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# INCIDENCE, PHYLOGENY AND MYCOTOXIGENIC POTENTIALS OF FUNGI ISOLATED FROM RICE IN NIGER STATE, NIGERIA

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## ABSTRACT

The study reports on the natural occurrence of fungi in 21 samples of field (10), stored (6) and marketed (5) rice (*Oryza sativa* L.) collected from Niger State, Nigeria. Fungal isolates were primarily identified based on morphological characteristics, while representative isolates were characterized genetically. An evolutionary tree was constructed from the resulting sequences of the isolated fungi. The toxigenic potentials of some of the isolated fungi were also determined. A total of 357 fungal isolates of nine genera including *Aspergillus*, *Fusarium*, *Sarocladium*, *Acremonium*, *Curvularia Botryosphaeria*, *Penicillium Alternaria* and *Ascomycota* in decreasing order of predominance were identified. The most frequent fungal contaminants of the rice samples were *A. flavus*, *A. fumigates*, *A. niger*, *A. parasiticus* and *F. proliferatum*. All strains of *A. flavus* (aflatoxins $B_1$ and $B_2$), *A. parasiticus* (aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$), *A. ochraceus* (ochratoxin A), *F. proliferatum* and *F. verticillioides* (fumonisins $B_1$ and $B_2$) tested, were excellent producers of their respective mycotoxins. Patulin was produced by *A. terreus*, whereas deoxynivalenol, zearalenone and T-2 toxin were produced by *F. chlamydosporum* and other *Fusarium* spp. The increased prevalence of toxigenic fungi in rice, a highly consumed food grain in Nigeria, poses serious health concerns to the general public.

## PRACTICAL APPLICATIONS

This study investigated the natural fungal occurrence of rice grown in Nigeria using polymerase chain reaction-based techniques as well as the toxigenic potentials of some of the identified fungi. The resultant fungal profile of rice, gene sequences of the fungi detected in the survey, which were deposited in the GenBank and the constructed evolutionary tree, will serve as reference data for the incidence of fungal species in rice and will help to evaluate the safety of Nigerian rice. It could be used in developing and predicting the degree of mycotoxin contamination in rice from Nigeria for effective mycotoxin control. The toxigenic fungi acquired from the work will be excellent microbial sources for production of mycotoxin standards namely aflatoxins $B_I$, $B_2$, $G_1$ and $G_2$, ochratoxin A and fumonisin $B_1$ and $B_2$.

## INTRODUCTION

Rice (*Oryza sativa*) is one of the world's most extensively cultivated crops and equally a staple food of over half of the world's total population (FAO 2002) with its consumption increasing significantly in Africa within the last few decades (Chang 1987). In Nigeria, this commodity is the sixth most cultivated crop after *Sorghum*, millet, cowpea, cassava and yam. Production of rice has increased over the years in Nigeria with an estimated 4.7 million tonnes produced in 2007, making it the second largest producer in Africa with Egypt as principal producer (FAO 2008). Paradoxically, Nigeria is the third major rice importing country after Iran and the Philippines in spite of such productivity. Accordingly, about 2 million tonnes of rice (USDA 2008) is imported and about 5 million tonnes consumed annually (USDA 2008a).

In Nigeria, rice is produced in all six agroecological zones of the country with the highest yield coming from the northern guinea savannah (Ezedinma 2005). Niger State, a generally hot and humid area with average annual temperature and relative humidity of 31.7C and 51.6%, respectively, falls within the guinea savannah region and after Kaduna State, it is the second largest producer of rice (Erenstein and Lancon 2003) contributing approximately 16% of total rice production (Ezedinma 2005). However, like for other food commodities, it is subjected to microbial contamination.

Toxigenic fungi can attack rice in the field and during storage resulting in increased mycotoxin levels in this commodity. *Aspergillus*, *Fusarium* and *Penicillium* are the predominant fungal genera associated with food grains during storage (CAST 2003). The ingestion of grains contaminated with mycotoxins especially those that are nephrotoxic, immunotoxic, teratogenic and mutagenic can provoke acute and chronic effects in man and animals ranging from disorders of central nervous, cardiovascular, pulmonary and intestinal tract systems to death (Hussein and Brasel 2001; Bhat and Vasanthi 2003). The involvement of these toxins in human hepatoma and esophageal cancer (Neal 1995; Richard 2007; Shephard 2008), increased susceptibility to diseases especially in children and childhood pre-five mortality with reduced life expectancy (Sherif *et al.* 2009), is of major concern with regards to public health.

Studies conducted to establish the prevalence of fungi and their toxins in foods and feedstuffs and ensure a healthy food supply to animals and humans are scanty in Nigeria unlike the case may be for other countries of the world. Very limited reports (Opadokun and Ikeorah 1979; Obidoa and Gugnani 1992; Ikeorah and Okoye 2005; Ayejuyo *et al.* 2008; Amadi and Adeniyi 2009) on fungi and mycotoxins contaminations of rice from some parts of Nigeria are available. Except for Makun *et al.* (2007), there is no information on fungi and mycotoxins in rice from Niger State, Nigeria. The previous survey (Makun *et al.* 2007) used conventional fungi identification technique that invariably necessitated more comprehensive surveys using accurate standardized methods. The present study was, therefore, conducted to examine the distribution and phylogeny of fungi in rice from Niger State as well as their ability to produce mycotoxins using polymerase chain reaction (PCR)-based technique and thin-layer chromatography (TLC), respectively. Knowledge on the toxigenicity of common fungal contaminants of rice is vital in elucidating animal and human mycotoxicoses expected from such a commodity and will equally be helpful in addressing the problem of mycotoxin control in Nigeria.

## MATERIALS AND METHODS

### Materials

All chemicals used were of analar grade unless otherwise stated.

(1) Antibiotics: streptomycin and chloramphenicol (Sigma, Aldrich).

(2) All chemicals for PCR analysis including the Fungal/Bacterial DNA extraction kit were purchased from Zymo Research Corporation, Irvine, CA.

(3) Strong anion exchange (SAX) cartridge (ANATECH, Gauteng, South Africa).

(4) Mycotoxins standards: Aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$, ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), T-2 toxin (T2) and patulin (PAT) reference standards were obtained from Sigma, St. Louis, MO. Fumonisin $B_1$ ($FB_1$), $B_2$ and $B_3$ were purchased from PROMEC, MRC, Tygerberg, South Africa.

### Sampling

Twenty-one representative rice samples from the fields (10), storage facilities (6) and market outlets (5) were randomly collected through donations and purchases in December 2008 from 21 villages in the traditional rice growing area of Niger State, Nigeria. Samples were collected from separate batches by thorough mixing of the contents of traditional storage facilities and market containers to obtain homogeneity and representative samples collected from the top, middle and bottom of the containers. In the case of field samples, between seven to ten bunches of rice from stalks at the front, middle and back of the farm were randomly collected and thoroughly mixed to give a sample. Samples (about 0.5 kg each) were put in sealed plastic bottles and transported to our laboratory in South Africa where they were finely milled to pass through a no. 20 sieve and stored in the deep freezer at −20C for a week until analyzed.

### Isolation and Identification of Fungi

The mycological analytical procedure involving four steps according to the method of Kaufman *et al.* (1963) was used including fungal isolation on potato dextrose agar (PDA) and Ohio agricultural and experimental station agar (OAESA), subculturing on PDA, malt extract agar (MEA) and Czapek yeast extract agar (CYA), macro- and microscopic identification and finally, phylogenetics of fungi. The phylogeny of fungi was determined following DNA extraction, PCR amplification, purification of PCR product, product quantification and DNA sequencing for a confirmation of various species of fungi. The culture media used (PDA, OAESA, MEA and CYA) were prepared as described by Atlas (2004).

Primarily, each milled sample was subjected to a six-level serial dilution technique in which 1 g was diluted in a 9-mL ringer solution, vortexed and subsequently, 1 mL of the suspension was transferred to a 9-mL ringer solution and vortexed, and so forth. One milliliter of each suspension was inoculated on solid PDA and OAESA in 90-mm Petri dishes and incubated at 25C for 7–14 days. Between the 5th and 7th day of incubation, all colonies were counted using a colony counter and results presented as number of fungal colonies per gram of sample calculated and expressed in colony-forming units per gram (cfu/g).

The fungi so screened were subcultured on CYA, MEA and PDA, incubated at 30C for 7–14 days and identified to species level where possible. In this case, the hyphae and conidia from each colony representing each fungal species were transferred aseptically on three spots diagonally on each Petri dish containing the medium. Identification to species level was done based on the macroscopic and microscopic characteristics of the isolates following the identification keys of Klich (2002) for *Aspergillus* spp. Pitt and Hocking (1997) for *Penicillium* and Nelson *et al.* (1983) for *Fusarium* spp. Isolates were subcultured on PDA slants and stored at 4C until further analyzed.

Eight of the 48 fungi isolated from the rice samples were precisely identified by this conventional method. These include *Aspergillus niger*, *A. parasiticus* and *A. penicillioides* and *A. flavus.* Isolates were further identified at the Inqaba Biotechnological Laboratories, Pretoria, South Africa by comparison of the nucleic acid profiles of individual fungal species as described by Samson *et al.* (2004). To this end, the mycelia of isolates on PDA slants were subcultured for 7 days as previously described and mycelia harvested, freeze-dried and then subjected to DNA extraction, PCR amplification, purification and quantification of PCR product, DNA sequencing and analysis as described by Samson *et al.* (2004) and Geiser *et al.* (2004) with some modifications. Accordingly, a Fungal/Bacterial DNA extraction kit (Zymo Research Corporation, Irvine, CA) was used for DNA extractions in addition to MSB Spin PCRapace (Invitek GmbH, Berlin, Germany) that was used for ultrafast purification and concentration of PCR-fragments. The freeze-dried cultures were thawed for 1 h and genomic DNA extracted. To this effect, 60 mg sample was weighed and resuspended in 200 μL phosphate-buffered saline (PBS) contained in a 1.5-mL ZR Bashing Bead™ lysis tube (Zymo Research Corporation, Irvine, CA). This tube was then placed in a genie disruptor for 5 min and then centrifuged at 10,000× g for 1 min. The supernatant was recovered in a Zymo-Spin™ IV spin filter placed in a 1.5 mL Eppendorf tube, again centrifuged at 7,000× g for 1 min, filtered into a collection tube and 1,200 μL of fungal/bacterial DNA binding buffer added and vortexed. Eight-hundred microliters of the mixture was transferred to a Zymo-Spin™ IIC column placed in a collection tube, centrifuged for 1 min at 10,000× g and the supernatant discarded (×2). A 200-μL aliquot of DNA pre-wash buffer I was added to the Zymo-Spin™ IIC column in a new collection tube, centrifuged at 10,000× g for 1 min and filtrate discarded, while retaining the column, which was then placed into a new tube. Into the Zymo-Spin™ IIC column, 500 μL fungal/bacterial DNA wash buffer II was added and again centrifuged at 10,000× g for 1 min. The Zymo-Spin™ column was transferred to a sterile 1.5 mL Eppendorf tube and 100 μL DNA elution buffer added directly to the column matrix, centrifuged at 10,000× g for 30 s and DNA eluted and preserved for PCR analysis.

The PCR procedure followed is covered by US Patents 4,683,195 and 4,683,202 (Hoffmann-LaRoche AG, Basel, Switzerland). The primers (ITS_1 and ITS_4) used were synthesized at a 0.01-μM scale and purified using reverse-phase cartridge purification (Inqaba). These primers were resuspended in 2 μM TE buffer prepared from a stock solution concentration of 100 μM. PCR was performed using the Fermentas 2 X PCR mix (Fermentas Life Science, Lithuania). The PCR mixture for each sample consisted of 25 μL of 2 X PCR mix, 1 μL each of 2 μM primers, 1 μL of DNA (final concentration of 10 μM), and constituted to a final volume of 50 μL with nuclease free water. A negative control, containing all of the reagents used except the DNA was also prepared. PCR was performed using an Eppendorf 96-well Thermocycler (Eppendorf, Westbury, NY). The PCR cycling conditions were set as follows: Pre-dwelling at 95C for 3 min, 35 cycles denaturation at 95C for 1 min, annealation at 58C for 45 s, extension at 72C for 1 min 30 s, post-dwelling at 72C for 10 min and held at 4C until samples were retrieved.

The PCR products were further analyzed on an ABI PRISM 3700 Genetic analyzer (AB, Applied Biosystems, Nieuwerkerk a/d Yssel, the Netherlands). The forward and reverse sequences of the PCR products were assembled with a DYEamic ET Terminator Cycle Sequencing Kit (Amersham, Bioscience, Roosendaal, the Netherlands) using the programs SeqMan and EditSeq from the LaserGene package (DNAStar, Inc., Madison, WI). DNA sequences and identities of fungi were obtained from Inqaba Finch server.

## Phylogenetic Analysis

Sequences of the rice fungal isolates in FASTA format obtained from Inqaba Finch server were further subjected to identification on the PCR amplification of the 16S and internal transcribed spacer (ITS_4) regions using the GenBank Blast. The evolutionary tree was constructed using PHYLIP package (version. 3.6) from http://evolution.genetics.washington.edu/phylip.html and the evolutionary distances matrix generated.

### Determination of Mycotoxin-Producing Potentials of Fungi Isolated

Each strain representing the fungal species of *Aspergillus*, *Penicillium* and other genera with the exception of *Fusarium* were further tested for toxigenicity to determine their ability to produce the following mycotoxins: aflatoxin $B_1$ ($AFB_1$); aflatoxin $B_2$ ($AFB_2$); aflatoxin $G_1$ ($AFG_1$); aflatoxin $G_2$ ($AFG_2$); OTA; and PAT. These isolates were cultured individually on solid yeast extract sucrose agar (YES) agar in a 90-mm Petri dish and incubated at 25C for 28 days according to the method of Singh *et al.* (1991). Mycotoxins synthesized by each fungus were extracted by dissolving 5 g of isolate including the medium in 10 mL of dichloromethane (DCM). The crude extract obtained was filtered through a Whatman no. 2V filter paper and the filtrate put in a screw-cap vial, dried under a stream of $N_2$ gas and stored at 4C until analyzed.

The mycotoxins in the crude extracts were detected by a two-dimensional TLC technique devised by Patterson and Roberts (1979). To this end, extracts were reconstituted with 200 µL DCM, vortexed and 20 µL of the extract solution spotted about 10 mm from the edge of a silica gel TLC plate. A similar procedure was followed for mycotoxin standards as a reference for detecting mycotoxins of interest. The developing solvents for the first and second runs were DCM/ethyl acetate/propan-2-ol (90:5:5, v/v/v) and toluene/ethyl acetate/formic acid (6:3:1, v/v/v), respectively. After the second run, plates were dried and visualized under ultraviolet radiation at wavelength of 365 nm. For PAT detection, plates were sprayed with 0.5% 3-methyl-2-benzothiazolinone hydrazone hydrochloride solution and heated at 120C for 3 min. PAT appears as a yellow spot with an orange fluorescence. Visual comparison of retention factor (RF) values and fluorescing color of spots of extracts to that of standards was the basis for identification of toxins.

The toxigenic potentials of the isolated *Fusarium* spp. in producing ZEA, DON, T-2, $FB_1$ and $FB_2$ were evaluated. Representative isolates of each *Fusarium* spp. were further plated on solid YES agar and incubated at 25C for 28 days. *Fusarium* mycotoxins were extracted according to the method of Hinojo *et al.* (2005) with some modifications following clean-up procedures depending on the type of mycotoxin to be determined. *Fusarium* toxins in 10 g of agar-containing mycelia was extracted into 25 mL $CH_3OH/H_2O$ (60/40, v/v) and shaken on a mechanical shaker for 1 h. The entire content was filtered through a Whatman no. 2V filter paper to obtain the crude extract for each fungus. For FB analysis, the extract was stored at 4C until used. ZEA, DON and T-2 toxins were extracted three times from the crude extracts with 25 mL of DCM. The bottom layer was passed through a bed of $Na_2SO_4$ anhydrous into a 500 mL round bottom flask and dried by rotary evaporation. The content was then reconstituted with 200 µL DCM into a 0.5-mL screw-cap vial, dried by passing through a stream of $N_2$ gas and then stored at 4C until analyzed.

Methanol was used for fumonisin extraction. The clean-up method of Sydenham *et al.* (1992) was adopted for FB analysis. The stored filtrate was passed through a SAX column cartridge (ANATECH) previously conditioned with 5 mL methanol followed by 5 mL methanol : water (3:1, v/v). The column was washed with 8 mL methanol : water (3:1, v/v) and then 3 mL methanol. The absorbed FB was then eluded with 10 mL 1% acetic acid in methanol. The eluent was evaporated to dryness and the residue stored in a screw-cap vial at 4C until analyzed.

A two-dimensional TLC technique (Patterson and Roberts 1979) as previously described was used for the detection of ZEA, DON and T-2 toxin. Here, dried extracts were reconstituted with 200 µL DCM and 20 µL was applied to the origin of a two-dimensional TLC plates as previously described but this time, those for ZEA analysis were run twice in DCM : acetone (9:1, v/v) and derivatized with cold dianisidine reagent prepared according to Malaiyandi *et al.* (1976). The mobile solvents for trichothecenes were DCM/ethyl acetate/propan-2-ol (90:5:5, v/v/v) and toluene/ethyl acetate/formic acid (6:3:1, v/v/v), respectively. Plates were derivatized with chromotropic acid (Baxter *et al.* 1983), while those for fumonisin analysis were developed in dicholoromethane/methanol/acetic acid (80:20:2 v/v/v) and butanol/water/acetic acid (12:5:3 v/v/v), respectively. Fumonisins were visualized as purple spots on dried plates that were sprayed with p-anisaldehyde reagent, followed by heating for 3 min at 120C. The retardation factors ($RF_1$ and $RF_2$) and color of the individual spots on TLC were calculated and compared with those of standard mycotoxins to aid in the identification of mycotoxins present.

### Statistical Analysis

Data on the cfu were subjected to statistically analysis. An analysis of variance was used to derive mean values and standard deviation using SigmaStat 3.5 for Windows (Systat Inc., San Jose, CA, 2006), which were compared by least significant difference. Mean values were deemed to significantly differ if $P \leq 0.5$.

## RESULTS

### PCR Analysis

The results of the evolutionary history and identities of the rice fungi based on 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence are presented in Fig. 1 and Table 1. PCR was successfully performed and bands (550 bp) were obtained. Each sequence was compared with those of the same species deposited at GenBank. Similarity distances ranged from 0.010 to 0.452. Total nucleotides amplified were 470–533 bp and corresponded to the ITS_4 complete regions; the 3, portion of the 18S gene, 5.8S complete sequence, and the 5, end of the 28S gene. The sequences and names of fungi processed by Inqaba Finch server were in complete conformity with our GenBank blasting results except in two instances. The sequence of HM 23, which was identified by Inqaba as *Curvularia* sp., was identified in the GenBank as *Curvularia affinis* (AF071335.1) with a query coverage of 51% and maximum identity of 99%. Similarly, the sequence data of HM 34 that was blasted as only *Fusarium* sp. by Inqaba was identified in the GenBank as *Pseudofusarium purpureum* strain MUCC 248 (EU301058.1) with an 84% query coverage and maximum identity of 99%. The identities obtained for these isolates from the GenBank were therefore adopted forthwith in this study. HM 22 failed to grow enough and HM 25 was a mixed culture, which did not allow for PCR analysis of the isolates so their identities as *Alternaria* and *Curvularia* spp. obtained via conventional methods based on morphological characterization were adopted.

## Fungal Contamination

The survey generated data on the fungal contamination of rice samples that are presented in Table 2. A total of 357 fungi belonging to nine different fungal genera including *Aspergillus*, *Fusarium*, *Penicillium*, *Acremonium*, *Alternaria*, *Ascomycota*, *Botryosphaeria*, *Curvularia* and *Sarocladium*, were isolated and identified in the survey. In overall, *Aspergillus* (62.75%) was the most predominant fungal genera identified followed by *Fusarium* (21.85%) in addition to the less frequent members of *Sarocladium* (3.92%), *Acremonium* (3.64%), *Curvularia* (3.08%), *Botryosphaeria* (1.68%), *Penicillium* (1.12%), the *Alternaria* (0.56%) and *Ascomycota* (0.56%) that were also isolated. The calculated mean ± standard deviation values of the cfu for the different types of samples, though not significantly different ($P \leq 0.05$), showed lower fungal contamination in field ($2.6 \times 10^2 \pm 5.0 \times 10^2$) than stored ($5.0 \times 10^2 \pm 1.6 \times 10^2$) and marketed ($5.0 \times 10^3 \pm 2.0 \times 10^2$) samples, an indication of increasing fungal contamination from field to storage and subsequently market.

Although, there was no clear distinction between field and storage fungi, some species were strictly field or storage fungi. All isolates each of *A. aculeatus*, *A. niveus*, two of *A. terreus*, *A. tubingensis*, *Alternaria* sp. and *Ascomycota* sp. were considered field fungi, whereas all isolates of *A. oryzae* (6), *A. sclerotiorum* (3), *Penicillium oxalicum* (4), *F. chlamydosporum* (2), *F. pseudonygamai* (2) and *F. verticillioides* (4) were found only in the stored samples (stored and marketed samples) as seen in Table 2.

*Aspergillus* members accounted for 227 of the total 357 fungi isolated with *A. flavus* being the most dominant. Fourteen species of *Aspergillus* were found among which *A. flavus* was recovered in a total of 19 samples analyzed, recording an incidence of 90.5%. The next sets of dominant members of the *Aspergillus* were *A. fumigatus* and *A. niger*, each isolated in 81% as well as *A. parasiticus* in 71% of samples. Other members belonging to this genus had low incidence rates and contamination by these species in decreasing order of predominance was *A. ochraceus*, *A. unguis*, *A. candidus*, *A. oryzae*, *A. pencillioides* and *A. terreus*. For those isolated in only one sample were *A. aculeatus*, *A. niveus* and *A. sclerotiorum*. The three strains of *Eurotium* found in this study were all belonging to *E. amstelodami* being recovered from two rice samples.

The genus *Fusarium* was also frequently isolated, accounting for 78 of the 357 fungal isolates from rice reported herein with *F. proliferatum* being the most dominant, occurring in 11 (52.4%) of the samples. In all, six members of this genus were isolated alongside others including *Fusarium* sp. (33.3%), *Pseudofusarium purpureum* (23.8%), *F. verticillioides* (14.3%), *F. chlamydosporum* (9.5%) and *F. pseudonygamai* (9.5%).

The third most dominant fungal genera found in the study after *Aspergillus* and *Fusarium* is the *Sarocladium* occurring in 10/21 samples analyzed. The two species found namely *S. attenuatum* and *S. oryzae* contaminated three and eight samples, respectively. Other genera were of low incidence. *Acremonium* sp. was found in 38% of the samples. Eleven isolates of two species of the *Curvularia* family contaminated 8 of the 21 rice samples. The species namely *Curvularia* sp. and *Curvularia affinis* had incidence rates of 38 and 14.3%, respectively. Six *Botryosphaeria dothidea* were isolated in four samples, while the four isolates of the only species of *Penicillium*, *P. oxalicum* were found in two samples. The two least predominant genera, *Ascomycota* (two isolates) and *Alternaria* (two isolates) contaminated two and one samples, respectively.

This mycological survey also showed that none of the samples analyzed was free of fungal contamination with samples having very high frequency of co-occurrences of between 5 and 14 fungal species. The commonest of such multioccurrences were the simultaneously contamination by *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. niger* and *F. proliferatum.*

## Mycotoxigenic Potentials of Fungal Isolates

Randomly selected representative isolates from each strain of the species were screened for their toxigenic potentials. Data on the toxigenicity of fungi tested in the present study are

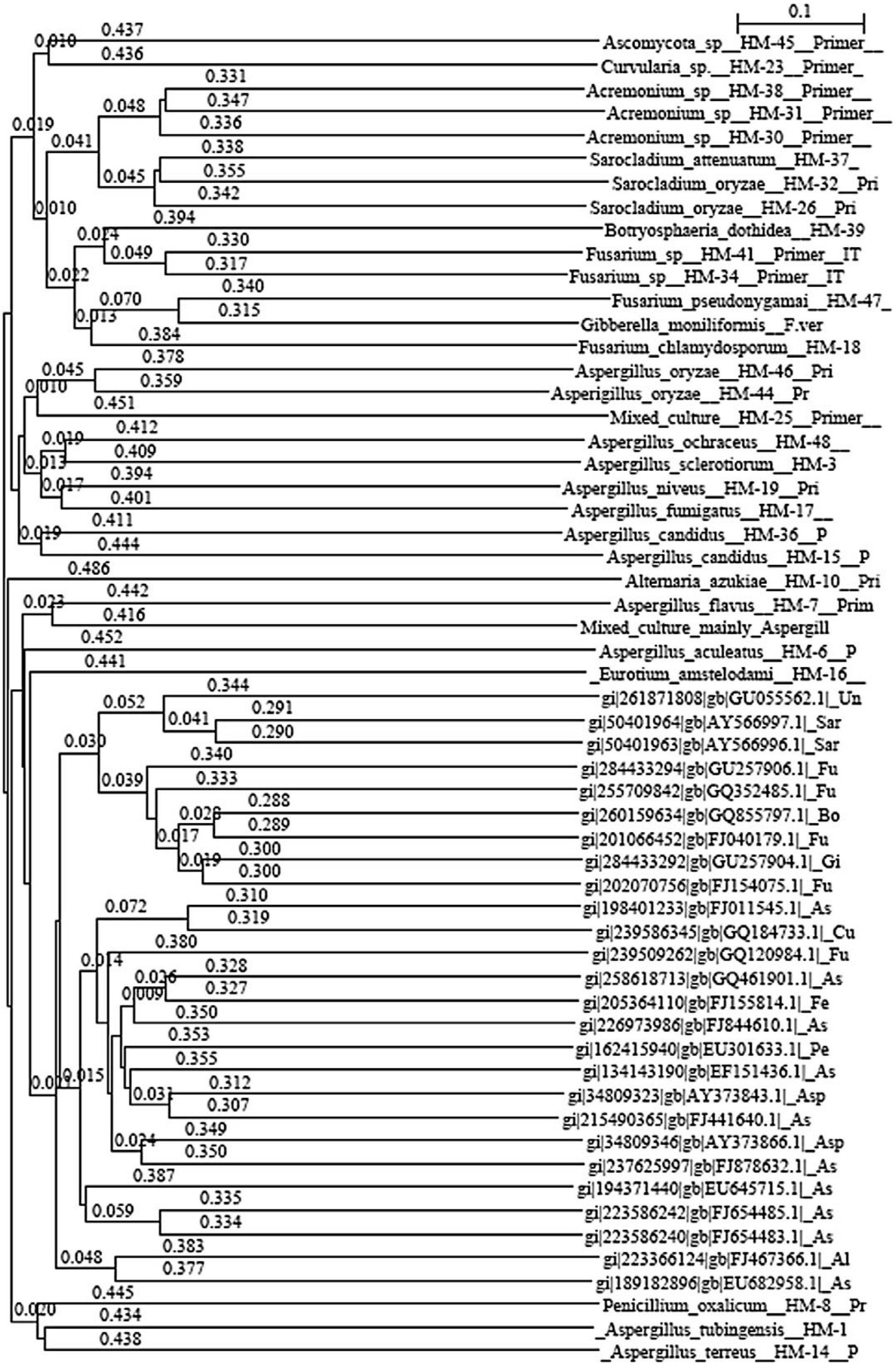

**FIG. 1.** PHYLOGENETIC TREE INFERRED FROM ITS-4 GENE SEQUENCE OF FUNGAL ISOLATES FROM RICE IN NIGER STATE, NIGERIA IN RELATION TO REFERENCE CULTURES IN THE LITERATURE

**TABLE 1.** LIST OF FUNGAL ISOLATES IN RICE FROM NIGERIA IN RELATION TO REFERENCE CULTURES

| Species name | Accession No | Geographic origin | Host plant/code | Reference |
|---|---|---|---|---|
| *Aspergillus* | | | | |
| *A. aculeatus* | HM 6 | Tasaba, Nigeria | *Oryzae sativa* F2 | |
| *A. aculeatus* | EU645715.1 | | Seafan Aspergillosis | Zuluaga *et al*. (unpublished) |
| *A. candidus* | HM 15 | Dama, Nigeria | *Oryzae sativa*, F8 | |
| *A. candidus* | HM 36 | Lafiyagi, Nigeria | *Oryzae sativa* M 20 | |
| *A. candidus* | FJ441640.1 | Xiamen, China | | Zhang and Huang, 2008 |
| *A. candidus* | AY373843.1 | USA | Dust samples | Haugland *et al*. 2004 |
| *A. flavus* | HM 7 | Maitumbi, Nigeria | *Oryzae sativa* F1 | |
| *A. flavus* | HM 20 | Tawi, Nigeria | *Oryzae sativa* S 13 | |
| *A. flavus* | HM 21 | Bida, Nigeria | *Oryzae sativa* M 19 | |
| *A. flavus* | FJ011545.1 | Southern China | | Xi *et al*. (unpublished) |
| *A. fumigatus* | HM 17 | Pigi, Nigeria | *Oryzae sativa* F3 | |
| *A. fumigatus* | HM 24 | Kwarkwata, Nigeria | *Oryzae sativa* S15 | |
| *A. fumigatus* | HM 28 | Innagi, Nigeria | *Oryzae sativa* M18 | |
| *A. fumigatus* | FJ844610.1 | China | | Zhang and Shi, 2009 |
| *A. niveus* | HM 19 | Dama, Nigeria | Oryzae sativa F8 | |
| *Fennellia nivea* | FJ155814.1 | Mexico | Orange peel | Solis *et al*. 2008 |
| *A. ochraeus* | HM 48 | Gbadadan, Nigeria | Oryzae sativa S14 | |
| *A. ochraceus* | FJ878632.1 | Greece | | Arabatzis and Velegraki (unpublished) |
| *A. oryzae* | HM 44 | Danzariya, Nigeria | *Oryzae sativa* S16 | |
| *A. oryzae* | HM 46 | Ekosa, Nigeria | *Oryzae sativa* M17 | |
| *A. oryzae* | FJ654485.1 | Indian Western Ghats | Megamai forest | Venkatesan and Muthuchelian (unpublished) |
| *A. oryzae* | FJ654483.1 | Indian Western Ghats | Megamai forest | Venkatesan and Muthuchelian (unpublished) |
| *A. sclerotiorum* | HM 35 | Lafiyagi, Nigeria. | Oryzae sativa M20 | |
| *A. sclerotiorum* | AY373866.1 | USA | Dust samples | Haugland *et al*. 2004 |
| *A. terreus* | HM 14 | Maitumbi, Nigeria | Oryzae sativa F1 | |
| *A. terreus* | GQ461901.1 | Greece | | Arabatzis and Velegraki (unpublished) |
| *A. tubingensis* | HM 13 | Dama, Nigeria | Oryzae sativa F8 | |
| *A. awamori* | EF151436.1 | China | | Xie and Jian (unpublished) |
| *A. unguis* | HM 27 | Shata, Nigeria | Oryzae sativa F6 | |
| *A. unguis* | FJ878626.1 | Greece | | Arabatzis and Velegraki (unpublished) |
| *E. amstelodami* | HM 16 | Gbadadan, Nigeria | Oryzae sativa F8 | |
| *E. amstelodami* | GQ120984.1 | Mediterranean Sea | Marine algae | Larriba *et al*. (unpublished) |
| *Fusarium* | | | | |
| *F. chlamydosporum* | HM 18 | Kwarkwata, Nigeria | *Oryzae sativa* F15 | |
| *Fusarium* sp | GQ352485.1 | Malaysia | | Sim *et al*. 2009 |
| *F. proliferatum* | HM 33 | Rimi, Nigeria | *Oryzae sativa*. F4 | |
| *F. proliferatum* | FJ040179.1 | China | *Oryzae sativa* | Wang *et al*. (unpublished) |
| *F. pseudonygamai* | HM 47 | Danzariya | *Oryzar sativa* S16 | |
| *F. pseudonygamai* | FJ154075.1 | China | Soil | Zhao and Gao (unpublished) |
| *Fusarium* sp | HM 34 | Kodoko, Nigeria | *Oryzae sativa* F7 | |
| *Pseudofusarium purpureum* | EU301058.1 | | | |
| *Fusarium* sp | HM 41 | Isheli, Nigeria | *Oryzae sativa* M21 | |
| *Fusarium* sp | GU257906.1 | | India | Chandra *et al*. (unpublished) |
| *F. verticillioides* | HM 29 | Kwarkwata, Nigeria | *Oryzae sativa* S 15 | |
| *F. verticillioides* | HM 40 | Ekosa, Nigeria | *Oryzae sativa* M17 | |
| *F. verticillioides* | HM 42 | Bida, Nigeria | *Oryzae sativa* M19 | |
| *Gibberella moniliformis* | GU257904.1 | India | | Chandra *et al*. (unpublished) |
| *Penicillium* | | | | |
| *P. oxalicum* | HM 8 | Innagi, Nigeria | *Oryzae sativa* M18 | |
| *Penicillium* sp | EU301633.1 | China | Forest soil | Zhou *et al*. (unpublished) |
| *Acremonium* | | | | |
| *Acremonium* sp | HM 30 | Kodoko, Nigeria | *Oryzae sativa* F7 | |
| *Acremonium* sp | HM 31 | Tawi, Nigeria | *Oryzae sativa* S13 | |
| *Acremonium* sp | HM 38 | Bida, Nigeria | *Oryzae sativa* M19 | |
| *Acremonium* sp | GU055562.1 | Austria | Agricultural soil | Klaubauf *et al*. (unpublished) |

**TABLE 1.** CONTINUED

| Species name | Accession No | Geographic origin | Host plant/code | Reference |
|---|---|---|---|---|
| *Alternaria* | | | | |
| *A. azukiae* | HM 10 | Maitumbi, Nigeria | *Oryzae sativa* F1 | |
| *A. azukiae* | FJ467366.1 | China | | Wu and Li (unpublished) |
| *Ascomycota* | | | | |
| *Ascomycota* sp | HM 45 | Chanchaga, Nigeria | *Oryzae sativa* F10 | |
| *Ascomycota* sp | EU682958.1 | China | | Sun *et al*. (unpublished) |
| *Botryosphaeria* | | | | |
| *B. dothidea* | HM 39 | Rimi, Nigeria | *Oryzae sativa* F4 | |
| *B. dothidea* | HM 43 | Isheli, Nigeria | *Oryzae sativa* M21 | |
| *B. dothidea* | GQ855797.1 | East China | Apple | Liu *et al*. (unpublished) |
| *Curvularia* | | | | |
| *Curvularia* sp | HM 23 | Gsada, Nigeria | *Oryzae sativa* F8 | |
| *Curvularia affinis* | AF071335.1 | | | |
| *Curvularia* sp | GQ184733.1 | China | | Zhang and Pan, 2009 |
| *Sarocladium* | | | | |
| *S. attenuatum* | HM 37 | Yikangbe, Nigeria | Oryzae sativa F5 | |
| *S. attenuatum* | AY566997.1 | | | Bills *et al*. 2004 |
| *S. oryzae* | HM 26 | Pigi, Nigeria | Oryzae sativa F3 | |
| *S. oryzae* | HM 32 | Tawi, Nigeria | Oryzae sativa s13 | |
| *S. oryzae* | AY566996.1 | | | Bill *et al*. 2004 |

The references and their sequences in this table were all obtained from the National Center for Biotechnology Information (NCBI) web site http://blast.ncbi.nlm.nih.gov/Blast.cgi.

presented in Table 3. As found, all 10 strains of *A. flavus* tested were excellent producers of $AFB_1$ and $AFB_2$. A similar observation was followed for *A. parasiticus* with all tested strains producing both the B and G types of aflatoxins ($AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$). OTA was recovered from cultures of *A. ochraceus* tested. Other ochratoxigenic fungi isolated were *A. niger* and *A. sclerotiorum* (HM 35). Two of the strains of *A. terreus* both originally from field rice samples were shown to produce PAT. Results of the toxicity screening of the *Fusarium* spp. indicate that all the strains of *F. proliferatum* (HM 33) and *F.verticillioides* (HM 29, 40, 40a and 42) produced $FB_1$ and $B_2$ ($FB_2$). The two strains of *F. chlamydosporum* (HM 18) with other *Fusarium* spp. (HM 41) were excellent producers of DON and ZEA, while only one strain of *Fusarium* spp. (HM 41) produced T-2 toxin. None of the species of *Penicillium*, *Acremonium*, *Sarocladium*, *Curvularia Alternaria*, *Eurotium*, *Ascomycota* and *Botryosphaeria* produced the toxins tested. However, many of them produced metabolites that could not be confirmed because of the lack of reference data.

## DISCUSSION

This study provides the first comprehensive documentation of the distribution and toxigenicity of fungal species contaminating rice from a major traditional rice producing region of Niger State in Nigeria. Fungal species belonging to nine genera viz: *Asperillus*, *Fusarium*, *Penicillium*, *Acremonium*, *Ascomycota*, *Alternaria*, *Botryosphaeria*, *Curvularia* and *Sarocladium* were found to be major fungal contaminants of rice from the 21 villages under study. It was also shown in the survey that fungal contamination increased from field to storage with co-occurrence of toxigenic fungi being highly frequent. Data from the toxigenic screening of the isolated fungi indicate that there are many toxigenic strains recovered from the rice samples that were producers of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$, OTA, ZEA, DON, T-2, $FB_1$ and $FB_2$ under laboratory conditions. The observed increase in fungal contamination from field to storage is expected as grains are infected by the ubiquitous fungi in the field and under favorable conditions, the pre-harvest fungi proliferate during storage (Ominski *et al.* 1994) with a resultant increase in fungal concentration and subsequent mycotoxin formation. The lack of distinction between field and storage with regards to the fungal strains recorded in the rice samples is also expected in tropical regions such as Niger State, where fungi belonging to especially the genus *Aspergillus* occur in a natural environment and colonize grains before harvest or during period of drought stress and insect damage (Moss 1987). Therefore, fungi present on grains in the field could easily persist during storage under water stress and insect damage conditions (Mclean and Berjack 1987) such that there is no definite demarcation between field and storage fungi. Grain infection in the tropics therefore, is a continuous process with available predominant species of fungi at any time depending upon prevailing micro-environmental conditions of the grain (Mycock and Berjack 1999). This explains the noted incidence of typical storage fungi *A. aculeatus*, *A. niveus*, *A. terreus* and *A. tubingensis* in the field

**TABLE 2.** ABSOLUTE FREQUENCIES OF THE DIFFERENT FUNGAL SPECIES IN RICE FROM NIGERIA

| S/no | Location of samples | Type of sample | cfu g/mL | *Aspergillus. aculeatus* | *A. candidus* | *A. flavus* | *A .fumigatus* | *A. niger* | *A. niveus* | *A. ochraceus* | *A. oryzae* | *A. parasiticus* | *A. penicillioides* | *A. sclerotiorum* | *A. terreus* | *A. tubingensis* | *A. unguis* | *Eurotium amstelodami* | *Penicillium .oxalicum* | *Fusarium .chlamydosporum* | *F. proliferatum* | *F. pseudonygamai* | *F.verticillioides* | *Fusaruim spp.* | *Pseudofusarium purpureum* | *Acremonium* sp | *Alternaria azukiae* | *Alternaria* sp | *Ascomycota*. sp | *Botryosphaeria.dothidea* | *Curvularia affinis* | *Curvularia* sp. | *Sarocladium attenuatum* | *Sarocladium oryzae* | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Maitumbi | Field | $4.2 \times 10^2$ | | | 10 | 6 | | | 1 | | 1 | | | 1 | 1 | 1 | 2 | | | 1 | | | | | 1 | 1 | 1 | | | 1 | 1 | | | 29 |
| 2 | Tasaba | Field | $2.6 \times 10^2$ | | | 1 | 6 | 1 | | 1 | | | 1 | | | | | | | | 11 | | | | | | | | | | | | | | 21 |
| 3 | Pigi | Field | $8.0 \times 10^2$ | 1 | | 2 | 2 | 1 | | | | 2 | | | | | | | | | 2 | | | | | | | | | | | 1 | | 1 | 12 |
| 4 | Rimi | Field | $1.3 \times 10^4$ | | | 4 | 3 | | | | | | | | | | | | | | 2 | | | 8 | 1 | | | | | 1 | | | | | 19 |
| 5 | Yikangbe | Field | $1.7 \times 10^4$ | | | 5 | 3 | 1 | | | | 1 | | | | | | | | | | | | | | | | | | 2 | | | | 2 | 14 |
| 6 | Shata | Field | $7.0 \times 10^3$ | | | 7 | | 1 | | 1 | | 1 | | | | | 3 | | | | | | | | | | | | | | | 1 | | | 14 |
| 7 | Kodoko | Field | $1.7 \times 10^4$ | | | 6 | 1 | | | | | | | | | | | | | | 1 | | | | | 1 | | | 1 | | | | 1 | | 11 |
| 8 | Dama | Field | $1.3 \times 10^4$ | | | | 3 | 3 | 1 | | | | | | 1 | 1 | 2 | | | | | | | | | 1 | | | | | | | 1 | | 13 |
| 9 | Gsada | Field | $5.0 \times 10^4$ | | 1 | 3 | 3 | 2 | | | | 2 | | | | | | | | | 2 | | | | | | | | 1 | | | 2 | | 1 | 17 |
| 10 | Chanchage | Field | $1.1 \times 10^4$ | | | 3 | 2 | 6 | | | | 3 | | | | | 1 | | | | 1 | | | | | 1 | | | | 1 | | | | 1 | 19 |
| 11 | Kodoko | Store | $1.1 \times 10^3$ | | | 4 | 1 | 2 | | | | 4 | | | | | | | | | | | | | | | | | | | | | | | 11 |
| 12 | Bupbe | Store | $5.0 \times 10^2$ | | | 8 | 1 | 5 | | | | 2 | | | | | | | | | | | | | | | | | | | | | | | 16 |
| 13 | Tawi | Store | $1.1 \times 10^3$ | | 1 | 6 | 2 | | | 2 | | 3 | | | | | | | | | 1 | | | | 1 | 5 | | | | | | | | 2 | 23 |
| 14 | Gbadadan | Store | $1.3 \times 10^4$ | | 4 | 3 | 2 | 2 | | 2 | | 1 | 1 | | | | 2 | 1 | | 1 | | | | | | | | | | | | | | | 19 |
| 15 | Kwarkwata | Store | $1.2 \times 10^4$ | | | | 3 | 1 | | | 2 | | | | | | | | 2 | 1 | 3 | | 1 | 2 | | | | | | | 1 | 2 | | | 18 |
| 16 | Danzariya | Store | $1.6 \times 10^4$ | | | 4 | | 1 | | | 1 | 2 | | | | | | | | | 7 | 2 | | 2 | 2 | | | | | | | | | 1 | 22 |
| 17 | Ekosa | Market | $8.0 \times 10^3$ | | | 1 | 1 | 3 | | | 1 | 1 | | | | | | | | | 7 | | 2 | | | | | | | | | | | | 16 |
| 18 | Innagi | Market | $8.9 \times 10^3$ | | | 2 | 1 | 2 | | | | 1 | | | | | | | 2 | | | | | 8 | | 1 | | | | | | | | | 17 |
| 19 | Bida | Market | $2.0 \times 10^4$ | | | 2 | 1 | 2 | | | 2 | | 1 | | | | | | | | | | 1 | 3 | | 2 | | | | | 1 | | | | 15 |
| 20 | Lafiyagi | Market | $1.3 \times 10^4$ | | 1 | 1 | | 2 | | 1 | | 3 | | 3 | | | | | | | | | | 2 | 1 | | | | | | | | 1 | 1 | 16 |
| 21 | Isheli | Market | $5.0 \times 10^3$ | | | 2 | | 2 | | | | 3 | | | | | | | | | | | | 1 | 1 | 1 | | | | 2 | | 1 | | 2 | 15 |
| Total | | | | 1 | 7 | 74 | 41 | 37 | 1 | 8 | 6 | 30 | 3 | 3 | 2 | 2 | 9 | 3 | 4 | 2 | 38 | 2 | 4 | 26 | 6 | 13 | 1 | 1 | 2 | 6 | 3 | 8 | 3 | 11 | 357 |

**TABLE 3.** MYCOTOXIGENIC POTENTIALS OF FUNGI ISOLATED FROM RICE IN NIGERIA

| Fungi | No. of strains isolated | No. of strains tested | No. of positive strains | Type of mycotoxins produced |
|---|---|---|---|---|
| *A. aculeatus* | 1 | 1 | – | Not detected |
| *A. candidus* | 7 | 5 | – | Not detected |
| *A. flavus* | 74 | 10 | 10 | $AFB_1$ and $AFB_2$ |
| *A. fumigatus* | 41 | 10 | – | Not detected |
| *A. niger* | 37 | 10 | 1 | OTA |
| *A. niveus* | 1 | 1 | – | Not detected |
| *A. ochraceus* | 8 | 8 | 8 | OTA |
| *A. oryzae* | 6 | 5 | – | Not detected |
| *A. parasiticus* | 30 | 10 | 10 | $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ |
| *A. penicillioides* | 3 | 3 | – | Not detected |
| *A. sclerotiorum* | 3 | 3 | 1 | OTA |
| *A. terreus* | 2 | 2 | 1 | Patulin |
| *A. tubingensis* | 2 | 2 | – | Not detected |
| *A. unguis* | 9 | 9 | – | Not detected |
| *P. oxalicum* | 4 | 4 | – | Not detected |
| *F. chlamydosporum* | 2 | 2 | 2 | ZEA and DON |
| *F. proliferatum* | 38 | 10 | 6 | $FB_1$ and $FB_2$ |
| *F. pseudonygamai* | 2 | 2 | – | Not detected |
| *F. verticillioides* | 4 | 4 | 4 | $FB_1$ and $FB_2$ |
| *Fusaruim spp.* | 28 | 15 | 5 | 4 (ZEA + DON) and 1 (T-2 toxin) |
| *Pseudofusarium purpureum* | 6 | 6 | – | Not detected |
| *Acremonium* sp | 13 | 5 | – | Not detected |
| *Alternaria azukiae* | 1 | 1 | – | Not detected |
| *Alternaria* sp | 1 | 1 | – | Not detected |
| *Ascomycota*. sp | 2 | 2 | – | Not detected |
| *Botryosphaeria.dothidea* | 6 | 5 | – | Not detected |
| *Curvularia affinis* | 3 | 3 | – | Not detected |
| *Curvularia* sp. | 8 | 5 | – | Not detected |
| *Eurotium amstelodami* | 3 | 3 | – | Not detected |
| *Sarocladium attenuatum* | 3 | 3 | – | Not detected |
| *Sarocladium oryzae* | 11 | 3 | – | Not detected |

and occurrence of acclaimed field fungi; *F. chlamydosporum*, *F. pseudonygamai* and *F. verticillioides* in stored samples. However, there were some few exceptions to such observations in this study. *Alternaria* spp. being strictly field fungi and *A. oryzae* (6), *A. sclerotiorum* (3), *P. oxalicum* found only in storage samples in the present work is in conformity with the classical grouping of fungi based on colonization period and moisture requirement by earlier scientists (Javis 1971; Lillehoj 1973).

*Aspergillus* spp. being the most dominant fungal contaminants of Nigerian food commodities as proven in this survey is well documented (Bankole and Adebanjo 2003; Bankole *et al.* 2003; Atehnkeng *et al.* 2008). Two reports of Makun *et al.* (2007) and Amadi and Adeniyi (2009) on fungi in Nigerian rice are also in agreement with the observations made herein. Although the distribution of members of the *Aspergillus* family varied with sample type, i.e., field, store and market samples, *A. flavus* was the most dominant species contaminating all rice samples. Similarly, high occurrence frequencies of this fungus have previously been reported in Nigerian foods including rice by the aforementioned authors. The very high incidence of *A. flavus* seen when compared with other members of *Aspergillus* can be explained by the corresponding high levels of *Aspergillus* section Flavi in the soil, plant debris and insects (Horn and Dorner 1999; Nesci and Etcheverry 2002; Jaime-Garcia and Cotty 2004), which serves as the reservoir of inoculum for infection of grains in the field. As reported, *A. flavus* is the most predominant member of *Aspergillus* section Flavi in soils in West Africa (Cardwell and Cotty 2002; Donner *et al.* 2006).

All the isolates of *A. flavus* screened for toxigenicity were found to produce their attendant mycotoxins and this is in agreement with the findings of Atehnkeng *et al.* (2008) who found significantly higher incidence of toxigenic strains of the species in Bida and Mokwa than in other parts of Nigeria. These are the same regions that were also sampled in the present study. The inherent high temperatures and drier conditions of Niger State favor grain infection by *A. flavus* with subsequent production of AF (Jones *et al.* 1981; Diener *et al.* 1987), which could explain the very high toxigenic potentials of the isolated *A. flavus* in this study. It could also be that the agricultural management practices in the study area have created unique ecological niches that promote the toxigenic potential of strains of this species (Bilgrami *et al.* 1981).

Other members of the *Aspergillus* reported in this study have all been shown as common fungal contaminants of rice from Nigeria (Amadi and Adeniyi 2009) and other parts of the world (Udagawa 1976; Park *et al.* 2005; Reddy *et al.* 2006). The *A. parasiticus* screened for toxigenicity were producers of both the B and G types of AF. This assessment distinguished it from other *Aspergilli* and served as a confirmation of its identity that there was no reason for it to be sent for confirmation via PCR-based method. Apart from the AF producers, three ochratoxigenic species belonging to the *Aspergilli* were also isolated. In this case, *A. niger*, *A. ochraceus* and *A. sclerotiorum* were shown in this work to be OTA producers as reported elsewhere in literature (Pitt and Hocking 1997; Klich 2002), whereas strains of *A. ochraceus* were profuse producers of OTA; 50% of *A. sclerotiorum* tested were equally toxigenic with only 1 of the 10 strains of *A. niger* screened found to be ochratoxigenic. *A. niger* is usually regarded as a benign fungus and toxin production does not seem to be common (Pitt and Hocking 1997), so the recorded very low toxigenic potential of the species is not surprising. Since the metabolic profile of fungi is subject to the growth medium (Kokkonen *et al.* 2005), it is also possible that the YES medium used is not very suitable for OTA production by *A. niger*. Contamination of Nigerian foods commodities by high levels of hepatocarcinogenic AF and nephrotoxic OTA (Bankole and Adebanjo 2003) is related to the abundant presence of aflatoxigenic and ochratoxigenic fungi as seen in this study, and is likely to be associated with increased incidences of human primary liver cancer (Olubuyide and Solanke 1990) and chronic renal failures (NAN 2008) experienced in the country.

*Fusarium* contamination of rice is documented in Nigeria (Ngala 1983; Makun *et al.* 2007; Amadi and Adeniyi 2009) and other parts of the world (Reddy *et al.* 2006). Ngala (1983) found *F. verticillioides* as the second major contaminant of Nigerian rice. Of the five species of *Fusarium* isolated in this study, four, namely *F. proliferatum*, *F. verticillioides*, *F. chlamydosporum* and *Fusarium* sp., were shown in this mycological study to be mycotoxigenic producing FB, ZEA, DON and T-2 on YES. *F. proliferatum* and *F. verticillioides*, which were shown herein as producers of $FB_1$ and $FB_2$, are not only important toxin-producing fungi associated with maize worldwide (Dutton 1996; Kpodo *et al.* 2000; Marasas 2001), but have been isolated from other food commodities (Reddy *et al.* 2006) including rice (Pitt and Hocking 1997; Pacin *et al.* 2002; Makun *et al.* 2007; Maheshwar *et al.* 2009). In fact, Park *et al.* (2005) found *F. proliferatum* as the most frequent *Fusarium* spp. in rice. The potentials of these fungi to synthesize $FB_1$, which is linked to increased incidence of human esophageal cancer in South Africa (Marasas *et al.* 1988; Sydenham *et al.* 1990) and China (Chu and Li 1994; Wang *et al.* 1995) and equine leukoencephalomalacia and porcine pulmonary edema (PPE) (Marasas 2001), should be of public health concern to the population of Nigeria. ZEA and the trichothecenes (TH)-DON and T-2 are also toxic metabolite products of the *Fusarium* spp. of the Nigerian rice samples. Though they are not acutely toxic *per se*, their presence in cereals has been associated with certain animal and human diseases. For example, ZEA, an estrogenic toxin that causes infertility in animals, 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 (Zinedine *et al.* 2007), while the TH, which are protein inhibitors, immunosuppressants, cause death due to internal hemorrhage in animals and man (Sudakin 2003).

The genus *Penicillium* was a rare group in the studied rice samples. And even the only species of this family, *Penicillium oxalicum* that was found in 2 of the 21 rice samples is the more ubiquitous member of the genus *Penicillium* and a normal representative of the mycobiota of the soil (Pitt and Hocking 1999). It can be reasonably inferred that its notable occurrence here and its widespread incidence in tropical commodities including rice (Pitt and Hocking 1997) is expected and normal. Though the metabolites of this fungus was not identified in this study, secalonic acid D is reported as the major metabolite of *P. oxalicum* and despite the fact that there has been contradictory experimental reports on its toxicity, its role in causing certain human and animal diseases is yet to be ascertained (Pitt and Hocking 1997). The practical rarity of *Penicilli* in this mycological study is supported by the findings of Amadi and Adeniyi (2009) who did not find any *Penicillium* spp. and their toxins in Nigerian rice from the same agroecological region studied herein. It can therefore be reasonably supposed that OTA in rice from Nigeria is produced by *A. ochraceus. Penicillium* spp. primarily produce OTA in temperate climates, whereas *A. ochraceus* are more commonly associated with warmer climates (Sweeney and Dobson 1998).

The near absence of *Penicilli* from Nigerian rice is the fundamental difference between the fungal profile of Nigerian rice and that from other major rice exporting countries of south east of Asia such as Thailand, the Philippines, Vietnam, Taiwan, Nepal, Sri Lanka, Bangladesh and Indonesia and Nepal. While all the fungal species isolated in this survey have been found in Asian rice, the *Penicillium* species especially *P. citreonigrum*, *P. islandicum* and *P. citrinum* alongside their toxins; luteoskyrin, cyclochlorotine and citreoviridin, which are common contaminants of Asian rice and linked to yellow rice disease (Uraguchi and Yamazaki 1978; Gangopayay and Chakraberti 1982; Garajapathy and Indira 1986; Rama Devi *et al.* 1988; Waghray *et al.* 1988; Jayaraman and Kalyanasundaram 1990; Misra *et al.* 1995; Desjardins *et al.* 1999;

Udagawa and Tatsuno 2004; Park *et al.* 2005; Tanaka *et al.* 2007; Reddy *et al.* 2008), may not likely be present in Nigerian rice.

The genus *Sarocladium* are known rice pathogens as demonstrated in this investigation. *Sarocladium oryzae* (Sawada) is a plant pathogen causing sheath rot disease in rice and also produces such phytoxins as helvolic acid and cerulin, which induce chlorosis and reduce seed viability and seedling health in the infected grains (Sakthivel *et al.* 2002). *Sarocladium attenuatum* has been shown to cause of rice grain spotting (dirty panicles) in Nigeria (Ngala 1983). Although their metabolites were not determined in this work, there is no association of this group of fungi with mycotoxin production or mycotoxicoses (Pitt and Hocking 1997). *Acremonium* spp., commonly referred to as *Cephalosporium*, are a ubiquitous, cosmopolitan fungi with wide distribution among cereals, maize being the most susceptible, have been shown not only in this study as contaminants of rice, but in other studies (Pitt and Hocking 1997). No mycotoxin has yet been ascribed to this family of fungi in literature. Frequently low to moderate infection of rice by *Curvularia* spp. as recorded in 7 of the 21 rice samples, is common in Nigeria (Ngala 1983; Makun *et al.* 2007) and elsewhere (Misra *et al.* 1995; Reddy *et al.* 2008). The mycotoxins synthesized by this species of fungi are not reported in this analysis but other reports indicate that they indeed produce curvularin, a phytotoxin that inhibits cell division by disrupting mitotic spindle formation (Kobayashi *et al.* 1988). Cytochalasins are another class of mycotoxins ascribed to *Curvularia* spp. that inhibit cytokinesis, protein synthesis and cause pulmonary hemorrhage and brain edema in mice (Visconti and Sibilia 1994).

*Botryosphaeria diothidea* is one important common tree pathogen associated with die back and canker diseases of woody plants, reducing the production of fruit crops such as apricot, peach and pistachio (Li *et al.* 1995; Smith *et al.* 1996; Ma *et al.* 2002; Slipper *et al.* 2004). While its ubiquitous nature particularly in forest areas could explain its interesting and unexpected presence in the present work, its low incidence could therefore, be related to the fact that cereals, rice inclusive, are not its natural habitats but orchard trees. The demonstration of *Alternaria* spp. as field rice pathogens reported in this study is in conformity with the reports of Makun *et al.* (2007) in the region under study and Manabe and Tsuruta (1975) in Japan, who also found them as field contaminants of rice. Members of this fungal family though not demonstrated here, are known to produce a wide spectrum of mycotoxins with only the mutagenic altertoxins, tenuazonic acid and cytochalasins providing adverse effects on animal and human health (Visconti and Sibilia 1994). *Ascomycota* or sac fungi, account for about 75% of all described fungi including those found in this study.

Finally, it is worth mentioning here that the much higher frequency of co-contamination of fungi within the same food matrix particularly those that produce AF, OTA and FB (*A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. niger* and *F. proliferatum* and other *Fusarium*) as found in this study is of concern. With such simultaneous occurrences therefore, it is highly likely to find natural co-occurrences of unrelated mycotoxins (Rizzo *et al.* 2004) in similar samples such as those already mentioned above. This is most likely the case especially when such fungi are toxigenic as has been observed herein. This will certainly increase the severity of health-related problems generated from consumption of such contaminated food products as consumption of multiple mycotoxin in foods may exert both synergistic and additive effects (Placinta *et al.* 1999; Casado *et al.* 2001; Creppy *et al.* 2004; Speijers and Speijers 2004; Luongo *et al.* 2008) in both animal and man. Rice is the main stable food consumed in Nigeria particularly in the urban settlements and data provided in this study show this commodity is of low quality with respect to fungal contamination. However, more number of samples from Niger State, Nigeria needs to be studied with regards to assessing fungal contamination both in the field and during storage, which may provide data that is critical in developing and predicting the degree of mycotoxin contamination in rice from the region for effective mycotoxin control. It is equally imperative to study similar samples based on their mycotoxin profiles as several of these samples were found to contain several toxigenic strains of fungi.

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