Original Research Article

Mycoflora and Mycotoxin Associated with Stored Wheat (*Triticumaestivum*) Retailed in Major Markets of Ondo Metropolis, Nigeria

ABSTRACT

Aim: The research was conducted to investigate the mycoflora associated with wheat, having high moisture from storage, sold in major markets in Ondo metropolis..
Methodology: Wheat samples were obtained from five major markets: *Okedibo, Sabo, Iyalaaje*, Post office and *Yaba* markets. These samples were analysed for aflatoxin content while the presence of fungal contaminants were determined by culture on Potato Dextrose Agar.
Results: The moisture content of the wheat samples was considered high (26.54±0.20% – 30.54±0.02%) andthe study revealed the presence of the following fungi: *Aspergillus flavus, A. niger, Mucor*, *Rhizopus*, *Geotrichum*, *Mucor*,and*Sporendonema. A.niger*showed the highest percentage occurrence of 27.4% while *Sporendonema*had the least occurrence (5%). Aflatoxin was found present only in samples that were collected from *Iyalaaje* and *Okedibo* with concentrations ranging from 30 – 48 ppb.
Conclusion: Though the presence of aflatoxin is observed in only a few of the samples, it still poses health risks, hence the need to proactively combat fungal contamination in stored products.

Key words: Mycoflora, Mycotoxins, Wheat, moisture content

1. INTRODUCTION

Cereals and their products remain essential parts of the daily diets in human and animal populations. The larger populations of the developing countries survive largely on cereal based diets which are rich in carbohydrate, thus serve as a main energy source in their diets. They are also rich in fibre and provide appreciable amounts of the water soluble vitamins and essential minerals that are required in nutrition[1,2]. Wheat is a staple crop, supplying 20-30% of the daily protein and energy in diets. Its adaptability to different geo-climatic conditions has contributed greatly to its survival ability in most regions where it is being cultivated[3]. Moreover, its major use in the production of baking flour and consequent preparation of pastry snacks, among other products has contributed an increase in its use and worldwide acceptability. In 2020, wheat was considered the third most consumed grain in Nigeria after rice and corn; the wheat consumed in Nigeria (almost 6 million metric tonnes) is mostly imported while the country produced about 420,000 metric tonnes. Wheat products including bread, semolina and pasta have become an integral part of the daily diet of many Nigerian homes. Moreover, the confectioneries and bakery industries are dependent majorly on wheat flour for their products.

Fungi are the major contaminants of wheat grains and it has been discovered that some of the contaminating fungi are mostly moulds which can produce toxic secondary metabolites under certain environmental conditions. These conditions include climate, harvest practices such as a wrong choice of harvesting method and time; postharvest practices including a retention of high moisture than is required for storage as well as poor processing and storage conditions [4].These varied conditions are also known to influence the microbial population of the wheat both in the field and during storage [5].

Comment [H1]: State the method used for aflatoxin analysis and fungal identification

Comment [H2]: Rich in carbohydrates and thus serve

Comment [H3]: Higher

The biochemical properties of mycotoxins are diverse, and their toxic effects are exceedingly variable. Mycotoxins are carcinogenic, haemorrhagic, genotoxic, teratogenic, nephrotoxic, hepatotoxic and immunotoxic in action[6]. Many groups of mycotoxins exist and are produced mainly by fungi of the genera *Aspergillus, Fusarium* and *Penicillium*which are organisms that have been associated with wheat. Aflatoxins, which are mainly produced by fungi of the genus *Aspergillus* especially in food crops can be classified into Aflatoxin B1, B2, G1 and G2 based on their chemical properties[7]. Moreover, various members of the genus *Fusarium* have been predominantly associated with wheat deterioration in many parts of the world. It has beenreported that toxins (aflatoxins) from fungi infection, contaminate about 25% of agricultural products, most especially cereals, leading to massive crop losses[8].The aim of this research is to identify the mycoflora and estimate mycotoxin contamination of wheat samples retailed in major markets in Ondo State, Nigeria.

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND PROCESSING

Wheat samples were purchased at five (5) major markets (*Sabo*, *Okedibo*, *Iyalaaje*, post office and *Yaba* markets) within Ondo town, Ondo State, Nigeria. They were collected aseptically inwhirl paksample bags and transported to the laboratory. The wheat samples were pulverized using an electric blender and kept in a clean, air-tight container for further analysis.

Comment [H4]: How many samples were collected from each market and how did you determine your sample size?

2.2 FUNGAL CULTURE

The collected wheat samples were subjected to ten-fold serial dilution and inoculated into Petri dishes by the pour plate method using Potato Dextrose Agar (PDA) supplemented with Streptomycin antibiotic. The plates were incubated at 28±2°C for 72 hours. Subcultures of the resulting mixed culture were done until a pure culture was obtained[9,10]. The isolated fungi were characterized by cultural and morphological features. The characteristics of the organisms were compared with those described in standard manual of fungi identification[11].

The frequency of occurrence of each fungal isolate from samples collected in each location, was expressed as a percentage of the total number of different organisms isolated over the period of study.

Comment [H5]: Was this a longitudinal study? If yes, please indicate the period of time the study was conducted.

2.3 MYCOTOXIN ANALYSIS

Methanol (70%; 100 ml) was added to 5g each of the blended wheat samples in a clear conical flask, and covered with parafilm. They were placed in an orbital shaker at 400 rpm for 15 minutes. The samples were filtered and filtrates (3 ml) were transferred into separating funnels. Distilled water (20 ml) and 25ml of dichloromethane were added to each of the filtrates in the separating funnels. The separating funnels were covered, gently agitated and the filtrate at the bottom was dispensed into sample cups through folded filter papers bedded with 20g of hydrous sodium sulphate. The layers containing the toxin extracts were dispensed into other sample cups. The cups were placed in a fume cupboard and allowed to dry by evaporation overnight. Dichloromethane (1 ml) was added to the toxins in the cups to dissolve them. The extracted sampleswere analyzed using thin layer chromatography with scanning densitometer [12,13].

Comment [H6]: 25 ml (space)

3. RESULTS AND DISCUSSION

The moisture content values of the samples ranged from 26.54±0.2 (*Iyalaaje*) to 30.54±0.02 (*Yaba*) across the sample locations. A moisture content of 30.10±0.01 was obtained for wheat sample collected in *Yaba*, 30.41±0.09 for sample collected in *Okedibo*and 30.50±0.005 for the sample collected in Post office market.

Comment [H7]: How was the moisture content measured? This was not mentioned in the methodology and in the abstract. They should be included in this places.

The results of the investigation for the occurrence of fungi in the collected samples showed the presence of the following fungi: *A.flavus, A.niger, Mucor*, *Rhizopus*S,*Geotricum*, *Mucor*, and*Sporendonema* (Table 1). *Rhizopus* was found in wheat samples obtained from all locations. *Mucor, A. flavus and A. niger,*were each isolated from three of the five markets. The least occurring fungus was *Sporendonema*, occurring only in samples from *Yaba* market.

Comment [H8]: Where does this belong?

Table 1:Occurrence of Fungal Isolates from Wheat Samples

Comment [H9]: There should be some form of evidence of the isolation of these fungal isolates.

The macroscopic and macroscopic descriptions should be documented and pictures of the isolates shown so as to authenticate the result.

Microorganisms/Sample Locations	Okedibo	Sabo	Iyalaaje	Post Office	Yaba
A. niger	+	+	+	-	-
A. flavus	+	+	+	-	-
Rhizopus	+	+	+	+	+
Geotricum	-	+	-	+	-
Sporendonema	-	-	-	-	+
Mucor	+	-	+	-	+

KEY: + = Present, - = Absent

The percentage mean occurrence of isolated fungi is shown in Table 2. In all the locations sampled from, *A. niger*was the most prevalent with an occurrence of 27.4%. *A. flavus* and *Rhizopus* both occurred at a percentage 20.9%. *Sporendonema*had the least percentage occurrence value of 5%. Based on location, 27.42% of all the fungal contaminants were isolated from *Sabo* market, 24.19% were obtained from each of *Okedibo* and *Iyalaaje* markets while the least occurrence of isolates were obtained in *Yaba* market with only 11.29% of the isolates.

Comment [H10]: "Lowest"

Comment [H11]: "Was"

Table 2 : PercentageFrequency of Occurrence of isolated fungi

Comment [H12]: This table can only be relevant if the number of samples collected from each area is more than one and is documented

Isolate	Frequency of Occurrence By Location (%)					Total	(%)
	Okedibo	Sabo	Iyalaaje	Post Office	Yaba		
A, niger	3.5	3	2	-	-	8.5	27
A. flavus	2	2	2.5	-	-	6.5	20.9
Rhizopus	1	1.5	1.5	2	0.5	6.5	20.9
Geotrichum	-	2	-	2	-	4	12.9
Sporendonema	-	-	-	-	1.5	1.5	5
Mucor	1	-	1.5	-	1.5	4	12.9
Total (%)	7.5 (24.19)	8.5 (27.42)	7.5 (24.19)	4 (12.90)	3.5 (11.29)	31 (100)	100

Comment [H13]: A.niger (change the comma to full stop)

KEY: - = Absent

Aflatoxin concentration of wheat samples obtained from all the locations ranged from 30 – 48ppb (Table 3). The least concentration was found in wheat samples collected from *Okedibo* market while the samples obtained from *Iyalaaje* market have the highest concentration of aflatoxin.

Comment [H14]: lowest

Comment [H15]: "had"

Table 3: Aflatoxin concentration of samples

Sample Location	Total Aflatoxin (ppb)
Okedibo 5	30
Iyalaaje 2	46
Okedibo 3	47
Iyalaaje 3	48
Okedibo 2	42

Comment [H16]: 1.What method was used to analyze the Aflatoxin?
2.TLC is a method used for qualitative analysis not necessarily quantitative.
3.Which of the aflatoxins was detected and quantified??

Comment [H17]: The names of sample location used here are introduced for the first time in this manuscript.
What do the numbers represent??

Data shown in table 1 shows that the wheat samples obtained from different locations are contaminated with several fungi including *A.flavus, A.niger, Mucor*, *Rhizopus*, *Geotrichum*, *Mucor*, *and Sporendonema*. In addition to other environmental conditions that favour their growth, fungi find wheat a natural growth substrate due to the presence of the nutrients required for growth. These organisms can get into wheat from the field during growth or harvesting, during postharvest processes and especially during storage [14]. The presence of the isolated organisms has been previously confirmed [15,16] and this has been associated with the environmental factors including the prevalent temperature and humidity[17]. In line with the results obtained in this research, isolates in the *Aspergillus* and *Rhizopus* genera were identified from stored wheat in Southeastern Ethiopia [18]. The predominance of the genus *Aspergillus* was also confirmed. Moreover, these organisms are regarded as storage fungi, hence their presence in wheat kept for retail. In agreement with this study, [19] also isolated *Geotrichum*sp from stored wheat.

Comment [H18]: With environmental. Remove "the"

Total aflatoxin concentrations (ppb) for the samples were high (30 – 47ppb). The samples containing aflatoxin were samples collected from *Okedibo* and *Iyalaaje* markets. It was observed from the results that 48.39% of all the isolates obtained were from these markets which also had the highest population of *Aspergillus flavus* (69.23% of all the *A. flavus* was obtained in wheat samples from these markets).The high occurrence of *A. flavus* in samples collected from these markets supports the presence of aflatoxin in samples obtained from those markets alone. As previously established, the major factors responsible for aflatoxin production are the fungus, a substrate, temperature and moisture among others, it can be reasonably concluded that while the fungus was identified on the substrate (wheat), the conditions of temperature and moisture present in the storage facilities at *Okedibo*and *Iyalaaje*markets were less than ideal,favoured the proliferation of the isolates and the production of aflatoxins by the fungal isolate[8].

Although only 20% of all the samples collected were contaminated by aflatoxinsand only 20.9% of the samples were contaminated by *A. flavus* isolates, the concentration of aflatoxin recorded was higher than the specified limits, posing a serious health risk to the potential consumers of such wheat products.

The moisture content of the wheat grains was observed to be quite high ranging between 26.54% and 30.54% as against lower ranges which are commonly reported for wheat including 11.40 – 13.80% [20] and 11.9% - 13.23% obtained from the flour of different varieties of commercial wheat samples [21]. This increase in moisture as recorded in this research can be attributed to the storage environment which led to the absorption of moisture by the grain. This condition is known to

accelerate the cell respiration of seeds during storage, resulting in the temperature increase of grain bulk witha consequent damage to the internal structure of endosperm and embryo as well as fungal growth [22]. With an increase in storage days, the presence of a high moisture content supports the proliferation of fungi isolates. This eventually leads to the quality deterioration of the grain.

Comment [H19]: remove

4. CONCLUSION

The presence of fungal contaminants in stored wheat is known to predispose the samples to aflatoxin contamination, which was observed in this study. Though the samples contaminated were minimal, the risk posed by the toxin is a cause for concern that must be addressed in all food samples.

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