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SCREENING FOR AFLATOXIGENIC FUNGI IN STORED AND OPEN MARKET GRAINS IN POTISKUM, NORTHEAST NIGERIA

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ABSTRACT

Fungal and aflatoxin contaminations of grains pose a significant risk to human health as well as animal health hence the need to screen for the presence of aflatoxigenic fungi in grains. Isolation and identification of fungi associated with grains was carried out on 50 samples including rice, millet, wheat, sorghum, barley, maize and sesame obtained from local grain markets and stores in Potiskum. Potato dextrose agar (PDA) and Neutral red desiccated coconut agar (NRCDA) were used for isolation and direct visual determination of aflatoxigenic fungi respectively. Fungal isolates were then screened qualitatively for aflatoxin B1 production by inoculating a pinch of mycelia onto the centre of NRDCA plates. Seven fungi species belonging to the four genera were identified. These include *Rhizoctonia, Botrytis* sp, *Fusarium, Aspergillus flavus, Aspergillus paraciticus, Aspergillus fumigatus and Aspergillus niger*. The most predominant fungal genus isolated from stored and open grains markets was *Aspergillus. Aspergillus flavus* had the highest detection frequency in both stored grain (4, 28.57%) and open grains (2, 40.0%). *A. flavus* isolated from stored sorghum, maize and sesame exhibited very bright blue fluorescence followed by *A. parasiticus* isolated from stored red sorghum which exhibited moderate bluish white fluorescence. The aflatoxigenic *Aspergillus* species 31.58% (n=6) were significantly correlated with qualitative results obtained from NRCDA plates. Isolates from samples with positive results for aflatoxin B1 detection and having blue fluorescence showed amplification of two target genes. Similarly, 14.29% (n=2) of the non-aflatoxigenic isolates were negative for the two genes.

Keywords: Aflatoxigenic fungi, Grains, *Aspergillus flavus, Aspergillus parasiticus,* Potiskum

INTRODUCTION

Mycotoxins are secondary metabolites produced by species of moulds belonging to the genera *Aspergillus, Penicillium* and *Fusarium.* They are global problem in grains and growers, traders, and processors are all faced with the difficulty of protecting customers from serious health issues (Meyer *et al.,* 2019). They deplete nutrients and pose a serious risk to the control of food quality and safety. Mycotoxins can be ingested by humans directly through foods with a plant origin, inadvertently through foods with animal origin, or through airborne exposure (both indoors and outdoors).

Estimation by Food and Agricultural Organization (FAO) revealed that as much as 25% of the global agricultural commodities are contaminated with mycotoxins, resulting in significant economic losses (Kabak *et al.,* 2006). These mycotoxins are associated with several toxic effects in humans and animal, hence, they are recognized as one of the most significant and disturbing problems about food safety (Sforza and Maechelli, 2006). Aflatoxins B1 (AFB1), Aflatoxins B2 (AFB2), Aflatoxins G1 (AFG1) and Aflatoxins G2 (AFG2) are known as the most significant mycotoxins in foods and feeds out of the four hundred known mycotoxins. Due to their high toxicity, carcinogenicity, teratogenicity, hepatotoxicity and mutagenic properties, they pose a threat to human health. When exposed to aflatoxin, hepatitis B and hepatitis C infected individuals have a significantly higher chance of liver cancer development (Costanzo *et al.,* 2015). The International Agency for Research on Cancer (IARC) has categorized AFB1 under group 1 human carcinogen due to its harmful effects.

Foodstuffs are prone to fungal contamination both before and after harvest (storage), especially when it is executed in poor conditions with high humidity (Piotrowski 2013). These facts may result in the contamination of food products, particularly cereals and items derived from cereals products with mycotoxin (Martins *et al.,* 2018). In developing countries cereal as well as cereal based products serve as the main source of energy. Ingestion of food products containing high doses of AF can result in certain disorders including edema, severe liver damage, hemorrhage and even death in severe cases (Martins *et al.*, 2018).

The mycotoxins contamination of agricultural products is influenced by a number of factors. The factors vary between the crops and the fungal species or strain. Fungal strains differ in their capacity to infest a given host, and also show differences between varieties of the same grain, since different varieties show differences in their levels of susceptibility or resistance to infestation by fungi (Christiane *et al.*, 2019). Additionally, environmental factors such as temperature and relative humidity have an impact on mycotoxin contamination and infestation of grains with mycotoxigenic fungus (Paterson and Lima, 2010).

Numerous mycotoxigenic fungus strains can infest crops, and the majority of these strains produce numerous types of mycotoxins. Contamination of agricultural commodities with several mycotoxins is regularly reported, and it becomes more likely when feed different raw materials are combined to make compound feed (Christiane *et al.*, 2019).

Tropical and subtropical countries have higher rates of food contamination, which can cause acute and chronic mycotoxicoses in both human and animals. According to Wild (1996), over 98% of the persons in West African nations tested positive for aflatoxin-DNA adducts indicating widespread exposure to aflatoxin. The significance of this situation is demonstrated by two outbreaks of aflatoxicosis in Kenya, one in 1982 caused by consumption of contaminated home-grown maize, which resulted in 12 fatalities and the other in 2004, when 317 people fell ill and 125 died in the central provinces (Wangikar *et al.,* 2005).

In many developed countries there are regulations for acceptable levels of mycotoxins in grains as well as their products since approximately 60% of the food produced and

consumed globally are derived from cereal crops mainly rice, corn, barley, wheat, rye, oats and sorghum. However, due to lack of food security, poverty and malnutrition, the risk of aflatoxin and other mycotoxin exposures persists in the developing nations (Gong *et al.,* 2004; Williams *et al.,* 2004). Open market is a practice where grains are sold by displacing them to customers. Grains in open market are prone to contamination by moulds especially the aflatoxin producers as a result of exposure to dust and hash weather conditions. Similarly, stored grains can easily become contaminated by aflatoxigenic moulds due to poor storage conditions and improper post-harvest practices. These can result in increased aflatoxin exposure for consumers, food insecurity and economic losses. These can be mitigated by implementing certain best practices such as regular aflatoxin screening as well as improved storage and handling practices.Therefore, this study was carryout to evaluate the occurrence of aflatoxigenic fungi in grains intended for human consumption in both storage and in open markets.

MATERIALS AND METHODS

Collection of grain samples

A sum total of 50 grain samples, each weighing 25g, were collected from grain markets, these samples included maize, millet, sorghum, wheat and rice were obtained from Grain markets in Potiskum, Yobe State. The samples were collected from stores and open market stores; samples were taken from the bottom, the middle and the top of each grain sack and placed in a clean polyethene bags. The samples were properly labeled and kept at ambient room temperature in the microbiology laboratory until further analyses.

Isolation of Fungal species

The grains were surface sterilized as described by Samson *et al*. (2010). Briefly, the grains were mixed thoroughly after which approximately 20 seeds each were selected randomly, washed in 350 mL of ethanol (0.5%) and then washed twice with distilled water. Using forceps, five to four kernels were randomly selected and placed onto Potato Dextrose Agar (PDA), and then incubated for seven days at room temperature (25ºC). The fungal species that emerged from the grains were subculture onto a new PDA media plate and incubated at ambient room temperature for a further seven days (Robert *et al*., 2020).

Microscopic identification based on morphological characteristics of the fungal isolates

Based on their macroscopic and microscopic characteristics, the fungal isolates were identified. Macroscopic identification was based on the pattern mycelia growth on PDA and the reverse (Samson *et al*., 2010). Using the slide culture technique, an agar block of correct dimensions using sterilized blade was sliced from the PDA media. It was then placed on a sterile microscope slide in a Petri dish containing cotton wools moistened with sterile distilled water. The four-part of the PDA block were then inoculated with isolates and covered using sterile coverslip. The plate was incubated for 5 days at 30°C. After the incubation period, the coverslip was removed and placed on a grease free microscope slide, stained with a drop of lactophenol and then observed under X40 objective lense for distinctive shape and arrangement of spores. The Larone Atlas of Mycology was used to identify the species both macroscopically and microscopically based on shape of the conidiophore, vesicle and conidia and the colour of the mycelia. (Daphne and Joel, 2013). The isolates were kept on PDA slants at 25°C for storage before further analysis.

Screening of the Isolates for Aflatoxigenic fungi

Preparation of Neutral Red Desiccated Coconut Agar (NRDCA) Medium

From the Kwangila market in Zaria, Kaduna, Nigeria, fresh coconuts were purchased. NRDCA was prepared in accordance to the procedure described by Atanda *et al* (2005), 200 grams of the coconut were steeped in 1 L of hot water (pH 4.77) for 30 minutes before being aseptically blended in a Waring blender (Torrington, CT, USA) for 5 minutes and filtered through four layers of cheese cloth. The filtered was mixed with 2% (2% agar, oxoid), heated to boiling, and then cooled to around 50ºC. Neutral red stain (pH 4.38) was diluted to 0.1-0.3%and added to the medium. The medium was then placed in autoclave at 121 ºC for 15 minutes, cooled, and poured (15ml) into sterile petri dishes (8.5cm) (Atanda *et al*., 2005)

Qualitative Detection of aflatoxigenic fungi

By placing a pinch of mycelia in the centre of the DCA plates, *Aspergillus paraciticus*, and *Aspergillus flavus* isolates were qualitatively screened for the formation of aflatoxin B1. The inoculated plates were then incubated at 25ºC for 5 days after which the fungi were examined for blue fluorescence under UV light at 364 nm wave length which indicated aflatoxin B1 production (Dahab *et al.,* 2016; Saleemi *et al.,* 2016). An un-inoculated plate was used as control.

Extraction of Fungal DNA

Genomic DNA of the fungal isolates was extracted according to the method described by (Sohrabi and Taghizadeh 2018). Fungal mycelia from freshly growing cultures in potato dextrose broth were used for the genomic DNA extraction. The mycelia were transferred in to a mortar and grinded with a pestle vigorously to fine powder. This was followed by the addition of lysis buffer: 1 M Tris-HCl (pH 7.5), 0.05 M EDTA, 0.9 M NaCl, 0.1 M Na2SO, and 1% sodium dodecylsulfate. The mixture was heat shock at 65°C for 20 min.

The resulting suspension was then centrifuged at 2,000 g for 5 min and the supernatant was transferred to a properly labelled microfuge tube. Chloroform: isoamyl alcohol (24:1) method was used to extract the DNA from the supernatant as follows: centrifugation for 15 min at 2,000 g followed by addition of equal volumes of isopropanol and centrifugation at 2,000 g for 5 min. The resulting pellets were suspended in 100 µL distilled water (Sohrabi and Taghizadeh 2018).

Polymerase chain reaction amplification

Clustered pathway genes involved in aflatoxin biosynthesis were amplified as described by Rahimi *et al.* (2016) with slight modifications in initial denaturation temperature and denaturation temperature to obtain optimum amplification. Previously used primers by Sohrabi and Taghizadeh (2018) were adopted. Table 1 illustrates the specifications of primers and target genes.

The two target genes were amplified separately following optimization. The PCR mixture contained 150 ng DNA template, 1.5 mM $MgCl_2$, and 10X PCR buffer composed of KCl (50 mM), dNTP (1 mM), taq polymerase (2.5 U), and each primer (0.3 mol), and then made up to 50 µl with sterile distilled water. Table 2 shows the optimized thermal cycling conditions of for each gene. The amplicons were electrophoresed on 1.2% agarose gel stained with ethidium bromide, and visualized under UV light using a gel documentation system (Gel Doc ODSSEY XF OXF-2016) (Sohrabi and Taghizadeh 2018).

Table 1: Nucleotide sequences of the primers used in the study

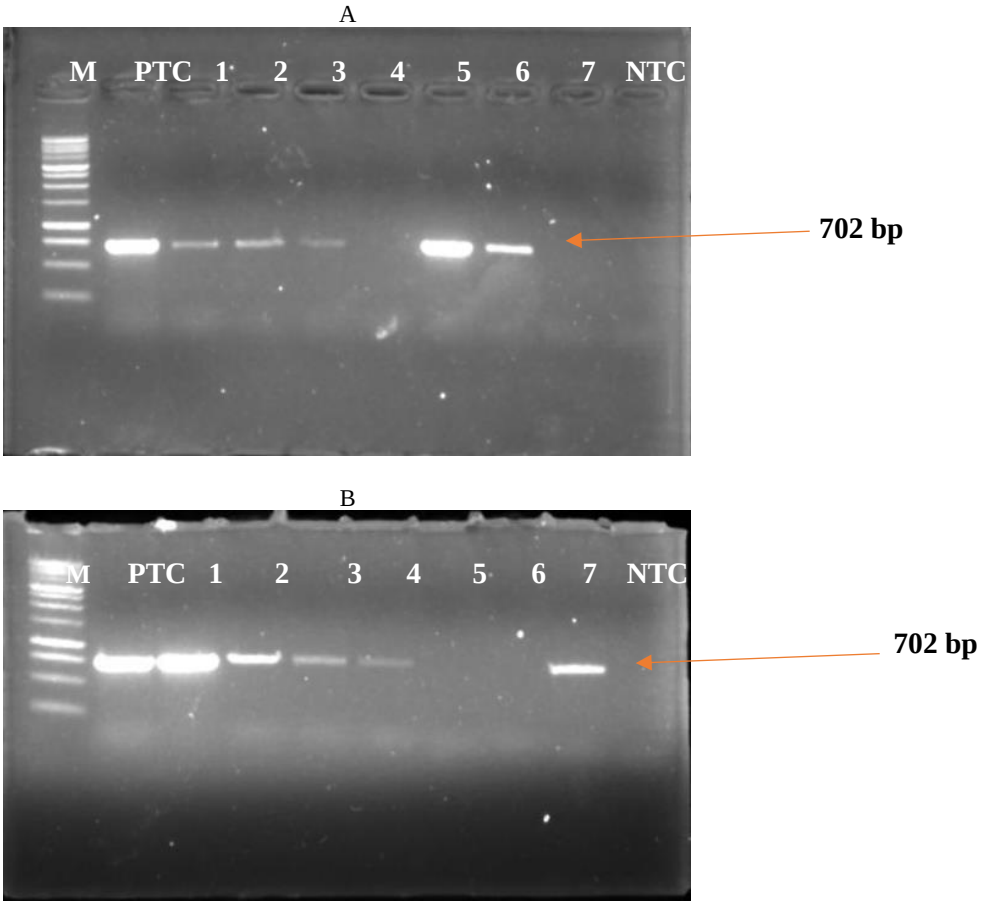
Gene name	Sequence	Amplicon size (bp)	Melting temperature
afl D (Nor-l)-F	5'-CTCATCACACCGCAGCATCGG-3'	702	62.8
afl D (Nor-l)-R	5'-AGATGCCTGCCACACTGTCT-3'		63.1
afl R-F	5'-AGAGCTACTGAACGTCCCAT-3'	1458	60.7
afl R-R	5'-ATCAGGTTGCACCGAACTGTCC-3'		61.1

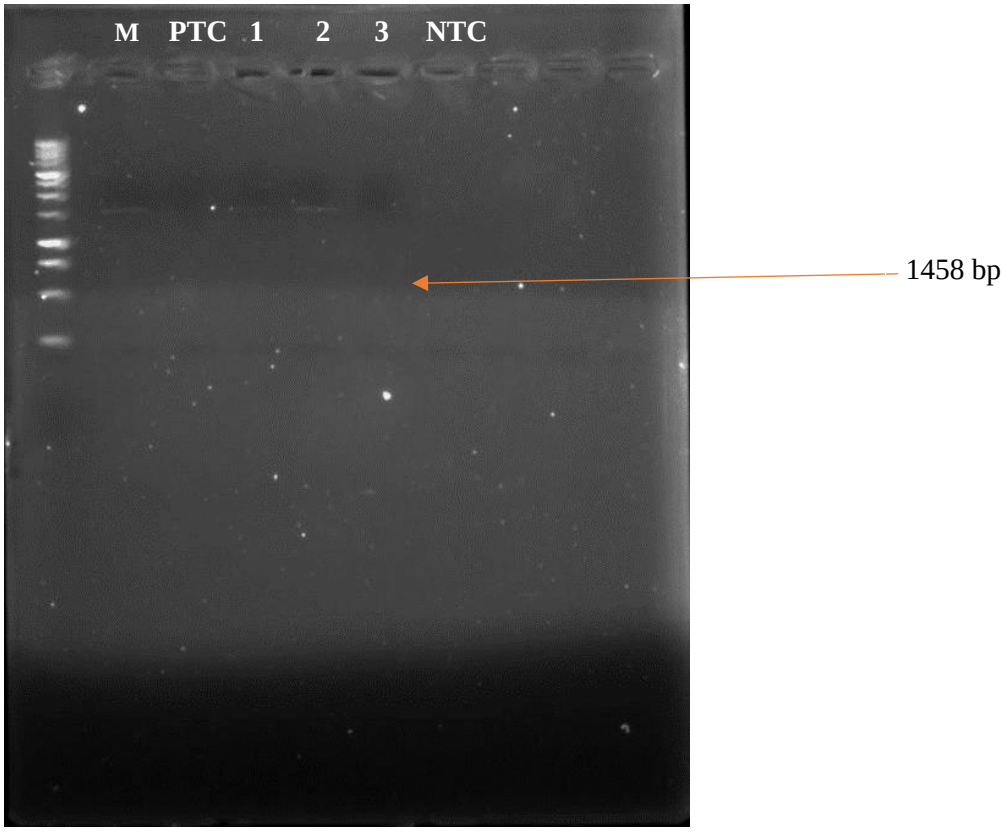
Table 2: Thermal cycling condition of polymerase chain reaction for each gene

Target gene	Initial denaturation	Denaturation	Annealing	Extension	Final Extension	No. of Cycles
aflD	94 ºC for 3 min	94 ºC for 30 s	61.4 ºC for 40 s	72 ºC for 30 s	72 ºC for 7 min	34
aflR	95 ºC for 2 min	95 ºC for 30 s	58 ºC for 45 s	72 ºC for 90 s	72 ºC for 7 min	34

Detection of specific regulatory genes *aflR* and *aflD* involved in the biosynthesis of AF using PCR

The a*flD*, and *aflR* genes were detected by PCR using two sets of primers. Figure 1 A-C depicts the electrophoresis picture of PCR products obtained for each target gene. The bands corresponding to *aflD*, and *aflR* gene could be seen at 702 bp and 1458 bp.





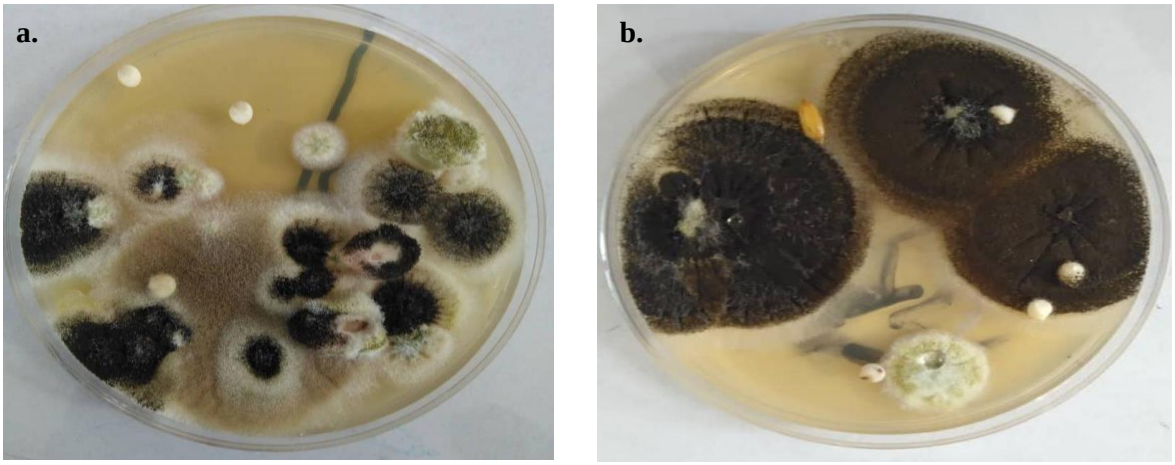
aflR (C) (1458 bp, lane 2 and 3). Lane M indicates DNA ladder size marker(1Kb)

Figure 1: Detection of *aflD* and *aflR* genes in genomic DNA extracted from *Aspergillus* species using a separated primer set. The amplified PCR products were analyzed by 1.2% agarose gel electrophoresis. The specific band corresponding to expected molecular size of *aflD* (A) (702 bp, Lane 1,2,3,5 and6), *aflD* (B) (702 bp, lane 1,2,3,4 and 7). *aflR* (C) (1458 bp, lane 2 and 3) were detected. Lane M indicates the 250 bp (A and B) and 1 Kb (C) DNA ladder size marker

RESULTS AND DISCUSSION

Isolation and Charaterization of Fungal Species from Grains

Plate 1 shows the macroscopic characteristics of the fungal species isolated from stored grains and open market grains on PDA. The microscopic characteristics of the fungal species isolated from stored grains and open market grains are shown on Plate 2.



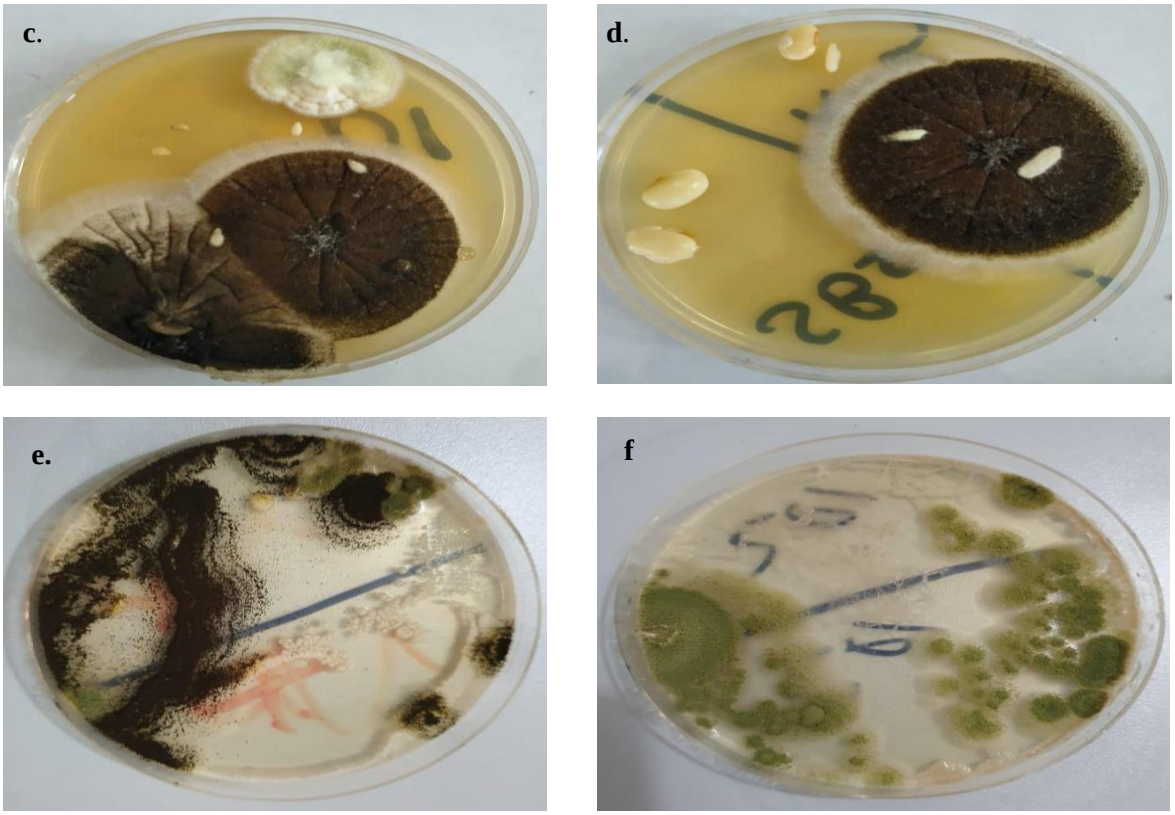
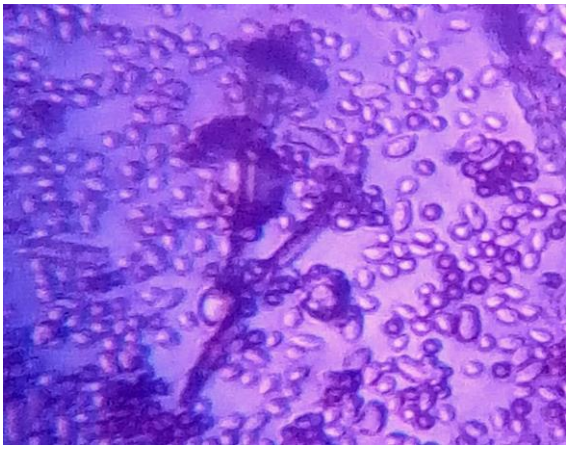


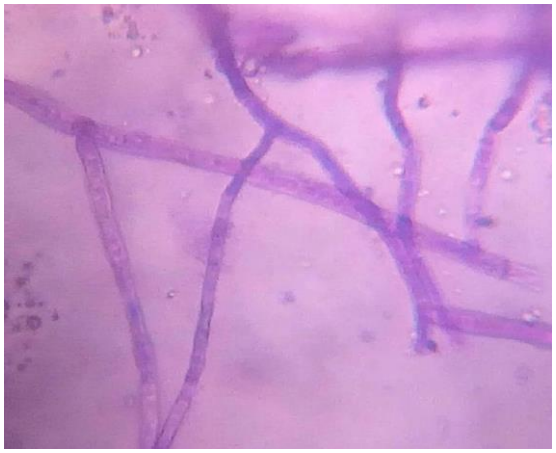
Plate 1: Macroscopic characteristics of fungal isolates from grains

Distribution and Occurrence of fungal isolates from the Grains

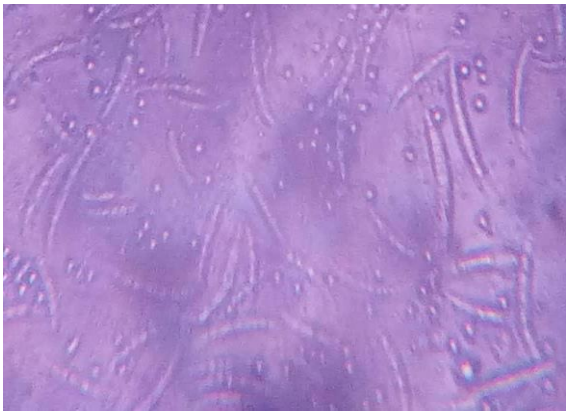
The distribution and occurrence of fungal isolates from the stored and open market grains is illustrated in Table 1. Fungi species were not isolated from soybeans, wheat, barley and biski. *Aspergillus flavus* and *Aspergillus fumigatus* were isolated from millet sample from open market while *Aspergillus flavus*, *A. niger* and *A. parasiticus* were isolated from stored white millet sample. *Fusarium* sp. and *A. niger* were isolated from brown beans.



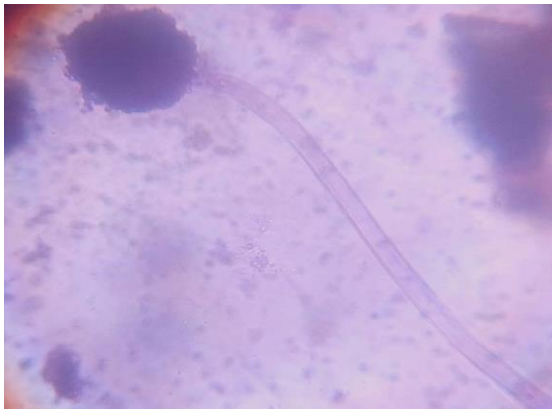
a. *Botrytis* sp.



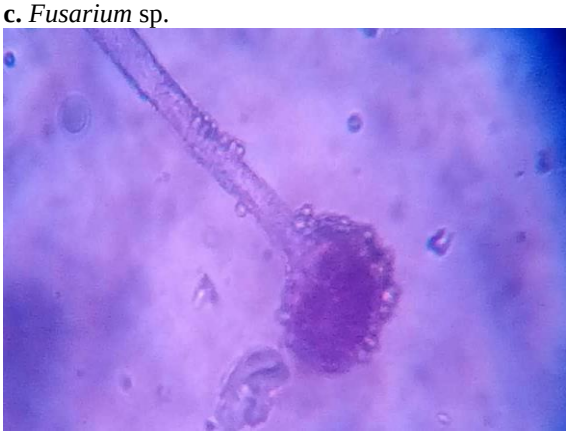
b. *Rhizoctonia* sp.



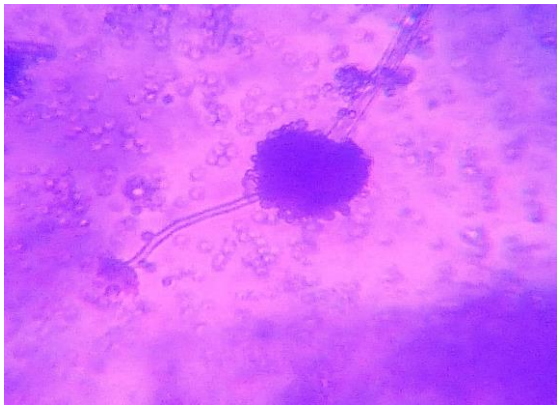
c. *Fusarium* sp.



d. *Aspergillus fumigatus*



e. *Aspergillus flavus*



f. *Aspergillus fumigatus*

Plate 2: Microscopic characteristics of fungal isolates from grains

Frequency and percentage of detection of fungal species in stored and open market grains

Aspergillus flavus had the highest frequency of detection in both stored grains (4, 28.57%) and open market grains (2, 40.00%). *Fusarium* sp. was not detected in stored grains while *A. niger*, *A. parasiticus* and *Rhizoctonia* sp. were not detected in open market grains (Figure 1).

Table 1: Distribution and Occurrence of fungal isolates from the grains

Grain type	Fungal Isolate													
	Fusarium sp.		*A. flavus*		*A. fumigatus*		*Rhizoctonia* sp.		*Botrytis* sp.		*A. niger*		*A. parasiticus*	
	SGs	OMGs	SGs	OMGs	SGs	OMGs	SGs	OMGs	SGs	OMGs	SGs	OMGs	SGs	OMGs
Millet	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Rice	0	0	0	1	0	0	0	0	0	1	0	0	0	0
White maize	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Soyabeans	-		-		-		-		-		-		-	
Barley	-		-		-		-		-		-		-	
Wheat	-		-		-		-		-		-		-	
Sesame	0	0	1	0	1	0	0	0	1	0	0	0	0	0
Groundnut	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Biski	-		-		-		-		-		-		-	
Brown beans	0	1	0	0	0	0	0	0	0	0	1	0	0	0
White beans	-		-		-		-		-				-	
Red sorghum	0	0	0	0	0	0	0	0	0	0	0	0	1	0
White sorghum	0	0	1	0	1	0	0	0	1	0	0	0	0	0
White millet	0	0	1	0	0	0	0	0	0	0	1	0	1	0

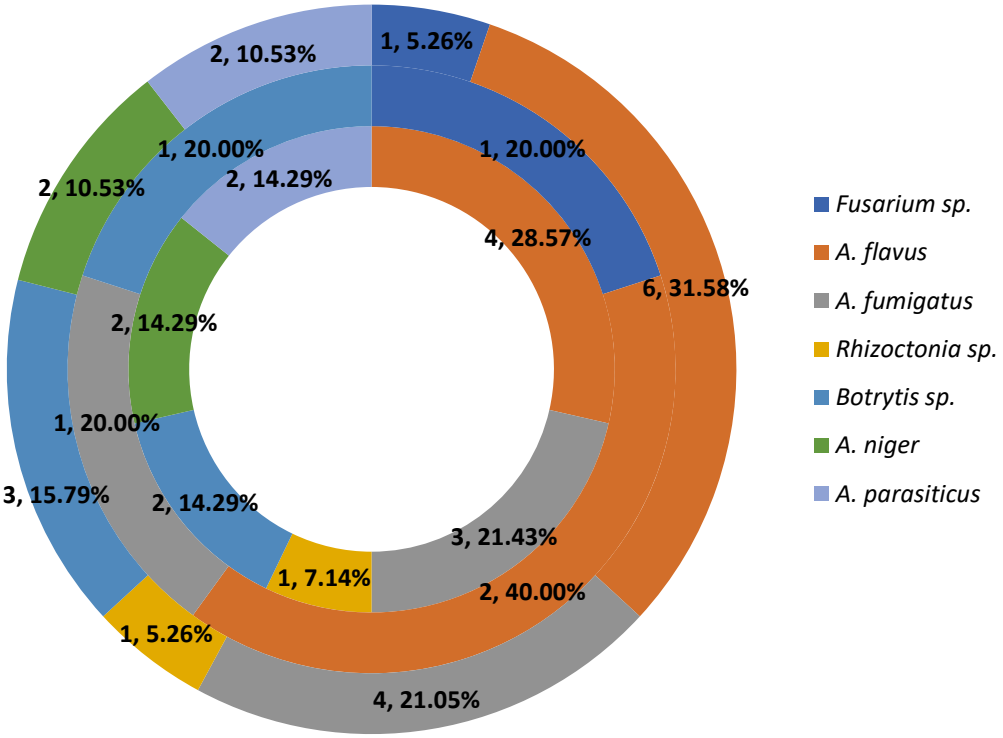
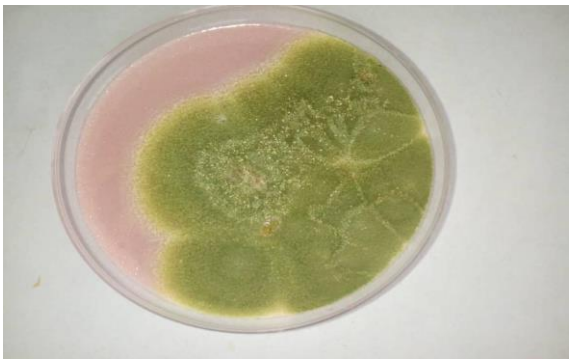


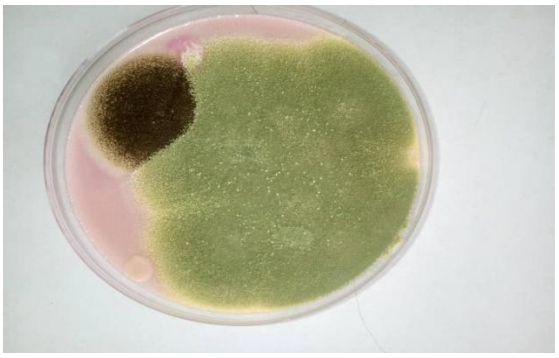
Figure 2: Frequency and percetage of detection of fungal species in stored and open market grains Inner ring, middle ring and outer ring represent frequency and percetage of fungal species in stored and open market grains, in stored grains only and in open market grains only respectively.

Screening of the Fungal Isolates for Aflatoxin Production

Plate 3 shows the macroscopic characteristic of the fungal isolates on Desiccated Coconut Agar. Blue fluorescence of the aflatoxigenic fungal isolates under UV light irradiation at 340mm is presented in Plate 4.



(a)



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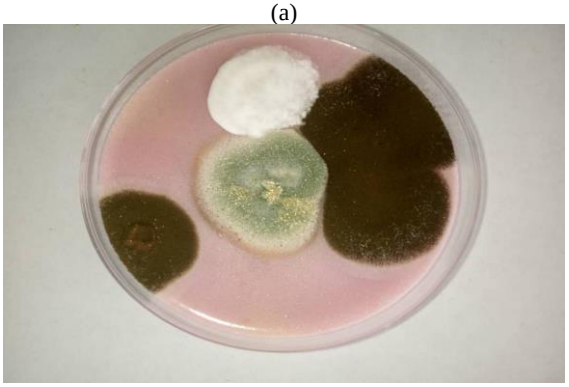
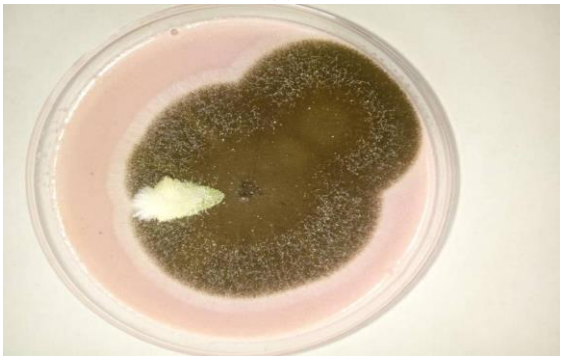
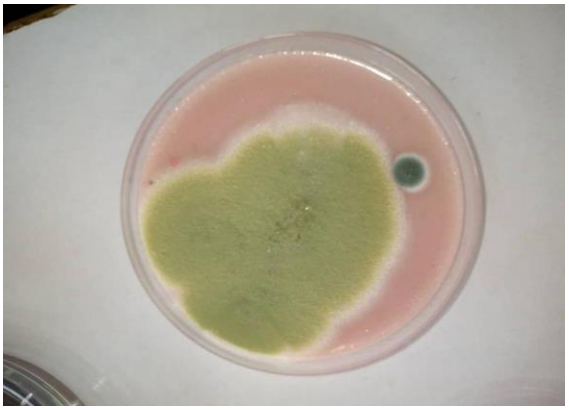


(c)

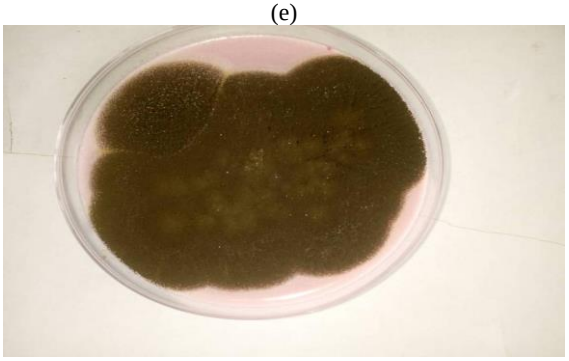


(d)

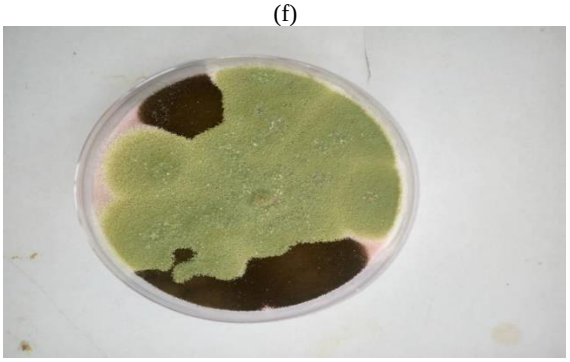


(e)

(f)



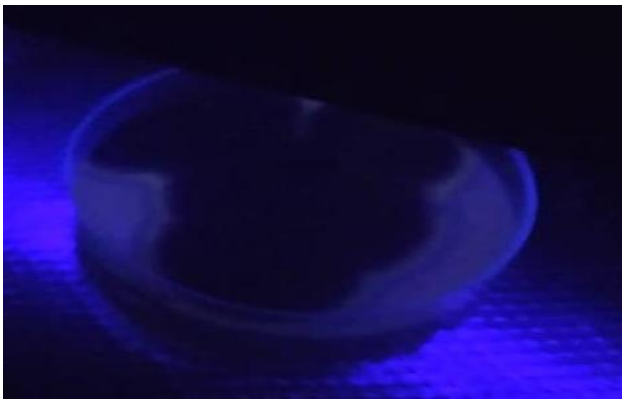
(g)



(h)

Plate 3: Macroscopic characteristic of fungal isolates on DCA

(a)



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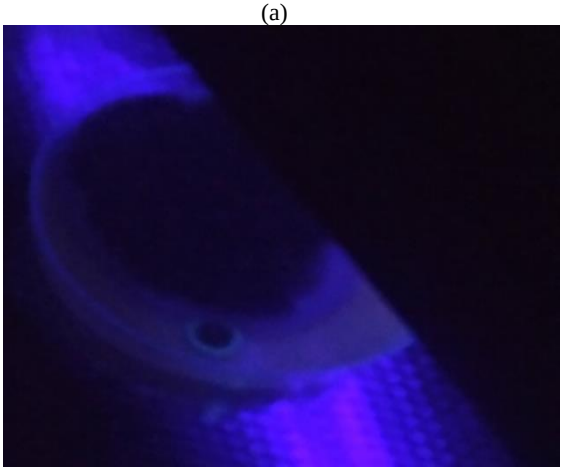

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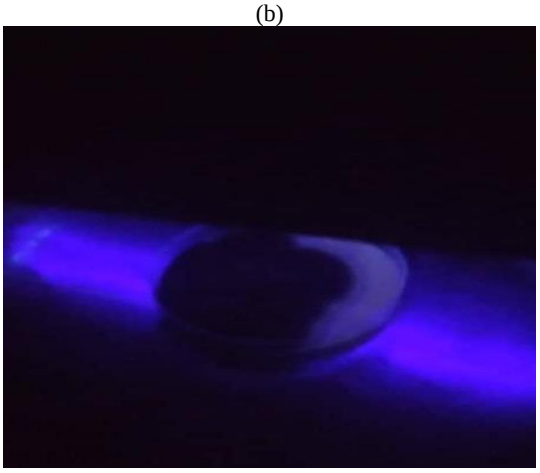

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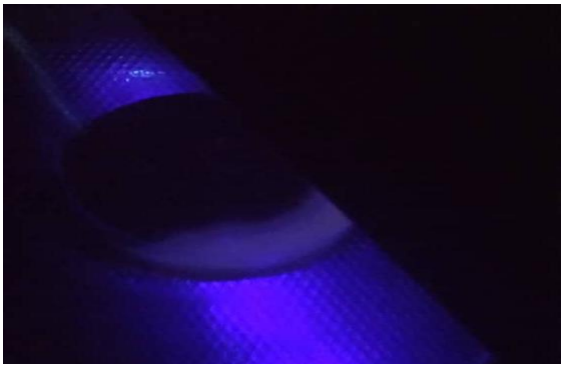

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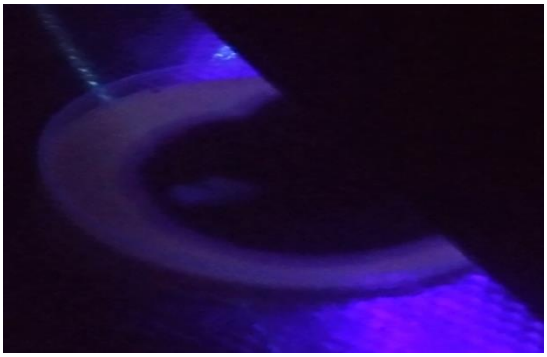

(f)

Plate 4: Blue florescence of aflatoxigenic fungi under UV light irradiation at 340mm

Distribution of Aflatoxigenic Fungi Isolated from Grains and Fluorescence Intensity Yield DCA under UV Light

Table 2 represents the distribution of aflatoxigenic fungi isolated from grains and fluorescence intensity yield DCA under UV light. None of the fungal species isolated from ground nut (0/1), white millet (0/3) and brown beans (0/1) were found to be aflatoxigenic. One isolate each from white sorghum (1/3), millet (1/2), white maize (1/2), red sorghum (1/1) and rice was found to be aflatoxigenic.

Table 2: Distribution of Aflatoxigenic fungi isolated from grains and fluorescence intensity yield DCA under UV light

Grain Type	No.	Toxigenic Species	Species associated with grains		Intensity of Fluorescence
		n %	Stored grains	Open market grains	
White sorghum	3	1 (33.33)	*A. flavus* *A. fumigatus* *Botrytis* sp.		+++
Millet	2	1(50.00)		*A. flavus A. fumigatus*	++
Sesame	3	1 (33.33)	*A. flavus* *A. fumigatus* *Botrytis* sp.		+++
Rice	2	0 (0.00)		*A. fumigatus* *Botrytis* sp.	++
White maize	2	1 (50.00)	*A. flavus* *A. fumigatus*		+++
Groundnut	1	0 (0.00)		*A. niger*	-
Brown beans	1	0 (0.00)	*A. niger*		-
Red sorghum	1	1 (100)	*A. parasiticus*		+
Soyabean	Nd		Nd	Nd	Nd
White millet	3	0 (0.00)	*A. flavus* *A. parasiticus* *A. niger*		++

Table 3: Comparison of the result of conventional and molecular assay in terms of aflatoxin production

Number	Species isolated	Source	DCA fluorescence	Detection by PCR	
				aflD	***aflR***
1	*A flavus*	White sorghum	+++	+	+
2	*A flavus*	sorghum	++	+	+
3	*A parasiticus*	Gnut	++	+	+
4	*A parasiticus*	Millet	++	+	+
5	*A fumigatus*	Rice	++	+	+
6	*A fumigatus*	White maize	++	+	+
7	*A niger*	Brown beans	-	-	-
8	*A niger*	White beans	-	-	-
9	*A orizea*	G/nut	-	+	+
10	*A orizea*	G/nut	-	+	+
11	*A canious*	Soya beans	-	+	+
12	*A canious*	Biski	+	+	+
13	*Fusarium spp*	Sesame	++	+	+
14	*Fusarium spp*	Red sorghum	++	+	+

Key: DCA fluorescence: +++ (Strong fluorescence), ++ (medium fluorescence), + (mild fluorescence), PCR: +(positive), -(negative)

Discussion

At least one fungal contaminant was detected in all the grain samples screened with the exception of soya beans, barley, wheat, biski and white beans. The climatic (warm to hot) conditions of Potiskum and other parts of Nigeria in addition to the poor agricultural practices favour the growth of molds and could be the reason incidence of fungal contaminants in grains (Abdus-Salaam *et al.,* 2016; Ezekiel *et al.,* 2020; Makun *et al.,* 2011). Mold contaminants in grains and other food products are recognized as significant contributor to the challenges of food safety and food insecurity globally (Avery *et al.,* 2019). The predominant fungal genus isolated from stored and open market grains in this study is *Aspergillus.* Similar to this finding, Ezekiel *et al.* (2020) and Ekpakpale *et al.* (2021) also report that *Aspergillus* is the predominant genus in grains. This is however contrary to the finding of Xing *et al.* (2017) and Ekwomadu *et al.* (2018), who observed that *Fusarium* is the predominant genus in maize.

Aspergillus flavus (6, 31.58%) and *A. fumigatus* (4, 21.05%) were the most prevalent fungal species in stored and open market grains in this study. So also, *A. niger* (2, 14.29%) and *A. parasiticus* (2, 14.29%) were detected in stored grains only while *Fusarium* species (1, 20.00%) was isolated from open market grains only. This has health implication in human and animals as a result of the mycotoxin production by *Aspergillus parasiticus and Apergillus flavus*. Higher incidence of *A. flavus* observed in this study might be linked to its ability to produce many enzymes that allows it to survive and degrade the grains. This observation is in line with the report of other researchers in Nigeria who reported *A. flavus* as the predominant species in grains and other food samples (Makun *et al.,* 2011; Ezekiel *et al.,* 2016; Oyedele *et al.,* 2017; Ekpakpale *et al.* (2021). However, in contrast to this finding, *Aspergillus aflatoxiformans* was reported as the predominant species in grains from Nasarawa and Niger state by Ezekiel *et al.* (2020).

Aspergillus flavus and *A. fumigatus* were isolated from stored maize; this is similar to the report by Hassan *et al* (2014) who also isolated *A. flavus* and *A. fumigatus* from stored maize collected from selected Iraqi governorates. However, contrary to their findings, *A. niger Fusarium* sp. and *Rhizoctonia* sp. were not isolated from stored maize in this study.

Five of the fungal isolates comprising of four *A. flavus* and one *A. parasiticus* were found to be aflatoxigenic. This finding is in line with the assertion of Mahuku *et al.,* 2019 and Sserumaga *et al*., 2020 who reported that *A. flavus* and *A. parasiticus* are the primary aflatoxins producing species among the 18 aflatoxins producing species of *Aspergillus* section flavi. While high levels of aflatoxins B and G are produced by *A. parasiticus* consistently, *A. flavus* only produces aflatoxins B at variable levels (Falade *et al.,* 2022). Another study by Diedhiou *et al* (2009), indicates that *Aspergillus flavus* is common colonizer of all types of grains at the time of harvest and during storage.

The occurrence of aflatoxigenic fungal isolates in grains is worrisome because aflatoxins are source of serious health threats and public health concern (Baranyi *et al*., 2013; Sserumaga *et al*., 2020). Their presence might be linked with improper processing practices and poor storage conditions.

The *A. niger* (2, 14.29%) and other non-aflatoxigenic moulds isolated from store and at open market in this study is an indication of poor harvest management practices, there presence could be an important indication of biodeterioration which will lead to poor food hygiene. The absence fungal contamination in Soya beans (0, 0.0%) and white beans (0, 0.0%) is an indication of non-susceptibility of the crops to aflatoxigenic contamination as compared to other crops such as maize, sorghum and millets. Similar study in Nigeria by Ezekiel *et al* (2013), reported that maize grains are predominant affected by aflatoxigenic fungi than leguminous crops.

Among the genes involved in the pathway of aflatoxin biosynthesis, *aflD* gene plays a vital role in the conversion of nosolorinic acid into averantin. *aflR* gene on the other hand plays a crucial role in the regulation of other genes involved in the pathway (Sohrabi and Taghizadeh, 2018). In this present study, 31.58% (n=6) of aflatoxigen producing *Aspergillus* species had consistent results regarding aflatoxin production on DCA and PCR detection. There was a complete correlation between the results of aflatoxin production and detection of the target genes. Similarly, 14.29% (n=2) of non aflatoxin producing strains had negative fluorescence and no bands in PCR reaction for the target genes. This finding is in line with those of the previous studies (Dolei *et al.,* 2015; Sohrabi and Taghizadeh, 2018). Yang *et al*. (2004) developed an optimized multiplex PCR condition for rapid detection of potential aflatoxigenic fungi in fermented foods and grains using avfA, omtA, and ver-1 genes. Hussain *et al.* (2015) reported that the detected of *Aspergillus* structural genes (i.e., Nor-1 and Ver-1) and regulatory gene (i.e., *aflR*) could be used as an early indicator of aflatoxin production. In this current study, we observed that most isolates positive for *aflR* gene were also positive for *aflD* gene. It was observed that *aflR* and *aflD* genes were amplified in all *A. flavus* and *A. parasiticus* isolates, suggesting that the presence of these genes is linked for aflatoxin production. This finding showed that these genes account for the aflatoxin production in the organisms. In this study some non-aflatoxigenic strains such as *A. canious* and *A. orizae* showed negative results in the conventional methods but had at least one of the *aflD*, or *aflR* genes as seen in table 4.5. Results of this study indicate that non production of aflatoxin in some aflatoxigenic species could be due to mutation (deletion or base replacement) of *aflR* or *aflD* genes (Sohrabi and Taghizadeh, 2018).

CONCLUSION

Agricultural commodities stored at various conditions in this study have significant levels of contamination by various species of aflatoxigenic fungi, attention should be paid to intrinsic factors such as drying to safe moisture level, aeration, prevention of insect damage and storage space to avoid fungal invasions which causes economic losses

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