Acute toxicity and total fumonisin content of the culture material of *Fusarium verticillioides* (SACC.) nirenberg (CABI-IMI 392668) isolated from rice in Nigeria.

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ABSTRACT

In a previous survey for mycotoxigenic fungi contaminating mouldy guinea corn and rice in Niger State, Nigeria, *Fusarium verticillioides* (Sacc.) Nirenberg was found to be one of the most toxic metabolite producing fungi isolated from the studied staples. The culture material of the fungus was therefore subjected to acute toxicity studies in chicks and mice in this work. The total fumonisin concentration of the culture material was also determined. When administered orally and intraperitoneally, the extract was acutely toxic to mice and chicks and the organs affected were liver, kidney and gastrointestinal tract. Oral administration of the fungal extract to mice and chicks caused mortality at 833.33mg/kg and 2500mg/kg body weight respectively. The intraperitoneal LD₅₀ values of the extract in both animals were between 45.40–87.90 mg/kg body weight with the mice being more susceptible. The total fumonisin content of the fungal residue as analyzed using veratox competitive direct enzyme linked immunosorbent assay (CD-ELISA) was 8.233ppm.

KEYWORDS: Fungi, mycotoxin, Fusarium verticillioides, toxicity, fumonisins,

INTRODUCTION

In the year 2000, a survey of the mycoflora, aflatoxin B1 (AFB1), ochratoxin A (OTA) and zearalenone (ZEN) contaminating mouldy field, marketed and stored guinea corn (Sorghum) and rice (Oryza sativa) samples collected during the dry, harmattan, dry-hot and rainy seasons from the twenty five local governments areas of Niger State, Nigeria, was conducted (Makun et al. 2007; Makun et al. 2009). A total of eight hundred and eighty four (884) fungi were isolated and identified from one hundred and sixty eight (168) 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. The fungal genera contaminating the two staples were species of Aspergillus, Penicillium, Fusarium, Mucor, Rhizopus, Alternaria, Phoma, Trichoderma, Arthrium, Helminthosporium, Curvularia, Collectritotichum, Chaetomium, Chrysosporium, Cladosporium, and Geotrichum. Others include Syncephalastrum, Rhodoturula, Scopulariopus, Torula, and Bipolaris,. AFB₁, ZEN and OTA in decreasing order were common contaminants of the grains.

Some one hundred and forty eight of these fungi isolated from both guinea corn (67) and rice (81) were screened for toxicity potential in white albino mice and ninety six of them 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*. (Makun *et al.* 2009^b). Data from this preliminary toxicity screening studies shows that *Fusarium verticllioides* (Sacc.) Nirenberg previously known as *Fusarium moniliforme* Sheldon caused lethality to mice at 40mg /kg body weight. It therefore produced one of the most toxic metabolites and of the thirteen most toxigenic fungi identified in guinea corn and rice in the State, available information on its toxicity in literature is the least in Nigeria. As the most toxigenic fungi found contaminating the two staples, it was necessary to determine the toxic effects to experimental animals and quantify the mycotoxins elaborated by this very toxic strain of the selected fungus of interest. This work was therefore carried out to determine the oral and intraperitoneal LD_{50} of the crude extract of the fungus in chicks and mice, the organs injured by the toxic extract and to identify and quantify the total fumonisins produced by the toxic fungus.

MATERIALS AND METHODS

Confirmation of identification of selected fungus. The selected fungus coded in our laboratory as HAM/Chibani/NG/01 and identified as a *Fusarium* species, was sent to International Mycological Institute (IMI) Laboratory of Centre for Agricultural Biosciences International (CABI) Laboratory, U.K for confirmation of identification. It was confirmed to be *Fusarium verticillioides* (Sacc.) Nirenberg, previously known as *F.moniliforme* Sheldon. The identification number of the isolate in CABI is IMI 392668.

Experimental Animals and Ethical Issues: Cockerels and broilers chicks and white albino mice were used for the acute toxicity studies. Poultry animals especially chicken, are the second most important source of protein after cows in Nigeria, and more convenient and cheaper for experimentation than cows hence the choice of chicks as experimental animal for this work. Mice were chosen as the second set of experimental animals because of their close similarity to human being with regards to metabolism. Good husbandry practices of avoidance of overcrowding, sanitizing the cages and sipper tube and water containers everyday were adhered to. Tap water, safe for human consumption was the drinking water of the animals. Commercially formulated animal feeds were stored in dry cupboards and monitored for microbial and chemical contamination to avoid diseases. Restraint and handling were in compliance with the guide to the care and use of experimental animals of Canadian Council on Animal Care (1997). The animal house is situated more than fifty metres from students' hostels, lecture halls and laboratories so noise was kept to a minimum around the animal room. Monitoring and assessment of animals, oral and health intraperitoneal dosing using feeding and injectable needles respectively, euthanasia of both chicks and mice by cervical dislocation, and post-mortem examinations on dead and sacrificed animals were performed by licensed, resident, expert animal veterinarians of the Niger State Government Clinic, Bosso, Nigeria. Ethical clearance (MLFD/VET-12/10/VOL_1) for the research was granted by the Ministry of Livestock and Fisheries Development, Niger State, Nigeria

Mice White male and female albino mice of 5-6 weeks old and weighing between 20-30g were used for this study. The animals were raised in our laboratory from parent animals that were purchased from National Veterinary Institute, Vom, Plateau State, Nigeria. Male and female animals were housed separately in conventional plastic-wire cages with sawdust bedding. They were kept in well ventilated, air conditioned room maintained at 25^oC and fed chick mash pellets and tap water *ad-libitum*. During the course of the experiment artificial lighting was provided throughout the day and night time. The cages were washed and the bedding changed every other day. They were kept for seven days before commencement of the experiment to stabilize.

Chicks Three day old cockerels and broilers were purchased from the Poultry Division of the Evangelical Church of West Africa (ECWA), Minna. They were housed in groups of ten in brooding chick cages equipped with electric bulb to provide warmth. The brooding temperature was 35^oC. Sawdust (wood shavings) was used for bedding. The animals were fed chick mash purchased from same ECWA poultry shop and were given tap water *ad-libitum*. The cages were cleaned and the bedding changed daily. The chicks were not used for toxicity studies until they were a week old.

Preparation of Crude Toxin

(a) Culturing of Fungus on Maize: Maize is a preferred natural substrate for growth and mycotoxin production by Fusarium verticillioides (Marasas, 2001), as compared to rice on which the fungus was isolated from, and so in order to generate more crude toxin, maize was substituted for rice. The culturing of the fungus and extraction of toxins from the maize culture material were carried out as described by Gbodi, (1986). To 500g of maize, 100ml of distilled water was added and mixed thoroughly in 2.5 litre Fernbach flask. This was left overnight for moisture equilibration. The maize substrate was then autoclaved for 20 minutes at 120°C and 15 psi pressure. After cooling it was aseptically inoculated with conidia or mycelium of five day old pure culture of Fusarium verticillioides grown

on potatoes dextrose agar (PDA) slant tubes. 5ml 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 litre Fernbach flask containing the maize substrate. The flasks were maintained in an incubator at 28°C as a static culture for 21 days.

(b) Extraction of Toxins: Fusarium mycotoxins were extracted in dichloromethane according to the method of Hinojo et al. (2005). Dichloromethane was the extracting solvent and 750ml of it was added to the Fernbach flask with the mouldy maize 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 in 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. The extract was preserved in the fridge until used for toxicity studies and CD-ELISA analysis.

Assay of Oral Toxicity of Residue in One-Week old Chicks: The toxicity studies in the chicks and mice which involved administration of toxins to animals and monitoring of the experimental animals were conducted by the method of Wallace, (2001). To determine whether the extract of the culture material was toxic to chicks at all, 2500mg/kg. 5000mg/kg, 7500mg/kg and 10,000mg/kg body weight were administered orally using a polyethylene cannula attached to 26 x 3/8 hypodermic needle, to four groups of five one week old cockerels per group; the fifth group received corn oil only. Corn oil was used as the vehicle. The chicks were fasted for six hours before administration of toxin. The extract was administered at a constant dose volume of 0.2ml (i.e., varying the dose concentration) throughout the toxicity aspect of the work. The animals were observed for 14 days and post-mortem examinations were carried out on dead chicks.

Assay of Intraperitoneal Toxicity of Extract in One Week Old Chicks: From the results of a pilot test where the doses that cause 0% and 100% mortality were determined, a range of doses at log interval of 0.08: 53.32, 64.00, 76.80, 92.16 and 110.60 mg/kg body weight were given to five groups of ten chicks (5 cockerels and 5 broilers) per group respectively of extract dissolved in dimethylsulphoxide. A control group received the highest dose of the solvent. The chicks dying in any group were subjected to postmortem examination including histopathological studies.

Assay of Acute Intraperitoneal Toxicity of Extract in Mice: Intraperitoneal (i.p) doses eliciting 0% and 100% mortality in the mice were determined and from the result of this pilot test, a range of doses were selected between 20mg/kg and 60mg/kg at log interval of 0.08. Thus the i.p doses were 32.00, 38.40, 46.09, 55.30 and 66.36mg/kg and were given to six groups of ten mice per group (5 males and 5 females). The control group of 10 mice received the highest dose of the solvent, dimethylsulphoxide. Mice dying in any group were subjected to post-mortem examination, while the histopathological studies were conducted later.

Assay of Acute Oral Toxicity of Extract in Mice: A range of doses was selected between 170mg/kg and 1400mg/kg (caused 0% and 100% mortality respectively) at a log interval of 0.60. Five doses of the PER at 177.3, 357.3, 427.3, 831.39 and 1438 mg/kg were administered to five groups of ten mice per group (5 males and 5 females) using a polyethylene cannula attached to 26 x 3/8 hypodermic needle. The sixth control group received the highest dose of the solvent, dimethylsulphoxide. The animals were fasted overnight before treatment. Post-mortem examinations were carried out on mice that died and samples of organs preserved for histopathological studies.

Estimation of Lethal Dose 50 of the PER in Chicks and Mice: The doses and percentage mortality values obtained from the oral and intraperitoneal toxicity tests above were subjected to four different methods of determination of LD₅₀ namely Thompson moving average method, graphical method of Muller and Tainter, Arithmetic method of Karber and Arithmetic method of Reed and Muench (Wallace, 1982; Wallace 2001).

Preparation of Tissues for Histopathological Studies

- (a) Tissue Processing and Sectioning Tissue processing, sectioning and staining were done according to standard laboratory method. Fresh samples of liver, lung, kidney, pancreas, brain, ovary, testis, stomach, duodenum and intestine of dead mice were fixed in 10% formal-saline. 3-4mm thick tissue was cut from each organ for processing. The cut tissues were transferred to the automatic tissue processor where the tissues were further fixed in 10% buffered formol-saline for two hours and dehydrated for two hours in each ascending grades of alcohol (70%, 90%, 100% v/v). The dehydrated tissues were then cleared in xylene for two hours and the tissues impregnated in molten paraffin wax for another two hours and left to cool. The sections were then trimmed and sectioned on the microtome at 5microns. The sections were floated out in a warm water bath, then attached to slides, and dried on a hot plate and stained.
- (b) Staining of the Sectioned Tissues The sections were dewaxed in xylene, and dehydrated in descending grades of alcohol – 100%, 90% and 70%. They were stained with haematoxylin for ten minutes, differentiated in 1% acid alcohol, blued in Scotts tap water and stained for three minutes. They were then rinsed, dehydrated in ascending grades of alcohol – 70%, 90% and 100% and finally cleared in xylene and mounted in PDX. The prepared slides were kept for histopathological examination.

Identification and Quantitation of Total Fumonisins: The crude dichloromethane extract obtained from the maize culture material as described above (extraction of toxin, b) was cleaned up by column chromatographic technique Gbodi, 1986) and the fumonisins eluted into aqueous methanol (methanol: water 3:1) as recommended in the WHO monograph (WHO, 2000). Briefly the chromatographic clean up involves column thoroughly mixing 1g portion of the crude extract with 10g of silica gel (Type 60 - 200 mesh). 5ml of acetone was added to the mixture and the acetone evaporated at 40°C in an oven. This dried toxin/silica gel mixture was applied to a silica gel column packed in hexane and fumonisins eluded in 500ml aqueous methanol (methanol: water: 3: 1v/v). The aqueous methanol fraction was evaporated at 60°C on rotary evaporator.

The dried elute was subjected to qualitative and quantitative analysis for total fumonisins ($FB_1 + FB_2 + FB_3$) using the veratox competitive direct enzyme

linked immunosorbent assay (CD-ELISA) quantitative test kit for fumonisin Lot.19104 (donated by Mycotoxin Laboratory, NAFDAC Central Laboratory, Oshodi, Lagos). The analysis was done in triplicates. The assay principle is based on free fumonisin in the sample and controls competing with enzyme-labelled fumonisin (conjugate) for the antibody binding sites. After a wash step, substrate is added, this reacts with bound conjugates to produce blue colour. The intensity of the blue colour gives a measure of the fumonisin content. The test is read in micro well reader to yield optical densities. The kit has a detection limit of 0.2ppm.

RESULTS

Toxicity Screening of PER given Orally to Chicks Table 1 shows the results of the effects of oral administration of extract to four groups of five chicks at high doses. This route of administration more than other routes confirms the toxicity of the toxin to animals because it mimics the real farm situation where animals ingest mycotoxins from contaminated foodstuffs.

Table 1. Toxicity of orally administered PER in Chicks

Groups	Oral dose	mg/kg). Number of dead/treated chicks
Control	corn oil	0/5
1	2500	1/5
2	5000	3/5
3	7500	3/5
4	10,000	5/5

The first death was recorded eight hours after treatment in the fourth group that received the highest dose. All the other animals in this group died within four days. Three of the five chicks that received 7500mg/kg of PER died within six days while those that died in the second group (5000mg/kg) did so within nine days. The only chick that died in the group that received the lowest dose did so on the sixth day. The clinical signs observed three hours after treatment were rapid breathing (tachypnoea) and increase in heartbeat above the normal rate (tachycardia). This was followed by decreased activity, loss of appetite with consequent reduction in feeding (anorexia), coma and death within eight hours to nine days depending on the dosage. Shaky movement and unsteady gait (ataxia) were also observed in majority of the dosed animals while diarrhoea was noted only in the groups that received the two highest doses.

The main post-mortem findings in dead chicks were haemorrhages of the digestive tract and liver.

Haemorrhages and degenerative necrosis of hepatocytes and, digestive tract and mucous membrane were the major histopathological changes elicited by the crude toxin. There was denudation of the mucosal lining and massive eosinophillic infiltration of the muscular coat of the stomach as shown in Plate 1. The fibro-vascular cores of the intestinal villi were heavily infiltrated by eosinophilic leucocytes. Slight periportal inflammation and necrosis of the liver was observed. These pathological changes were obvious in chicks that were administered high doses of extract. No significant changes were observed in the kidney during both post-mortem and histopathological studies.

Acute intraperitoneal (IP) Toxicity of PER in one week Old Chicks The mortality rate and i.p LD_{50} of extract of *Fusarium verticillioides* in chicks are given on tables 2 and 3. The i.p LD_{50} values obtained from the different methods of estimation ranged between 81mg/kg and 87.9mg/kg. The standard deviation and fiducial limits calculated for the Thompson moving average were: 54.40 and 3.3975 ± 0.4511 mg/kg respectively. The correlation coefficient of the regression curve, plot of dosage against mortality was 0.97 and this relationship was significant at P<0.05. The slope for the curve was 1.79.

The main clinical signs of toxicity include tachypnoea, tachycardia, anorexia, somnolence, coma and death. Death was recorded seven hours after administration of 64mg/kg and 110.60 mg/kg. The last death was recorded six days after treatment. The main post mortem findings were congestion of muscle and heart. There was inflammation of the kidney. Petechiae and eccymotic haemorrhage at the mesenteric edges vessels in the intestine were also observed. No death was recorded in the control aroup. The liver was the most affected. Histopathological studies show periportal necrosis and wide spread feathery degeneration of the hepatocytes with hyperchromasis and periportal inflammation (Plate 2).

Acute intraperitoneal Toxicity of PER in Mice The Table 3 gives the values of intraperitoneal lethal dose 50 of the extract of *Fusarium verticillioides* in mice. The fiducial limit for the Thompson moving average method was between 14.03 to 132.33mg/kg while the slope for the graphical method was 2.92. The correlation coefficient of the dose response curve was 0.99 and this was significant at 95% level of significance. Dyspnea (abdominal breathing), catalepsy. prostration and somnolence were the clinical symptoms of toxicity observed. Death was recorded three hours after administration at the following dose levels 38.40mg/kg, 55.30mg/kg and 66.36mg/kg. The last death was recorded eight days after administration of extract. The post-mortem results were congestion of heart, thoracic region, liver and mesenteric vessels. Haemorrhages were observed in the mesenteric vessels and liver. In one of the dead animals of the highest dose group, there was an area of paloid of the cranial visceral aspect of the kidney. When the mice administered with lower doses (26.66mg/kg and 32.00mg/kg) were sacrificed, the only significant gross post-mortem findings were the enlargement of liver, kidney, heart, lungs and spleen. Microscopic examination of the kidney showed wide spread tubular necrosis and intra renal micro-thrombi formation (plate 3). The liver had periportal inflammation with piece-meal necrosis and some regenerative nodules (Plate 4). Compare with normal liver (Plate 9) and kidney (Plate 10). There was no remarkable response by the digestive tract to toxin administered through the i.p route.

Acute oral toxicity of PER in mice: Table 3 also shows results of oral LD₅₀ determination of PER in mice, using the Thompson moving average method. The extract had an oral LD_{50} of 1092.82.18 \pm 153.20mg/kg with fiducial limits of 162 \pm 1339.68mg/kg. Using the graphical method of Miller and Tainter, the PER had an LD_{50} value of 1060mg/kg and a correlation coefficient of the regression curve of 0.98. The coefficient was significant at p<0.05. The slope for the curve was 0.13. Deaths were recorded within the fifth and twelfth days after administration of extract.

The clinical signs of toxicity include dyspnoea (deep laboured inspiration accompanied by wheezing sound); tachypnoea, somnolence, decreased activity and anorexia. The postmortem findings in dead mice were congestion of the liver and its subsequent enlargement and congestion of the messentric vessels. Also haemorrhage was noted in the messentric vessels and livers. When the surviving mice were sacrificed, the only significant gross postmortem lesions were the enlargement of liver, distended spleen and alopecia (loss of abdominal hair). Piece-meal necrosis, cellular oedema and intra hepatic bile stasis indicating hepatitis with cholestasis was the major pathological changes noted in animals given doses of above 833.33mg/kg body weight (Plate 5). Also observed in this category of animals

was mucosal ulceration and submucosal oedema with inflammatory infiltrates indicating superficial gastric erosion (Plate 6-8).

Quantification of Total Fumonisin The results for the determination of total fumonisin in the fungal extract are shown on table 4. Using the optical densities of varying concentrations of fumonisin standards, the curve was plotted and concentration of the sample extrapolated from it. The standard curve has a slope of -1.7110 and correlation coefficient of 0.9915. Three attempts were made to measure the total fumonisin ($FB_1 + FB_2 + FB_3$) content of the sample and the concentrations obtained were between 7.9ppm to 8.8ppm (7900ug/kg – 8800ug/kg) with a mean value of 8.233 ± 0.162ppm. (8233 ±162ug/kg).

T	B.B 174		3.1			1.00		administration.
I ania 7	Mortality	/ ratae in ch	neke and mic	o aivon PF	R through	dittoront	routes of	administration
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	i.p in	chicks	i.p ir	n mice	oral in I	mice	
Group	Dose mg/kg	Number of dead/treated chicks	Dose mg/kg	Number of dead/treated mice	Dose mg/kg	Number of dead/treated mice	
1.	Control	0/10	Control	0/10	Control	0/10	
2.	53.22	0/10	32.00	0/10	694.41	0/10	
3.	64.00	1/10	38.40	1/10	833.33	1/10	
4.	76.80	4/10	46.08	4/10	1000.00	4/10	
5.	92.16	8/10	55.30	7/10	1199.00	8/10	
6.	110.60.	10/10	66.36	10/10	1438.80	10/10	

Table 3. LD₅₀ values of PER extract of *Fusarium verticillioides* in chicks and mice estimated by different methods.

	Lethal Dose 50 (mg/kg)		
Method	i.p in chicks	i.p in mice	oral in mice
Thompson Moving Average	84.00	51.71	1092.82
Graphical Method of Miller and Tainter	81.00	49.50	1060.00
Arithmetic Method of Karber	81.05	45.40	1054.96
Arithmetic Method of Reed and Muench	87.90	51.58	1111.73

Table 4. Neogen Veratox Software Log/Logit Details for total fumonisin determination.

Sample	Description	OD	Result(ppm)
1	0 ppm	1.179	0.0
2	1 ppm	0.762	0.9
3	2 ppm	0.586	2.1
4	4 ppm	0.430	4.4
5	6 ppm	0.398	5.4
6	Sample 1 st trial	0.321	7.9
7	Sample 2 nd trial	0.321	8.0
8	Sample 3 rd trial	0.302	8.8

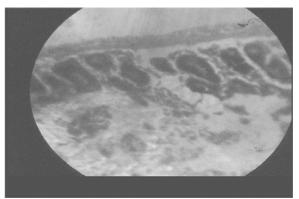


Plate 1: Micrograph of chick intestine after oral administration of 5000 mg/kg body weight of extract. (x 400 H & E). Mucosal denudation of intestine.

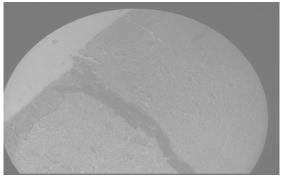


Plate 3: Micrograph of mice kidney at X 100 H & E magnification after dosing interperitoneally with 66.36mg/kg body weight of extract of *F. verticillioides.* Showing wide spread intra renal tubular necrosis with micro thrombi formation.

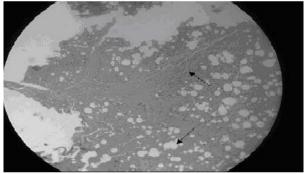


Plate 5 Micrograph of mice liver at X 100 H & E Magnification after a single oral dose (833.33mg/kg body weight) of fungal extract.

Showing wide spread fatty degeneration as evidence by empty clear vacuoles and broad fibrosis appearing as septae signifying early cirrhosis

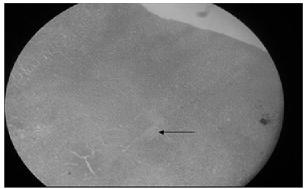


Plate 2: Micrograph of liver of three-day old chicks after dosing interperitoneally with 110.60 mg/kg body weight of fungal extract (100 H & E magnification). Fine periportal fibrosis; a component in liver cirrhosis.

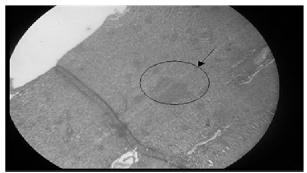


Plate 4: Micrograph of mice liver after administration of a single interperitoneal dose (55.30mg/kg body weight) of extract of *F. verticillioides*.

Showing a regenerating nodule signifying healing from necrosis and probable formation of nodularity in cirrhosis.

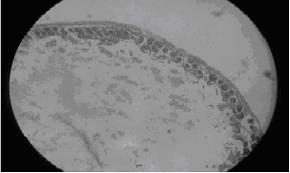


Plate 6 Micrograph of mice liver at X 100 H % Emagnification after administeration of a single oral dose(1438.80 mg/kg body weight) of extract Showing massive eosinophillic leucocytes infilteration and aggregation in the mucosa. This implies eosinopillic graniloma resulting from intense inflammation caused by injury.

DISCUSSION

Fusarium verticllioides (Sacc.) Nirenberg (CABI, IMI 392668) was isolated from mouldy chaffed rice stored for almost a year in local thatched mud storage barn, 'rumbu' (Hausa) in Chibani, Tafa Local Government Area of Niger State, Nigeria during the cold Harmattan season when the temperature was as low as 15^oC. This rice sample was obtained from the bottom of the barn. *Sorghum* and maize were intermittently stored in same barn in previous years. There is a stream passing around the barn which is situated under many big trees. The airy, cool slightly forested almost temperate microclimatic conditions of the location of the barn that also permeates the 'rumbu' are quite conducive for growth of *Fusarium* species.

Acute toxicity studies are usually undertaken to define the important and basic toxicity of a substance, to assess the susceptible species, identify target organs, and provide data for risk assessment in case of acute exposure to the chemical and information for the design and selection of dose levels for prolonged studies (Wallace, 2001). In line with the objectives of such study, this work has shown that chicks and mice are susceptible to the toxins produced by Fusarium verticillioides and the organs mostly affected were digestive tract, liver and kidney. In terms of toxicity, mycotoxins are categorized into three classes namely, extremely toxic, very toxic and toxic mycotoxins (Uraguchi and Yamazaki, 1978). According to this classification, the extremely toxic mycotoxins are those with a lethal dose of below 1mg/kg body weight, very toxic ones have a lethal of 1-10mg/kg and the toxic mycotoxins exhibit lethal doses of between 10-100mg/kg. Rubratoxin B and cycochlorotine belong to the first group while aflatoxin B₁ and trichothecenes are in the second group. Ochratoxin A and sterigmatocystin are examples of toxic mycotoxins. The i.p LD 50 values obtained in this study indicate that the fungus elaborates metabolite(s) in the toxic group of mycotoxins.

The common clinical signs of toxicity of the extract in chicks and mice were; ataxia, anorexia, diarrhoea, dyspnoea, tachycardia, tachypnoea and somnolence. Ataxia is the inability to control and coordinate movement and such toxic sign suggests effect on the sensory and autonomic central nervous system (Wallace, 1982; Wallace, 2001). Similarly, diarrhoea, tachycardia (increase heart beat), tachypnoea (quick and shallow respiration), and somnolence are signs of toxicity of the central nervous system (Wallace, 2001) and it implies that the toxin(s) produced by

Fusarium verticillioides is/are likely to be neurotoxins. Mycotoxins are known to have adverse effects on the various body organs and systems. Many of them have affinity for certain tissues and consequently are so classified. (e.g as hepatoxins, nephrotoxins, dermatoxins). Just as it was observed in this work, the liver is the organ most commonly affected by mycotoxins. Gastrointestinal tract and the kidney are the other organs damaged by the toxins of the *Fusarium verticillioides*. The culture material of this fungus is therefore neurotoxic, hepatotoxic, nephrotoxic and gastrotoxic to chicks and mice.

Fumonisins especially FB1 elicits hepatoxic and nephrotoxic changes in mice. Damages to the liver, bone marrow, adrenals and kidneys were caused in mice administered FB1 at gavage doses ranging from 1 to 75mg/kg body weight per day for 14 days (Bondy et al. 1997). The hepatosis was characterized by hydropic swelling, hyaline droplet accumulation, single-cell necrosis, increased mitotic figures, lipid accumulation and fibrosis of the liver. In addition to these hepatic changes, fatty changes and necrosis of the kidney were recorded (Voss et al. 1995). Many reports have been published implicating fumonisins in poultry animal toxicosis and the main clinical features of the disease often include diarrhoea, weight loss, increased liver weight, poor performance and suppression of the immune system (Voss et al. 1995). These scientific reports on the hepatoxicity and nephrotoxicity of fumonisins to mice and poultry animals support the acute toxicity findings and imply that these toxins are possible components of the culture material of the fungus. This inference from the findings and other reports (Marasas, 2001) support the presence of fumonisins in the extract which was confirmed by the results of the CD ELISA qualitative and quantitative test.

Doses between 1 and 75mg/kg body weight of pure FB₁ have not been found by Bondy *et al.*(1997) and Sharma et al. (1997), to be acutely toxic to mice. Meanwhile, the observed average intraperitoneal LD₅₀ value of the culture extract of the Fusarium verticillioides in mice which contains only 8.233ppm of total fumonisins is 49.58mg/kg body weight. This means that the fungal extract is more toxic than pure FB1 and likely to contain FB1 in synergism with one or more toxins. Other fumonisins especially FB₂ and FB₃, fusarins, moniliformin, beauverin or deoxynivalenol that exhibit toxic synergisms in diet with FB₁ and are usually present in culture materials of Fusarium verticillioides (WHO, 2000) are possibly the other synergistic component(s) of the extract.

Further physico-chemical analysis would be required to completely elucidate the toxins in the methanol: water fraction of the extract.

The importance of this study borders on its public health implications as it has been associated with human and animal diseases. The toxic effects of fumonisins with particular reference to FB1 in animal and human health are well documented. The toxin is hepatotoxic to mice, rats, equids, rabbits, pigs and non-human primates, and nephrotoxic to pigs, rats, sheep, mice and rabbits. Renal toxicity occurs at lower doses than hepatotoxicity in rats and rabbits (Prelusky and Rotter, 1994). Horses are extremely sensitive to low amounts of fumonisins which causes leukoencephalomalacia (liquefaction of the brain) (Marasas et al. 1988) and in swine, FB1 causes pulmonary oedema as well as liver and pancreatic lesions (WHO, 2000). High concentrations of FB1 has been shown to correlate with increased incidence of oesophageal cancer in China and South Africa. and upper gastrointestinal tract cancer among male humans with high alcohol and maize consumption in Northern Italy. It is classified as category II-B carcinogens (Marasas, 2001). FB1 is also phytotoxic, causing damages to cell membrane, and disruptions of chlorophyll and sphingolipids biosynthesis in maize infected by fumonisin-producing Fusarium species (WHO, 2000).

The natural occurrence of *Fusarium verticillioides* and its toxin, FB_1 in Niger State as shown by this studies and in other parts of Nigeria (Bankole and Mabekoje, 2003; Bankole and Adebanjo, 2004) coupled with their adverse impact on human health, livestock production, and national and international trade, makes it imperative for mycotoxin regulations to be enforced in the country.

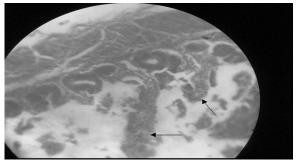


Plate 7 : Micrograph of mice intestine at 100 H & E magnification after administration of a single oral dose (833.33 mg/kg body weight) of extract of *F.verticillioides*. Mucosal denudation of the intestine.

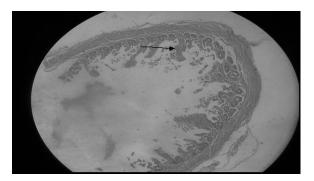


Plate 8: Micrograph of mice intestine at 100 H & E magnification after administration of a single oral dose (1438.8 mg/kg body weight) of extract of *F. verticillioides*. Intestinal eosinophillia at a lower magnification.

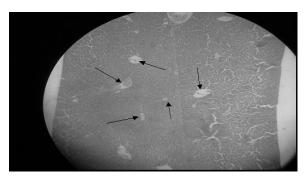


Plate 9: Micrograph of control (normal liver) x 100 H & E magnification. Normal hepatic architecture showing maintenance of central vein and portal tract interphase with hepatic plates separated by sinusoids.

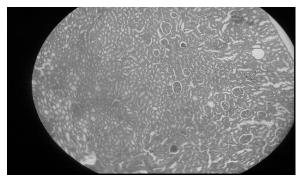


Plate 10: Micrograph of control (normal kidney) x 100 H & E magnification. Normal glomerular apparatus and tubules outlined.

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