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Full Length Research Paper

Toxicologic screening of fungi isolated from millet (*pennisetum spp*) during the rainy and dry harmattan seasons in Niger state, Nigeria

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A survey of fungi contaminating mouldy field, stored and marketed millet samples collected during rainy and dry harmattan seasons of the year 2000 from the twenty five local government areas of Niger State, Nigeria, was conducted. Some of the fungal isolates from the two groups of samples were screened for their mycotoxin producing potentials in mice. Aflatoxin B_1 content of the rainy season millet samples was determined. *Aspergillus niger* was the predominant fungi found in millet during the rains. Twelve out of the 49 wet season millet samples were contaminated with AFB_1 at concentrations between 1370.28 and 3495.10 ug/kg. *Penicillium* spp. was the commonest contaminant of millet during the dry harmattan season. Thirty five of the fifty five fungal isolates screened for toxicity were found to produce toxic metabolites that were lethal to mice and were isolates of *Aspergillus*, *Fusarium*, *Penicillium*, Rhizopus, *Mucor, Syncephalastrum*, and *Helminthosporium*.

Key words: Fungi, aflatoxin B_1, mycotoxins, millet, Niger State, Nigeria.

INTRODUCTION

Millet *(Pennisetum* spp.) is resistant to drought and so has been extensively cultivated in arid regions. Millet ranks the sixth most important grain in the world, sustains one third of the world's population and is produced mainly by China, India and Nigeria. Over the past 50 years, the percentage of the world millet production in China has significantly decreased, while Nigeria has increased (African Crops, 2006). Over five million tonnes of millet were produced on yearly basis in the 1990s in the Nigeria (Shimada, 1999) and it contributes about 20% share of the main staples in Nigerian average food consumption in calories term (Akande, 2002). It is commonly consumed as pap, porridge, local cake ("masa"), millet meal ("tuwo"), gruel-like drink ("kunu – zaki"), and "fura" in the Northern Nigeria where it is mostly cultivated.

The susceptibility of millet to fungal growth and mycotoxin contamination has been demonstrated in other parts of Nigeria (Okoye, 1992) but little is known about the situation in Niger State. Meanwhile Niger State, which is generally warm and humid throughout the year (with an average rainfall of 1,400 mm and temperatures of between 20-30°C; Umoh, 1997) especially between May and October, has suitable climatic conditions to support fungal growth and mycotoxin production on cultivated cereals. Niger state is also a major national producer of cereals. Millet is the fourth most commonly grown and consumed grain after sorghum, rice and maize in Niger State and about 148,710 tonnes were produced in the State in 2004 (ADP, 2004).

The variation of incidence of fungi is climate dependent (Ominski et al., 1994) and therefore the fungi indigenous to Niger State may differ from those of other studied areas hence the need to generate information on mycotoxicology for Niger State. The survey for mycotoxigenic fungal contaminants of the highly cultivated and consumed millet in a state strategic for grain production would no doubt give an insight into the possible mycotoxins which can be produced in nature and their toxicological roles in national food safety and human and animal health. The purpose of this study, therefore, was to investigate and provide information on the mycoflora` contaminating field, stored and marketed millet grains during the rainy and dry harmattan seasons in Niger Sta-

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te and to screen some of the isolated fungi for their toxic metabolite producing potential. The aflatoxin B_1 content of the millet samples of the rainy season was also determined.

MATERIALS AND METHODS

Sample collection

Forty nine (49) mouldy samples of millet grains were collected during the rainy season between the 29th of June, 2000 and the 8th of July, 2000, from all the twenty five local government areas (LGAs) of the State. The rainy season samples were representative of stored samples from the previous year's farming season and were collected from various storage places and markets. Seven, fifteen and twenty-seven samples were collected from "rumbu", sacks and markets, respectively. "Rumbu" is the name in Hausa for a locally built mud storage barn. The samples obtained were placed in polythene bags, securely tied, labeled and transported to the laboratory. One kilogram of the samples were collected and divided into two. One half was stored in the freezer for mycotoxin analysis and the other half was used immediately for fungal isolation studies.

Similarly millet grains were sampled during the dry harmattan period between the 16th December and 23rd December 2000 from all the twenty-five LGAs of the State. The samples collected were mostly taken from the field shortly before or at harvest. Thirty four samples were collected during this season, and of these, 20 were collected from fields, 5 from storage in sacks and 9 from markets. About 500 g of each sample was collected for fungal isolation.

Isolation and identification of fungi

About 10 grains of millet were washed aseptically with ten successive 100 ml volumes of sterile distilled water and was surfaced-sterilized using 5.25% sodium hypochloride solution (NaOCl), and then rinsed with sterile distilled water. Ten grains were placed at random in each of the Petri-dishes containing potato dextrose agar (PDA) and chloramphenicol (500 mg/litre). The dishes were incubated at room temperature and examined daily for 5 days. Fungi from plated contaminated grains were isolated in pure cultures, and transferred to PDA slant bottles and to fresh PDA in Petri-dishes for identification.

The identification of isolates was carried out by the Microbiology Unit of the Biological Sciences Department, Federal University of Technology, Minna, Nigeria.

Culturing of fungi

To 20 g of Akkad rice produced in Thailand, 8 ml of distilled water was added and mixed thoroughly in a 50 ml conical flask and left over night for moisture equilibration. The rice substrate was then autoclaved for 20 min, at 120°C and 15 psi pressure. After cooling it was aseptically inoculated with conidia or mycelia of four to seven days old pure culture of fungi from millet that were grown on PDA in slant tubes and incubated for 12 – 14 days under ambient temperature. The culture was scratch with sterile inoculation wire loop to suspend the spores. The cooled conical flask were inoculated with suspended spores and maintained in an incubator at 28°C as a static culture for 14 days.

Extraction of toxin

The 20 g cultured rice were homogenized for ten minutes in 100 ml methylene chloride using blender, it was then filtered through fast filter paper. 2 ml of cotton seed oil was added to the filtrate in a round bottom flask of 55°C and the methylene chloride was distilled off. When the methylene chloride was distilled off, clean empty distillate flask collector was used to replace the methylene chloride collector and rotary evaporator turned on for another 10 min to ensure complete removal of methylene chloride vapour. The cotton-seed oil toxin extract was transferred into a dram vial and kept in deep freezer at –25°C until used for toxicity testing in mice.

Experimental animals

Four to six weeks old male white mice weighing 20 - 25 g were used for the screening of toxin-producing fungi isolated. They were purchased from National Veterinary Research Institute, Vom, Plateau State, Nigeria. The animals were housed in conventional plastic wire cages with synthetic bedding. They were given mice pellets (composed of maize, sorghum, wheat, soybeans (full fat and cake), groundnut cake, fish meal and rice brand) and tap water. The temperature of the room was usually between 25 - 30°C and extra lighting was provided in the night. The animals were randomly allotted to cages and three animals were kept in a cage for seven days prior to commencement of the toxicity testing. The cages were washed daily and bedding changed.

Screening culture extracts of fungi for toxicity in mice

A total of 55 isolates of the 158 fungi isolated from the studied grains were screened for toxicity. Twenty two of the eighty seven fungal isolates from millet samples of the rainy season tested for toxicity were *Helminthosporium* spp (1), *A. niger* (1), *Aspergillus flavus* (2), *Aspergillus parasiticus* (1), *Aspergillus fumigatus* (1), *Aspergillus glaucus* (1), *Penicillium* spp. (2) and *Rhizopus stolonifer* (3). Others include *Fusarium* spp. (1), *Fusarium equiseti* (1), *Fusarium trincitum* (1) *Syncephalastrum* spp. (3), *Mucor* spp. and *Phoma* spp. Seventy one isolates were cultured and identified from millet of the dry harmattan season. Of these, thirty three were screened for mycotoxin producing potential in mice and they include an isolate each of the 6 *Aspergillus species*, and *Fusarium nwale*. The other fungal contaminants of millet screened for toxicity include *Arthroconidia* spp. (2), *Cladosporium* spp. (2), *Penicillium* spp. (2), *Mucor* spp. (2), *Penicillium rubrum* (2) *Rhizopus* spp. (2), *Phoma* spp. (2), *Syncephalastrum* spp. (2), *Fusarium verticillioides* (2), and *Fusarium* spp. (8).

Each mouse received by interperitonial injection 0.2 ml of cotton – seed oil extract of fungi culture. The mice were observed for signs of toxicity for 14 days. The mice which received pure cotton-seed oil after methylene chloride was evaporated and used as control. The toxicity of the extract was arbitrarily classified into four categories:

(1) Very toxic (If all of the three extract treated mice died).
(2) Moderately toxic (two of the three of the extract treated mice died).
(3) Mildly toxic (If the one of the three mice was killed).
(4) Non – toxic (If none of the three extract treated mice died).

Extraction and identification of aflatoxin B_1

The millet samples of the rainy seasons were the only ones assayed for Aflatoxin B_1. The AFB_1 standards were obtained from Makor Chemical Limited, Jerusalem, Israel. The toxin was extracted from the samples into methylene chloride-phosphoric acid as described by Ehrlich and Lee (1984). In the method, 50 g of pulverized samples were weighed into 500 ml Erlenmeyer flask and 25 ml 1 M-phosphoric acid and 250 ml of methylene chloride were

Table 1. Incidence of fungi isolated from millet samples collected from "rumbu", sacks and markets during the rainy season.

Fungi species	Incidence			
	"Rumbu"	Sack	Market	Total infected samples
Aspergillus flavus	1/7*	3/15*	7/27*	11/49*
Aspergillus fumigatus	-	1/15	-	1/49
Aspergillus glaucus	-	-	-	1/49
Aspergillus niger	3/7	9/15	10/27	22/49
Aspergillus parasiticus	-	2/15	7/27	9/49
Fusarium equiseti	-	-	1/27	1/49
Fusarium spp	1/7	2/15	6/27	9/49
Fusarium trincintum	1/7	-	-	1/49
Helminthosporium spp	-	-	1/27	1/49
Penicillium spp	2/7	6/15	11/27	19/49
Penicillium verrucosum	-	-	1/27	1/49
Phoma spp	1/7	1/15	1/15	3/49
Mucor	-	-	2/27	2/49
Rhizopus stolonifer	-	-	3/27	3/49
Syncephalastrum spp	-	1/15	2/27	3/49
Total	**9/7**	**25/15**	**53/27**	**87/49**

Results are presented as number of infected samples/number of samples collected.

* = number of samples collected.

added. The flask was shaken for 30 min using a shaker and the content filtered under pressure on Buchner funnel fitted with 18 cm circle rapid filter paper. About 50 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_1 assay.

AFB_1 was analyzed in the 50 ml aliquot using the method of the Association of Official Analytical Chemists (Ehrlich and Lee, 1984). 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.

Quantitation

The quantitation of the mycotoxins was done by visual comparison of the intensities of the standards and samples. This involved the comparison of the fluorescence intensities of the spots of same Rf values of the mycotoxins in the samples with those of corresponding standard and determine which of the sample spot matches any of the standard. The corresponding aliquot volumes were then recorded and the concentrations of the mycotoxins in the samples in ug/kg were then calculated as follows: mycotoxin content (ug/kg) = SYV/ WZ, where S = volume of standard with same colour intensity as sample (µl), Y = concentration of mycotoxin standard used in µg/ml, V = volume of solvent required to dilute sample contained in final extract, W = effective weight (g) of original sample contained in final extract, and Z = volume of spotted sample equivalent to standard (µl).

RESULTS

A total of a hundred and fifty eight fungal isolates were cultured and identified from eighty three mouldy grain samples studied in the state. Eighty seven isolates were identified from 49 millets samples collected during the rainy season while 71 fungal isolates were found in 34 millet samples of the dry harmattan season. Ten genera of fungi namely *Aspergillus* (70), *Penicillium* (43), *Fusarium* (23), *Rhizopus* (6), *Mucor* (5), *Syncephalastrum* (4), *Phoma* (4), *Cladosporium* (1), *Arthroconidia* (1) and *Helminthosporium* (1) in order of decreasing predominance were the identified fungal contaminants of millet in the State in 2000. Of the 158 fungi isolated in this work, 55 (22 and 33 of the samples were collected during the rainy and dry harmattan seasons, respectively) were screened for toxicity. The extracts of thirty five (63.6%) of the isolates tested for toxicity were found to be lethal to mice and they include 10 isolates of *Aspergillus*, 13 of *Fusarium*, 5 of *Penicilli*, 3 of *Rhizopus*, 2 of *Syncephalastrum*, and one isolate each of *Helminthosporium* and *Mucor*.

Rainy season millet samples

Table 1 shows the incidence of fungal isolates identified in stored and marketed millet from Niger State during the rainy season. Eighty seven fungal isolates of fifteen different species were cultured and identified. *A. niger* (22/49), *Penicillium* spp. (19/49), *A. flavus* (11/49), *A. parasiticus* (9/49) and *Fusarium* spp. (9/49) were the common fungi contaminating millet in the state during the rains. Other fungal contaminants of millet include *Syncephalastrum* spp. (3/49), *R. stolonifer* (3/49), *Phoma* spp.

Table 2. Toxicity of extracts of some fungi isolated from millet during the rainy season.

Very toxic (1)	Incidence	Moderately toxic (4)	Incidence	Mildly toxic 10)	Incidence	Non-toxic (7)	Incidence
Helminthosporium spp.	1/1	*Aspergillus niger*	1/4	*Aspergillus flavus*	1/10	*Mucor* spp.	2/7
		Penicillium spp.	1/4	*Aspergillus fumigatus*	1/10	*Penicillium* spp	1/7
		Rhizopus stolonifer	2/4	*Aspergillus glaucus*	1/10	*Penicillium verrucosum*	1/7
				Aspergillus parasiticus	1/10	*Phoma* spp.	1/7
				Fusarium equiseti	1/10	*Rhizopus stolonifer*	1/7
				Fusarium spp.	2/10	*Syncephalastrum* spp.	1/7
				Fusarium trincintum	1/10		
				Syncephalastrum spp.	2/10		

Very toxic: all three mice killed (3/3) = 1 isolate
Moderately toxic: two out three mice killed (2/3) = 4 isolates
Mildly toxic: one mouse out of three mice killed (1/3) = 10 isolates
Non-toxic: Non of the mice was killed (0/3) = 7 isolates

Table 3. Incidence and concentrations (µg/kg) of Aflatoxin B_1 in millet collected during the rainy season.

Mode of storage	Positive/total samples (%)	Toxin level (µg/kg)
"Rumbu"	1/7 (14.3%)	1370.28
Sack	1/15 (6.7%)	3427.31
Market	10/27 (37.0%)	2964.81±79.53 (1521.13 - 3495.10)
Total	12/49 (24.5%)	2587.470 ± 783.23a (1370.28 – 3495.10)

Results of toxin level are represented as mean ± standard deviation (range).

(3/49) and *Mucor* (3/49). *A. fumigatus, A. glaucus, F. trincintum, Helminthosporium* spp., *Penici-llium verrucosum* and *Fusarium equiseti* had the least incidence of (1/49) each. The marketed samples had the highest incidence of fungi and consequently level of contamination. The samples stored in "rumbu" had the least contamination rate followed by samples from sacks.

Toxicity screening results of fungal isolates from millet samples of the wet season are presented in Table 2. Fifteen (68.2%) of the 22 screened isolate produced toxic metabolites. The *Helminthosporium* spp. were highly toxic (4.5%) while the *A. niger, Penicillium spp* and *R. stolonifer* were moderately toxic (18.2%). The ten fungal isolates that produced mildly toxic metabolites were *A. flavus, A. fumigatus, Aspergillus glaucus, A. parasiticus, F. equiseti, Fusarium* spp., *F. trincintum* and *Syncephalastrum* spp. Seven isolates were found to be non-toxic (31.8%) and they include two isolate of *Mucor* and *Penicillium* spp., and one each of *Phoma* spp., *R. stolonifer* and *Syncephalastrum* spp.

Table 3 shows the incidence and AFB_1 content of the wet season samples in Niger State. Twelve (12) of the total 49 samples analyzed contained the toxin at levels between 1370.28 µg/kg and 3495.10 µg/kg which are above the current internationally set safe limit of 20 µg/kg or 20 ppb. Marketed samples had the highest number of contaminated samples (10/12) while samples stored in "rumbu" and sacks had a contaminated sample each.

Millet samples of the dry harmattan season

Seventy one isolates of seventeen different species of fungi were isolated from millet during the dry harmattan season (Table 4). *Penicillium* spp. (22/34) was the most frequent contaminant of millet during the season followed by *A. flavus* (14/34) and, then *Fusarium* spp. (6/34), *A. niger* (5/34) and *Fusarium verticillioides* (5/34). Other fungal contaminants isolated in millet include species of the following genera *Aspergillus, Fusarium, Penicillium, Cladosporium, Phoma* spp., *Rhizopus* spp., *Mucor* spp., *Syncephalastrum* and *Anthroconidia* spp.

The storage fungi contaminating millet samples in Niger State during the dry harmattan season (Table 4) include

Table 4. Incidence of fungi found in millet samples from fields, sacks and markets during the dry harmattan season.

Species	Incidence			
	Field	Sack	Market	Total infected samples
Aspergillus flavus	8/20	2/5*	4/9*	14/34*
Aspergillus nidulans	3/20			3/34
Aspergillus niger	3/20	2/5		5/34
Aspergillus glaucus	1/20			1/34
Aspergillus parasiticus	1/20			1/34
Aspergillus versicolor	2/20			2/34
Arthroconidia spp.			1/9	1/34
Cladosporium spp.		1/5		1/34
Fusarium nwale	1/20			1/34
Penicillium spp.	10/20	5/5	7/9	22/34
Mucor spp.	1/20		2/9	3/34
Penicillium rubrum	1/20			1/34
Rhizopus spp.	2/20		1/9	3/34
Phoma spp.	1/20			1/34
Syncephalastrum spp.	1/20			1/34
Fusarium verticillioides	4/20	1/5		5/34
Fusarium spp.	3/20	1/5	2/9	6/34
Total	43/20	11/5	17/9	71/34

*Number of sample collected.

Table 5. Toxicity of extracts and incidence of fungi isolated from millet during the dry harmattan season in mice.

Very toxic (9)		Moderately toxic (3)		Mildly toxic (8)		Non- toxic (13)	
Aspergillus nidulans	1/9	Fus*arium nwale*	1/3	*Aspergillus flavus*	1/8	*Aspergillus versiscolor*	1/13
A. niger	1/9	*Rhizopus* spp.	1/3	*A. glaucus*	1/8	*Arthroconidia* spp.	2/13
A. parasiticus	1/9	*Penicillium* sp.p	1/3	*Fusarium verticillioides*	2/8	*Cladosporium* spp.	2/13
Fusarium spp.	4/9			*Fusariun* spp.	2/8	*Phoma* spp.	2/13
Penicillium.rubrum	2/9			*Mucor* spp.	1/8	*Syncephalastrum*	2/13
				Penicillium spp.	1/8	*Rhizopus* spp.	1/13
						Mucor spp.	1/13
						Fusarium spp.	2/13

Very toxic: all three mice killed (3/3) = 9 isolates.
Moderately toxic: two of the three mice were (2/3) = 3 isolates.
Mildly toxic: one of the three mice was killed (1/3) = 8 isolates.
Non-toxic: Non of the mice was killed (0/3) = 13 isolates.

A. flavus, A. niger, Penicillium spp.*, Arthroconidia* spp.*, Rhizopus* spp.*, Mucor* spp.*, F. verticillioides* and *Fusarium* spp. All the fungi species isolated from millet during this season, with the exception of *Arthroconidia* spp., were identified as field fungi of millet.

Extracts of twenty (60.61%) of the thirty three fungal isolates from millet of this season that were screened for toxic metabolite producing potential were toxic to mice while 13 (39.39%) were found not to produce toxic metabolites (Table 5). The six very toxic ones includ *Aspergillus nidulans, A. niger, A. parasiticus, Fusarium* spp. and *Penicillium rubrum*. Three isolates namely *F. nwale, Rhizopus* spp. and *Penicillium* spp. were found to be moderately toxic. Eight isolates were found to produce mildly toxic metabolites and of these, two were *Aspergillus* species, four *Fusarium* spp. and one each of M*ucor* and *Penicillium* spp.

DISCUSSION

Species of *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* , *Mucor*, *Syncephalastrum*, *Phoma*, *Cladosporium, Arthroconidia* and *Helminthosporium* found contaminating millet in Niger State, have been found in the same crop and

other cereals in other parts of the country (Gbodi, 1986; Okoye, 1992). Based on their growth requirements, fungi are classified into two main groups namely field and storage fungi (Ominski et al., 1994). The field fungi (*Aspergillus, Fusarium, Penicillium, Mucor, Phoma, Syncephalastrum* and *Rhizopus*) and storage fungi (*Aspergillus, Fusarium, Penicillium, Helminthosporium, Cladosporium, Phoma, Rhizopus, Mucor, Syncephalastrum* and *Arthroconidia*) found in the work have been shown as same in other studies (Elegbede, 1978; Gbodi, 1986; Uraguchi and Yamazaki, 1978; Ominski et al., 1994; Bankole and Adebanjo, 2003). Grains are exposed during sales in markets in the study area and such exposure could account for the higher incidence of fungi and AFB_1 in marketed samples than in stored and field samples.

The results of toxicity screening demonstrated that fungal isolates of *Aspergillus*, *Fusarium*, *Penicillumi*, *Rhizopus*, *Mucor Syncephalastrum*, and *Helminthosporium* from the cereal crops studied produced toxic metabolites that were deleterious to mice. The presence of these toxigenic fungi in highly consumed millet points to potential serious hazards to man and animals in Niger State and indeed Nigeria as a whole. The *Aspergillus* species which were the commonest fungal contaminant of the two crops are known to produce a host of mycotoxins that are hepatocarciongenic (aflatoxins, penicillic acid and sterigmatocystin), neurotoxic (fumitremorgens A and B, and fumigaclavines) and nephrotoxic (ochratoxn A and citrinin) to man and animals (Prelusky et al., 1994). Kojic acid and malformins, which are also toxins of *Aspergillus*, are lethal to mice and rats, respectively, while secalonic acid also produced by same genera of fungi is lethal, cardiotoxic, teratogenic and a lung-irritant to mice (Reddy and Hayes, 2001). Aflatoxin B_1 which was found in some mouldy millet samples in this study is the *Aspergillus* mycotoxin of greatest public health concern because it has been associated with high incidence of liver cancer in certain parts of the world (Bankole and Adebanjo, 2003).

Penicillium species and their toxins have been associated with the yellow rice diseases of Japan which are characterized by cardiac beri-beri, liver and kidney damage (Uraguchi and Yamazaki, 1978). The mycotoxins elaborated by these fungi include but not limited to ochratoxin A, citrinin, patulin, roquefortine C, verrucosidin, penicillic acid and cyclopiazonic acid (Scott, 1994). Patulin, roquefortine C and verrucosidin are potent neurotoxins while cyclopiazonic acid has been reported to be toxic to gastrointestinal tract, liver, heart, kidney and skeletal muscle, and a secondary immunosuppressant (Prelusky et al, 1994).

Several *Fusarium* species occurring worldwide on cereals are capable of accumulating, in infected kernels, several mycotoxins some of which have notable impact on human and animal health. The main groups of *Fusarium* toxins commonly recognized in grains are: trichothecenes, including T-2 toxin (T2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), fusarenone X (FUS), and nivalenol (NIV); zearalenones, essentially zearalenone (ZEN); and fumonisins, in particular fumonisin B_1 (FB_1) (Bottalico, 1998). In addition, moniliformin (MON), beauvericin (BEA), and fusaproliferin (FUP) were also found in *Fusarium* infected cereal ears (Bottalico, 1998). Trichothecenes are potent immunosuppressants and inhibitors of protein synthesis and the commonly encountered trichothecenes are deoxynivalenol, and to lesser extents, nivalenol, T-toxin, HT-2 toxin, and, rarely, diacetoxyscirpenol (Beardall and Miller, 1994). This group of toxins (especially T-2 toxin) cause general gastrointestinal disorder and haemorrhages of internal organs in poultry animals, swine, dogs, cows, goats and horses and man (Beardall and Miller, 1994). Zearalenone is the cause of the oestrogenic syndrome known as vulvovaginitis in swine, and is considered as the possible causative agents of the outbreaks of precocious pubertal changes in young children between six months and eight years) in Puerto Rico and has been suggested to have a possible involvement in human cervical cancer (JECFA, 2000). Fumonisins are the most recently discovered mycotoxins. They cause equine leukoencephalomalacia (ELEM), a neurologic syndrome characterized by extensive brain damage, and porcine pulmonary oedema which results in the rapid death of field pigs from massive pulmonary hydrothorax and liver damage (WHO 2000). Epidemiological data are highly suggestive that the most abundant and toxic fumonisin, FB_1 is the causative agent of oesaphageal cancer in the Transkei district of South Africa, Linxian district of China (Prelusky et al, 1994) and North –Eastern Italy (Doko and Visconti, 1994). Bottalico (1998) made a comprehensive review of the toxicity of moniliform (MON), beauverin (BEA) and fusaproliferin from which were drawn the effects of these three toxins on man and animals. MON is a cytotoxic compound that can cause reduced performance, hematologic disorders, myocardial hypertrophy, and mortality in rodents, chicks, ducklings, and pigs. It was suspected to cause the Keshan disease, a myocardic human impairment occurring in rural areas of China and South Africa (Transkei) where there is high maize consumption. BEA was found to be cytotoxic to mammalian cell tissues, and was reported to cause apoptosis on both murine and human cell lines. In addition, BEA showed toxic effects on the contractility of guinea pigs smooth muscle. FUP which has been found to be cytotoxic to human B lymphocytes and has teratogenic impact on chicken embryo causes high mortality in broiler chicks.

Rhizopus and *Mucor* produce rhizonin A, which elicits degenerative necrosis of hepatocytes and renal tubular epithelium in mice and rats (Wilson et al., 1984). *Helminthosporium* species elaborate sterigmatocystin, an intermediary metabolite of aflatoxin biosynthetic pathway and like AFB_1 is also a hepatoxic and nephrotoxic carcinogen but exhibits lower toxicity than the former (Scott, 1994).

Cytochalasins are also secreted by *Hel-minthosporium* species and these mycotoxins inhibit cytokinesis and protein synthesis and have been shown to cause pulmonary haemorrhage and brain oedema in mice (Visconti and Sibilia, 1994). No toxin is ascribed to *Syncephalastrum*, however it causes allergy to man (Mycotoxin Reference, 2005).

The demonstrated presence of mycotoxigenic fungi and AFB_1 in mouldy millet in this study has public health implications because low grade, cheap, mouldy grains are consumed by animals and humans in the country and other parts of Sub Saharan Africa region resulting to high risk of human and animals mycotoxicoses with adverse effects on crop and livestock production, and therefore national economy and trade. This makes regulation of mycotoxins in our foods and feedstuffs, an imperative.

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