

## Fungi and Some Mycotoxins Found in Mouldy *Sorghum* in Niger State, Nigeria

<sup>1</sup>A. Makun Hussaini, <sup>1</sup>A. Gbodi Timothy, <sup>1</sup>H. Akanya Olufunmilayo,  
<sup>2</sup>A. Salako Ezekiel and <sup>3</sup>H. Ogbadu Godwin

<sup>1</sup>Department of Biochemistry, Federal University of Technology, Minna, Nigeria

<sup>2</sup>Department of Crop Production, Federal University of Technology, Minna, Nigeria

<sup>3</sup>Sheda Science and Technology Complex, Federal Ministry of Science and Technology, Abuja, Nigeria

**Abstract:** Mouldy Sorghum samples were collected at three different seasons (dry harmattan season, November-January; hot, dry season, February-April; and rainy season, May-October) from twenty-five local government areas of Niger State and screened for their fungal, aflatoxin B<sub>1</sub>-AFB<sub>1</sub>, ochratoxin A-OTA and zearalenone-ZEN contamination. Eight hundred and eighty four (884) fungi were isolated from one hundred and sixty eight (168) mouldy Sorghum samples collected. The three major fungal contaminants of Sorghum in the state were *A.niger*, *Rhizopus oryzae* and *A.flavus*. The commonest fungi found in Sorghum during the dry, harmattan and rainy season was *Rhizopus oryzae* while *A.niger* was the most frequent fungal contaminant of Sorghum in the hot, dry season. AFB<sub>1</sub> was found in 91 out of the 168 samples analyzed while ZEN was detected in 60 out of the 168 samples examined. Twenty three of the 112 were analyzed for ochratoxin A, contained the toxin.

**Key words:** Fungi • Mycotoxins • Aflatoxin • Ochratoxin • Zearalenone • Guinea corn • Sorghum • Niger State and Nigeria

### INTRODUCTION

Four million hectares of land in Nigeria are under Sorghum cultivation making it the most cultivated and consumed cereal in the country [1]. Nigeria is the world highest Sorghum producer, it produced over 10% (7.5 million) of the about 60 million metric tonnes of world Sorghum produced annually in the 1990s [2] and of this amount; Niger State contributes 13.76% (691,354 tonnes) on the average annually [3]. Sorghum is used in many farms as an important starchy food for human and animal consumption particularly in Northern Nigeria. Nigerians produce ogi or koko, a traditional fermented thin porridge from Sorghum. Fermented breads called masa and unleavened bread called 'waina' are also produced in Nigeria from it. 'Tuwo' is a stiff porridge produced in the country from adding Sorghum flour to boiling water and continuous stirring.

Sorghum has high phenol and tannin contents [2] and these principles make it resistant to mould infestation, diseases and damage. Despite its inherent resistance to

mould infestation, Sorghum grain mould constitutes one of the most important biotic constraints to Sorghum improvement and production worldwide. It is estimated that annual economic losses in Asia and Africa as a result of grain mould are in excess of US\$ 130 million [4]. The mycoflora and mycotoxins contaminating Sorghum in Nigeria and many parts of the globe have been documented [5,6]. Niger state is a major producer of Sorghum in the country, meanwhile, apart from the work of Dada [7] nothing seems to have been done on the fungi and their toxins in Sorghum in the State.

Since fungi and their toxins cause obvious reduction in crop and animal livestock production and diseases in human, the survey for AFB<sub>1</sub>, OTA, ZEN and mycotoxigenic fungi contaminating Sorghum in Niger state, a leading cereal producer would be of great importance with respect to public health, agricultural and economic growth of Nigeria. Based on the foregoing, this study was undertaken to determine the fungi and some mycotoxins contaminating Sorghum in Niger State, Nigeria with a view to rationally speculate

**Corresponding Author:** Dr. Hussaini A. Makun, Department of Biochemistry, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria  
Current Address: Food, Environment and Health Research Group, Faculty of Health Sciences, University of Johannesburg, South Africa

the types of mycotoxicoses expected following consumption of the studied cereal in Niger State and by implication in Nigeria.

## MATERIALS AND METHODS

**Collection of Samples:** In the year 2000, visibly mouldy samples of Sorghum were collected during the dry harmattan (November-January), hot, dry season (February-April) and rainy season (May -October) from twenty five local government areas of Niger State: Agaie, Agwara, Bida, Borgu, Bosso, Edati, Gbako, Gurara, Katcha, Kontagora, Lapai and Lavun. Others include Magama, Mariga, Mashegu, Minna, Mokwa, Munya, Paikoro, Rafi, Rijau, Tafa, Shiroro, Suleja and Wushishi. The local government areas fall into four microclimatic zones. The wettest zone (zone 1) has an annual rainfall of above 1400 mm and comprises of Suleja and Tafa. Borgu and Magama make up the wet zone (zone 11) with an annual rainfall of 1200-1400 mm. The dry zone (zone 111) is the largest comprising of 18 LGAs (Agaie, Agwara, Bida, Bosso, Edati, Gbako, Gurara, Katcha and Kontagora. Others are Lapai, Lavun, Mashegu, Minna, Mokwa, Munya, Paikoro, Rijau and Shiroro) and has rainfall of 1000-1200 mm. Mariga, Rafi and Wushishi make up the zone 1V which is the driest of the four with an annual rainfall of below 1000 mm.

In each of the season stored and marketed samples were collected while the field samples were only obtained in the dry harmattan season shortly before harvest period. The stored samples were collected from locally built mud barns called rumbu in Hausa. About two kilograms of the samples were collected, labeled, packaged in sterile polythene bags and taken to the laboratory. In the laboratory, the samples were divided into two halves. One half was grounded and stored for mycotoxin analysis and the other half was used immediately for fungal isolation studies.

**Isolation and Identification of Fungi:** Fungi were isolated and cultured according to the method described by Halfon-Meir and Barkon-Golan [8]. About 10 gm of the grains were surface sterilized using 5% sodium hypochlorite solution and washed with ten successive 100 ml volume of sterile distilled water. Ten grains were placed at random in each of the Petri-dishes containing Potato Dextrose Agar (PDA) and chloramphenicol (500 mg per litre). The dishes were incubated at room temperature and examined daily for five days. Fungi from plated grains

were transferred to PDA slant media bottles and subsequently to fresh Petri-dishes containing PDA for identification. Identification of isolates was carried out based on morphological and microscopic characteristics at the Microbiology Department of Federal University of Technology, Minna and Department of Crop Protection, Ahmadu Bello University, Zaria.

**Analysis of Mycotoxins:** The samples were screened and analyzed for aflatoxin B<sub>1</sub>, ochratoxin A and zearalenone. The AFB<sub>1</sub> and OTA standards were obtained from Makor chemicals Ltd, Jerusalem, Israel and the zearalenone standards from the USDA Southern Regional Centre. A multi-mycotoxin assay method [9] was used for the mycotoxins analysis. In the method, dichloromethane and phosphoric acid are used for the simultaneous extraction of AFB<sub>1</sub>, OTA and ZEN. A separate portion of the initial dichloromethane/phosphoric acid extract was subjected to a specific clean-up procedure for each mycotoxin. Each of the procedure was a modification of a published procedure for each toxin as described below.

**Extraction and Identification of Mycotoxins:** Fifty gram of pulverized samples were weighed into 500 ml Erlenmeyer flask and 25 ml 1M-phosphoric acid and 250 ml of dichloromethane were added. The flask was shaken for 30 minutes using a shaker and the content filtered under pressure on Buchner funnel fitted with 18 cm circle rapid filter paper. About 200 ml of the filtrate was collected and from this, 50 ml aliquots were placed in separate 100 ml Erlenmeyer flasks with glass stoppers, for AFB<sub>1</sub>, OTA and ZEN assay.

AFB<sub>1</sub> was analyzed in one of the 50 ml aliquot using the method of the Association of Official Analytical Chemists [10]. The plates were developed in ether-methanol-water (96:3:1 by volume) and were estimated by visual comparison of fluorescence intensity of samples with that of standards. Aflatoxin was confirmed by spraying the thin layer chromatographic plates with aqueous sulphuric acid (50:50, v/v), dried and viewed under long wave and the spots fluoresced yellow. The recovery percentage was 97%.

OTA was quantified by a modification of the method of Paulsch *et al.* [11], with 1M-phosphoric acid substituted for 4 M-phosphoric acid and dichloromethane substituted for chloroform. The intensities of the standards and samples were compared visually with a recovery percentage of 93%. To confirm the presence of OTA, the thin layer chromatographic

plates were sprayed with alcoholic aluminum chloride ( $AlCl_3$ ) (20 gm/100 ml alcohol) and also by exposure to ammonia vapour and viewed under long wave. The fluorescence changed from blue green to bright blue to confirm OTA spots.

ZEN was assayed by a modified method of Ware and Thorpe, [12] as described by Gbodi *et al.* [13] and had a recovery percentage of 100%. In this method phosphoric acid and dichloromethane were used for extraction in place of chloroform. Plates for ZEN determination were developed first in benzene -hexane (75:25) followed by developing in methylene-ethanol (97:3). ZEN was confirmed by spraying the plates with Alcoholic aluminum chloride and viewed under short wave when the fluorescence intensity increased.

**Statistical Analysis:** Mean $\pm$ standard deviation and analysis of variance (student's-test) of data generated were calculated using SPSS software. The statistical level of significance was fixed at  $P < 0.05$  (95%).

## RESULTS

**Fungi Isolated:** Table 1 shows the list and incidence of fungi isolated from field, marketed and stored mouldy Sorghum collected at the three different seasons of the year. Eight hundred and eighty four fungal isolates were cultured and identified from a total of a hundred and sixty eight samples studied. The results indicate that the genera of fungi contaminating Sorghum in Niger State in order of frequency were *Aspergillus*, *Rhizopus*, *Penicillium*, *Mucor*, *Fusarium*, *Alternaria*, *Phoma*, *Trichoderma*, *Helminthosporium* and *Cladosporium*. Others include *Curvularia*, *Colletotrichum*, *Chrysosporium*, *Scopularia*, *Torula*, *Arthrium* and *Rhodotorula*. The thirteen most frequent fungi species infecting Sorghum in the State were *A.niger*, *Rhizopus oryzae*, *A.flavus*, *Mucor spp*, *Penicillium spp*, *Rhizopus spp*, *Trichoderma spp* and *Alternaria alternate*. Others include *Helminthosporium spp*, *Phoma spp*, *Phoma sorghina*, *Fusarium spp* and *Cladosporium spp*.

Table 1: Incidence of fungi in mouldy sorghum collected during the three seasons of the year in Niger State

Dry-Cold Harmattan (Nov-Dec.)			Hot, Dry Season (March-May)			Rainy Season (June-Oct.)			Total Incidence
Fungus	No. of samples Infected	No. of samples Studied	Fungus	No. of samples Infected	No. of samples Studied	Fungus	No. of samples Infected	No. of samples Studied	
<i>Alternaria alternata</i>	5	56	<i>Alternaria alternata</i>	12	56	<i>Alternaria alternata</i>	16	59	33
<i>Alternaria spp</i>	9		<i>Alternaria spp</i>	3		<i>Alternaria spp</i>	13		25
<i>Arthrium spp</i>	1								1
<i>Aspergillus flavus</i>	28		<i>Aspergillus flavus</i>	30		<i>Aspergillus flavus</i>	29		87
			<i>A. fumigatus</i>	6		<i>A. fumigatus</i>	9		15
<i>A. glaucus</i>	2					<i>A. nidulans</i>	3		2
						<i>A. niger</i>	39		3
<i>A. niger</i>	34		<i>A. niger</i>	31		<i>A. ochraceus</i>	3		104
						<i>A. parasticus</i>	18		3
<i>A. parasiticus</i>	1		<i>A. parasiticus</i>	4		<i>A. versicolor</i>	2		23
<i>A. versicolor</i>	3		<i>Chaetomium</i>	2					5
						<i>Chrysosporium tropicam</i>	3		2
<i>Cladosporum spp</i>	5		<i>Cladosporum spp</i>	8		<i>Cladosporum spp</i>	13		3
						<i>Cladosporum werneckil</i>	2		26
<i>Collectrotichum spp</i>	5					<i>Colletotrichum spp</i>	10		2
<i>Curvularia lunata</i>	8		<i>Curvularia lunata</i>	6		<i>Curvularia lunata</i>	10		15
<i>Fusarium spp</i>	3		<i>Fusarium spp</i>	6		<i>Fusarium spp</i>	16		24
<i>F. equseti</i>	12		<i>F. equseti</i>	3		<i>F. equseti</i>	1		25
<i>F. oxysporum</i>	9					<i>F. oxysporum</i>	2		16
						<i>F. semitectum</i>	9		11
						<i>F. solani</i>	3		9
<i>Helminthosporium spp</i>	11		<i>Helminthosporium spp</i>	4		<i>Helminthosporium spp</i>	13		3
<i>Mucor spp</i>	31		<i>Mucor spp</i>	23		<i>Mucor spp</i>	17		28
<i>Penicillium spp</i>	18		<i>Penicillium spp</i>	19		<i>Penicillium spp</i>	31		71
						<i>P. citrinum</i>	4		68
						<i>P. notatum</i>	1		4
									1

Table 1: Continued

<i>P. rubrum</i>	2			<i>P. rubrum</i>	2	4
<i>P. verrucosum</i>	6			<i>P. verrucosum</i>	6	12
<i>Phoma sorghina</i>	12	<i>Phoma sorghina</i>	4	<i>Phoma sorghina</i>	11	27
<i>Phoma</i> spp	8	<i>Phoma</i> spp	13	<i>Phoma</i> spp	7	28
<i>Rhizopus oryzae</i>	47	<i>Rhizopus oryzae</i>	25	<i>Rhizopus oryzae</i>	29	101
<i>Rhizopus</i> spp	9	<i>Rhizopus</i> spp	10	<i>Rhizopus</i> spp	39	58
				<i>Rhodoturula rubra</i>	1	1
				<i>Scopulariopsis</i>	3	3
				<i>Torula</i> spp	2	2
<i>Trichoderma</i> spp	11	<i>Trichoderma</i> spp	3	<i>Trichoderma</i> spp	25	39
	280		212		392	884

Table 2: Incidence of fungi in mouldy sorghum in Niger State, Nigeria in accordance to microclimatic zones

Fungus	Zone I		Zone II		Zone III		Zone IV		Total Incidence		
	No. of samples	No. of samples	Fungus	No. of samples	No. of samples	Fungus	No. of samples	No. of samples			
<i>Alternaria alternata</i>	8	42	<i>Alternaria alternata</i>	9	42	<i>Alternaria alternata</i>	11	45	5	42	33
<i>Alternaria</i> spp	9		<i>Alternaria</i> spp	6		<i>Alternaria</i> spp	8		2		25
<i>Arthrium</i>	1										1
<i>Aspergillus flavus</i>	23		<i>Aspergillus flavus</i>	21		<i>Aspergillus flavus</i>	24		19		87
<i>A. fumigatus</i>	6		<i>A. fumigatus</i>	3		<i>A. fumigatus</i>	6				15
<i>A. glaucus</i>	2										2
<i>A. nidulans</i>	1		<i>A. nidulans</i>	1				<i>A. nidulans</i>	1		3
<i>A. niger</i>	27		<i>A. niger</i>	23		<i>A. niger</i>	28		26		104
<i>A. orchraceus</i>	2					<i>A. orchraceus</i>	1				3
<i>A. parasiticus</i>	5		<i>A. parasiticus</i>	4		<i>A. parasiticus</i>	11		3		23
<i>A. versicolor</i>	2		<i>A. versicolor</i>	1		<i>A. versicolor</i>	2				5
<i>Chaetomium</i> spp	1		<i>Chaetomium</i> spp	1							2
<i>Chrysosporium tropicam</i>	1		<i>Chrysosporium tropicam</i>	1		<i>Chrysosporium tropicam</i>	1				3
<i>Cladosporium</i> spp	6		<i>Cladosporium</i> spp	10		<i>Cladosporium</i> spp	5		5		26
<i>Cladosporium verneckil</i>	2										2
<i>Collectrotichum</i> spp	5		<i>Collectrotichum</i> spp	6		<i>Collectrotichum</i> spp	3		1		15
<i>Curvularia lunata</i>	3		<i>Curvularia lunata</i>	6		<i>Curvularia lunata</i>	14		1		24
<i>Fusarium</i> spp	9		<i>Fusarium</i> spp	9		<i>Fusarium</i> spp	3		4		25
<i>F. equseti</i>	4		<i>F. equseti</i>	2		<i>F. equseti</i>	7		3		16
<i>F. oxysporum</i>	2		<i>F. oxysporum</i>	4		<i>F. oxysporum</i>	3		2		11
<i>F. semitectum</i>	1		<i>F. semitectum</i>	2		<i>F. semitectum</i>	4		2		9
<i>F. solani</i>	1					<i>F. solani</i>	2				3
<i>Helminthosporium</i> spp	8		<i>Helminthosporium</i> spp	9		<i>Helminthosporium</i> spp	4		7		28
<i>Mucor</i> spp	12		<i>Mucor</i> spp	16		<i>Mucor</i> spp	23		20		71
<i>Penicillium</i> spp	15		<i>Penicillium</i> spp	14		<i>Penicillium</i> spp	17		22		68
<i>P. citrinum</i>	1		<i>P. citrinum</i>	3							4
						<i>P. notatum</i>	1				1
<i>P. rubrum</i>	1		<i>P. rubrum</i>	1		<i>P. rubrum</i>	1		1		4
<i>P. verrucosum</i>	4		<i>P. verrucosum</i>	4		<i>P. verrucosum</i>	2		2		12
<i>Phoma sorghina</i>	8		<i>Phoma sorghina</i>	5		<i>Phoma sorghina</i>	5		9		27
<i>Phoma</i> spp	8		<i>Phoma</i> spp	9		<i>Phoma</i> spp	7		4		28
<i>Rhizopus oryzae</i>	24		<i>Rhizopus oryzae</i>	23		<i>Rhizopus oryzae</i>	26		28		101
<i>Rhizopus</i> spp	12		<i>Rhizopus</i> spp	16		<i>Rhizopus</i> spp	13		17		58
						<i>Rhodoturula rubra</i>	1				1
<i>Scopulariopsis</i>	1		<i>Scopulariopsis</i>	1		<i>Scopulariopsis</i>	1				3
<i>Torula</i> spp	2										2
<i>Trichoderma</i>	7		<i>Trichoderma</i>	12		<i>Trichoderma</i>	12		8		39
	224			223			245		192		884

During the dry harmattan season (November-February) *Rhizopus oryzae* (47/56) was the most common fungi followed by *A. niger* (34/56), *Mucor* spp (31/56) and *A. flavus* (Table 1). On same table, the studies reveals that *A. niger* (31/56), *A. flavus* (30/56), *Rhizopus oryzae* (25/56) and *Mucor* spp (23/56) were the four commonest fungi during the dry, hot season. During the rainy season,

*Rhizopus oryzae* (39/59) and *A. flavus* (39/59) that were of equal occurrence predominated followed by *Penicillium* spp (31/59), *Rhizopus oryzae* (29/59) and *A. flavus* (29/59). There was higher fungal contamination (average fungal isolate per sample) during the rainy season (6.64) than the dry harmattan season (5.00). The least number of isolates were cultured in the dry, hot period (3.78).

Table 3: Incidence of fungi in field, marketed and stored mouldy sorghum in Niger State

Fungus	Field (28)		Market (84)		Store (84)		Total Incidence		
	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples			
<i>Alternaria alternata</i>	2	16	<i>Alternaria alternata</i>	5	75	<i>Alternaria alternata</i>	26	80	33
<i>Alternaria spp</i>	5		<i>Alternaria spp</i>	9		<i>Alternaria spp</i>	11		25
<i>Arthrium spp</i>	1								1
<i>Aspergillus flavus</i>	10		<i>Aspergillus flavus</i>	32		<i>Aspergillus flavus</i>	45		87
			<i>A. funigatus</i>	1		<i>A. fumigatus</i>	14		15
<i>A.glaucus</i>	1					<i>A. glaucus</i>	1		2
			<i>A. nidulans</i>	1		<i>A. nidulans</i>	2		3
<i>A.niger</i>	13		<i>A. niger</i>	39		<i>A. niger</i>	52		104
			<i>A. ochraceus</i>	1		<i>A. ochraceus</i>	2		3
<i>A. parasiticus</i>	1		<i>A. parasiticus</i>	12		<i>A. parasticus</i>	10		23
<i>A. versicolor</i>	2		<i>A. versicolor</i>	1		<i>A. versicolor</i>	2		5
						<i>Chaetomium</i>	2		2
						<i>Chrysosporium tropicam</i>	3		3
<i>Cladosporum spp</i>	4		<i>Cladosporum spp</i>	6		<i>Cladosporum spp</i>	16		26
			<i>Cladosporum werneckil</i>	1		<i>Cladosporum werneckil</i>	1		2
<i>Collectrotichum spp</i>	5		<i>Collectrotichum spp</i>	3		<i>Colletrotrichum spp</i>	7		15
<i>Curvularia lunata</i>	6		<i>Curvularia lunata</i>	7		<i>Curvularia lunata</i>	11		24
<i>Fusarium spp</i>	3		<i>Fusarium spp</i>	11		<i>Fusarium spp</i>	11		25
<i>F. equseti</i>	3		<i>F. equseti</i>	6		<i>F. equseti</i>	7		16
<i>F. oxysporum</i>	3		<i>F. oxysporum</i>	1		<i>F. oxysporum</i>	7		11
			<i>F. semitectum</i>	3		<i>F.semitectum</i>	6		9
						<i>F.solani</i>	3		3
<i>Helminthosporium spp</i>	8		<i>Helminthosporium spp</i>	6		<i>Helmintho Sporum spp</i>	14		28
<i>Mucor spp</i>	10		<i>Mucor spp</i>	26		<i>Mucor spp</i>	35		71
<i>Penicillium spp</i>	4		<i>Penicillium spp</i>	27		<i>Penicillium spp</i>	37		68
						<i>P. citrinum</i>	4		4
<i>P. rubrum</i>	1					<i>P. notatum</i>	1		1
<i>P. verrucosum</i>	2		<i>P. verrucosum</i>	3		<i>P. rubrum</i>	3		4
<i>Phoma sorghina</i>	5		<i>Phoma sorghina</i>	8		<i>P. verrucosum</i>	7		12
<i>Phoma spp</i>	5		<i>Phoma spp</i>	8		<i>Phoma sorghina</i>	14		27
<i>Rhizopus oryzae</i>	12		<i>Rhizopus oryzae</i>	41		<i>Phoma spp</i>	15		28
<i>Rhizopus spp</i>	5		<i>Rhizopus spp</i>	24		<i>Rhizopus oryzae</i>	48		101
						<i>Rhizopus spp</i>	29		58
						<i>Rhodoturula rubra</i>	1		1
						<i>Scopulariopsis spp</i>	3		3
						<i>Torula spp</i>	2		2
<i>Trichoderma spp</i>	3		<i>Trichoderma spp</i>	13		<i>Trichoderma spp</i>	23		39
	114			295			475		884

Table 2 presents the incidence of fungi according to zones. More fungi were obtained from the dry zone 111 (245) followed by the wettest zone 1 (224), wet zone 11 (223) and the driest zone 1V (192) respectively.

Table 3 shows the field, market and storage fungi of Sorghum in the state. The commonest field fungi were *A.niger*, *Rhizopus oryzae*, *Mucor spp*, *A.flavus* and *Helminthosporium spp* while the predominant storage fungi were *A.niger*, *Rhizopus oryzae*, *A.flavus*,

*Penicillium spp* and *Mucor spp*. *Rhizopus oryzae*, *A. niger*, *A. flavus*, *Penicillium* and *Mucor spp* were the most frequent contaminants of marketed Sorghum. The field (7.1) samples had higher fungal contamination than the stored (5.9) and marketed (3.9) samples. The occurrence of fungi in Sorghum and their incidence in the twenty five local government areas of the state is presented below in Table 4 and it shows that the highest fungal contamination was observed in Borgu, Gurara and Suleja local government areas.

Table 4: Incidence of fungi in mouldy sorghum samples collected from the twenty-five local government areas of Niger State during the three seasons

Fungus	Agaie	Agwara	Bida	Borgu	Bosso	Edati	Gbako	Gurara	Katcha	Kontogara	Lapai	Lavun	Magama
<i>Alternaria alternata</i>		2		5	1			4		2			3
<i>Alternaria spp</i>	2			3			1	1			1	1	3
<i>Arthrium spp</i>													
<i>Asperillus clavatus</i>	3	3	3	4	3	3	3	5	4	4	3	3	3
<i>A flavus</i>	1	1		1				2		1			2
<i>A fumigatus</i>								1					
<i>A glaucus</i>				1				1					
<i>A niger</i>	5	2	3	7	4	5	4	5	5	3	2	6	5
<i>A. ochraceus</i>								1		1			
<i>A. parasiticus</i>	1	1		2			1	2	1		1		2
<i>A terreus</i>						1							1
<i>A.versicolor</i>				1									
<i>Bipolaris spp</i>				1									
<i>Cladosporium spp</i>	1			6				3					4
<i>C. werneckil</i>								1					
<i>Cryptococcus neoformans</i>	1			2				2					4
<i>Curvularia lunata</i>	1	1	1	2	1	1		2			2		2
<i>Fusarium oxysporum</i>				5				2		1		1	3
<i>F. semitectum</i>	1			1		1		2				2	1
<i>F. solani</i>				2		1			1				2
<i>Fusarium spp</i>				1		1	1		1				1
<i>F. verticillioides</i>			1								1		
<i>Geotrichum candidum</i>	1		1	3	1		1	1	1		1		5
<i>Gilocladium spp</i>	3	3	3	2	5	1	4	2	3	2	3	1	3
<i>Helmintho sporium spp</i>	2	1	3	4	3	4	3	5	3	2	3	5	4
<i>Mucor spp</i>				1									3
<i>Nocardia brasiliensis</i>											1		
<i>Penicillium citrium</i>				1	1			1					
<i>P. cyclopium</i>		1		1			1					1	1
<i>P expansum</i>	1	1	1	1		2	1		1	1	1		1
<i>Penicillium spp</i>	1	1	1	2	1	1	2					1	3
<i>P. viridicatum</i>	5	4	3	2	2	4	2	7	5	5	4	7	7
<i>Rhizopus spp</i>	1	2	1	5	2	3	1	4	2	3	1	2	3
<i>Rhodotrui rubra</i>													
<i>Syncephalastrum spp</i>				1				1					
<i>Trichoderma spp</i>				1									1
Total incidence	1	1	1	6	1	1	1	2	1	1	1	1	1
% Incidence	31	24	22	74	25	29	26	56	29	26	25	31	68
	3.5	2.7	2.5	8.4	2.8	3.3	2.9	6.3	3.3	2.9	2.8	3.5	7.7

Table 4: Continued

Fungus	Mariga	Mashegu	Minna	Mokwa	Munya	Paikoro	Rafi	Rijau	Shiroro	Suleja	Tafa	Wushishi	Frequency
<i>Alternaria alternata</i>		2	2	1	1	2	2	1	1	1	3		33
<i>Alternaria spp</i>				1				1	1	3	5	2	25
<i>Arthrium spp</i>												1	1
<i>Asperillus clavatus</i>	4	4	3	3	3	3	5	3	5	4	3	3	87
<i>A flavus</i>			1	1				1		2	2		15
<i>A fumigatus</i>										1			2
<i>A glaucus</i>												1	3
<i>A niger</i>	4	5	3	4	5	3	3	3	3	5	4	6	104
<i>A. ochraceus</i>											1		3

Table 4: Continued

<i>A. parasiticus</i>	1	2		1	1	2	1	1		2	1		23
<i>A. terreus</i>			1							1	1		5
<i>A. versicolor</i>										1			2
<i>Bipolaris spp</i>							1					1	3
<i>Cladosporium spp</i>	1	1	2			1	1			2	2	2	26
<i>C. werneckii</i>											1		2
<i>Cryptococcus neoformans</i>	1	1					1			1	2		15
<i>Curvularia lunata</i>	2			2	1	3	2				1		24
<i>Fusarium oxysporum</i>							1		2	4	3	3	25
<i>F. semitectum</i>	1	1			2		2			1	1		16
<i>F. solani</i>	1	1		1						1	1		11
<i>Fusarium spp</i>			1				1		1	1			9
<i>F. verticillioides</i>											1		3
<i>Geotrichum candidum</i>	1	1		1			1	1		3	3	2	28
<i>Gilocladium spp</i>	3	4	5	3	4	4	2	3	2	2	2	2	71
<i>Helmintho sporium spp</i>	2	2	3	2	3	2	3	1	2	3	2	1	68
<i>Mucor spp</i>													4
<i>Nocardia brasiliensis</i>													1
<i>Penicillium citrium</i>			1										4
<i>P. cyclopium</i>	1			1	1		1			1	1	1	12
<i>P. expansum</i>	3	1		1	1	1	1		1	4	1	2	27
<i>Penicillium spp</i>		2	2	1	1	1	2			3	2	1	28
<i>P. viridicatum</i>	2	5	3	4	2	3	5	5	3	5	6	1	101
<i>Rhizopus spp</i>	1	3	3	3	2	1	5	1	1	2	1	5	58
<i>Rhodotruiia rubra</i>								1					1
<i>Syncephalastrum spp</i>										1			3
<i>Trichoderma spp</i>													2
Total incidence	1	3	1	2	1	1	1	1	2	1	3	3	39
% Incidence	29	38	31	30	28	28	39	26	24	56	54	35	884
	3.3	4.3	3.5	3.4	3.2	3.2	4.4	2.9	2.7	6.3	6.1	3.9	

Table 5: Incidence and concentrations ( $\mu\text{g kg}^{-1}$ ) of aflatoxin B<sub>1</sub>, ochratoxin A and zearalenone in mouldy sorghum during the dry harmattan, dry-hot and rainy seasons in Niger State, Nigeria

Mycotoxin	Incidence (concentration)	Dry harmattan	Dry, hot season	Rainy season	Total
Aflatoxin B <sub>1</sub>	Occurrence	28/56	31/56	34/56	93/168
	Mean + SD	141.11 + 203.18x	197.96 + 292.20	259.46 + 267.02x	199.51 + 259.90
	(Range)	(0 - 728)	(0 - 1164)	(0 - 728)	(0 - 1164)
Ohratoxin A	Occurrence	13/56	10/56	NA	23/112
	Mean + SD	80.78 + 194.67b	16.93 + 45.23b		48.86 + 144.29
	(Range)	(0 - 712)	(0 - 226)		(0 - 712)
Zearalenone	Occurrence	29/56	16/56	17/56	62/168
	Mean + SD	223.82 + 371.08	170.92 + 315.33	159.52 + 296.22	184.76 + 328.31
	(Range)	(0 - 1454)	(0 - 1164)	(0 - 1164)	(0 - 145)

Note: values with similar superscript are significantly different at P<0.05

NA means not assayed for.

**Aflatoxin B<sub>1</sub>:** The results of aflatoxin B<sub>1</sub> analyses of the mouldy Sorghum samples are presented in Table 5. The toxin was found as a contaminant of Sorghum all year round with a higher incidence during the rainy season (34/56) than the dry, hot (31/56) and dry harmattan (26/56).

However, higher concentrations of the mycotoxin were observed during the rainy season (259.46  $\mu\text{g kg}^{-1}$ ) than in the dry, hot (197.96  $\mu\text{g kg}^{-1}$ ) and dry harmattan (141.11  $\mu\text{g kg}^{-1}$ ) seasons. More stored samples (49/77) were contaminated with AFB<sub>1</sub> than marketed (39/75) and

Table 6: Occurrence and concentrations ( $\mu\text{g kg}^{-1}$ ) of aflatoxin B<sub>1</sub>, ochratoxin A and zearalenone in field, marketed and stored mouldy sorghum samples in Niger State, Nigeria

Mycotoxin	Occurrence and Concentration( $\mu\text{g kg}^{-1}$ )	Field	Market	Store	Total
Aflatoxin B <sub>1</sub>	Occurrence	5/16	39/75	49/77	93/168
	Mean + SD	9.88 + 17.73	174.97 + 233.61	262.82 + 288.09	199.51 + 259.90
	(Range)	ab(0 - 54)	ac(0 - 1139)	bc(0 - 1164)	(0 - 1164)
Ochratoxin A	Occurrence	2/16	8/48	13/48	23/112
	Mean + SD	28.50 + 102.85	16.17 + 50.68	88.33 + 200.99	48.86 + 144.29
	(Range)	(0 - 412)	a(0 - 242)	a(0 - 712)	(0 - 712)
Zearalenone	Occurrence	9/16	23/75	30/77	62/168
	Mean + SD	211.50 + 394.46	132.39 + 273.64	230.21 + 358.16	184.76 + 328.31
	(Range)	(0 - 1454)	a(0 - 1164)	a(0 - 1454)	(0 - 1454)

Note: values with similar superscript are significantly different at  $P < 0.05$

Table 7: Incidence and concentrations ( $\mu\text{g kg}^{-1}$ ) of aflatoxin B<sub>1</sub>, ochratoxin A and zearalenone in mouldy sorghum from the four microclimatic zones of Niger State, Nigeria

Mycotoxin	Occurrence (concentration)	Zone 1	Zone 11	Zone 111	Zone IV	Total
Aflatoxin B <sub>1</sub>	Occurrence	27/42	23/42	24/42	19/42	93/168
	Mean + SD	224.98 + 236.93	209.79 + 236.45	164.91 + 266.22	198.38 + 331.53	199.51 + 259.90
	(Range)	(0 - 728)	(0 - 712)	(0 - 721)	(0 - 1164)	(0 - 1164)
Ochratoxin A	Occurrence	5/28	8/28	3/28	7/28	23/112
	Mean + SD	28.57 + 104.09	94.00 + 210.71	12.86 + 41.84	60.00 + 156.81	48.86 + 144.29
	(Range)	x(0 - 536)	xy(0 - 712)	yz(0 - 162)	z(0 - 1169)	(0 - 712)
Zearalenone	Occurrence	17/56	22/56	11/56	12/56	62/168
	Mean + SD	118.10 + 224.15	255.95 + 330.78	215.67 + 402.36	149.31 + 324.75	184.76 + 328.31
	(Range)	xy(0 - 876)	x(0 - 1164)	y(0 - 1454)	(0 - 1454)	(0 - 1454)

Note: values with similar superscript are significantly different at  $P < 0.05$

field (5/16) samples (Table 6). The concentrations of the mycotoxins were significantly ( $p < 0.05$ ) higher in the stored grains than the marketed and field samples. AFB<sub>1</sub> was also a common contaminant of Sorghum in the four microclimatic zones (Table 7) with higher values observed in samples from zone 1, zone 11, zone 1V and zone 111 in decreasing order. Table 8 indicates that aflatoxin was detected in samples from all the twenty five local government areas except Mokwa and Mashegu local government areas.

**Ochratoxin A:** Of the one hundred and twelve samples analyzed for OTA twenty three contained the toxin with higher incidence and toxin content found in dry harmattan (13/56, 80.78  $\mu\text{g kg}^{-1}$ ) than dry, hot (10/56, 16.93  $\mu\text{g kg}^{-1}$ ) season samples (Table 5). Rainy season samples were not analyzed for OTA because we ran out of stock of Ochratoxin A standard. In the samples analyzed (Table 6) the toxin had higher incidence in stored samples (13/47)

than marketed (8/47) and field grains (2/16). OTA was also a common contaminant of the four zones (Table 7). The concentrations were significantly lower in zone 111 and zone 1 than in samples from zone 1V and zone 11 and occurred in ten local government areas (Table 8).

**Zearalenone:** Zearalenone was detected in sixty of the one hundred and sixty eight samples analyzed at maximum levels of 1454  $\mu\text{g kg}^{-1}$ . The incidence and concentrations of the toxin were highest in the dry harmattan as compared to the other seasons (Table 5). Stored samples (230.21  $\mu\text{g kg}^{-1}$ ) had the highest ZEN content followed by field (211.50  $\mu\text{g kg}^{-1}$ ) and marketed (132.39  $\mu\text{g kg}^{-1}$ ) samples (Table 6) while the wet zone 11 had the highest ZEN incidence and mycotoxin contents followed by the dry zone 111, driest zone 1V and wettest zone 1 in decreasing order (Table 7). ZEN was found contaminating Sorghum in sixteen of the twenty five local government areas (Table 8).



Table 8: Incidence of aflatoxin B<sub>1</sub>, Ochratoxin A and Zearalenone in mouldy sorghum from the twenty five local government areas of Niger State

Mycotoxin	Agai	Agwara	Bida	Borgu	Bosso	Edatei	Gbako	Gurara	Katcha	Kontogara	Lapai	Lavun	Magama
Aflatoxin B <sub>1</sub>	1	2	2	11	2	1	3	11	-	1	1	1	12
% Incidence	1.1	2.2	2.2	11.8	2.2	1.1	3.2	11.8	0	1.1	1.1	1.1	12.9
Ochratoxin A	-			5		1		3		1			3
% incidence				21.8		4.4		13.1		4.4			13.1
Zearalenone	1	2		12	1		1	10	1		1		10
% incidence	1.6	3.2		19.2	1.6		1.6	16.1	1.6		1.6		16.1

Table 8: Continued

Mycotoxin	Mariga	Mashegu	Minna	Mokwa	Munya	Paikoro	Rafi	Rijau	Shiroro	Suleja	Tafa	Wushishi	frequency
Aflatoxin B <sub>1</sub>	9	-	2	-	3	1	9	1	2	7	10	1	93
% Incidence	9.7	-	2.2	-	3.2	2.2	9.7	1.1	2.2	7.5	10.8	1.1	
Ochratoxin A	5				1		2			1	1		23
% incidence	21.8				4.4		8.7			4.4	4.4		
Zearalenone	9			3			2	1		2	5	1	62
% incidence	14.5			4.8			3.2	1.6		3.2	8.1	1.6	

**Co-occurrence of Mycotoxins Isolated:** This studies has shown that aflatoxin B<sub>1</sub> (91/168) was the predominant mycotoxin of the three toxins contaminating Sorghum in the state and was followed by zearalenone (60/168) and lastly ochratoxin A (23/112). Six samples were contaminated with the three mycotoxins while eighteen contained both AFB<sub>1</sub> and ZEN. AFB<sub>1</sub> and OTA occurred concurrently in two mouldy Sorghum samples. Only four samples contained both OTA and ZEN. These multiple contaminations occurred in six, fourteen, two and four local governments respectively.

## DISCUSSION

Seventeen fungal genera were found in this study to contaminate Sorghum in the state. Many of these families of fungi have also been shown to cause spoilage to Sorghum in others parts of the globe. As observed in this study, Elegbede, [14], Dada, [7] and Salifu, [15] identified the following fungal genera as the main contaminants of Sorghum in Northern Nigeria: *Aspergillus*, *Fusarium*, *Penicillium*, *Phoma*, *Alternaria*, *Chaetomium* and *Helminthosporium*. The other genera represented in their reports were *Curvularia*, *Colletrotrichum*, *Rhizopus* and *Mucor*, as well as bacterial colonies. Uruguchi and Yamazaki, [16] reported the incidence of the above mentioned fungi in Japanese Sorghum as well as *Cladosporium*, *Trichoderma* and *Scopulariopsis*. Other workers [5,17,18] have demonstrated the presence of all these fungi in the grain.

Fungi isolated from cereals are generally classified as field and storage fungi [19]. The field fungi identified from Sorghum in this study were species of *Alternaria*, *Arthrium*, *Aspergillus*, *Cladosporium*, *Collectritrochum*,

*Curvularia*, *Fusarium*, *Mucor*, *Penicillium*, *Phoma*, *Rhizopus* and *Trichoderma*. These fungi were also shown as field fungi [17,19]. The storage fungi found on Sorghum in Niger State in this work included the species isolated from the field and those of *Chaetomium*, *Scopularia*, *Chrysosporium*, *Rhodoturula* and *Torula* which are in accordance with the report of Uruguchi and Yamazaki, [16] except for the last three.

Fungi are only second to insects as a cause of deterioration and loss of grains and seeds [20] and their invasion of cereals decrease the quality, grade and market value of these agricultural products which in most instances are rendered unsafe for human and animal consumption. Conditions favouring fungal growth are numerous and not well understood but it is now obvious that hot, humid conditions enhance the development of these organisms on foods and feedstuffs [21]. These conditions which are more prevalent in wet seasons, zones, local government areas and traditional storage facilities may account for the higher fungal contaminations recorded during the rainy season, wetter zones 1, 11 and 111 and wet local government areas (Borgu, Gurara and Suleja) than drier seasons (harmattan and dry-hot seasons), zones 1V and local government areas. The exceptions to this rule as observed in our findings, where drier zone 111 had higher fungal content than the wetter zones could be because of the interactions of other factors that enhance fungal growth in the grain ecosystem. Such factors like higher insect infestation, mechanical damage, aeration, microbial load and longer storage time when they occur in the grain ecosystem even in drier places are likely to cause higher fungal contamination than in grains without these conditions in their ecosystem and in hot, humid climate [20].

Of the hundreds of mycotoxins occurring in nature aflatoxin, ochratoxin A and zearalenone are among the five agriculturally-important fungal mycotoxins [22] which are found in abundance in foods especially grains including Sorghum and pose a vast array of scientific problems and challenges to food production, public health and international trade. The other two are deoxynivalenol and fumonisins. Several investigations including this study have detected aflatoxin B<sub>1</sub>, OTA and ZEN from Sorghum in Nigeria and other parts of the globe [6,16]. Niger State has a tropical climate that is conducive for growth of *Aspergillus*, producers of aflatoxin and this explains why these species of fungi and their toxin aflatoxin B<sub>1</sub> are the most predominant fungal and mycotoxin contaminants of Sorghum in the state. The abundance of *Aspergillus spp* in the Sorghum samples correlated with the incidence of AFB<sub>1</sub>. This study also shows that zearalenone and to a lesser extent ochratoxin A occurs in mouldy Sorghum samples from different parts of Niger State. Both toxins were presumed by earlier scientists [23] to occur mainly in temperate countries. Fewer samples were subjected to OTA analysis as compared to AFB<sub>1</sub> and ZEN. This might account for the lower incidence of OTA in the studied samples than AFB<sub>1</sub> and ZEN.

Ochratoxin A is known to be produced mainly by *P. verrucosum* and *Aspergillus ochraceus* but of recent *Penicillium cyclopium*, *P. viridicatum*, *A. versicolor*, *A. glaucus* and *A. flavus* [24] and *Aspergillus niger* [25]. All these species of OTA producers were isolated from field, marketed and stored mouldy Sorghum in Niger State. *A. ochraceus* and *P. viridicatum* produce OTA between 0 and 31°C [20]. The upper limit of this temperature range is not unusual during the dry, harmattan and rainy seasons in the state. The incidence and concentration of the toxin were indeed higher in the dry harmattan period than the dry-hot season. In addition some studies have shown that *Penicillium spp* can grow and elaborate mycotoxins over a broader range of temperatures than *Aspergillus* [20] which implies that the earlier presumed temperate fungi, *Penicillium spp* and their secondary metabolites are capable of contaminating crops in the tropics as clearly demonstrated in this work. Many scientists [26,27] have also isolated OTA from foods and feedstuffs in the tropics.

Three ZEN producing species namely *F. equiseti*, *F. oxysporum* and *F. semitectum* [28] were isolated in the mouldy Sorghum samples studied. In general, zearalenone production by *Fusarium spp.* is greater in mouldy samples and is favoured by wet climates (high rainfall) and

especially by wet, cool weather [29]. These conditions are obtainable in the State particularly during the harmattan and rainy seasons. Our study conforms to these earlier findings as zearalenone incidence was higher during the harmattan, rainy and dry-hot seasons (in order of decreasing prevalence). *Fusarium* contaminated samples were also more during the cool, wet season than dry, cool harmattan and dry-hot periods respectively. Others workers [6,27,30] have shown zearalenone as crop contaminant in tropical climate.

Crops are infected in the field by fungi and these field fungi persist and proliferate with consequent increase in mycotoxin formation during storage when favourable conditions prevail [31]. Therefore mycotoxin incidence and contents are likely to be higher in badly stored grains than those on the field as observed in this work where the incidence and concentrations of the three studied mycotoxins were consistently higher in storage (store and market) samples than those from the fields. The more enhancing humid conditions [21] in the wet zones (I and II) than the dry zones (III and IV) was responsible for the higher fungal and mycotoxin (AFB<sub>1</sub>, OTA and ZEN) contamination of samples from the former group than the later. Similarly, except for ZEN, the wet season had higher mycotoxin contents than the dry seasons.

This study was a biased one because only mouldy samples of Sorghum were sampled for the work. Though such studies cannot give useful incidence data like unbiased study, biased analysis can give the natural fungal and mycotoxin profile of an area. Such biased studies are also becoming increasingly important in public health hazards analysis in Sub Saharan Africa because cheaper, low grade, mouldy grains are fed to animals. Scarcity of food, disasters and poverty leave many people in this region of the world with little or no option but to purchase and consume low grade cereals. The consumption of mouldy grains by man and animals has severe consequences on public health particularly that the mycotoxins are found at unsafe levels in the mouldy grains.

*Aspergillus* species the most frequent fungal contaminant isolated in this study produce many mycotoxins including aflatoxins and ochratoxins [34]. The involvement of these toxins in many human [30] and animal [33] maladies has been documented. Of greatest concern is the presence in our foods of aflatoxin B<sub>1</sub>, one of the most potent naturally occurring carcinogens. Ochratoxin A causes kidney and liver impairment in animals and man especially pigs [35,36].

*Penicillium* species also elaborate a host of mycotoxins including but not limited to ochratoxin, patulin and citrinin [34]. Patulin is neurotoxic while Citrinin is nephrotoxic [32]. *Fusarium spp* produce zearalenone, fumonisins and trichothecenes and many other fusariotoxins that have adverse impact on man and animals. Zearalenone adversely affects the reproductive system of animals and has been associated with precocious pubertal changes in children and human cervical cancer [29]. Diseases caused by the other fusariotoxins are well documented [37].

*Aspergillus*, *Chaetomium* and *Helminthosporium* which were also isolated in this work are known producers of sterigmatocystin [34], an intermediary metabolite of aflatoxin biosynthetic pathway and like AFB<sub>1</sub>, it is also a hepatotoxic and nephrotoxic carcinogen but exhibits lower toxicity than the former [32]. *Rhizopus* and *Mucor spp* were also abundantly found in Sorghum in our work. These genera of fungi produce rhizinin A which has deleterious effects on kidney and liver of mice and rats [38].

Moulds of the genus *Alternaria* are known to secrete a host of mycotoxins but the ones of toxicological significance to man and animals are altertoxins, tenuazonic acid and cytochalasins [35]. According to the authors altertoxins induce mutagenesis and transformation in mammalian cells while tenuazonic acid inhibits protein synthesis causing salivation, emesis, anorexia, erythema, gastrointestinal haemorrhages and convulsion in guinea pigs, mice, rabbits, dogs and monkeys. The same authors reported that cytochalasins inhibit cytokinesis and protein synthesis and have been shown to cause pulmonary haemorrhage and brain oedema in mice. Species of *Helminthosporium*, *Curvularia*, *Phoma* and *Trichoderma* are also producers of cytochalasins [39].

Some species of *Cladosporium* are known to elaborate emodin, a toxin that is cytotoxic and mutagenic to hepatic cells which causes haemolytic jaundice, renal failure and eventually death in mice [40]. No toxic diseases have been documented to date against *Chrysosporium*, *Scopulariopsis*, *Torula* and *Rhodotorula* [41].

The presence of mycotoxigenic fungi and the studied mycotoxins in mouldy Sorghum in Niger State as demonstrated in this study and the possible synergistic effects [42] of these fungi and toxins implies that Sorghum could be an important causative agent of several human and animal mycotoxicoses including those discussed above in Nigeria. The consumption of such mouldy grains therefore has adverse consequences on national

livestock production, human health and economy [43] and so enlightenment programmes on the hazards of mouldy crops to the Nigerian populace especially the crop and animal farmers and the enforcement of the 1987 Nigeria mycotoxin regulatory guidelines in the country have become imperative.

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