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Full Length Research Paper

Health implications of toxigenic fungi found in two Nigerian staples: guinea corn and rice

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A total of one hundred and forty eight fungi isolated from both guinea corn (67) and rice (81) in a previous fungal and mycotoxin survey in Niger State, Nigeria, were tested for toxicity potential in white albino mice. Of all these, 64.2% were found to produce toxic metabolites that were lethal to mice and were mainly species of *Aspergillus spp*, *Fusarium spp*, *Penicillium spp* and *Trichoderma spp*. Others include *Syncephalastrum spp, Alternaria spp, Phoma spp, Curvularia lunata, Colletotrichum spp, Geotrichum candidum* and *Helminthosporium spp, Cladosporium werneckil,* and *Mucor spp* and the bacteria *Cryptococcus neoformis.* The novel, most toxigenic fungi found contaminating these two staples were *Fusarium verticillioides (*Sacc.) Nirenberg, previously known as *F.moniliforme* Sheldon (CABI Biosciences is IMI 392668).The extract of the fungus caused lethality to mice at 40 mg /kg b. wt. The health implications of these toxic microbes in our diets were discussed.

Key words: Guinea corn, rice, Nigeria, toxigenic fungi, mycotoxins.

INTRODUCTION

A survey for fungi, aflatoxin B_1 (AFB_1), ochratoxin A (OTA) and zearalenone (ZEA) contaminating mouldy field, marketed and stored guinea corn (*Sorghum*) [Makun, 2007] and rice (*Oryza sativa*) [Makun et al., 2007] during the dry harmattan (November – February), hot dry (March – May) and rainy (June – October) seasons in the four microclimatic zones of Niger State, Nigeria, was previously conducted. Of the three studied mycotoxins, AFB_1 was the commonest contaminant of the grains followed by ZEN and OTA in decreasing order. Eight hundred and eighty four (844) fungal isolates were cultured and identified from a total of a hundred and sixty eight mouldy guinea corn samples while one thousand and sixty two fungi (1062) were isolated and identified from one hundred and ninety six mouldy rice samples analysed. The fungi found in the studied staples were species of twenty three genera namely; *Aspergillus, Penicillium, Fusarium, Mucor, Rhizopus, Alternaria, Phoma, Trichoderma, Arthrium, Helminthosporium, Curvularia, Collectritotichum, Chaetomium, Chrysosporium, Cladosporium, and Geotrichum*. Others include *Syncephalastrum, Rhodoturula, Scopulariopus, Torula, Bipolaris, Gilocladium* and *Nocardia*.

The screening studies determined only aflatoxin B_1, ochratoxin A and zearalenone in the mouldy samples. It is possible that many more mycotoxins may contaminate grains in the state given that quite a number of moulds isolated from the grains are known to produce mycotoxins. Apart from those which toxigenic strains are commonly associated with production of AFB_1 (e.g A. flavus; A. parasiticus), OTA (e.g A.ochraceus) and ZEN (F. oxysporum), there were several which toxigenic strains are known to produce other mycotoxins e.g *Phoma sorghina*, *P. citrium*, as well as those which are not completely associated with mycotoxin production. In order to have a better insight into the potential health implications of *Sorghum* and rice infection by various fungi, it is necessary to determine the mycotoxigenic potentials of the fungal isolates which would be indicative

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of the likely mycotoxicoses arising from consumption of these staples in Niger State, a leading cereal producing state in Nigeria.

This study was therefore undertaken to screen some fungi found in guinea corn and rice in the State for their toxigenicity using mice with a view to selecting a novel toxigenic fungi and determining the possible mycotoxin contaminants of the staples. Multiples of isolates of each species found in the studied grains were selected for the toxicity screening.

MATERIALS AND METHODS

Culturing of fungi from guinea corn and rice

The culturing and toxicity screening of fungi isolated and acute toxicity testing of the thirteen most toxic extracts were carried out as described by Gbodi [1986]. To 20 g of Akkad rice imported from Thailand, 8 ml of distilled water was added and mixed thoroughly in a 50 ml conical flask and left overnight 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 mycelium of five day old pure cultures grown on Potatoes Dextrose Agar (PDA) slant tubes and incubated for 14 days at 28°C.

Extraction of toxins

The 20 g rice cultures were homogenized for 2 min in 100 ml dichloromethane using blender. The homogenate was then filtered through fast fluted filter paper. Two millimetres of corn oil was added to the filtrate in a round bottomed flask and concentrated in a rotatory evaporator at water bath temperature of 55°C. When the dichloromethane was distilled off, clean empty distillate flask collector was used to replace the dichloromethane collector and rotatory evaporator turned on for another ten minutes to ensure complete removal of dichloromethane vapour. The corn oil-toxin extract was transferred into a vial and kept in deep freezer at -25°C until used for toxicity testing in mice.

Screening the sorghum and rice fungal culture extracts for toxicity in mice

Five to six week old male white albino mice weighing 20-30 g were used for the toxigenicity screening of the isolates. The mice used for this experiment were raised from the parent animals purchased from National Veterinary Research Institute, Vom, Plateau State, Nigeria. The animals were housed in conventional plastic wire cages with sawdust bedding. They were given chick mash pellets and tap water *ad-libitum*. No extra lighting was provided in the night. Three mice were kept in a cage. For the toxicity screening test and pilot acute toxicity assay, there were three mice per group. The cages were washed and the bedding changed every other day. The mice were kept for seven days to acclimatize prior to the toxicity assay.

Three mice each received by intraperitoneal (IP) injection 0.2 ml of the corn oil extract of culture of fungal extract from the grains. The mice were observed for 14 days for any signs of toxicity. Five mice which received corn oil were used as controls. The toxicity of the extracts was arbitrarily categorized into four classes viz:-
Very toxic (if all three of the extract treated mice died)
Moderately toxic (If two of three of the mice died)
Mildly toxic (If one of the three mice died) and
Non toxic (If none of the mice died.

Mass culturing of the selected very toxic fungi

From the above toxicity screening, the thirteen fungi (*Aspergillus. flavus, A.niger, A.parasiticus, Curvularia. lunata, Fusarium spp, Fusarium nwale, Fusarium verticillioides, Helminthosporium* spp*, Penicillium spp, Penicillium rubrum, P.verrucosum* and *Trichoderma spp)* that were found to produce the most toxic fungal metabolites were selected and further investigated with a view to finding a new, very toxic fungus contaminating the two staples. Fresh PDA tubes were inoculated with each selected fungus from stock cultures maintained on PDA tube and incubated for five days at 28°C
For mass culturing of the thirteen most toxigenic fungi, 5 ml of Triton X-100 (Searle Hopkin and Williams, Chalwell Health, Essex, England-) treated sterile distilled water (one drop Triton X-100 to 200ml sterile distilled water) was added to each culture tube and the surface of the culture scratched with sterile inoculating wire to suspend the spores. The suspensions were then used to inoculate a 2.5 L Fernbach flask containing 250 g of Akkad rice, the moisture content of which had been equilibrated as earlier described. The flasks were autoclaved for 25 min and pressure of 15 psi. The cooled flasks were inoculated with the suspended spore and maintained in an incubator at 28°C as a static culture for 21 days.

Extraction of toxins from culture material of the selected very toxic fungi

For extraction of toxins from the thirteen selected isolates, 750 ml of dichloromethane were added to the Fernbach flask with the mouldy rice chopped into small fragments and blended for three minutes. The homogenate was filtered through a bed of Hyflosuper-cell in Buchner funnel fitted with fast flow filter paper. The filtrate was again filtered through anhydrous sodium sulphate to remove the moisture and clarify the extract. The clarified clear solvent was removed at 50°C by rotatory vacuum evaporator.

The oily viscous residue was added to chilled swirling petroleum ether (1:15 residue/ petroleum ether v/v) and the mixture kept overnight in a deep freezer at -15°C to complete the precipitation of the toxins. The crude toxin was recovered by filtration and dried in a fan oven at 50°C for three hours before being used for pilot toxicity studies.

Acute toxicity testing with thirteen very toxic extracts

A pilot test was carried out using the extracts of thirteen most toxigenic fungi selected from the screening test. To determine the dose of different extracts that would cause 0 to 100% death in mice, five groups of three mice each were used for each extract. The extract was dissolved in dimethyl sulphoxide (DMSO). A log interval of 0.60 was used to select a range of i.p doses, 40, 160, 640 and 2560 mg/kg body weight. A group of three mice which served as control received the highest dose of the DMSO administered to extract treated mice. The animals were observed for 14 days for signs of toxicity. From this pilot acute toxicity test, *Fusarium vertillioides* was shown to be the very toxic and relatively novel fungus of the fungal contaminants of the two staples.

Confirmation of identification of selected fungus

The selected fungus was sent to CABI Biosciences Laboratory, Surrey, U.K (former Commonwealth Mycological Institute, Kew, Surrey, U.K) for confirmation of identity. It was confirmed to be *F. verticillioides (*Sacc.) Nirenberg, previously known as *F. moniliforme* Sheldon. The identification number of the isolate in CABI Biosci-

Table 1. Toxigenicity potential of fungi isolated from *sorghum* in Niger State using mouse as test animal.

Very toxic	(12)	Moderately toxic	(17)	Mildly toxic	(14)	Non-toxic	(24)
Helmintthosporium spp	(1/12)	*Aspergillus flavus*	(3/17)	*A.fumigatus*	(1/14)	*A.parasiticus*	(2/24)
Fusarium spp	(2/12)	*A.niger*	(1/17)	*A.nidulans*	(1/14)	*A.versicolor*	(1/24)
A. niger	(3/12)	*A.glaucus*	(1/17)	*A.niger*	(1/14)	*Arthrium spp*	(1/24)
Penicillium rubrum	(1/14)	*A.fumigatus*	(1/17)	*A.glaucus*	(1/14)	*A. alternate(*	1/24)
A.flavus	(2/12)	*A.versicolor*	(1/17)	*A.parasiticus*	(1/14)	*F. equiseti*	(3/24)
A. parasiticus	(1/12)	*Colletrotrichum*	(2/17)	*A.ochraceus*	(1/14)	*F.oxysporum*	(1/24)
P. verrucosum	(1/12)	*P.citrinum*	(1/17)	*Alternaria spp*	(1/14)	*F.semitectum*	(1/24)
F.nwale	(1/12)	*Syncephalastrum spp*	(1/17)	*Phoma spp*	(1/14)	*Cladosporium spp*	(1/24)
		Trichoderma spp	(2/17)	*Phoma sorghina*	(1/14)	*C. werneckil*	(1/24)
		Mucor spp	(1/17)	*Trichoderma spp*	(1/14)	*Chaetomium spp*	(2/24)
		Curvularia lunata	(2/17)	*F.solani*	(1/14)	*Mucor spp*	(2/24)
				P.notatum	(1/14)	*Penicillium spp*	(2/24)
				Syncephalastrum spp	(1/14)	*Rhizopus spp*	(2/24)
				Penicillium spp	(1/14)	*Rhodotorula rubra*	(1/24)
						Scopulariopsis	(1/24)
						Torula spp	(1/24)
						Trichoderma spp	(2/24)

Values in parenthesis indicate the number of the isolates of the species in the group.
Summary/key: Very toxic, all three mice died (3/3)= 12; Moderately toxic, two of three mice died (2/3) = 17 ; Mildly toxic, one of the three mice died (1/3)= 14; Non-toxic = no mice died (0/3) = 24.

Biosciences is IMI 392668 (Appendix 2).

RESULTS

Toxigenicity of fungi isolated from sorghum and rice

One hundred and forty eight fungal isolates from both guinea corn (67) and rice (81) were tested for toxicity (Tables 1 and 2). Of all these, 95 were found to produce toxic metabolites and were *Aspergillus spp* (41), *Fusarium spp* (14), *Penicillium spp* (10), *Trichoderma spp* (8), *Syncephalastrum spp* (4), three each of *Alternaria spp, Phoma spp* and *Curvularia lunata.* Others include two each of *Colletotrichum spp, Geotrichum candidum* and *Helminthosporium spp,* and one each of *Cladosporium werneckil, Cryptococcus neoformis* and *Mucor spp.* A few of the fungi which have not been known to produce mycotoxins were found to be toxigenic. For example *Syncephalastrum spp* isolates from both guinea corn and rice were found to be moderately and mildly toxic. Similarly, *G. candidum* and *C. neoformans* isolated from rice were demonstrated to be mildly toxic.

Different strains of the same fungal species infect *Sorghum* in Niger State. For example, among the fungi from *Sorghum* that were tested for toxicity there were at least two strains of *A. flavus* (Table 1). One was very toxic while the other is moderately toxic respectively. Similarly, three strains of *A. niger* isolated from mouldy *Sorghum* were very toxic, moderately toxic and mildly toxic, respectively. Other fungi from *Sorghum* that had multiple strains isolated include *A. parasiticus* (3), *A. glaucus* (2), *A. fumigatus* (2), *A.versicolor* (2), *Syncephalastrum* spp (2), *Trichoderma* spp (3), *Mucor* spp (2) and *Penicillium* spp (2). Thirteen fungal species isolated from rice had more than one strain contaminating the grain in Niger State (Table 2).The fungi and the numbers of strains found were *A. flavus* (3), *A. niger* (2), *A. glaucus* (2), *A.parasiticus* (3), *A.terreus* (2), *A.versicolor* (2), *Fusarium spp* (3), *F.solani* (2), *F.verticillioides* (2), *Penicillium* spp (2), *Syncephalastrum* spp (2) and *Trichoderma* spp (2).

Many fungal species which are known to produce mycotoxins were found to be non-toxigenic in this work (Tables 1 and 2). These include species known to produce aflatoxin (*A.parasiticus*), cyclopiazonic acid and sterigmatocystin (*A.versicolor* and *Bipolaris*), patulin (*A.clavatus, P.expansum*), 3-nitropropionic acid (*Arthrinium* spp), cytochalasins (*Alternaria alternate*), trichothecenes and zearalenone (*F.equseti, F.oxysporum, F semitectum*), fumonisins and monili-formin (*F.verticillioides*), gliotoxin (*Gliocladium* spp), emodin (*Cladosporium*), chaetomin (*Chaetomium* spp), ochratoxins, penicillic acid and rubratoxin (*Penicillium* spp), satratoxins, gliotoxin and T-2 toxin (*Trichoderma* spp).

The mycotoxins associated with the demonstrated toxigenic fungi isolated from *Sorghum* and rice they include aflatoxins, Sterigmatocystin, Fumitremorgens A and B, ochratoxin A, citrinin, patulin, cytochalasins, tenuazonic acid, gliotoxin, emodin, curvularin, trichothecenes (deoxynivalenol, niva-lenol, T-2 toxin, diacetoxiyscripenol (DAS) and related

Table 2. Toxigenicity potential of fungi isolated form mouldy rice in Niger State, using the mouse as test animal.

Very toxic (14)	Moderately toxic (19)	Mildly toxic (19)	Non-toxic (29)
Curvularia lunata (1/14)	*A. flavus* (1/19)	*A. flavus* (2/19)	*A. clavatus* (1/29)
Helmintthosporium spp (1/14)	*A.niger* (2/19)	*A.fumigatus* (2/19)	*A.glaucus* (1/29)
Trichoderma spp (1/14)	*A.glaucus* (1/19)	*A.nidulans* (1/19)	*A.parasiticus* (3/29)
Fusarium spp (2/14)	*A.terreus* (1/19)	*A.terreus* (1/19)	*A.versicolor* (1/29)
A. niger (3/14)	*A.versicolor* (2/19)	*Alternaria spp* (1/19)	*Arthrium spp* (1/29)
Penicillium rubrum (2/14)	*A.ochraceus* (2/19)	*C. werneckil* (1/19)	*Bipolaris spp* (1/29)
A.flavus (2/14)	*F.solani* (1/19)	*C. neoformans* (1/19)	*Fusarium spp* (2/29)
A. parasiticus (1/14)	*Penicillium spp* (1/19)	*G. candidum* (2/19)	*F.oxysporum* (1/29)
F.verticillioides (1/14)	*P.citrinum* (1/19)	*Phoma sorghina* (1/19)	*Gilocladium spp* (1/29)
	Syncephalastrum spp (2/19)	*A. alternate* (1/19)	*Cladosporium spp* (1/29)
	Trichoderma spp (2/19)	*Trichoderma spp* (2/19)	*A.parasiticus* (1/29)
	Fusarium spp (3/19)	*F.solani* (1/19)	*Mucor spp* (3/29)
		F.semitectum (2/19)	*Nocardia brasiliensis* (1/29)
		P.cyclopium (1/19)	*P.expansum* (1/29)
			P.viridicatum (1/29)
			Penicillium spp (1/29)
			Rhizopus spp (2/29)
			Syncephalastrum spp (1/29)
			Rhodotorula rubra (1/29)
			F.verticillioides (4/29)

Values in parenthesis indicate the number of the isolates of the species in the group.
Summary/key: Very toxic: all three mice died (3/3)= 14; Moderately toxic = two of three mice died (2/3) = 19; Mildly toxic = one of the three mice died (1/3) = 19; Non-toxic = no mice died (0/3) = 29.

Table 3. Results of the preliminary acute toxicity assay of the crude toxin extracts of the thirteen most toxigenic fungal isolates.

Mortality rate (number dead per 3 mice)				
Source of crude toxin extract	**Dose 1**	**Dose 2**	**Dose 3**	**Dose 4**
Aspergillus niger	1/3	2/3	2/3	3/3
A.niger	0/3	0/3	1/3	3/3
A.parasiticus	0/3	1/3	1/3	2/3
Fusarium oxysporum	0/3	0/3	1/3	3/3
Curvularia lunata	0/3	0/3	2/3	3/3
Helminthosporium spp	0/3	0/3	3/3	3/3
Trichoderma spp	1/3	0/3	0/3	3/3
Fusarium spp	0/3	1/3	2/3	3/3
Penicillium rubrum	0/3	0/3	1/3	3/3
Fusarium verticillioides	1/3	2/3	2/3	3/3
Penicillium verrucosum	1/3	1/3	2/3	3/3
Penicillium spp	0/3	0/3	2/3	3/3
Helminthosporium spp	0/3	0/3	0/3	1/3

Dose 1, 40 mg toxin extract/kg b.wt; dose 2, 160 mg/kg/b.wt; dose 3, 640 mg/kg b.wt; dose 4, 2560 mg/kg/b.wt. Each dose is given i.p in DMSO.

ted compounds), fusarenon-X, zearalenone, fumonisins, moniliformin, fusarin C, rhizonin A, cyclopiazonic acid,- penicillic acid, rubratoxin, penitrem A and B, satratoxin H, trichodermol and related trichodermal trichothecenes.

Preliminary acute toxicity testing

Twenty six fungal isolates from both guinea corn and rice (Tables 1 and 2) were found to be very toxic to mice but

there were different strains of same fungi species and so the isolate of a particular species that killed the mice fastest was selected for preliminary acute toxicity test as the representative fungal isolate for that species. Thirteen fungal isolates emerged and were used for the pilot studies.

The results of the preliminary acute toxicity assay of the crude extracts of the 13 most toxigenic fungi are summarized on Table 3. At 40 mg/kg body weight four isolates (*A.niger, Trichoderma spp, Fusarium. verticillioides* and *Penicillium verrucosum*) killed a mouse each out of the three used for the test. Four fungal isolates (*A.niger, A.parasiticus, F.verticillioides* and *Penicillium verrucosum*) caused death at 160 mg/kg body weight. All the thirteen fungal isolates except *Helminthosporium* and *Trichoderma spp* were lethal to mice at 640 mg/kg body weight. With the exception of *A. parasiticus* and *Helminthosporium spp*, all other extracts caused 100% mortality at 2560 mg/kg body weight.

From this result, *A. niger* and *F. verticillioides* caused the highest lethality in mice even at low concentration and therefore were the two most toxic fungi found in guinea corn and rice. *F. verticillioides* was selected as the novel most toxic fungi contaminating guinea corn and rice in Niger State because less information about its toxicity is available in literature as compared to *A. niger.*

DISCUSSION

The results of the toxicity screening tests showed that many of the fungal isolates contaminating guinea corn and rice in Niger State produced toxic metabolites that were lethal to mice and this is toxicologically significant. From the data, the profiles of more than thirty possible additional mycotoxins that may contaminate the studied grains in the State have been deduced, and their toxic effects in animals and man are well documented [Gbodi and Nwude, 1988; Prelusky and Rotter, 1994; Beardall and Miller, 1994; Peraica et al., 1999; Bankole and Adebanjo, 2004; Mycotoxin, 2005]

Of the toxic fungal isolates, *Aspergillus spp, Penicillium, Fusarium* and *Trichoderma* were the most prevalent and so the mycotoxins they are likely to produce would be of major health concern. Aflatoxin B_1 was the commonest toxin found during the mycotoxin screening studies [Makun, 2007] and this correlates with the toxicity screening which shows that *Aspergillus* spp, AFB_1 producers, are the most predominant toxigenic fungi found in the State. A host of mycotoxins [Uraguchi and Yamazaki, 1978; Scott, 1994; Mold-Help, 2004], some of which are of public health significance are elaborated by species of *Aspergillus* [Gbodi and Nwude, 1988; Prelusky and Rotter, 1994; Beardall and Miller, 1994; Peraica et al., 1999; Bankole and Adebanjo, 2004; Mycotoxin, 2005] but of major concern are the aflatoxins and sterigmatocystin which are naturally occurring hepatocarcinogens and have been linked to high incidence of liver cancer in some parts of the world where foods are frequently contaminated with aflatoxins [Bankole and Adebanjo, 2004; ProMED, 2004]. They are known to exacerbate HIV/AIDS [Lane, 2005], impair growth of children [Pier AC and McLoughlin 1985; Gong et al., 2002; Gong et al., 2004] and serve as anti-nutritional factors [IARC, 1976; Hendrickse, 1991; Carlos et al., 2004].

Several of the mycotoxins ascribed to *Aspergillus* species are also *Penicillium* mycotoxins. However, the major *Penicillium* toxins are ochratoxin A, citrinin, patulin, penicillic acid, roquefortine, cyclopianonic acid, verrucosidin, rubratoxin, cyclochlorotine and luteoskyrin [Scott, 1994].The toxicological significances of these mycotoxins to human health, livestock production and trade have been reviewed by many scientists [Gbodi and Nwude, 1988; Prelusky and Rotter, 1994; Beardall and Miller, 1994; Peraica et al., 1999; Bankole and Adebanjo, 2004; Mycotoxin, 2005]. Apart from aflatoxins, the three main *Aspergillus* and *Penicillium* mycotoxins that pose the greatest public health are ochratoxin A, patulin and citrinin. Ochratoxin A causes kidney and liver impairment in animals and man especially pigs [Wafa et al., 1998]. This mycotoxin has been proposed as the causative agent of endemic nephropathy that occurs among rural populations in Croatia, Bosnia and Herzegovina, Yugoslavia, Bulgaria, and Romania, where it has been estimated that about 20,000 people are either suffering from or are suspected to have the disease [Peraica et al., 1999]. The toxin is also associated with urothelial tumours of pelvis and ureter in Egypt, Croatia, Bulgaria and Yugoslavia and chronic interstitial nephropathy in Tunisia [Peraica et al., 1999; JECFA, 200025]. Patulin and citrinin are neurotoxic and nephrotoxic respectively [Peraica et al., 1999].

A good number of known zearalenone producing *Fusarium* species were shown in this work to be toxic to mice. Zearalenone, an oestrogenic toxin causes infertility in animals and is associated with outbreaks of precocious pubertal changes in children in Puerto Rico and has been suggested to have a possible involvement in human cervical cancer [Miller and Trenholm, 1994]. The other mycotoxins elaborated by *Fusarium spp* are; trichothecenes, culmorins, enniatins, fusarins, fumonisins moniliformin, butenolide and chlamydosporol [Marasas, 2001]. Trichothecenes are protein inhibitors with consequent immunosuppressive effects causing severe damage to digestive tract and death due to intestinal haemorrhage [Beardall and Miller, 1994]. The commonest trichothecenes are deoxynivalenol (DON) and T-2 toxin. DON was the causatic agent of a large-scale incident of human toxicosis in the Kashmir Valley, India in 1988, and acute toxicosis of DON has been reported in China, Japan, and Korea among other countries [Beardall and Miller, 1994]. Fumonisins especially FB_1 cause liver and kidney cancer, and neural

tube defects in rodents, leukoencephalomalacia in equine and pulmonary oedema in pigs [Wilson et al., 1984]. The association of FB_1 with elevated incidence of human oesphageal cancer in parts of South Africa, North Eastern Iran and China, upper gastrointestinal tract cancer in Northern Italy and neural tube defects in human babes [Wilson et al., 1984] is a major public health concern. The International Agency for Research on Cancer classifies fumonisins as possible human carcinogens (category II-B).

Trichoderma spps have been found as fungal contaminants of *Sorghum*, maize, acha and rice in Nigeria and Japan [Gbodi, 1986; Uraguchi and Yamazaki, 1978]. The mycotoxins produced by *Trichoderma spp* are numerous and they include alamethicins, chrysophanol, emodin, ergokonin, gliotoxin, gliovirin, G-protein, harzianum A, heptelidic acid, isocyanocyclopentenes, koninginins A,B,C,G, aracelsin, saturnisporin, suzukacillin, trichodermin, trichorzianines A and B, Trichothecenes, Trichotoxin, Trichoviridin and Viridin [Mycotoxin, 2005; Uraguchi and Yamazaki, 1978]. However, satratoxin H, trichodermol, trichodermin and T-2 are the most elaborated and toxic. Satratoxin H is an immunosuppressant that causes abortogenicity in animals while the other three are inhibitors of protein synthesis and cause damage to the gastrointestinal tract and haemoglobin of animals and man [Prelusky and Rotter, 1994; Miller and Trenholm, 1994].

The high incidence of *Mucor* and *Alternaria* spp in both mouldy guinea corn and rice, and their proven toxicity in mice suggest the likely presence of metabolites of these fungi in the grains. Rhizonin A secreted by *Mucor* spp has deleterious effects on the kidney and liver of mice and rats [Visconti and Sibilia, 1994]. Moulds of the genus, *Alternaria* elaborate many toxins but mainly cytochalasins and tenuazonic acid [Mycotoxins, 1997] which have been implicated in human haemorrhage disease, 'Onyalia' in South Africa [Beardall and Miller, 1994].

Literature search reveals that no mycotoxin has been associated with *Colletritrochum spp, Geotrichum candidum, C. neoformins* and *Syncephalastum spp,* however, *Syncephalastrum* spp are known to cause allergy to man [Mycotoxin, 2005] while *G. candidum* can cause geotrichosis, a secondary infection in association to tuberculosis which is a rare disease that causes lesions of the skin, bronchi, mouth, lung and intestine [Beardall and Miller, 1994]. Fifty three fungal isolates mainly species of *Aspergillus, Fusarium, Penicillium, Alternaria* and *Trichoderma* which are known mycotoxin producing fungi were found to be non toxic in this study. These could be the non toxigenic strains of the species that do not have the genetic capacity to produce mycotoxins [WHO, 1999]. Since sub-culturing can cause mutation which could lead to loss of ability to produce toxin [Gbodi, 1986], it is possible that some of the potential toxic fungi lost their potency as a result of sub-culturing. They might also be the temperate strains of the species, of which the tropical climate of Niger State could have been unsuitable for optimal mycotoxin synthesis.

Data from the preliminary acute toxicity studies shows that *F. verticllioides* (Sacc.) Nirenberg produced one of the most toxic metabolites and that of the thirteen most toxic fungi identified in guinea corn and rice in the State, available information on its toxicity in literature is the least. The fungus is a known producer of mainly fumonisins however there are reports that it also elaborates trichothecenes [Mold-Help, 2004]. The toxic effects of these mycotoxins have been discussed in previous paragraphs. Fumonisins and any of the trichothecenes acting singly do not cause lethality in mice at low concentration of 40 mg per kilogram body weight [Visconti and Sibilia, 1994; WHO, 2000; Seleye-Fubara and Jebbin, 2007] as demonstrated in this work. It implies that the culture material of this strain of *F. verticillioides* is more toxic than pure fumonisin B_1, DON or T-2 toxin and so might contain a new toxin or multiple of the above mentioned toxins in synergistic effect. Further work on its toxic effects on experimental animals and the nature and number of toxins elaborated by this very toxic strain of fungus is therefore necessary.

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