxins were analyzed on Agilent Technologies 1200 series HPLC with UV detection at wavelength of 365 nm. The octadecylsilyl group (ODS) column 4.6 mm  $\times$  150 mm  $\times$  5  $\mu$ m was used at ambient temperature of 25°C. Acetonitrile: water and acetic acid in ratio 10:50:40 v/v/v respectively was used as mobile phase at flow rate of 0.8 ml/min and injection volume of 20  $\mu$ l. The analyses were carried out with aflatoxins standards (Sigma chemical Company, St. Louis, MO, USA) of known concentrations with AFB1, AFB2, AFG1 and AFG2 eluting at a distinct retention time.

## Results and Discussion

The result presented in Table 1 shows the fungi species isolated from different fresh and dried vegetable samples with most isolates being *Aspergillus spp.* They are known to produce aflatoxins to varying extents when grown on media. This mycological finding is similar to some earlier reports [7,14]. The variation between the fungi contaminants with that found in other existing literature arises due to differences in country of origin, related environmental factors, sample variability, the method of processing and storage facilities/practices in a locality [15].

Table 2 shows result for aflatoxins contamination in both fresh and dried tomato samples with AFB2 being the major contaminant. There were no significant ( $P>0.05$ ) difference in the level of contamination between the dried tomato and fresh tomato samples. This current investigation revealed aflatoxins contamination levels, which agrees with previous report of Muhammad et al. [16] who detected aflatoxins on fresh tomato from Sokoto market, Nigeria, and it stands in contrast with that of Hell et al. [13] who did not detect aflatoxins in dried tomato. The presence of aflatoxins in fresh and dried tomato samples are risk factors that calls for public interests since tomato is a delicacy in most part of the country. Spinaches were contaminated with aflatoxin (0.07-7.32  $\mu$ g/kg) with a relatively low concentration. Fresh pumpkin leaf sample were contaminated with aflatoxins at relatively low amounts. This result agrees with Lagauskas et al. [17] and Nagmus et al. [18] that reported aflatoxins in pumpkin leaves, this report shows that vegetables can be a good substrate for mycotoxins production.

Comparative studies of the four vegetables studied, show a relatively higher level of aflatoxin contaminant on dried bitter leaf samples than all other sample types. Also, fresh vegetables had relatively lower aflatoxin contamination than their dried leaf samples, this could be due to presence of antifungal potent phytochemicals present in the fresh plants. According to Abosi and Raseroka [19] and Sayed et al. [20] flavonoids, saponins and alkaloids have activities against fungi contamination; these phytochemicals are ideally higher in fresh vegetable samples than dried samples. Domsch et al. [21] postulated that contamination of foodstuffs with spoilage fungi was the result of natural extraneous pollution with dust particles containing spores during storage. Our results agree with earlier reports on fungi and mycotoxins contamination in bitter leaf conducted in Ibadan, Nigeria where isolates of aflatoxin B1 was detected from mouldy vegetable material offered for sale with the highest concentration of 94  $\mu$ g/kg [22]. Efuntoye [1] also reported the presence of aflatoxin B1, aflatoxin B2 and aflatoxin G1 as contaminants of herbal plants such as bitter leaf. In Ethiopia, Fufa and Urga [23] had previously reported that aflatoxin B1 within the range of 100-525  $\mu$ g/kg contaminated shiro and ground red pepper, while Hell et al. [13] reported levels of upto 3.2  $\mu$ g/kg and 6.0  $\mu$ g/kg aflatoxin B1 as contaminant of dried hot chili and okra respectively within Benin, Mali and Togo. Makun et al. [14] found 60% of red chili pepper contaminated with aflatoxins upto 19.45  $\mu$ g/kg but detected no aflatoxin in baobab leave and okra leaf.

The occurrence of aflatoxin is highly pivoted to the climatic condition of Minna. The study area is warm (average annual temperature of 31.7°C) and humid (average annual humidity of 51.6%) most time of the year, which are favourable conditions for growth of aflatoxigenic fungi and toxin synthesis [24-26]. Minna market, where movement of people and vehicles are in confined spaces may result in high dust and potential microbial spore formation with little aeration [27] that would accelerate fungi and mycotoxins development. The methods of processing, packaging and storage can also have great effect on fungal contamination and levels of infestation. Chourasia [28] reported that dried vegetables and spices stored in gunny bags and on bare ground are highly contaminated with fungi as compared to those stored in wooden boxes, metal or glass containers. The level of fungi infestations can be compounded by insect damage, stress condition at both pre-harvest, harvest and post-harvest stages of vegetable production [29]. Other possible source of fungi infestation can result from inappropriate handling and storage methods often associated with poor hygiene. Another compelling factor that heightens aflatoxin levels in foods particularly in sub-saharan Africa like Nigeria, is the common man ignorance of the existence of mycotoxins.

Fungal species	Vegetables						Total isolates	Frequency of isolation (%)
	Dry bitter leaf	Fresh bitter leaf	Dry tomato	Fresh tomato	Spinach	Pumpkin		
<i>A. flavus</i>	3	4	3	8	10	3	31	18.8
<i>A. niger</i>	4	8	9	15	11	19	66	40.0
<i>A. parasiticus</i>	2	-	6	4	3	6	21	12.7
<i>Mucor spp.</i>	6	1	-	-	-	6	13	7.8
<i>P. brevicopactum</i>	1	-	-	-	6	7	14	8.5
<i>P. chrysogenum</i>	-	-	-	-	8	-	8	4.8
<i>F. culmorum</i>	-	-	-	6	6	-	12	7.3
Range	0-6	0-8	0-9	0-15	0-11	0-19	8-66	
Total isolates	16	13	18	33	44	41	165	
Number of fungi species	5	3	3	4	6	5		

Table 1: Total fungi isolates, occurrence frequency per fungi species in different dried and fresh vegetables.

Sample Name	Sample No.	AF type	+ve samples	Range µg/kg	Mean ± SEM	% Contamination
Pumpkin leaf	50	B1	6	2.48-10.66	0.57 ± 0.26*	12
	50	B2	1	0.00-3.44	0.06	2
	50	G1	1	0.00-12.74	0.25	2
	50	G2	1	0.00-12.07	0.24	2
Dried bitter leaf	25	B1	10	2.41-9.51	2.39 ± 0.68**	40
	25	B2	11	1.45-16.45	3.00 ± 0.92**	44
	25	G1	13	7.12-56.04	13.30 ± 3.36***	52
	25	G2	1	0.00-3.86	0.15	4
Fresh bitter leaf	25	B1	-	-	-	
	25	B2	-	-	-	
	25	G1	5	7.69-37.65	4.51 ± 2.09**	20
	25	G2	-	-	-	
Dried tomato	25	B1	-	-	-	
	25	B2	5	0.72-8.00	0.66 ± 0.34*	20
	25	G1	1	0.00-0.30	-	4
	25	G2	1	0.00-9.12	-	4
Fresh tomato	25	B1	-	-	-	
	25	B2	6	0.57-7.13	0.56 ± 0.31*	24
	25	G1	-	-	-	
	25	G2	-	-	-	
Spinach	50	B1	5	0.08-1.49	0.13 ± 0.07*	10
	50	B2	7	0.07-7.32	0.37 ± 0.29*	14
	50	G1	2	0.15-0.47	0.02 ± 0.01*	4
	50	G2	5	0.55-5.39	0.68 ± 0.38*	10

Table 2: Aflatoxins contamination in fresh and dried vegetable samples.

Since aflatoxins are deadly mycotoxins [30], chronic ingestion of foods that are aflatoxins contaminated leads to great health risk.

The presence of aflatoxins at unsafe concentrations in these vegetables necessitates the involvement of good agricultural practices, processing, handling and storage practices. There is also a need for public enlightenment on the hazards, the contribution of stakeholders is necessary along the commodity chain to improve on quality control. Finally, there is a call to strict enforcement of aflatoxin legislations.

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