African Journal of Biotechnology Vol. 6 (2), pp. 099-108, 18 January 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Fungi and some mycotoxins contaminating rice (*Oryza Sativa)* in Niger State, Nigeria

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Accepted 17 November, 2006

Study on the fungi and some mycotoxins (Aflatoxin B_1 - AFB_1, ochratoxin A - OTA and zearalenone - ZEN) contaminating rice (*Oryza sativa)* in Niger State of Nigeria was carried out. One thousand and sixty two fungi were isolated and identified from one hundred and ninety six mouldy rice samples collected from the state. The major fungal genera contaminating rice were *Aspergillus, Penicillium, Fusarium, Alternaria, Mucor, Rhizopus, Trichoderma, Curvularia, Helminthosporium* and *Cladosporium*. The most prevalent fungal species on rice were .*Penicillium* spp.*, A. flavus, A. parasiticus, A. niger, Mucor* spp.*, Rhizopus* spp. and *Alternaria* spp. The commonest fungal contaminants of the dry, harmattan; dry-hot and rainy seasons were *A. niger, Penicillium* spp. and *A. flavus* respectively. AFB_1 was detected in 97 of the samples analyzed at concentrations between 20-1642 ug/kg. Fifty six of the one hundred and forty samples analyzed for OTA contained the toxin (24 – 1164 ug/kg). Zearalenone was found in ninety three of the one hundred and ninety six mouldy rice samples analyzed at concentrations of between 24 and 1169 ug/kg. Twenty two samples were concurrently contaminated with the three toxins while seven others were found to contain both AFB_1 and OTA. AFB_1 and ZEN occurred together in twelve samples, and eight samples contained both OTA and ZEN.

Key words: Fungi, mycotoxins, rice, aflatoxin B_1 ochratoxin A, zearalenone.

INTRODUCTION

In terms of cultivation and consumption, rice (*Oryza sativa)* is the world's most extensively cultivated crop after wheat and a staple food of above 50% of the total world population (FAO, 2002). About 593 million tonnes (Mt) is produced annually globally (FAO, 2002). Nigeria is one of the nine major rice-importing countries. Others include Cote d'Ivoire, Philippines, Iran, Saudi Arabia, Brazil, Senegal, Japan, and Indonesia (Hynes, 2005). Nigeria produces 3.13 million tonnes on the average annually and of this quantity Niger State, one of the thirty six states of the federation contributes about 0.474 (15.14%) million tonnes yearly (ADP, 2004).

Rice is used for a variety of food and nonfood products. The foods include cooked rice, breakfast cereals, desserts, and rice flour. It is also used in local beer. The inedible rice hull is used as fuel, fertilizer, and insulation, while the bran is a source of cooking oil. Straw from the leaves and stems is used as bedding for animals and for weaving roofs, bricks, hats, baskets, and sandals. In Nigeria, rice is commonly eaten as boiled rice .In the northern parts of the country it is taken as paste, "tuwo", fermented breads ('masa') and as unleavened bread ('waina'). The Hausa use it in preparation of a local snack called "nakiya". The consumption of rice in the country particularly in the urban settlements is so high that the domestic output is inadequate and therefore importation is adopted as supplementary measure.

Rice is also one of the important cereals which favour mycotoxin production. Many scientists in Nigeria (Okoye, 1992) and from several other parts of the world (Uraguchi and Yamazaki, 1978; Jelinek et al., 1989; BFSA, 2002; Taligoola et al., 2004) have studied and documented the fungi and mycotoxins contaminating this cereal but there does not seem to be reports of the fungal and mycotoxin profile of rice in Niger State, a state with favourable climate (average annual temperature of 31.7°C and aver-

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age humidity of 51.6% and it is hot and humid for most part of the year especially between May and October (29.5°C and 73.1%) for fungal growth and mycotoxin production on foods.

Since rice is highly consumed by Nigerians and to a lesser extent by animals, a survey of the fungi and mycotoxins contaminating this grain in a major cereal producer like this State, would give the basis for rational speculation as to the types of animal and human diseases expected from our diets and to what extent these microbes and their toxins contribute to public health hazards in the country. Based on these reasons this study was conducted to determine the kinds of fungi contaminating rice and some mycotoxins which these fungi can produce in rice under natural conditions in Niger State.

MATERIALS AND METHODS

Collection of samples

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

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

Isolation and Identification of fungi

About 10 g of the grains were surface sterilized using 5% sodium hypochlorite solution and then washed with ten successive 100 ml volume of sterile distilled water. Ten grains were placed at random in each of the Petri-dishes containing potato dextrose agar (PDA) and chloramphenicol (500 mg per litre). The dishes were incubated at room temperature and examined daily for five days. Fungi from plated grains were transferred to PDA slant media bottles and fresh PDA in Petri-dishes for identification. Identification of isolates was carried out at the Microbiology Department of Federal University of Technology, Minna and Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria.

Analysis of mycotoxins

The samples were screened and analyzed for aflatoxin B_1, ochratoxin A and zearalenone. The AFB_1 and OTA standards were obtained from Makor chemicals Ltd, Jerusalem, Israel, and the zearalenone standards from the USDA Southern Regional Centre. A multimycotoxin assay method (Ehrlich and Lee, 1984) was used for the mycotoxins analysis. In the method, methylene chloride and phosphoric acid are used for the simultaneous extraction of AFB_1, OTA and ZEN. A separate portion of the initial methylene chloride / phosphoric acid extract was subjected to a specific clean-up procedure for each mycotoxin. Each of the procedure was a modification of a published procedure for each toxin as described below.

Extraction and identification of mycotoxins

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 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 200 ml of the filtrate was collected and from this, 50 ml aliquots were placed in separate 100 ml Erlenmeyer flasks with glass stoppers, for AFB_1, OTA and ZEN assays.

AFB_1 was analyzed in one of the 50 ml aliquot using the method of the Association of Official Analytical Chemists (AOAC, 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.

OTA was quantified by a modification of the method of Paulsch et al. (1982), with 1 M phosphoric acid substituted for 4 M phosphoric acid and methylene chloride substituted for chloroform. The intensities of the standards and samples were compared visually. To confirm the presence of OTA, the thin layer chromatographic plates were sprayed with alcoholic aluminum chloride ($AlCl_3$) (20 g/100 ml alcohol) and also by exposure to ammonia vapour and viewed under long wave. The fluorescence changed from blue green to bright blue to confirm OTA spots.

ZEN was assayed by a modified method of Ware and Thorpe, (1978) as described by Gbodi et al. (1986). In this method phosphoric acid and methylene chloride were used for extraction in place of chloroform. Plates for ZEN determination were developed first in benzene: hexane (75:25) followed by developing in methylene: ethanol (97:3). ZEN was confirmed by spraying the plates with alcoholic aluminum chloride and viewed under short wave when the fluorescence intensity increased.

Statistical analysis

Mean+standard deviation and analysis of variance (students’ t-test) of data generated were calculated using SPSS software. The statistical level of significance was fixed at $P<0.05$ (95%).

RESULTS

Fungi isolated

Tables 1, 2 and 3 show the lists and occurrence of fungi isolated from mouldy rice collected from field, market and

Table 1. Incidence of fungi in mouldy rice collected during the three seasons of the year in Niger State, Nigeria.

Fungus	Dry - Cold Harmattan (Nov-Dec.)[a]	Hot, Dry Season (March - May)	Rainy Season (June - Oct.)	Total Incidence
Alternaria alternata	-	12[b]	7	19
Alternaria spp	47		21	68
Arthrium spp	8		2	10
Aspergillius clavatus	4		3	7
A .flavus	51	29	36	116
A .fumigatus	12	4	6	22
A. glaucus	-	4		4
A .niger	52		36	88
A. ochraceus	12	6	2	20
A. parasiticus	29	30	36	95
A. terreus	-	4		4
A. versicolor	6	1		7
Bipolaris spp	3	3		6
Cladosporum spp	-	3	4	7
Cladosporium werneckil	5	-	4	9
Cryptococcus neoformans	-	-	2	2
Curvularia lunata	19	16	11	46
Fusarium oxysporium	13	4	4	21
F. semitectum	11			11
F. solani	5			5
Fusarium spp	28	17	18	63
F. verticillioides	6		14	20
Geotrichum candidum	1	1	2	4
Gilocladium spp	-	1		1
Helminthosporium spp	4	16	12	32
Mucor spp	28	26	28	82
Nocardia brasiliensis	-	-	1	1
Penicillium citrium	8		3	11
P. cyclopium	1			1
P .exapnsium	4		2	6
Penicillium spp	46	36	34	116
P. viridicatum	7		5	12
Rhizopus spp	45	21	13	79
Rhodotorula rubra	1		2	3
Syncephalastrum spp	2	2	3	7
Trichoderma spp	26	13	18	57
Total	**484**	**249**	**329**	**1062**

store at the three different seasons from the four microclimatic zones of Niger State. One thousand and sixty two fungal isolates were cultured and identified from a total of a hundred and ninety six mouldy rice samples. The fungal genera found contaminating rice in the state in order of decreasing predominance were *Aspergillus, Penicillium, Fusarium, Alternaria, Mucor, Rhizopus, Trichoderma, Curvularia, Helminthosporium* and *Cladosporium*. Others include *Arthrinium, Syncephalastrum, Geotrichum, Bipolaris, Rhodotorula, Cryptococcus, Gilocladium* and *Nocardia.* The commonest fungi species contaminating rice in Niger State were *Penicillium* spp., *A. flavus, A. parasiticus, A. niger, Mucor spp, Rhizopus* spp. and *Alternaria* spp. Others were *Fusarium* spp., *Trichoderma* spp., *Curvularia lunata, Helminthosporium* spp., *F. oxysporium and A. ochraceus.*

Four hundred and eighty four fungal isolates were cultured and identified from the eighty four samples collected during the dry harmattan period (November-February) and *A. niger* (52/84), *A. flavus* (51/84), *Alterna-ria* spp. (47/84), *Penicillium* spp. (46/84) and *Rhizopus* spp. (45/84) were the most frequent fungal contaminants of

Table 2. Incidence of fungi in mouldy rice in Niger State, Nigeria in accordance to microclimatic zones

Fungus	Zone I	Zone II	Zone III	Zone IV	Total Incidence
	No. of samples Infected	No. of samples Infected	No. of samples Infected	No. of samples Infected	
Alternaria alternata	5	6	6	2	19
Alternaria spp	14	23	17	14	68
Arthrium spp	4	2	2	2	10
Aspergillius clavatus	4	1	1	1	7
A. flavus	33	29	26	28	116
A. fumigatus	4	5	7	6	22
A. glaucus	1	1	2	-	4
A. niger	24	17	22	25	88
A. ochraceus	6	6	4	4	20
A. parasiticus	23	21	22	30	96
A. terreus	1	-	2	1	4
A. versicolor	-	3	1	3	7
Bipolaris spp	1	4	1	-	6
Cladosporium spp	1	2	2	2	7
Cladosporium werneckil	1	2	1	5	9
Cryptococcus neoformans		1	-	-	2
Curvularia lunata	11	13	9	13	46
Fusarium spp	22	17	17	7	63
F. oxysporium	7	7	5	2	21
F. semitectum	4	4	2	1	11
F. solani	-	-	2	3	5
F. verticillioides	3	41	7	6	20
Geotrichum candidum	1	1	2	-	4
8Gilocladium spp		-	-	1	1
Helminthosporium spp	9	8	5	10	32
Mucor spp	20	22	19	21	82
Nocardia brasiliensis	-	1	-	-	1
Penicillium spp	30	28	30	28	116
P.citrium	3	1	1	6	11
P. cyclopium	1	-	-	-	1
P.exapnsium	1	3	2	-	6
P. viridicatum	1	3	6	2	12
Rhizopus spp	22	18	21	18	79
Rhodotorula rubra	-	2	1	-	3
Syncephalastrum spp	3	-	2	2	7
Trichoderma spp	8	13	20	16	57
Total	**268**	**268**	**267**	**259**	**1062**

the period. During the dry, hot period (March-May), two hundred and forty nine fungal isolates were found contaminating fifty six mouldy samples collected during the period and of these fungi, the predominant ones were *Penicillium* spp. (36/56), *A. parasiticus* (30/56), *A. flavus* (29/56), *Mucor* spp. (26/56) and *Rhizopus* spp. (21/56). Three hundred and twenty nine fungi were isolated from fifty six mouldy rice samples collected during the rainy season (June–October). During this period the commonest fungi growing on rice were *A. flavus, A. niger* and *A. parasiticus* with equal incidence of 36/56, followed by *Penicillium* spp. (34/56) and *Mucor* (26/56).

Table 2 shows the incidence of fungi in the four microclimatic zones of the State. The wetter zones (Zone 1 =268/49 and zone 11= 268/49) had more fungal infection i.e. more fungal isolates per sample than the drier zones (zones 11 1 = 267/49 and zone 1V = 259/49). Fungi contaminating grains are broadly categorized into field and storage fungi. Table 3 presents the field and storage fungi infecting rice in the state and also gives the fungi

Table 3. Incidence of fungi in field, marketed and stored mouldy rice in Niger State

Fungus	Field (28)	Market (84)	Store (84)	
	No. of samples Infected	No. of samples Infected	No. of samples Infected	Total Incidence
Alternaria alternata		9	10	19
Alternaria spp	14	18	36	68
Arthrium spp	4	2	4	10
Aspergillius clavatus	1	2	4	7
A. flavus	12	41	63	116
A. fumigatus	1	5	16	22
A. glaucus	-	2	2	4
A. niger	8	34	46	88
A. ochraceus	-	4	16	20
A. parasiticus	7	45	43	95
A. terreus	-	3	1	4
A. versicolor	-	4	3	7
Bipolaris spp	-	2	4	6
Cladosporum spp	-	1	6	7
Cladosporium werneckil	1	4	4	9
Cryptococcus neoforman	-	1	1	2
Curvularia lunata	3	13	30	46
Fusarium spp	8	33	22	63
F. oxysporium	6	8	7	21
F. semitectum	4	3	4	11
F. solani	1	3	1	5
F. verticillioides	2	4	14	20
Geotrichum candidum	-	1	3	4
Grilocladium spp	-	-	1	1
Helminthosporium spp	1	19	12	32
Mucor spp	7	32	43	82
Nocardia brasiliensis	-	-	1	1
Penicillium spp	7	40	69	116
P. citrium	2	6	3	11
P. cyclopium	-	-	1	1
P.exapnsium	1	-	5	6
P. viridicatum	-	5	7	12
Rhizopus spp	17	21	41	79
Rhodotorula rubra	-	1	2	3
Syncephalastrum spp	1	4	2	7
Trichoderma spp	1	26	30	57
Total	**109**	**397**	**556**	**1062**

contaminating marketed rice. The commonest field fungi were *Rhizopus* (17/28), *Alternaria* spp. (14/28), *A. niger* (8/28), *Fusarium* spp. (8/28), *A. parasiticus* (7/28) and *Penicillium* spp. (7/28). The eighty four mouldy rice sampled from the store were mostly contaminated by *Pencillium* (69), *A. flavus* (63), *A. niger* (46), *A. parasiticus* (43), *Mucor* (43) and *Rhizopus* spp. (41). The major fungal contaminants of marketed rice in the state were *A. parasiticus* (45/84), *A. flavus* (41/84), *Penicllium* spp. (40/84), *A. niger* (34/84) and *Fusarium* spp. (33/84). Higher ratio of isolates per sample was observed in stored (6.6) samples as compared to marketed (4.7) and field (3.9) samples. Suleja, Borgu, Magama and Gurara in increasing order had the highest fungal contamination in comparison with the other local government areas.

Aflatoxin B_1

Of the one hundred and ninety six mouldy rice samples analyzed for AFB_1, ninety seven were contaminated with

Table 4. Occurrence and concentrations (ug/kg) of aflatoxin B_1 , ochratoxin A and zearalenone in field, marketed and stored mouldy rice in Niger State, Nigeria.

Mycotoxin	Occurrence (Concentration)	Field	Market	Store	Total
Aflatoxin B_1	Incidence Mean + SD (Range)	11/28 35.89 + 91.77[ab] (0 – 464)	39/84 183.67 + 307.86[a] (0 – 1169)	47/84 271.49 + 360.56[b] (0 - 1642)	97/196 200.19 + 320.98 (0 – 1642)
Ochratoxin A	Incidence Mean + SD (Range)	8/28 76.21 + 158.54[ab] (0 – 624)	20/56 168.89 + 305.20[a] (0 – 1164)	28/56 182.91 + 263.60[b] (0 - 1164)	56/140 155.96 + 250.54 (0 – 1164)
Zearalenone	Incidence Mean + SD (Range)	16/28 129.64 + 250.44[ab] (0 – 1164)	37/84 214.83 + 381.93[a] (0 – 1169)	40/84 227.08 + 343.53[b] (0 - 1164)	93/196 207.91 + 349.37 (0 – 1169)

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

Table 5. Occurrence and concentrations (ug/kg) of aflatoxin B_1, ochratoxin A and zearalenone in mouldy rice samples collected during the three seasons in Niger State, Nigeria.

Mycotoxin	Occurrence (concentration)	Dry harmattan	Dry, hot season	Rainy season	Total
Aflatoxin B_1	Incidence Mean + SD (Range)	45/84 187.19 + 389.68 (0 – 1164)	24/56 171.43 + 288.26 (0 – 1139)	28/56 248.46 + 389.96 (0 – 1642)	97/196 200.19 + 320.98 (0 – 1642)
Ochratoxin A	Incidence Mean + SD (Range)	34/84 149.23 + 238.36 (0 – 1164)	22/56 166.07 + 305.31 (0 – 1139)	NA	56/140 155.96 + 250.54 (0 – 1164)
Zearalenone	Incidence Mean + SD (Range)	40/84 183.08 + 330.36 (0 – 1169)	22/56 206.09 + 353.16 (0 – 1169)	31/56 246.98 + 375.29 (0 – 1169)	93/196 207.91 + 349.37 (0 – 1169)

Note: values with similar superscript are significantly different at P<0.05
NA : Not assayed

the toxin at concentrations of between 20-1642 ug/kg with a mean value of 200.19 ug/kg. The incidence of the mycotoxin was lowest in the field samples (11/28) when compared with the marketed (39/84) and stored (47/84) samples. Similarly AFB_1 contents were significantly (P<0.05) lower in field samples as compared to marketed and stored samples. However, there were no significant differences between the AFB_1 concentrations of the two later groups. The occurrence of AFB_1 in rice was highest during the harmattan season (45/84) with lower incidentces observed during the rainy season (28/56) and dry, hot season. The concentrations of the toxin did not differ significantly (P>0.05) in the samples collected during the three seasons (Table 4). AFB_1 was a common contaminants of rice from all the four microclimatic zones of the state (Table 5) and it occurred at virtually same incidence rate in all the zones (zone 1= 23, zone 11= 22, zone 111 =24 and zone 1V = 24). The mycotoxin contents were significantly lower in samples from zone 1 than those from the other zones. There were no significant (P>0.05) differences between AFB_1 concentrations in samples from zone 11, 111 and 1V. AFB_1 was detected in samples from twenty two local government areas.

Ochratoxin A

Table 4 shows the incidence and concentrations respectively of OTA contaminating field, marketed and stored mouldy rice sampled during the dry- harmattan, dry-hot and rainy periods from the four microclimatic zones of the state. The samples collected in the rainy season were not analyzed for OTA. Fifty six of the one hundred and forty samples analyzed contained the toxin (24–1164 ug/kg). Field samples had significantly (P<0.05) lower incidence and OTA contents than the marketed and stored samples. While there were fewer OTA contaminated rice samples in the dry-hot period (22/56) than the harmattan season (34/84), their OTA concentrations were not significantly (P>0.05) different (Table 4). Similarly the OTA concentrations in samples from the zones (Table 5) were not significantly different however higher incidence

Table 6. Incidence and concentrations (ug/kg) of aflatoxin B_1, ochratoxin A and zearalenone in mouldy rice samples collected from the four microclimatic zones of Niger State, Nigeria.

Mycotoxin	Incidence (concentration)	Zone 1	Zone 11	Zone 111	Zone IV	Total
Aflatoxin B_1	Occurrence Mean + SD (Range)	24/49 93.47 + 173.62^{xyz} (0 – 712)	23/49 201.59 + 358.98^{x} (0 – 1642)	25/49 310.25 + 369.69^{y} (0 – 1169)	25/49 95.49+314.39^{z} (0 – 1169)	97/196 200.19 + 320.98 (0 – 1642)
Ochratoxin A	Occurrence Mean + SD (Range)	17/35 171.57 + 273.94 (0 – 1164)	14/35 156.83 + 296.16 (0 – 1164)	13/35 137.43 +250.54 (0 – 1164)	12/35 158.96 +266.22 (0 – 931) n = 35	56/140 155.96 + 250.54 (0 – 1164)
Zearalenone	Occurrence Mean + SD (Range)	30/49 272.14 + 389.94^{xy} (0 – 1169)	24/49 183.22 + 372.75 (0 – 1169)	20/49 214.88 + 313.05^{x} (0 – 1169)	19/49 161.41+349.37^{y} (0 – 1169)	93/196 207.91 + 349.37 (0 – 1169)

Note: values with similar superscript are significantly different at P<0.0

of the toxin was recorded in Zone 1 (17/35) and 1V (14/35) than zones 111(13/35) and 11 (12/35). Seventeen local government areas produced the samples that were contaminated with OTA.

Zearalenone

The results for zearalenone analyses are presented in Tables 5 - 6. Zearalenone was detected in ninety three of the one hundred and ninety six mouldy rice samples analyzed at concentrations of between 24 ug/kg to 1169 ug/kg. The toxin was more frequent (% incidence) in field (57.1%) samples than those from the store (47.6%) and market (44.1%) but at significantly (P<0.05) lower concentrations (field = 129.64 ug/kg) than in samples from the other collection points (Store=227.08 ug/kg and market = 214.83 ug/kg). Zearalenone was common to the harmattan (40/84), dry-hot (22/56) and rainy seasons (31/56) at varying concentrations that were not signify-cantly (P>0.05) different. The toxin was found as a more frequent contaminant of rice from the dry zone 11 (30/49) and wettest zone 1 (24/49) than the driest zones1V (20/49) and the wet zone 11 (19/49) occurring at higher concentrations in zone 1,111, 11 and 1V in decreasing order. Zearalenone contaminated samples from all the local government areas except Rijau.

Co-occurrence of mycotoxins Isolated

Of the three studied mycotoxins, AFB_1 (% incidence = 49.5) was the commonest contaminants of rice in the state followed by ZEN (47.5%) and OTA (40%). Twenty two samples were concurrently contaminated with the three toxins while seven others were found to contain both AFB_1 and OTA. AFB_1 and ZEN occurred together in twelve samples, and eight samples contained both OTA and ZEN. Aflatoxin B_1, ochratoxin A and zearalenone occurred together in rice from fifteen local government areas. Seven local government areas produced samples that contained both AFB_1 and OTA. Aflatoxin and zearalenone occurred concurrently in samples from twelve local government areas while the multiple contaminations of grains with OTA and ZEN were observed in five local government areas.

DISCUSSION

Some of the eighteen fungal genera found in this study as the contaminants of rice in Niger State have also been reported in same crop by other investigators. Uraguchi and Yamazaki, (1978) also described the following fungi as mycoflora of Japanese rice: *Aspergillus, Penicillium*, *Fusarium, Phoma, Curvularia, Helminthosporium, Cladosporium Arthrinium* and *Alternaria*. Garcia, (1986) and Rama Devi et al. (1988) reported species of *Trichoderma* and *Chaetomium* as fungal contaminants of rice in Asia. *Rhizopus* and *Mucor* are surface fungi of many cereal crops including rice (Uraguchi and Yamazaki, 1978). The other fungal (*Syncephalastrum, Geotrichum, Bipolaris, Rhodotorula, Cryptococcus, Gilocladium* and *Nocardia*) genera demonstrated in rice in this study are spoilage fungi of quite a numbers of cereals (Uraguchi and Yamazaki, 1978; Traczyk et al., 2001; Miroslava, 2003) and rice in Uganda (Taligoola et al., 2004).

Species of Rhizopus*, Alternaria, Aspergillus, Fusarium* and *Penicillium* were the most frequently encountered field fungi in rice as demonstrated in this work. *Alternaria* and *Fusarium* require high relative humidity and water contents and are not competitive in storage conditions (Ominski et al., 1994) and so are common field fungi of cereals. *Penicillium, Aspergillus,* and *Rhizopus* that thrive under storage conditions occur in sub tropical and tropical fields during periods of drought and insect damage (Ominski et al., 1994) and have actually been isolated from newly harvested rice and other crops (Uraguchi and Yamazaki, 1978; Garcia, 1986). *Penicil-*

lium, Aspergillus, Mucor and *Rhizopus* are common storage fungi (Ominski et al., 1994; Taligoola et al., 2004) as shown in this work.

Adequate water (13–25%) for fungal growth may result from penetration of rain into storage bin or the field and stored grain gains water by absorption from warm moist air (Agboola, 1992). This important requirement is adequately fulfilled more during rainy seasons and in wetter areas than in drier seasons and places with resultant higher fungal contamination of grains in wet conditions than dry ones. This explains why lower fungi incidences were recorded in dry seasons and zones as compared to the wet season, zones and local government areas (Borgu, Gurara and Suleja) respectively. The exception to this rule was observed where more fungi were isolated during the dry, harmattan season (484) than the rainy season (329). Mechanical damage by insects, rodents and birds on the field which is common sight in the state (field samples were all collected during the harmattan season) and sharp temperature changes of the harmattan season resulting in condensation of water vapour to dew facilitate fungal growth (Agboola, 1992) and could account for the exception.

Fungi infect crops on the field and they persist and proliferate in storage resulting in increased fungal and mycotoxin contamination with duration of storage (Bainton et al., 1980). Therefore the fungal contents of field samples are likely to be lower than those of storage (stored and marketed) samples as shown in this study. Similarly, AFB_1, OTA and ZEN incidence and concentrations were higher in stored and marketed samples than field samples.

Aflatoxins, ochratoxin A and zearalenone are among the five most significant and abundant mycotoxins contaminating foods and feedstuffs in the world (Bhat and Vasanthi, 2003), and have also been shown in this work to be major contaminants (AFB_1, ZEN and OTA in decreasing order of predominance) of rice in Niger State, Nigeria. Our results show that species of *Aspergillus* were the dominant fungi on rice in the state. The incidence of these fungi correlated with the occurrence and contents of AFB_1 in the sampled mouldy rice. *Fusarium* and *Penicillium* species, presumed temperate fungi (Ominski et al., 1994) and producers of ZEN and OTA respectively were found in lower incidence than *Aspergillus* spp hence the lower occurrence of the two toxins in comparison with AFB_1.

The following OTA producers: *A. ochraceus*, *Penicillium cyclopium, P. viridicatum, A. versicolor, A. glaucus* and *A. flavus* (Czerwiecki et al., 2002)*, A. niger* (Pardo et al., 2004) were isolated in this work which accounts for the presence of ochratoxin A in the analyzed mouldy rice. The isolation of three ZEN producers, *F. oxysporum, F. semitectum* and *F. verticillioides* (Pallaroni, 2003) explains the contamination of the samples by the toxin. These findings are indications that the presumed temperate *Fusarium* and *Penicillium* species can grow and elaborate their toxins in certain tropical areas and are in accordance with the works of Adebajo et al. (1994) and Adebajo and Popoola, (2003).

This study was a biased one because only mouldy samples of rice were collected for fungal isolation and mycotoxin analysis. Though such studies cannot give useful incidence data like unbiased study, biased analysis can give the natural fungal and mycotoxin profile of an area. Such biased studies are becoming increasingly important in public health hazards analysis in Sub Saharan Africa because mouldy grains are fed to animals. During the course of sampling we observed that grains are now graded at the local markets. The more mouldy ones are cheaper than the non-mouldy grains. The fact that they are sold in our markets implies that mouldy crops are a part of our human and animal food chain. The implications of this are numerous and enormous.

Aspergillus species the most abundant fungi isolated in this study elaborates many types of mycotoxins including aflatoxins, ochratoxins, tremorgens and more than a dozen other toxins (Scott, 1994). These toxins are involved in many human (Peraica et al., 1999) and animal (Gbodi and Nwude, 1988) maladies. Of major concern is the presence in our foods of aflatoxin B_1, one of the most potent naturally occurring carcinogens. The positive association between the incidence of human hepatocellular carcinoma and frequency of aflatoxin contaminated diets has been established in hepatoma endemic areas of the world and adequately reviewed (National Data Network, 2002). Ochratoxin A causes kidney and liver impairment in animals and man especially pigs (Thuvander et al., 2001; Carlos et al., 2004).

Penicillium species also produce a wide variety of mycotoxins including (but not limited to) ochratoxin, patulin, and citrinin (Scott, 1994). The last two toxins are neurotoxic and nephrotoxic respectively (Peraica et al., 1999). Zearalenone, fumonisins and trichothecenes are metabolites of *Fusarium spp* and the adverse impact of these fusariotoxins are well documented by Bottalico, (1998). Zearalenone 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 (JECFA, 2000).

Sterigmatocystin is an intermediary metabolite of aflatoxin biosynthetic pathway and like AFB_1 it is also a hepatoxic and nephrotoxic carcinogen but exhibits lower toxicity than the former (JECFA, 2000). The following fungal genera that produce this compound were also isolated in this study: *Aspergillus* and *Helminthosporium* (Scott, 1994)*. Rhizopus* and *Mucor spp* were also abundantly found in rice in our work. These genera of fungi produce rhizonin A which has deleterious effects on kidney and liver of mice and rats (Wilson et al., 1984). The workers showed that this mycotoxin elicits degenera-tive necrosis of hepatocytes in experimental animals. The liver-tissue architecture was changed by disassociation of

liver cell cords and there was periportal bile-duct proliferation. Renal tubular epithelium showed changes ranging from degeneration to necrosis.

Moulds of the genus *Alternaria* are known to secrete a host of mycotoxins namely alternariol, alternariol monomerthylether, L- tenuazonic acid, Altertoxins I, II, III, Altenuene, Brefeldin A, Cytochalasins A and B, Destruxin B, Desmethyldestruxin B, Homodestruxin B, and Zinniol (Visconti and Sibilia, 1994). According to their review, alternariols and alteruenes have no significant biological activities in man and animals while altertoxins induced mutagenesis and transformation in mammalian cells. They also reported that tenuazonic acid inhibits protein synthesis causing salivation, emesis, anorexia, erythema, gastrointestinal haemorrhages and convulsion in guinea pigs, mice, rabbits, dogs and monkeys. In the same review, it was reported that cytochalasins inhibit cytokinesis and protein synthesis and has been shown to cause pulmonary haemorrhage and brain oedema in mice. The other *Alternaria* toxins are phytotoxins. Species of *Helminthosporium, Curvularia* and *Trichoderma* are also producers of cytochalasins (Visconti and Sibilia, 1994).

Some species of *Cladosporium* are known to cause haemolytic jaundice and renal failure and eventually death in mice (Daunter and Greenshields, 1973). According to same authors, the toxin elaborated by this family of fungi is emodin which is cytotoxic and mutagenic to hepatic cells and is responsible for the observed toxicity in the mice. No toxic diseases have been documented to date against *Rhodotorula* (Mycotoxin reference, 2005).

The *Arthrinium spp* are known to elaborate 3-nitropropionic acid (3-NPA), the causal agent of mouldy sugar poisoning which occurred in 13 provinces in China between 1972 and 1988 (Beardall and Miller, 1994). Eighty four out of the reported 847 cases died. The disease results in torsion spasms and might leave the patient permanently disabled. *Bipolaris spp* are producers of the hepatotoxic and nephrotoxic carcinogen, sterigmatocystin (Mycotoxin Reference, 2005). The species *Geotrichum candidum* has been shown to be a common contaminant of grains and can cause a secondary infection (geotrichosis) in association with tuberculosis. This rare disease can cause lesions of the skin, bronchi, mouth, lung and intestine. Adenocarcinoma of the glandular stomach, angioendothelioma of the thoracic wall, fibro sarcoma of the liver and epithelial dysplasia lesions in the esophagus and fore stomach were induced in rats fed extracts and concentrated liquid from pickled vegetables contaminated by *G.candidum* (Beardall and Miller, 1994).

Gliocladium is a fungus which is structurally similar to *Penicillium* sp and elaborates gliotoxin and trichothecenes (Mycotoxin Reference, 2005). Gliotoxin is an immunosuppressive toxin while the trichothecenes are protein synthesis inhibitors that cause severe damage to the digestive tract and death due to internal haemorrhage (Beardall and Miller, 1994). *Nocardia brasiliensis* causes tumorous lesions in cutaneous and subcutaneous tissues usually the foot (Mycotoxin Reference, 2005). No toxin is ascribed to *Syncephalastrum,* however it causes allergy to man (Mycotoxin Reference, 2005).

The co-occurrence of toxigenic fungi in same samples was common in this study. Similarly, the mixture of the mycotoxins on same commodity was also observed. The implications of this observation are complex. The natural combination of different fungi and mycotoxins in same crop could be synergistic or antagonistic in host organisms. For instance the effect of pure zearalenone is antagonized by the presence of deoxynivalenol at low concentrations while the effects of vomitoxin are enhanced in combination over a range of zearalenone concentrations (Miller, 1995). The synergistic effects of the studied toxigenic fungi and mycotoxins on animals and man can only be imagined.

The public health implications of the presence of AFB_1, ZEN, OTA and many mycotoxigenic fungi found in rice in Niger State as discussed above are cause for concern particularly if these compounds and fungi are synergistic with each other. This makes regulation of mycotoxins in our foods and feedstuffs, an imperative.

ACKNOWLEDGEMENTS

This project was partly funded by a grant from the University Board of Research of Federal University of Technology, Minna, Niger State, Nigeria. We wish to express our profound gratitude to Mallam Kudu of the Microbiology Departmental Laboratory, F.U.T, Minna and Mallam A.G. Mohammed of Crop Protection Department, A.B.U, Zaria for their assistance in identification of fungi.

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