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Isolation of Fungal Species and Aflatoxin Detection from Grinded Cereals Sold in Dutsin-Ma Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aflatoxins are the most common and potent mycotoxins produced mainly, by *Aspergillus* species, often found contaminating cereals. This study was aimed at isolating fungal species and detecting aflatoxins from grinded cereals sold in Dutsin-ma metropolis of Katsina State, Nigeria. Forty (40) cereal samples (Maize, Millet, Sorghum and Wheat), 10 of each were collected randomly from 3 different locations in Dutsin-ma metropolis. Standard microbiological procedures were used to isolate and identify fungal species on Potato Dextrose Agar (PDA) and fungal ATLAS [1,2] respectively. The predominant fungi isolated were species of *Aspergillus, Mucor* and *Fusarium*. *Aspergillus niger* had the highest incidence of 32.1%, *Aspergillus flavus* had 20.2%, followed by *Mucor* spp. with 13.1%, *Aspergillus fumigatus* with 11.9%, *Aspergillus parasiticus* had 8.3%, then *Aspergillus lentulus* and *Fusarium oxysporum* had 7.14% each. Presence of Aflatoxins were tested

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(out of the four aflatoxin types) in the samples using High-Performance Liquid Chromatography (H.P.L.C) (UV-VIS detector) by comparing the retention time of standards to the time on the peaks obtained from the samples during the HPLC analysis. Aflatoxins were detected in all three samples that were tested with the maize sample having the highest % height of B1 (48.10%), Millet had the highest % height of G1 (29.88%). G2 was not detected on the millet samples. All the other 2 samples had all three aflatoxins detected. Percentage height of aflatoxin peaks ranged from 16.60% – 48.10. This study revealed high contamination of grinded cereals by species of *Aspergillus*, *Mucor* and *Fusarium*. Aflatoxins B1, G1 and G2 were also detected, which makes their consumption a public health risk. Proper pre-harvest and post-harvest management of cereals coupled with proper cooking of cereal products before eating are recommended so as to reduce contamination and prevent possible intake of these toxins.

Keywords: Grinded cereals; fungal species; aflatoxin; dutsin-ma; HPLC.

1. INTRODUCTION

Grasses producing grains for consumption are called cereals, they are the most staple food in the world [3]. They are consumed widely for their nutritional complementary value in Africa and are known to be a remedy for malnutrition, though they are highly prone to Mycotoxin contamination [4].

Fungi are plant pathogens mostly found on every plant, so they cause spoilage to foodstuff and foods. They reduce yield in plants and affect quality of the plant produce thereby causing significant economic loss. This contamination when caused by mycotoxins which are secondary metabolites can be poisonous when consumed with grains and cereals [5]. Fungal infestation can lead to discolouration, production of off-odours and can result in mycotoxins contamination [6].

Aflatoxins are class of mycotoxins produced as secondary metabolites of mold belonging to the genus *Aspergillus* that contaminates nuts that are stored improperly. The species responsible for this contamination are mainly *Aspergillus flavus* and *Aspergillus parasiticus.* Studies later revelated that other *Aspergillus* species could also produce aflatoxins underfavourable conditions. The species of *Aspergillus* include: *A. stamari*, *A. nominus* and *A. pseudotamari* [7]. Aflatoxins are usually found hidden as a slow poisonous substance in human biological pathways and are known for their adverse effects especially in young children where it might lead to developmental delay, damage to liver, stunted growth and liver cancer [8]. The unpredictable behavior and climate conditions of the fungus unfortunately makes it very difficult to eradicate these fungi and subsequently the toxins [9]. There are four major aflatoxins commonly isolated from foods which include aflatoxin B1, B2, G1 and G2. There is also aflatoxin M1 and M2 which are considered to be hydroxylated metabolites of aflatoxin B1 and B2 respectively and are found in milk products and milk producing animals [10].

The health hazards of Aflatoxins and other mycotoxins with their significant economic importance affect developing countries especially those with adequate food storage capabilities is of enormous concern. To curtail this issue, the international trade and animal and human health monitors fungal and Mycotoxin contamination periodically so as to meet international and national mycotoxin regulatory standards [5].

Therefore, the present study was aimed to identify fungal species and detect the presence of aflatoxins in grinded cereals.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Dutsin-ma town, Dutsin-ma local government area of Katsina State, with coordinates of $12^{o}27^{l}18^{ll}$N, $7^{o}29^{l}29^{ll}$E. The experiment was conducted in the Microbiology Laboratory, Federal University Dutsin-Ma, Dutsin-Ma Local Government Area of Katsina State, Nigeria. The local government has an area of 527 km^2 and a population of 169, 671 people as of 2006 census [11]. It is inhabited mostly by Hausa and Fulani speaking tribes and most of them are involved in farming and rearing of animals.

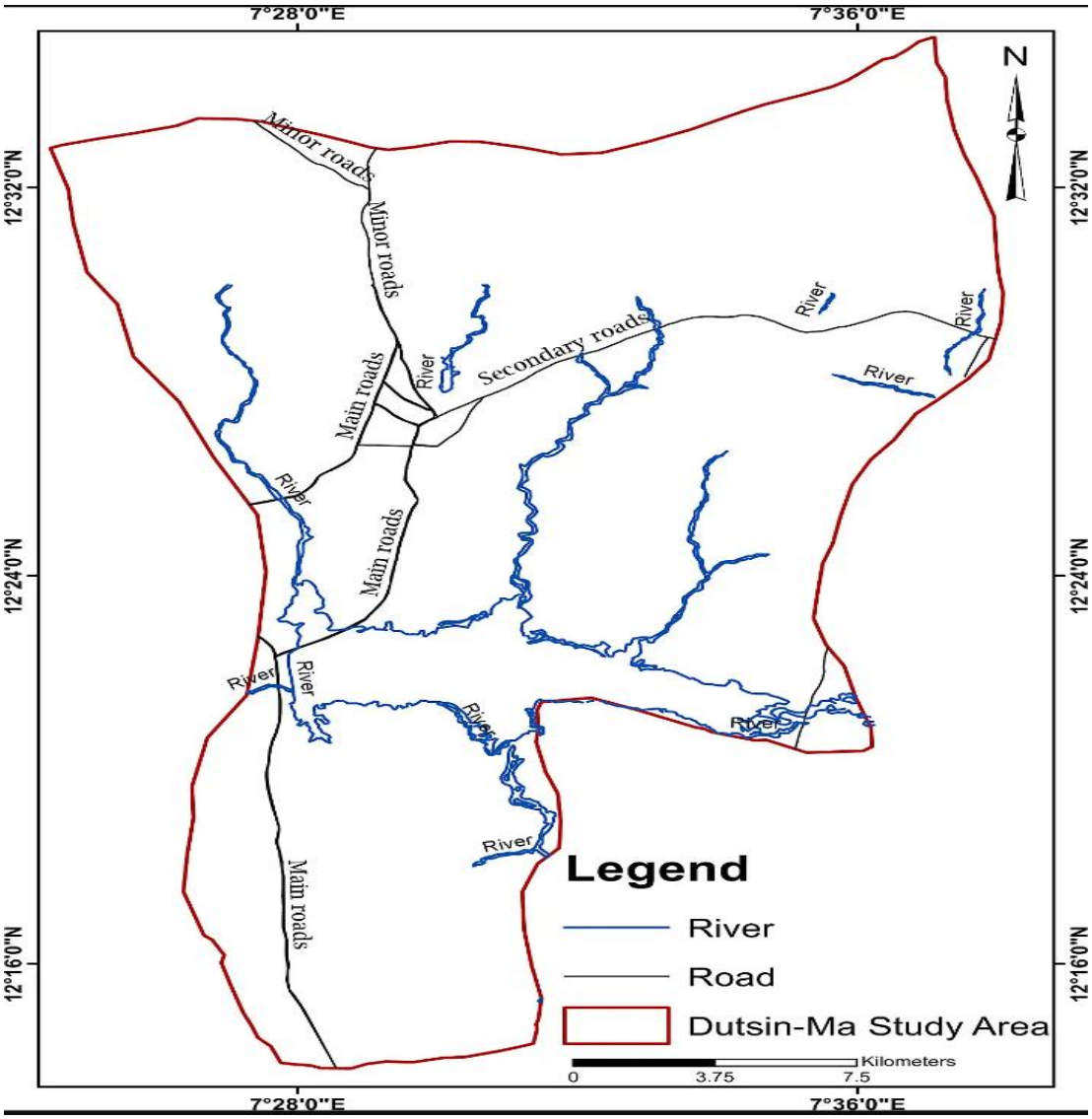

Fig. 1. Map showing Geographical Location of Study Area, Dutsinma of Katsina State

2.2 Sample Collection

Forty samples of grinded cereals in total were collected randomly from three different locations in Dutsin-ma local government area of Katsina state. The locations are: Wednesday market, Hayin gada and Hospital road. The samples were collected for two weeks from each location making a total of forty (40) samples and stored aseptically in sterile polythene bags.

2.3 Isolation and Identification of Fungi

Total 1g of each sample was placed in separate test tubes containing 9ml peptone water. The test tubes were then shaken and 1 ml of the preparation was transferred into petri dishes containing potato dextrose agar (PDA) medium containing streptomycin (100ppm) for each sample [12]. The petri dishes were then incubated at room temperature for 5-7 days for possible fungal growth. After incubating for about 7 days, the growth rate and other colonial characteristics of each sample were examined macroscopically. After 7 days, individual colonies were sub-cultured on PDA plates by picking and streaking the colonies using sterile wire loop on already prepared PDA plates.

2.4 Microscopy

A drop of Lacto-phenol cotton blue was placed on a clean glass slide and a portion of the fungal colony was collected aseptically using a sterile

needle and covered with a cover-slip. The prepared slide was observed under microscope by using X10 and X40 objective. Morphological characterization was done based on morphologcal characters and compared with standard reference keys (ATLAS) for possible identification [13].

2.5 Aflatoxin Detection

The high-performance liquid chromatography (HPLC) method was used to detect and quantify the aflatoxin present in the grains. All solvents used in HPLC experiments were “HPLC grade”. An HPLC instrument (Shimadzu Prominence) equipped with a UV–VIS detector (SPD-20-AV), Aproma (Promasil) C18 250 mm×4.6mm, 5 µm pores column and CTO-20AC column oven. Sample was eluted using an isocratic mode at wavelength of 273 nm. A Mixture of methanol: water 50:50 (%, v/v) was used as the mobile phase with a flow rate was set to 0.8 ml/min and the injection volume was 20 µL while column temperature was maintained at 40 ◦C. Aflatoxins were identified by matching retention time (RT) of sample peaks with standard peaks retention time. Samples were analysed using the same HPLC chromatographic conditions. Three of the four types of aflatoxins were detected (B1, G1 & G2).

2.6 Standard Preparation

Aflatoxin standards, B1, G1 and G2 were obtained from Sigma Aldrich. Mixed standard was prepared by weighing 1 mg of each standard was mixed with 10 ml toluene/acetic acid (99:1, v/v) in a clean beaker. Working standard solutions were prepared by diluting the stock standard solution with methanol/acetonitrile (50:50, v/v).

2.7 Sample Extraction and Cleanup of Column

Ten (10) grams of the test sample was extracted with 40 mL methanol and water (80:20, v/v) solution by shaking vigorously for 30 min in a sealed flask. One gram of sodium chloride (NaCl) and 20 mL of n-hexane were added prior to the extraction. The extract was filtered through Whatman No. 5 filter paper. The filtrate was centrifuged for 15 min at 4000 x g and the upper hexane phase was discarded while the lower methanol phase was used for column cleanup. An aliquot of 4 mL of the filtrate was diluted to 20 mL with phosphate-buffered saline (PBS) solution pH 7.4. After conditioning the column with 8 mL PBS, 20 mL of the diluted filtrate was passed through it at a flow-rate of 1.2 mL/min. The column was washed with 50% methanol thereafter.

3. RESULTS

Colonies. The results of present study revealed that Out of the 40 grinded cereal samples collected and analyzed, 35 (87.5%) tested positive for various fungal speciesbelonging to the genera *Aspergillus, Mucor* and *Fusarium.* The samples, the colony characteristics, microscopy and the possible colony of the observed fungal colonies are presented in Table 1.

It was observed that all maize samples (10 samples) were contaminated with at least one fungal species, while, the Millet samples had the least contaminations with most of the samples having just 2 different fungal contaminants. *Aspergillus niger* and *Aspergillus flavus* were the most occurring species among the different samples while, *Fusarium oxysporum* had least occurrence.

Out of the 40 samples of grinded cereals analyzed, fungal species occurred 84 times in the 35 samples that tested positive for fungal species, of which, *Aspergillus niger* had the highest occurrence on 27 with an incidence of 32.1% followed by *Aspergillus flavus* (an aflatoxin producing species) with 17 (20.2%). *Mucor* spp*., Aspergillus fumigatus*, *Aspergillus parasiticus* (another aflatoxin producing species) are next with 11 (13.1%), 10 (11.9%) and 7 (8.3%) respectively. *Fusarium oxysporum* and *Aspergillus lentulus* had the lowest incidence among the seven most abundant species isolated with occurrence of 6 and incidence of 7.14% each (Table 3).

Table 4 shows the retention time of the aflatoxin standards B1, G1 and G2 having 7.100, 5.949 and 5.368 respectively.

The result of the detected aflatoxins with their percentage (%) heights showed that B1 had the highest % height in all three samples. The % height ranged from 43.39% – 48.10%, followed by G2 with a % height range ranging from 20.24% – 20.68% and was detected in only two of the three samples (sample 5 & 37). G1 had the lowest % height range of 16.60% – 19.23%.

Table 1. Macroscopic and Microscopic characteristics of fungal isolates and their possible identity

Sample	Colony colour	Macroscopy shape	Elevation	Micros copy	Possible identity
Ma1	L.G, D.G and black colonies	Oval	Elevated	L.C. on aerial and septate hyphae.	*A. flavus, A. parasiticus & A. niger*
Ma2	L.G & white colonies3	Oval, irregular	Elevated	L.C on aerial and septate hyphae.	*A. flavus & Mucor* sp.
Ma3	L.G, black and G.G colonies	Oval	Elevated	L.C, S.C on aerial andseptate hyphae conidiophore.	*A. flavus, A. Niger & A. fumigatus*
Ma4	L.G, black, B.L, W.P and G.G colonies	Oval and irregular	Elevated	L.C on aerial and septate hyphae, short unbranched conidial heads & S.C.	*A. flavus, A. niger Mucor* sp., *A. lentulus, & A. fumigatus*
Ma5	L.G, black, G.G & W.P. colonies.	Oval	Elevated	L.C, S.C on aerial andseptate hyphae conidiophore short unbranched conidial heads	*A. flavus, A. niger & A. fumigatus & A. lentulus*
Ma6	Black & D.G. colonies	Oval	Elevated	L.C. on septate hyphae.	*A. niger and A. parasiticus.*
Ma7	L.G, black & W.P. colonies	Oval	Elevated	L.C. on septate hyphae & short unbranched conidial heads.	*A. flavus, A. Niger & A. lentulus.*
Ma8	Black, G.G & B.L. colonies	Oval & irregular	Elevated	L.C & S.C on septate hyphae.	*A. niger, A. fumigatus & Mucor* sp.
Ma9	W.F colonies.	Irregular	Elevated	Oval to kidney shaped M.C.	*Fusarium oxysporum.*
Ma10	L.G, black & B.L. colonies	Oval & irregular.	Elevated	L.C on septate hyphae.	*A. flavus, A. niger & Mucor* sp..
M1	Black colonies	Oval	Elevated	L.C on septate hyphae.	*A. niger*
M2	W.F. colonies	Irregular	Elevated	Oval to kidney shaped M.C.	*Fusarium oxysporum.*
M3	L.G & black colonies	Oval	Elevated	L.C on septate hyphae.	*A. flavus & A. niger.*
M4	Black & B.L. colonies	Oval & irregular	Elevated	L.C on septate hyphae.	*A. niger & Mucor* sp.
M5	L.G & black colonies	Oval	Elevated	L.C on septate hyphae.	*A. flavus & A. niger.*
M6	Black & B.L. Colonies	Oval & irregular	Elevated	L.C on septate hyphae.	*A. niger & Mucor* sp.
M7	L.G and B.L colonies	Oval & irregular	Elevated	L.C on septate hyphae.	*A. flavus & Mucor* sp.
M8	W.F. colonies	Irregular	Elevated	Oval to kidney shaped M.C.	*Fusarium oxysporum*
M9	-	-	-	-	-
M10	Black & B.L. colonies	Oval & irregular	Elevated	L.C on septate hyphae.	*A. niger & Mucor* sp.
S1	L.G, black, G.G, D.G.	Oval	Elevated	L.C, S.C on septate hyphae.	*A.flavus, A. niger, A. fumigatus & A. parasiticus*
S2	W.F. Colonies	Irregular	Elevated	Oval to kidney shaped M.C.	*Fusarium oxysporum*
S3	L.G, G.G, black& W.P	Oval & irregular	Elevated	L.C, S.C on septate hyphae & short	*A. flavus, A. niger, A. fumigatus & A.*

Sample	Colony colour	Macroscopy shape	Elevation	Micros copy	Possible identity
	colonies			unbranched conidial heads.	*lentulus.*
S4	L.G, black & D.G colonies	Oval	Elevated	L.C on septate hyphae.	*A. flavus, A. niger & A. fumigatus.*
S5	Black, D.G, B.L colonies	Oval & irregular	Elevated	L.C on septate hyphae.	*A. niger, A. parasiticus & Mucor* sp.
S6	W.F. colonies.	Irregular	Elevated	Oval to kidney shaped M.C.	*Fusarium oxysporum*
S7	L.G, black & G.G colonies	Oval	Elevated	L.C & S.C on septate hyphae.	*A. flavus, A. niger & A. fumigatus*
S8	L.G, black, D.G & B.L colonies	Oval	Elevated	L.C on septate hyphae.	*A. flavus, A. niger, A. parasiticus & Mucor* sp.
S9	Black, G.G & B.L colonies	Oval & irregular	Elevated	L.C, S.C on septate hyphae.	*A. niger, A. fumigatus & Muco* sp.
S10	Black colonies	Oval	Elevated	L.C on septate hyphae.	*A. niger.*
W1	–	–	–	–	–
W2	W.P colonies.	Oval	Elevated	Short unbranched conidial heads	*A. lentulus*
W3	Black, G.G & W.P. colonies	Oval	Elevated	L.C, S.C on septate hyphae & short unbranched conidial heads.	*A.niger, A. fumigatus & A. lentulus*
W4	–	–	–	–	–
W5	Black, D.G & B.L. colonies	Oval & irregular	Elevated	L.C on septate hyphae.	*A. niger, A. parasiticus & Mucor* sp.
W6	L.G & black colonies	Oval	Elevated	L.C on septate hyphae.	*A. flavus & A. niger*
W7	L.G, black & G.G colonies	Oval	Elevated	L.C, S.C on septate hyphae.	*A. flavus, A. niger & A. fumigatus.*
W8	W.F. colonies	Irregular	Elevated	Oval to kidney shaped M.C.	*Fusarium oxysporum*
W9	-	-	-	-	-
W10	Black colonies	Oval	Elevated	L.C on septate hyphae.	*A. niger*

Key: *L.G −Light Green, D.G – Dark Green, G.G – Greyish Green, W.P – Whitish - Pink, B.L – Brown− Like W.F− White Filamentous, L.C – Long Conidiophore, S.C – Short Conidiophore, M – Millet, Ma – Maize, S – Sorghum, & – and, W – Wheat*

Table 2. Fungal species isolated from Cereal samples

S/N	Sample	*A. flavus*	*A. niger*	Fungal *A. fumigatus*	Pathogen *A. parasiticus*	*A. lentulus*	*Mucor* spp.	*F. oxysporum*
1	Maize	+	+	−	+	−	−	−
2	Maize	+	−	−	−	−	−	−
3	Maize	+	+	+	−	−	−	−
4	Maize	+	+	+	−	+	+	−
5	Maize	+	+	+	−	+	−	−
6	Maize	−	+	−	+	−	−	−
7	Maize	+	+	−	−	+	−	−
8	Maize	−	+	+	−	−	+	−

S/N	Sample	*A. flavus*	*A. niger*	Fungal *A. fumigatus*	Pathogen *A. parasiticus*	*A. lentulus*	*Mucor* spp.	*F. oxysporum*
9	Maize	−	−	−	−	−	−	+
10	Maize	+	+	−	−	−	+	−
11	Millet	−	+	−	−	−	−	−
12	Millet	−	−	−	−	−	−	+
13	Millet	+	+	−	−	−	−	−
14	Millet	−	+	−	−	−	+	−
15	Millet	+	+	−	−	−	−	−
16	Millet	−	+	−	−	−	+	−
17	Millet	+	−	−	−	−	+	−
18	Millet	−	−	−	−	−	−	+
19	Millet	−	−	−	−	−	−	−
20	Millet	−	+	−	−	−	+	−
21	Sorgh.	+	+	+	+	−	−	−
22	Sorgh.	−	−	−	−	−	−	+
23	Sorgh.	+	+	+	−	+	−	−
24	Sorgh.	+	+	−	+	−	−	−
25	Sorgh.	−	+	−	+	−	+	−
26	Sorgh.	−	−	−	−	−	−	+
27	Sorgh.	+	+	+	−	−	−	−
28	Sorgh.	+	+	−	+	−	+	−
29	Sorgh.	−	+	+	−	−	+	−
30	Sorgh.	−	+	−	−	−	−	−
31	Wheat	−	−	−	−	−	−	−
32	Wheat	−	−	−	−	+	−	−
33	Wheat	−	+	+	−	+	−	−
34	Wheat	−	−	−	−	−	−	−
35	Wheat	−	+	−	−	−	+	−
36	Wheat	+	+	−	−	−	−	−
37	Wheat	+	+	+	+	−	−	−
38	Wheat	−	−	−	−	−	−	+
39	Wheat	−	−	−	−	−	−	−
40	Wheat	−	+	−	−	−	−	−

Key: *Sorgh: Sorghum, A: Aspergillus, +: positive, spp: species, F: Fusarium – : Negative*

Table 3. Incidence of fungal species in grinded Cereals

S/N	Fungal Species	Occurrence	Incidence (%)
1	*Aspergillus flavus*	17/84	20.2*
2	*Aspergillus niger*	27/84	32.1
3	*Aspergillus fumigatus*	10/84	11.9
4	*Aspergillus parasiticus*	7/84	8.3*
5	*Aspergillus lentulus*	6/84	7.14
6	*Mucor* spp.	11/84	13.1
7	*Fusarium oxysporum*	6/84	7.14
	Total	84	100

Note:Differentiates the aflatoxin producing species from the other fungal species*

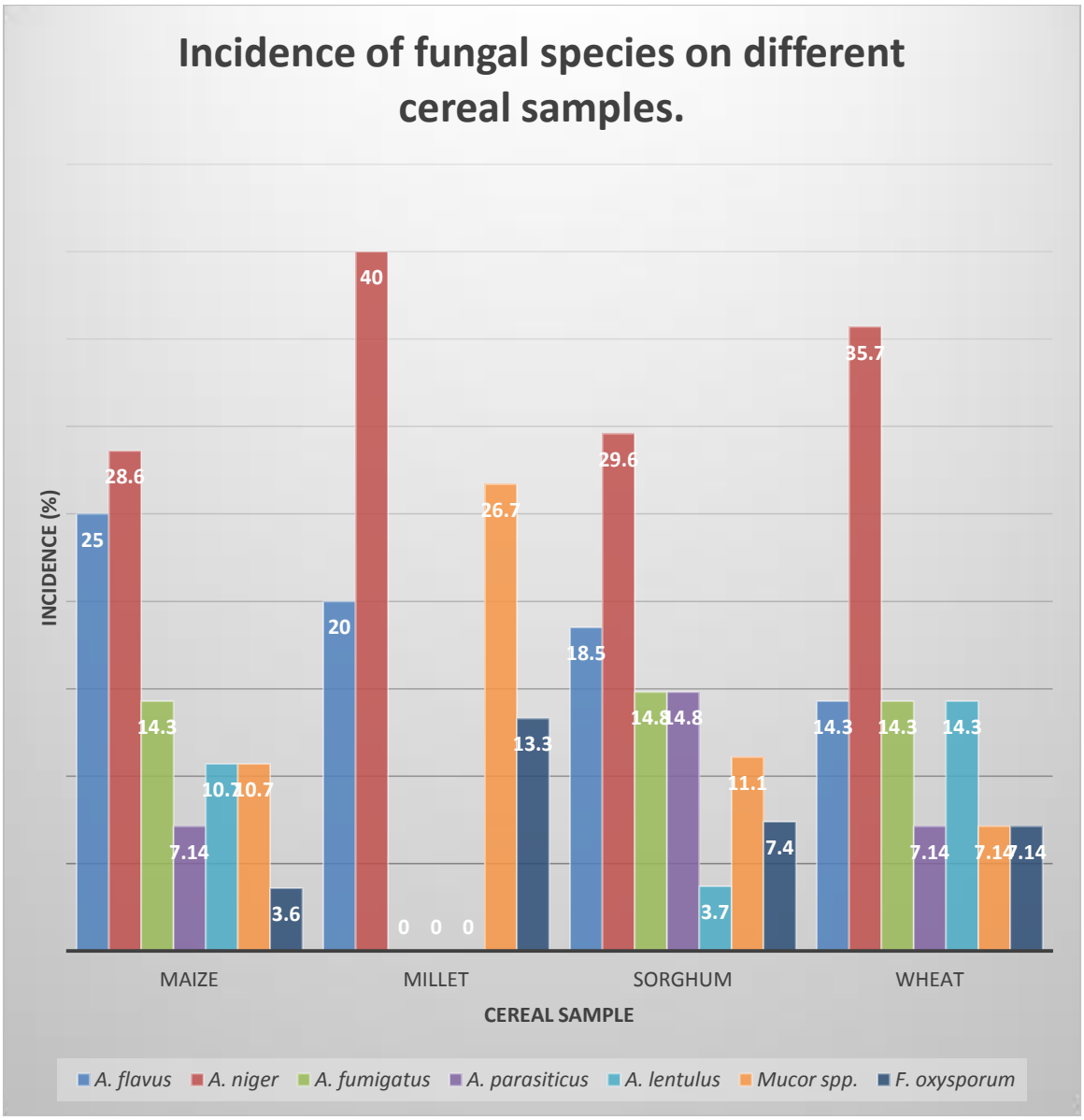

Fig. 2. Incidence of fungal species on different Cereal samples

Table 4. Aflatoxins and their retention times

S/N	Aflatoxins	Retention time (Min.)
1	G2	5.368
2	G1	5.949
3	B1	7.100

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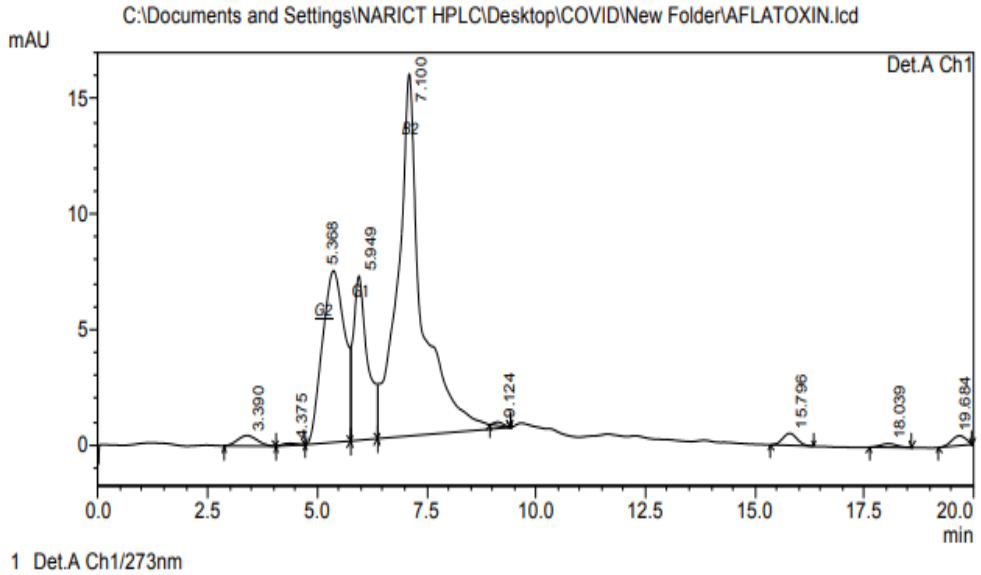


PeakTable

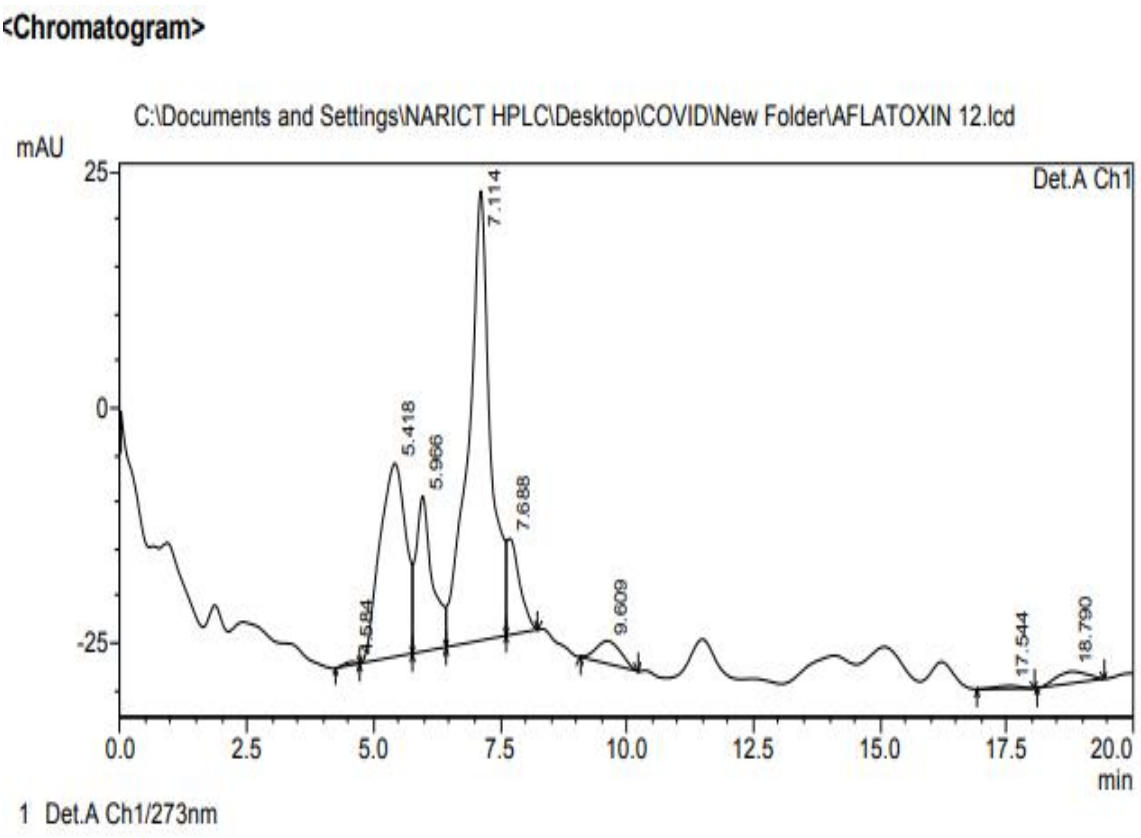
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.390	13812	449	1.280	1.400
2	4.375	2008	97	0.186	0.302
3	5.368	260298	7439	24.126	23.213
4	5.949	164707	7116	15.266	22.204
5	7.100	610332	15693	56.569	48.969
6	9.124	2234	152	0.207	0.475
7	15.796	11991	525	1.111	1.638
8	18.039	4069	154	0.377	0.481
9	19.684	9470	422	0.878	1.317
Total		1078922	32047	100.000	100.000

Fig. 3. Chromatogram of aflatoxin types

Table 5. Detected aflatoxins and their percentage heights

S/N	Sample	Aflatoxin producing specie found	Aflatoxin	%Height
1	Sample 5 (Maize)	*Aspergillus flavus*	B1	48.10
			G1	16.60
			G2	20.68
2	Sample 13 (Millet)	*Aspergillus flavus*	B1	43.52
			G1	29.88
			G2	–
3	Sample 37 (wheat)	*Aspergillus flavus, A. parasiticus*	B1	43.39
			G1	19.23
			G2	20.24



PeakTable

Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.584	5801	329	0.201	0.331
2	5.418	735889	20537	25.485	20.687
3	5.966	369472	16485	12.795	16.606
4	7.114	1448471	47753	50.162	48.102
5	7.688	182509	10223	6.321	10.297
6	9.609	82350	2404	2.852	2.422
7	17.544	10617	304	0.368	0.306
8	18.790	52460	1239	1.817	1.248
Total		2887570	99275	100.000	100.000

Fig. 4. Chromatogram of sample 5

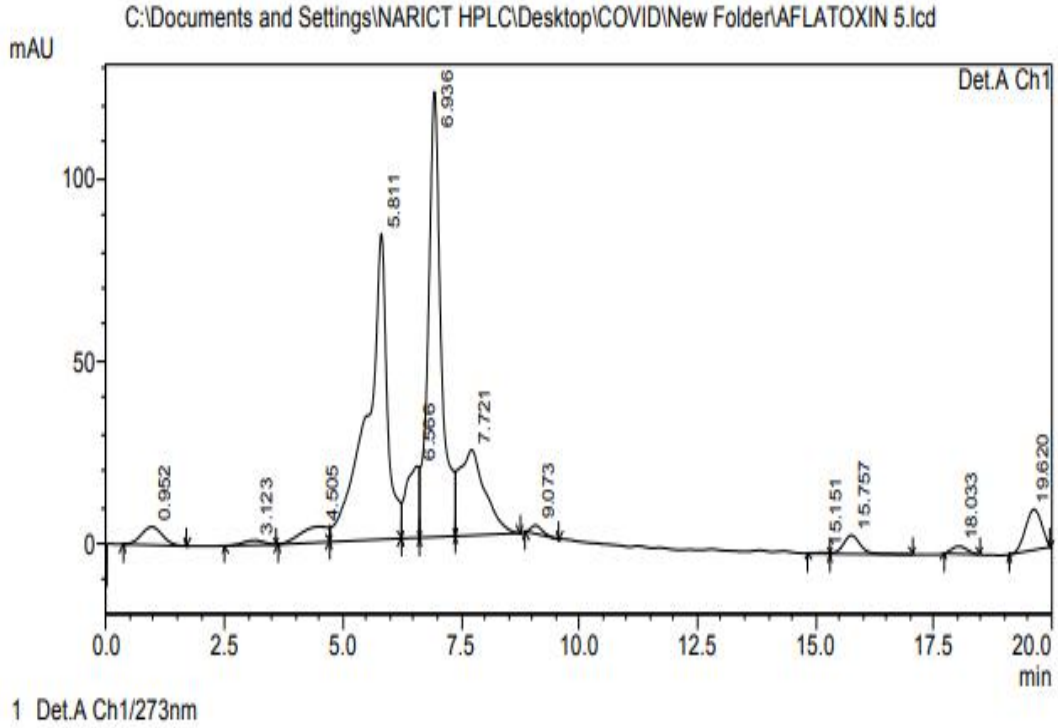
4. DISCUSSION

This study was aimed at determining fungal species and aflatoxin detection in grinded cereals sold in Dutsin-Ma metropolis. Forty samples of four different grinded cereals of maize, millet, sorghum and wheat were examined for fungal contamination and detection of mycotoxins specially aflatoxins. The prevalence of fungal species on grinded cereals was 87.5% (35/40). Fungi isolated include species of *Aspergillus, Fusarium and Mucor. Aspergillus* species were the most occurring with 79.6 % incidence.

Species isolated and identified are: *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus lentulus, Mucor* spp. and *Fusarium oxysporum.*

Aspergillus niger had the highest incidence of 32.1%, followed by *Aspergillus flavus* which is an aflatoxin producing species with 20.2%. *Mucor* spp. had an incidence of 13.1%, while, *Aspergillus fumigatus* had 11.9%, *Aspergillus parasiticus* (8.3%) while, both *Fusarium oxysporum* and *Aspergillus lentulus* had an incidence of 7.1%. Similar results were also repoted by Mzungu et al. [13] who also carried out their research in Dutsin-ma and had a total prevalence of *Aspergillus* spp. to be 60% as to 79.6% in this research. Ojonoka [14] in Enugu got the incidence for *Aspergillus* species to be 33.3% which showed a lower incidence of these species. Muthomi et al. [15] also recorded higher occurrence of *Aspergillus* spp. from grains in semi- arid regions (of which the study area belongs) than the humid regions.

<Chromatogram>



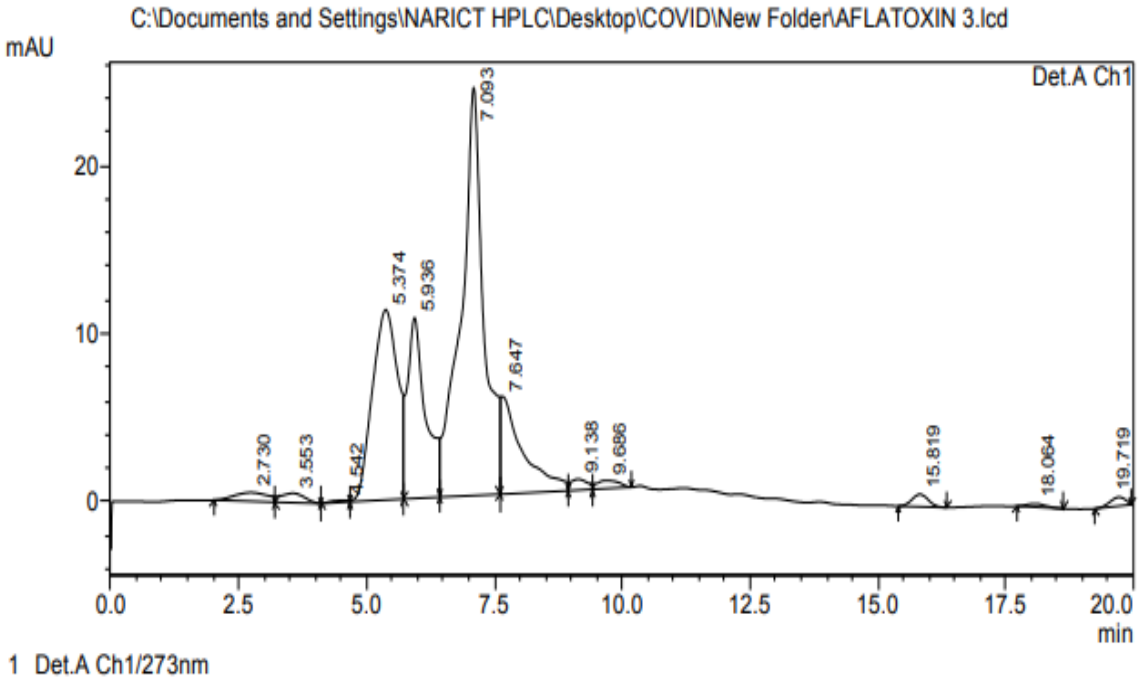
PeakTable

Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.952	155090	4992	2.254	1.780
2	3.123	38258	1313	0.556	0.468
3	4.505	172722	4330	2.511	1.544
4	5.811	2447530	83809	35.575	29.888
5	6.566	382007	19636	5.553	7.003
6	6.936	2317366	122049	33.683	43.525
7	7.721	888292	23398	12.911	8.344
8	9.073	38244	2356	0.556	0.840
9	15.151	3778	219	0.055	0.078
10	15.757	123805	5097	1.800	1.818
11	18.033	47406	2079	0.689	0.741
12	19.620	265394	11133	3.858	3.970
Total		6879891	280411	100.000	100.000

Fig. 5. Chromatogram of sample 13

:Chromatogram>



PeakTable

Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.730	23906	518	1.414	0.925
2	3.553	17759	557	1.050	0.995
3	4.542	3109	119	0.184	0.212
4	5.374	394420	11341	23.327	20.243
5	5.936	260762	10778	15.422	19.238
6	7.093	751715	24309	44.458	43.390
7	7.647	177673	5813	10.508	10.375
8	9.138	14566	646	0.861	1.154
9	9.686	14306	478	0.846	0.852
10	15.819	16266	736	0.962	1.313
11	18.064	5096	199	0.301	0.356
12	19.719	11256	530	0.666	0.947
Total		1690835	56026	100.000	100.000

Fig. 6. Chromatogram of sample 37

The maize and sorghum had all of their samples contaminated with one or more of the fungal species discussed above. Millet had one sample that was not contaminated and wheat had three samples not contaminated while, all maize samples were found contaminated. Only millet samples do not yield all the 7 species isolated. The fungi namely, *Aspergillus fumigatus, A. parasiticus* and *A. lentulus* were not found in the millet samples. Out of the ten maize samples, 7 samples had *Aspergillus flavus,* 8 samples had *A. niger,* 4 samples had *A. fumigatus,* 2 samples had *A. parasiticus,* 3 samples had *A. lentulus* and *Mucor* spp. and only 1 plate was contaminated with *Fusarium oxysporum*. Of the ten millet samples, 3 samples had *Aspergillus flavus,* 6 samples had *A. niger,* 4 samples had *Mucor* spp. and 2 samples had *Fusarium oxysporum*. No sample was found associated with *A. parasiticus, A. lentulus and A. fumigatus*.

Drought conditions can cause high incidence of *Aspergillus flavus* [16]. Out of the ten sorghum samples, 5 samples had *Aspergillus flavus*, 8 had *Aspergillus niger*, 4 had *Aspergillus fumigatus* and *Aspergillus parasiticus,* 1 sample had *Aspergillus lentulus*, 3 samples had *Mucor* spp. and 2 samples had *Fusarium oxysporum*. Of the 10 wheat samples, 2 samples had *Aspergillus flavus*, 5 samples had *Aspergillus niger,* 2 samples had *Aspergillus fumigatus*, 1 sample had *Aspergillus parasiticus*, 2 samples had *Aspergillus lentulus*, 1 sample had *Mucor* spp. and *Fusarium oxysporum.* A total of 20 aflatoxin producing species were detected from the 40 examined samples. Ojonoka [14] in her work reported that 4 out of all 4 samples showed aflatoxin producing species as compared to 20 out of the 40 samples reported in this research work. The differences in contamination of these cereals might be due to differences in major constituents of the organic and inorganic carbohydrate nutrient in these cereals as well as the moisture content attributed to storage conditions and agro ecological conditions of the field.

During the aflatoxin detection only three standards out of four were used (B1, G1 & G2) in the test. The retention time for the three aflatoxin was: B1 – 7.100min., G1 – 5.949min., & G2 – 5.368min. Maize, wheat and millet were tested for aflatoxins, 1 for each sample making a total of three samples. Aflatoxin B1 had the highest peaks in all three samples (43.39% – 48.10%) then aflatoxin G1, which had a peak range of 16.60% – 29.88%, G2 had peaks of 20.24% and 20.68% on wheat and maize samples respectively. G2 was not detected on the millet sample. Mzungu et al. [11] used ELISA and detected aflatoxin producing potential from majority of the aflatoxin producing species isolated from cereals in the same study area. The similarities in the results can largely be attributed to abiotic factors like temperature, humidity etc, which are similar among study areas and the differences observed in Ojonoka [14] result can be said to be due to the differences in the abiotic factors from the two study areas. The presence of aflatoxigenic moulds in agricultural produce may be traced to inadequate storage and handling of these moulds and the environmental factors [17].

5. CONCLUSION

This study revealed high contamination of grinded cereals by moulds of the genera *Aspergillus* with 79.8% incidence rate*, Mucor* spp with an incidence of 13.1% and *Fusarium* spp with 7.4% incidence. The contamination may most likely be due to poor methods of handling, storage and processing. Aflatoxin detection also revealed that the grinded cereals had aflatoxins B1, G1 and G2 in them, which makes their consumption a public health risk. The following are therefore.

6. RECOMMENDED

- Farmers should be encouraged on the use of standard methods of storage and processing to prevent fungal infestation (pre and post-harvest management).
- Early harvest and proper drying of grains to avoid growth of fungal pathogens should be encouraged.
- Surveillance and awareness creation should also be enhanced to curtail the menace of aflatoxins.
- A further research can be carried out to determine methods of preventing aflatoxins or detoxifying cereals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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