Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 6 Issue 6 June 2022

Research Article

Isolation and Molecular Identification of Fungi Associated with Stored Grains Sold at Dawanau and Rimi Markets of Kano State, Nigeria

Sultan, Z.*, Nuhu, A.A,, Aminu, I.M,, Kamal, A.A. and Sumayya, S.S

Department of Biological Science, Yusuf Maitama Sule University, Kano, Nigeria

***Corresponding Author:** Sultan Z, Department of Biological Science, Yusuf Maitama Sule University, Kano, Nigeria. **E-mail:** drzafarsultan1@gmail.com

DOI: 10.31080/ASAG.2022.06.1145

Received: March 14, 2022

Published: May 27, 2022

Abstract

Plant pathogenic fungi belong to the Kingdom Fungi comprising of over 100,000 registered species grouped into about 4,300 genera. Many of these fungi infect a variety of cereals particularly stored condition. A study was conducted in the Dawanau and Rimi Markets of Kano state of Nigeria to quantify the number, types and frequency of occurrence of Plant pathogenic fungi associated with stored grains in these markets. The total fungal load and pathogenicity test was also conducted besides subjecting the fungal load for exact identification using the Molecular Biology Technique. A total of seven (7) fungal isolates were isolated from the collected grains. It was found that Genus *Aspergillus* was the most frequent isolate (44.8%) from the two markets combined while *Alternaria* spp. was the least reported fungi (6.9%) from the grains collected from these two markets. Some species of Aspergillus are known to produce Aflatoxins. A combination of many different isolates was present on the grains. The molecular identification method using Polymerase Chain Reaction (PCR) helped in the identification of fungi at the species level with precision and in the least possible time. The study concludes that there is need for having a National Level, Germplasm collection centre and detailed database of all the naturally occurring post-harvest Plant pathogenic fungi in Nigeria for easy intervention and timely overcoming of situations in case of any emergency.

Keywords: Fungi Identification; PCR; Aflatoxins; Plant Pathogenic Fungi; Stored Grains

Introduction

The name "Fungi" is a Greek word and simply represents the most popular member of the group known as Mushroom. Although Fungi are not easily defined in a single term by most mycologists, they can be described as a diverse congregation of eukaryotic, achlorophyllous, chemo-organotrophic, spore-bearing living organisms that were formerly regarded as plants. They were therefore initially grouped with algae in the division "thallophyta" probably because of their enormous similarities, especially in reproductive behaviours [17]. However, they possess many differences from the typical plants hence, in the five-kingdom classification; the fungi are separated and grouped into a separate kingdom (kingdom fungi), comprising of about 50,000 to 100,000 registered species usually grouped into about 4,300 genera [1].

Cereals and derived products represent an important nutrient source for mankind world-wide. In addition, they are the most important dietary food for North African populations [22]. They are the major source of calories and proteins for the people of Nigeria [19]. The major cereals cultivated in Nigeria are sorghum, millet, rice and maize. Cereal grains constitute an important group of substrates for fermented foods in tropical Africa. The common cereals which are used include maize, sorghum and millet. Fermented foods from cereals include: Ogi produced from millet, sorghum or maize; Kenkey, a staple food in Ghana produced from maize, Tuwo,

the staple food in Northern Nigeria produced from both maize and sorghum. In West Africa, crop production is mostly concentrated in the drier parts of the region owing to its drought tolerance. It is mainly grown in the Northern areas of Nigeria and the Republic of Niger [4,16].

Unfortunately, cereals are naturally contaminated with fungi in the field, during drying, processing, transportation and subsequent storage and it may be difficult to completely prevent mycotoxins formation in contaminated commodities, particularly those that are produced in tropical and subtropical climates, in countries where high temperature and humidity promote the growth and proliferation of fungi [9]. Thus, they are often colonised by fungi, including species from the genus Aspergillus, Penicillium and Fusarium, which cause significant reductions in crop yield, quality and safety due to their ability to produce mycotoxins. Mycotoxins commonly occurring in cereals and cereal products include zearalenone, fumonisins, trichothecenes (as deoxynivalenol and T2-HT2), ochratoxin and aflatoxins [15]. It was reported that 25-50% of harvested world crops have been contaminated with mycotoxins [23]. A number of surveys have been carried out to identify a general pattern of toxigenic fungi and mycotoxins contamination in crops that are dried prone to contamination.

Cereals are attacked by a number of fungi at every stage of development [25]. Reported that fungi cause about 50-80% damage to farmers' grain during the storage period or when conditions are favourable for their development resulting in significant loss both quantitatively and qualitatively. In addition, fungi produce mycotoxins which are hazardous to man and animals. Various reports have shown yield losses of up to 67% [5-7,20]. The storage fungi damage the grains in several ways; they reduce the germinability, produce undesirable odour and kernel discolouration, depletion in seed viability, hardness, colour, size and shape, grain weight and various biochemical parameters; protein, carbohydrate and vitamins decrease the food value and also produce toxins injurious to the health of consumers [12,14].

The present study was designed to quantify the number, type and frequency of fungal isolates in the two markets- Dawanau and Rimi of Kano state in Nigeria. The grains were further subjected to pathogenicity test and molecular level identification using PCR technique for identification of different isolates from a group of isolates parasitizing upon the same grains.

Materials and Method

Reagents used

- 1% aqueous sodium hypochlorite
- 10% sodium hypochlorite solution
- Lysis buffer M
- Binding buffer 1
- 70% ethanol
- Elution buffer E
- Master mixes (dNTPs, polymerase enzymes, Magnesium chloride and reaction buffer)
- PCR grade water
- ITS1 and ITS4 primers
- 1% agarose gels
- Ethidium bromide
- Distilled water

Samples collection

Samples of grains (Maize, millet and Sorghum) were purchased randomly from the two major grain markets in Kano state namely, Dawanau and Rimi in the 2019 dry season. Samples collected from Dawanau market were found spread on sacks for many days while that of Rimi market were found in large metallic containers. The samples were packed in polyethylene bags, brought to the laboratory and labelled accordingly for further processing.

Figure 1: Political map of Kano State of Nigeria.

Sample preparation

The grains were surfaced sterilized by dipping into 1% aqueous sodium hypochlorite solution for 1 min, followed by three successive rinses in sterile distilled water. The grains were blotted dry in between sterile Whatman No. 1 filter paper and plated on Potato Dextrose Agar (PDA) at the rate of 10 grains per plate and incubated at a temperature of 25 ± 2°C for 7 days as described by Hussein and Solomy, (2012) [8]. Mixed growth was then sub-cultured to obtain axenic cultures.

Morphological identification of fungal isolate

The isolated fungi were identified according to colony morphology and microscopic examination as described by Barnet and Hunter, 1987 [2] Nelson., *et al.* (1983) [18] Pitt and Hocking, (1997) [21] Leslie and Summerall, (2006) [11].

Pathogenicity test

A pathogenicity test was carried out to prove Koch's postulate. All fresh samples were separately washed in 10% sodium hypochlorite solution followed by three successive rinses in distilled water and allowed to dry. A sterilized needle were used to streaked fungal hyphae from mixed culture and then placed on fresh samples of millet and sorghum. Controls were inoculated with sterile distilled water. Sterilized forceps was used to remove portions from the disease areas on the 4th day and placed on freshly prepared PDA plates and incubated at 25.7 ± 2°C for 3 days. Fungal growth that appeared was recorded.

Molecular identification of fungal isolate

Extraction of DNA

Cell pellet extraction

Fungal mycelium was produced in 20 ml of Potato Dextrose Broth (PDB). Mycelium was harvested by pouring the media onto 1.7 ml Eppendorf tube and freeze centrifuged for 5 mins, 8°C at 14,000 rpm, the sediment was then pipetted unto 1.7 ml microcentrifuge tube.

Lysate preparation

600 ml of lysis buffer M was added to the cell pellet and was briefly vortexed. The lysate was then transferred to an RNase- free microcentrifuge tube, incubated for 10 mins at 65°C, and 100 ml of binding buffer 1 was added, brief vortexed thoroughly and incubated for 5 mins on ice. The lysate was then pipetted unto filter column (clear O-ring), spun for 2 mins, at 8°C and 14,000 rpm, the clear supernatant from the flow-through was pipetted unto a DNase free microcentrifuge tube. An equal volume of 70% ethanol was added and the lysate was vortexed for 2 mins at 8°C, 14,000 rpm.

Binding nucleic acid to column

The lysate was transferred unto a spin column (grey O-ring), 600 ml of clarified lysate with ethanol was applied unto the column and was centrifuged for 1 min at 8°C, 6,000 rpm.

Column Wash

500 µl of solution WN was applied to the column, centrifuged for 1 min at 8°C, 20,000 rpm, the flow-through was discarded and the column was re-assembled with the collection tubes. 500 µl of wash solution A was applied to the column, centrifuged for 1 min at 14, 000 rpm and the flow through was discarded and the column was re-assembled with the collection tubes. The steps were repeated to wash the column a second time with wash solution A, followed by spinning the column for 2 mins, at 8°C 14,000 rpm to mix and dry the resin. The collection tube was discarded.

Nucleic acid elution

The wash column was placed into a 1.7 ml elution tube, 75 µl of elution buffer E was added to the column and the column was centrifuged for 2 mins at 14,000 rpm, 8°C followed by 1 min spin under the same conditions.

PCR amplification

The PCR amplifications were carried out in a total volume of 20 µl, containing 10 µl of master mixes (dNTPs, polymerase enzymes, Magnesium chloride and reaction buffer), 5 µl of PCR grade water, 3 µl of DNA and 1 µl of ITS1 and ITS4 primers respectively. The sequences of the ITS1 and ITS4 primers were 5'-TCCGTAGGTGAACCTGCGG-3' and 5'-TCCTCCGCTTATTGATATGC-3' respectively. The PCR amplification was carried out according to the following temperature profile: An initial step of 5 min at 94°C, 40 cycles of 50 sec at 94°C, 30 sec at 56°C, 30 sec at 72°C and a final step of 5 mins at 72°C.

Gel electrophoresis

Electrophoresis of PCR-amplified products was performed in 1% agarose gels (low melting) for 45 mins at 7.0 V cm^{-2}. The PCR

products were stained with 10 µl of ethidium bromide and visualized with 305 nm ultraviolet light.

DNA sequencing

DNA sequencing was sent to Macrogen Incorporation, Seoul, South Korea.

Data analysis

The data obtained were analyzed using Microsoft excel. Two-way Analysis of Variance (ANOVA) was conducted to see if differences exist between the two sampling sites (Dawanau and Rimi markets) and different species of fungal isolates. (Level of significance $\alpha = 0.05$).

Results and Discussion

Fungal isolates	Dawanau sample Maize millet sorghum			Rimi sample Maize millet sorghum			Total	Incidence (%)
A. Niger	1	1	3	2	ND	2	9	31.0
A. flavus	1	1	ND	ND	1	1	4	13.8
Alternaria spp.	*ND	ND	ND	ND	1	1	2	6.9
Cladosporium	2	1	ND	1	1	ND	5	17.2
Fusarium	ND	1	1	ND	4	ND	6	20.6
Rhizopus	1	ND	ND	1	1	ND	3	10.3
Total	13			16			29	100

Table 1: Frequency of Fungal isolates identified in samples obtained from Dawanau and Rimi Markets.

*ND = Not Detected

Fungal isolates	Dawanau	Rimi total	
A.niger	5	4	9
A.flavus	2	2	4
Alternaria	0	2	2
Cladosporium	3	2	5
Fusarium	2	4	6
Rhizopus	1	2	3

Table 2: Total incidence of fungal isolates identified in samples obtained from Dawanau and Rimi Markets.

Total 13, 16, 29.

*Analysis of Variance have shown no significant difference between the sampling sites and different fungal isolates, $P > 0.05$ at 5% level of significance.

Fungal isolates	Dawanau Maize millet sorghum			Rimi Maize millet sorghum		
A.niger	+	+	+	+	-	+
A.flavus	+	+	-	-	+	+
Alternaria	-	-	-	-	+	+
Cladosporium	+	+	-	+	+	-
Fusarium	-	+	+	-	+	-
Rhizopus	+	-	-	+	+	-

Table 3: Pathogenicity test result on fresh/ apparently healthy seeds.

Detected = "+", Not detected = "-".

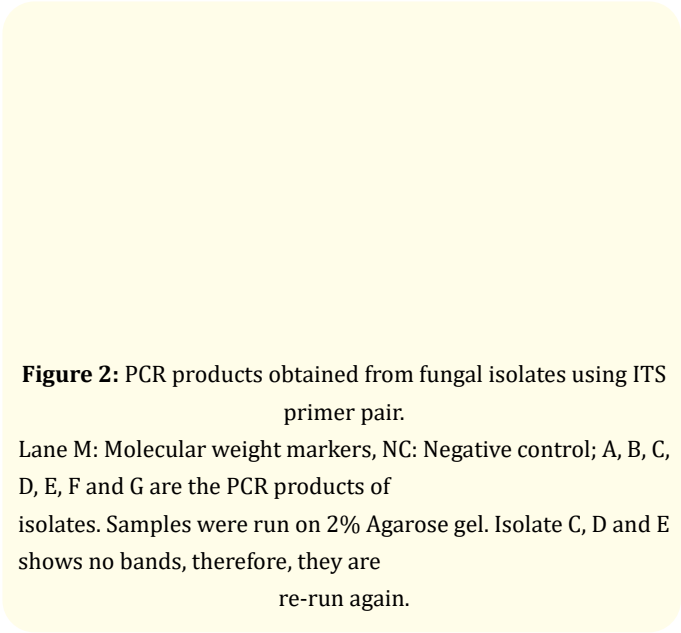

Figure 2: PCR products obtained from fungal isolates using ITS primer pair.

Lane M: Molecular weight markers, NC: Negative control; A, B, C, D, E, F and G are the PCR products of isolates. Samples were run on 2% Agarose gel. Isolate C, D and E shows no bands, therefore, they are re-run again.

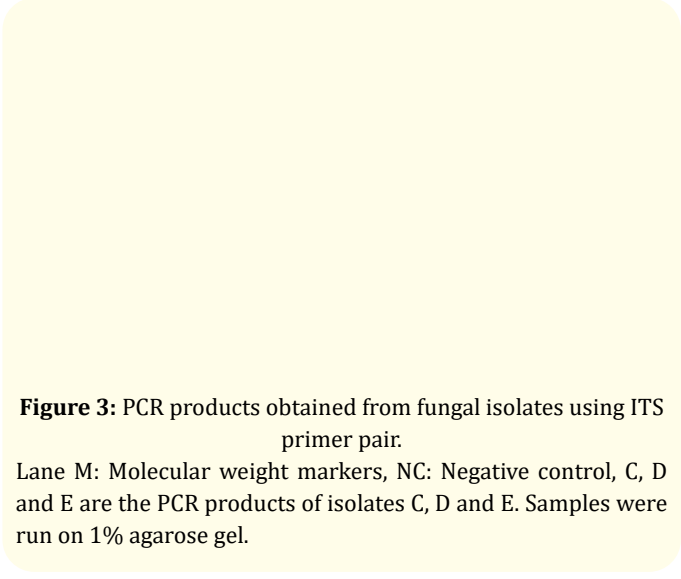

Figure 3: PCR products obtained from fungal isolates using ITS primer pair.

Lane M: Molecular weight markers, NC: Negative control, C, D and E are the PCR products of isolates C, D and E. Samples were run on 1% agarose gel.

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Accession
Aspergillus japonicus CBS114.51 hypothetical protein (B086DRAFT 302044), partial mRNA	39.2	39.2	37	9.3	100.0	XM025667517.1
Aspergillus uvarum CBS 121591 hypothetical protein (B082DRAFT 284251), partial mRNA	39.2	39.2	37	9.3	100.0	XM025631450.1

Table 4: Sequence producing significant alignments for isolate A.

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Accession
Syncephalastrum racemosum strain SJ5 internal transcribed spacer 1 and 5.8S ribosomal RNA, p	342	342	34	1e-89	96.60	KX530450.1
Syncephalastrum racemosum culture-collection MUT <ITA> 2770 internal transcribed spacer 1, partial s	737	737	78	0.0	95.11	KM668151.1
Syncephalastrum racemosum strain SWC 18 internal transcribed spacer 1, partial sequence; 5.8S, ribos	787	787	84	0.0	94.85	KY065367.1
Syncephalastrum monosporum var. *monosporum* CBS 569.91 internal transcribed spacer 1, part	785	785	86	0.0	94.35	MH862279.1
Syncephalastrum monosporum strain EML-BTS-1 internal transcribed spacer 1, partial sequence; 5.8S	713	713	78	0.0	94.22	KY047152.1
Syncephalastrum monosporum var. Cristatum strain CBS 568.91 internal transcribed spacer partial se	782	782	86	0.0	94.17	MH862280.1
Synvephalastrum monosporum pluriproliferum CBS 569.91 internal transcribed spacer region, from TYPE material	780	780	86	0.0	94.15	NR160185.1
Syncephalastrum monosporum strain CBS 569.91 internal transcribed spacer 1, par	780	780	86	0.0	94.15	MH86228.1
Syncephalastrum spp H23 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, co	780	780	86	0.0	94.15	KV058977.1
Syncephalastrum monosporum strain UBOCC-A-101373 18S ribosomal RNA gene, partial sequence, in	780	780	86	0.0	94.15	KF225035.1

Table 5: Sequence producing significant alignments for isolate B.

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Accession
Rhizopus stolonifer MJU-5 internal transcribed spacer 1, partial sequence;5.8S ribosomal RNA gene	49.1	49.1	53	0.024	91.18	MN413688.1
Aspergillus niger isolate KUSR1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	49.1	49.1	53	0.024	91.18	MN416235.1
Rhizopus stolonifer MJU-4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	49.1	49.1	53	0.024	91.18	MN413687.1

Table 6: Sequence producing significant alignments for isolate C.

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Accession
Aspergillus niger Vom18 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	48.2	48.2	50	0.022	93.55	MK640638.1
Uncultured fungus clone S138 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	48.2	91.8	50	0.022	93.55	KY978370.1
Penicillium crustotum isolate 32/a21endo internal transcribed spacer 1, partial sequence; 5.8S ribosomal	47.3	47.3	54	0.077	90.91	KP714285.1
Aspergillus tubingensis isolate B3181 small subunit ribosomal RNA gene, partial sequence; internal tran	43.7	43.7	50	0.94	90.32	MT043791.1
Aspergillus niger haplotype ZmH27 18S small subunit ribosomal RNA gene, partial sequence; internal tran	43.7	43.7	50	0.94	90.32	MG228419.1
Aspergillus niger haplotype ZmH26 18S small subunit ribosomal RNA gene, partial sequence; internal tran	43.7	43.7	50	0.94	90.32	MG228418.1
Aspergillus niger haplotype ZmH25 18S small subunit ribosomal RNA gene, partial sequence; internal tran	43.7	43.7	50	0.94	90.32	MG228417.1
Aspergillus niger haplotype ZmH24 18S small subunit ribosomal RNA gene, partial sequence; internal tran	43.7	43.7	50	0.94	90.32	MG228416.1

Table 7: Sequence producing significant alignments for isolate D.

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Accession
Fusarium equiseti strain AM83 18S ribosomal RNA gene, partial sequence: internal transcribed spacer	41.9	41.9	64	1.9	84.38	MG583741.1
Fusarium sp isolate AM83 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer	41.9	41.9	64	1.9	84.38	MH259186.1
Uncultured fungus clone 036A20180 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	41.9	41.9	64	1.9	84.38	JX378544.1
Uncultured fungus clone 039A50611 ribosomal RNA gene, partial sequence; internal transcribed spacer	41.9	41.9	64	1.9	84.38	JX374427.1
Fusarium equiseti isolate F192 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	41.0	41.0	68	1.9	82.35	KP267111.1
Uncultured fungus clone 037A38622 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	39.2	39.2	66	6.8	81.82	JX355520.1
Uncultured fungus clone 106A57747 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	39.2	39.2	66	6.8	81.82	JX324064.1
Fusarium equiseti isolate JG58 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA	40.1	40.1	72	6.8	80.56	KJ412505.1

Table 8: Sequence producing significant alignments for isolate E.

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Accession
Aspergillus flavus isolate L-218/2013 internal transcribed spacer 1, partial sequence;5.8S ribosomal RNA	48.2	48.2	60	0.015	93.94	MN533862.1
Aspergillus flavus GGV B703 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA	50.0	93.6	60	0.004	93.75	KC907367.1
Aspergillus toxicarius CBS 822.72 internal transcribed spacer region; from TYPE material	50.0	50.0	60	0.004	93.75	NR138297.1
Aspergillus oryzae strain AO- KS0309 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA	50.0	50.0	60	0.004	93.75	HM753601.1
Aspergillus flavus isolate AM- 05 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	46.4	90.0	60	0.054	93.75	MN031597.1
Aspergillus flavus Therm internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	44.6	44.6	56	0.19	93.55	KF946095.1
Aspergillus flavus internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal	46.4	46.4	56	0.054	93.33	KJ831193.1
Aspergillus flavus isolate A453 13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	46.4	46.4	60	0.054	91.18	JX501410.1
Aspergillus nomius isolate C internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	43.7	43.7	60	0.66	90.91	MK440000.1
Aspergillus flavus haplotype ZmH21 18S small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer	43.7	43.7	60	0.66	90.91	MG228413.1

Table 9: Sequence producing significant alignments for isolate F.

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Accession
Aspergillus tubingensis internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, compl	51.8	51.8	58	0.001	93.94	MF952445.1
Uncultured fungus clone S138 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA	51.8	99.1	58	0.001	93.94	KY978370.1
Aspergillus tubingensis isolate 12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	48.2	48.2	55	0.018	93.53	HQ905469.1
Aspergillus tubingensis isolate HSA54 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA	47.3	47.3	58	0.063	90.91	MN839772.1
Aspergillus tubingensis HSA51 small subunit ribosomal RNA gene, partial sequence, internal	47.3	47.3	58	0.063	90.91	MN839771.1
Aspergillus tubingensis CNUML 003 small subunit ribosomal RNA gene, partial sequence, internal	47.3	47.3	58	0.063	90.91	MN818622.1
Aspergillus spp strain K2 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence	51.8	51.8	67	0.001	89.47	MH201394.1
Aspergillus tubingensis Sh3 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer	48.2	95.4	67	0.018	86.84	KY615746.1
Aspergillus niger isolate Saba3 18S ribosomal RNA gene, partial sequence	48.2	48.2	67	0.018	86.84	KX073814.1

Table 10: Sequence producing significant alignments for isolate G.

Discussion

Storage fungi associated with grain samples from the two prominent grain market in Kano state; Dawanau and Rimi were isolated and identified. The results showed that all the grains (Maize, Millet and Sorghum) obtained from the two markets were infested with various degrees of fungi. The result indicated the isolation and identification of five fungal genera with incidence: *Aspergillus* (44.8%), *Alternaria* (6.9%), *Cladosporium* (17.2%), *Fusarium* (20.6%) and *Rhizopus* specie (10.3%). The most common genera isolated were Aspergillus and Fusarium. Among *Aspergillus* specie, the strains identified were *A. flavus* strain which are known to produce aflatoxins and *A. niger* which are known to produce ochratoxin A could pose a risk to consumer health. It is observed that a total of 29 isolates were discovered throughout the study from all the samples obtained from Dawanau and Rimi markets. However, of these isolates, the most frequently occurring was *Aspergillus niger* (31.0%) and the least was *Alternaria* (6.9%). The fungal isolates were very much associated with grain samples obtained from both markets with samples obtained from Rimi market with a high number of fungal occurrences. The results have demonstrated the invasion of grains with mycotoxin producing fungi sold in Kano markets. These results are similar to works of Kutama and Aliyu, 2008 [10] who isolated three fungal genera from local groundnut samples sold in Kano markets [26] who isolated six *Aspergillus* strains from millet and sorghum.

The pathogenicity test confirmed Koch's postulate for the identification of the causative agent of a particular disease. The pathogens were present in all cases of the disease. The same pathogens were isolated from the diseased host and re-grown in pure culture when inoculated into a healthy grain sample of millet and sorghum the pathogen from the pure culture caused the same disease. The same pathogen was re-isolated from the new host and shown to be the same as the originally isolated pathogen.

The data obtained were analyzed using Microsoft excel statistical package. The two-way ANOVA results revealed that the p-value is 0.11 for the different fungal isolates, which is greater than 0.05 level of significance. Similarly, the p-value for the two sampling sites is 0.41 which is also greater than the 0.05 level of significance. The results obtained from the statistical analysis have shown no significant difference between the two sampling sites and different fungal isolates, as P-value > 0.05 in both cases.

Seven fungal isolates were amplified on the basis of their molecular characteristics. The amplification of the 18S rRNA gene with ITS1 and ITS4 primers has been successfully performed and the 18S rRNA gene was chosen as a target for PCR amplification because the sequence data is widely used in the molecular analysis to reconstruct the history of the organism.

Sequencing of the seven amplicons was successfully performed by Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa. Sequence analysis of the nuclear-encoded rDNA showed significant alignments (100%) identity in isolate A for *Aspergillus japonicus* CBS114.51 (GenBank accession No. XM025667517.1), this fungal specie was classified under the genus *Alternaria* at the morphological level of identification. (96.60%) identity in isolate B for *Syncephalastrum racemosum* strain SJ5 (GenBank accession No. KX530450.1). this fungal specie was classified under the genus *Rhizopus* at the morphological level of identification. (91.18%) identity in isolate C for *Rhizopus stolonifer* MJU-5 (GenBank accession No. MN413688.1), This fungal sample was classified under the genus *Cladosporium* at the morphological level of identification. (93.55%) identity in isolate D for *Aspergillus niger* Vom18 (GenBank accession No. MK6406381), This fungal sample was identified as *Aspergillus flavus.* (84.38%) identity in isolate E for *Fusarium equiseti* strain AM83 (GenBank accession No. MG583741.1), This fungal sample was classified under the genus *Fusarium* at the morphological level of identification. (93.94%) identity in isolate F for *Aspergillus flavus* isolate L-218/2013 (GenBank accession No. MN533862.1), this fungal sample was classified as *Aspergillus niger* at the morphological level of identification and (93.94%) identity in isolate G for *Aspergillus tubingensis* (GenBank accession No. MF952445.1), this fungal sample was identified as *Aspergillus niger* at the morphological level of identification.

These results agreed with the results of Danazumi., *et al.* 2015 [3] who obtained four fungal genera from sorghum samples and similar to that of Omaima., *et al.* 2018 [24] that isolated five fungal genera in different grain samples from all Egyptian governorates. The fungal organisms isolated from the grain samples in this study are known to be spoilage organisms associated with many agricultural products including cereals, fruits and nuts Muhammad., *et al.* 2014) [16]. These fungi might have colonized the grains during production in the field, transportation or storage. The variation in the frequency of their occurrence may have reflected differences in

the inoculum density in the area or the prevailing environmental conditions favouring the growth of the fungi.

Conclusion

Seven fungal species were isolated from three-grain samples obtained from the two prominent grain markets in Kano state. The isolated species were identified morphologically as; *Alternaria, Aspergillus flavus, Aspergillus niger, Cladosporium, Fusarium* and *Rhizopus* species. The fungal samples were then identified through molecular techniques by sequencing of 18S rRNA gene using ITS1 and ITS4 primers and were identified as; *Aspergillus flavus, Aspergillus japonicus, Aspergillus niger, Aspergillus tubingensis, Fusarium equiseti, Rhizopus stolonifer* and *Syncephalastrum racemosum.*

The molecular techniques used in this study, has added great benefits to the process of distinguishing between similar species of fungi in comparison with the classical techniques.

Recommendations

- Molecular classification procedure should be encouraged as its a fast procedure, requires minimal management of pathogens and also helps in distinguishing morphologically similar species.
- The presence of fungi in the sample grains is an indication that there is a need for effective monitoring of fungal contamination and raising awareness on the hazards of fungi and their mycotoxin on human and animal health.
- Testing seeds health of major crops should be introduced in the national control system, and proper harvesting, packaging and storage conditions should be promoted.
- The use of resistant cultivars should also be encouraged.

Bibliography

1. Ali S., *et al.* "The assessment and management of *Tinea capitis* in children". *Pediatric Emergency Care* 23.9 (2007): 662-668.
2. Barnett HL and Hunter BB. "The illustrated genera of Fungi. 3rd edition". Burgress publishing Company, Minnesota (1987): 241.
3. Danazumi IB., *et al.* "Seed-borne fungi isolated from five selected varieties of Sorghum (*Sorghum bicolor.* L)". *International Journal of Current Science and Research* 17 (2015): E43-49.
4. Food and Agriculture Organisation FAO. Database, Food and Agriculture Organisation, Roma, Italy (2002).
5. Gachomo EW., *et al.* "Diversity of fungal species associated with peanuts in storage and levels of aflatoxins in infected samples". *International Journal of Agriculture and Biology* 6 (2004): 955-959.
6. Gwary DM., *et al.* "Assessment of leaf anthracnose caused by *Colletotrichum sublineolum* on sorghum geneotypes in the Sudan Savanna". *Agricultural Tropica et Subtropica* 35 (2002): 53-59.
7. Halt M. "*Aspergillus* flavus and Aflatoxin B1 in Flour Production". *European Journal of Epidemiology* 10.5 (1994): 555.
8. Hussein HZ and Slomy AK. "Detection of Fusarium graminearum in maize seeds and determination of isolates produced toxin and evaluating the activity of some compounds against the fungus on cultural media". *The Iraqi Journal of Agricultural science* 43 (2012): 95-102.
9. Kumar KV., *et al.* "Mycotoxins research and mycoflora in some commercially important agricultural commodities". *Crop Protection* 27 (2008): 891-905.
10. Kutama AS and Aliyu BS. "Fungal contamination of local Groundnut varieties in Northern Nigeria". *International Journal of Bioscience* 3 (2008): 2.
11. Leslie JF and Summerall BA. "The Fusarium Laboratory Manual 1st Edition". Blackwell Publishing, Iowa, USA (2006).
12. Magan N., *et al.* "Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain". *European Journal of Plant Pathology* 109 (2003): 723-730.
13. Marley PS. "Sorghum Pathology: In: Cropping Scheme Meeting Report, Cereal Research Programme". Institute of Agricultural Research, Ahmadu Bello University of Zaria, Nigeria (1996) 24-27.
14. Matthew Shiju., *et al.* "An evaluation on the Impact of Fungi on the Post-Harvested Stored Wheat Grains". *International Journals of Biotechnology and Biochemistry* 6 (2010): 9951002.
15. Miller JD. "Mycotoxins in small grains and maize: old problems, new challenges". *Food Additives and Contaminants* 25 (2008): 219-230.

16. Muhammad S., *et al.* "Survey of the market diseases and aflatoxin Contamination of cereals in Wammako market, Sokoto State, North western Nigeria". *Nutrition and Food Science* 34.2 (2014): 72-76.

17. Muhammad Yusha'u and Ahmed Shehu Kutama. "Introduction to Mycology". Lap Lambert Academic Publishing, Deutschland, Germany (2016): 1-25.

18. Nelson PE., *et al.* "Fusarium species: An illustrated manual for identification. 1st Edition". Pennysylvania State University Press, University Park, PA., USA (1983): 226.

19. Nkama I and Ikwelle MC. "Assessment of food quality of millet grain. In: Emechese, A.M. Ikwelle, M.C., Ajayi D., *et al.* "Pearl Millet in Nigerian Agriculture: Production, Utilization and Research Priorities. Proceedings of the Pre-season National Coordination and Planning Meeting of the Nationally Coordinated Research Programme on pearl millet, Maiduguri, April 21-24, 1997". Lake Chad Research Institute, Maiduguri, Nigeria (1998): 177-178.

20. Pandey SN and Trivedi PS. "A textbook of botany volume I". Vikas publishing house PVT limited, India (2008).

21. Pitt JI and Hocking AD. "Fungi and food spoilage. 3rd Edition". Blackie Academic Proffessional, London (1997).

22. Riba A., *et al.* "*Aspergillus* section Flavi and aflatoxins in Algerian wheat and derived products". *Food and Chemical Toxicology* 48 (2010): 2772-2777.

23. Ricciardi C., *et al.* "Development of a micro cantilever-based immuno sensing method for mycotoxins detection". *Biosensors Bioelectronics* 40 (2012): 233-239.

24. Omaima A., *et al.* "Isolation and molecular identification of Fusarium fungi from some egyptian grains". *Asian Journal of Plant Sciences* 17 (2018): 182-190.

25. Salfelder K., *et al.* "Atlas of Fungal Pathology (Current Histopathology, 17), Springer; 1990th edition" (1990): 121-142.

26. Yahya SM., *et al.* "Incidence of *Aspergillus* species on the stored millet (Pennisetum glaucum) and Sorghum (Sorghum bicolor) at Dawanau and Sharada Markets". *Dutse Journal of Pure and Applied Sciences (DUJOPAS)* 3 (2017).