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Assessment of Micro Flora, Deoxynivalenol (Don) and Fumonisin Contamination of Grains sold in Local Markets, Nigeria

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***Abstract*:** Fusarium the major deoxynivalenol (DON) and fumonisin producing species of fungi grow as a corn endophyte often without causing disease symptoms in plant. Climate changes resulting in appropriate weather conditions helps in Fusarium growth causing seedling blight, stalk rot and ear rot. Thus most grain contamination of Fusarium comes from the farm. This study was carried out to assess the micro flora and level of DON and fumonisin contamination in Zea mays, Sorghum bicolor, Triticum aestivum and Pennisetum americanum sold in Ota. Forty samples comprising ten of each grain type was analyzed, Fumonisin at concentration ≥4.0 mg/kg (ppm) was detected in eight samples of Zea mays and two samples of Triticum aestivum while DON at concentrations ≥1.25mg/kg (ppm) was detected in all the wheat samples using the Rida® Quick Fumonisin and DON test kits. The total aerobic plate count for the samples ranged from 2.0×104 to 8.4×106 cfu/g, fungal count ranged from 1.0×104 to 6.0×106 cfu/g while coliform count ranged from < 10 to 2.0×103 cfu/g. The predominant microbial isolates from the grains included species of Fusarium, Aspergillus, Mucor, Penicillium, Bacillus, Klebsiella, and Pseudomonas. Infection of grains by fungal species and contamination with mycotoxins can generally be influenced by favourable weather conditions. Measures to address climate changes, effective hazard analysis and critical control point (HACCP) and good storage system are advocated to prevent mould contamination and deleterious mycotoxin production in grains.

Keywords: Fumonisin, Deoxynivalenol, Micro flora, Mycotoxins, Coliform, Climate changes, HACCP

Introduction

Fungal contamination of food and feeds causes considerable economic losses due to damage to crops, discoloration, off-odors, off-flavors, reduced yields, loss of nutritive value and mycotoxin production. Mycotoxins are secondary metabolites of fungi some of which are harmless and even helpful, serving as antibiotics and other clinical and industrial chemicals (Magen and Aldred, 2005). Some mycotoxins are

toxic to animals and man having predilection for some organs of the body. The Food and Agriculture Organization estimates that 25% of the world food crops are affected by mycotoxins during growth and storage under a diverse range of climatic conditions and situations (FAO, 1979; Miller and Trenholmed, 1994). The accumulation of mycotoxins in foods and feeds represents a major threat to human and animal health as they are responsible for much different toxicity including the induction of cancer, mutagenicity, estrogenic and gastrointestinal disorders, urogenital, vascular, kidney, liver and nervous disorders (Pitt and Hocking, 2009).

Fumonisins constitute a group of carcinogenic metabolites mainly produced by Fusarium verticilloides, Fusarium proliferatum and Fusarium nygamai, as well as Alternaria spp, It has been associated with toxicities such as hepatotoxin and nephrotoxin in all animal species tested, it is implicated in elevated human esophageal cancer incidence in Africa, Asia and Central America (Franceschi et al., 1990; Marasas et al., 1991; Rheeder et al., 1992; Chu and Li, 1994) and with high incidence of liver cancer, neural tube defects and apoptosis in the liver. Fumonisins is associated with several diseases in animals including liver and kidney tumors in rodents, equine leukoencephalomalacia (ELEM) in horses, and acute pulmonary edema in pigs (Wild and Gong, 2010).

Deoxynivalenol toxicity in man is associated with abdominal pain, vomiting, headache, dizziness, fever, intestinal wall necrosis, anorexia and impaired antibody and immunoglobulin levels. In animals DON toxicity is usually linked with feed refusal, decreased feed conversion, altered nutritional efficiency, weight loss, vomiting, severe dermatitis, abortion, bloody diarrhea, hemorrhaging, and abnormal feathering, lesions at the edges of bird beaks, decreased egg production, decreased weight gain, anorexia, and death (Pestka et al., 2005;Yoshizawa and Morooka, 1977; Herrman et al., 2002; Forsyth et al., 1977; Rotter et al., 1994; Trenholm et al., 1984; Young et al., 1983).

The main objective of this research is to determine the microbial contaminants specifically mycoflora and fumonisin and deoxynivalenol contamination levels of some grains sold in Ota and also create awareness to the public at large.

Materials and Methods

Sample collection and preparation

Samples were collected from the popular Oja-Ota and Sango Ota markets, these outlets were chosen because they are the major market and are highly patronized by members of the public in the sampling area. Ten samples each of Corn (Zea mays), Millet (Pennisetum americanum), Wheat (Triticum aestivum) and Sorghum/Guinea corn (Sorghum bicolor) were purchased randomly from food vendors. The samples were aseptically collected in sterile polyethylene bags and transported same day to the laboratory for further analysis. Two hundred and fifty gram (250 g) of each grain samples was ground using warring blender, this served as stock for all other analyses.

Microbiological analysis

One gram of the samples was homogenized in normal saline and

diluted 10-1 to 10-5. Aliquot 0.1mL of the sample homogenates was spread inoculated onto Saboraud Dextrose agar plus Chroramphenicol (SDA+C) for fungal isolation; Nutrient agar was inoculated for total aerobic plate count (TAPC) and MacConkey agar for coliforms. One gram each of the ground samples was inoculated into lactose broth in caped test tubes with inverted Durham's tube, for coliform test. SDA + C was incubated at room temperature 28± 2°C for 3 to 5 days while other cultures were incubated for 24-48 h at 37°C.

Assay for Deoxynivalenol (DON) and Fumonisin

The RIDA® QUICK Deoxynivalenol (DON) and Fumonisin kits (R-Biopharm, UK) was employed. Following the manufacturer's instruction, the test kits were brought out of fridge and allowed to assume room temperature of 28±2°C. Aliquot 5 g and 1g of ground samples was respectively weighed into test tubes and 20 mL and 40 mL of extraction buffer (as contained in each test kits either for Fumonisin or DON) added and then manually shaken vigorously for 1-2 minutes and in a laboratory shaker (Jenway, UK) for another 2-3 minutes. The solution was filtered after 3-5 minutes sedimentation. The filtrates were used for the assays as applicable (Fumonisin or DON) and following the manufacturer's test instruction manual. The RIDA® QUICK DON and Fumonisin test is an antigen- antibody reaction, an immunochromatographic assay based on the principle of specific DON / Fumonisin antibody recognizing DON / Fumonisin molecules in the samples. The results are read visually following development of coloured bands. The test band is only visible in the presence of DON / Fumonisin in the sample. The control band is not influenced by DON / Fumonisin in the sample and should be present in all cases in order to prove that the test strip is valid. Results are considered positive that is the sample is contaminated with Fumonisin/DON if the control band/line is visible and the test band is also visible, mycotoxin level ≥ detection limit. Negative result is recorded if that the sample is free of DON/Fumonisn or below detection limit, if only the control band/line is clearly visible but test band invisible. Result is invalid if test band is clearly visible but control band is invisible.

Determination of Moisture Content of Samples

The standard procedure as described by AOAC (2000) was used. Samples were oven dried at 105°C for 2 hours to constant weight. Triplicate readings were recorded and the mean value was obtained.

Results

The mean microbial count of the samples and contamination of the samples by DON and Fumonisin is presented in table 1. It shows that all the samples had microbial contaminants. TAPC ranges from 2.0x 104 in Millet to 8.4x106 in Sorghum. Fungal count ranges from 1.0x104 in millet to 6.0x106 in Wheat. Coliform count ranged from < 10 to 2.0x103. However, only one maize sample had faecal coliform contamination. The table also shows that eight samples of maize and two samples of wheat were contaminated with Fumonisn at concentrations ≥4.0 mg/kg while ten

wheat samples had DON contaminations at levels ≥1.250 mg/kg. The sorghum and millet samples were neither contaminated with DON nor fumonisins. Table 2 shows the microbial isolates from the samples. Fungi specifically Aspergillus and Fusarium are the most prevalent in all the samples, while bacillus spp is the predominant bacterial contaminants. The percentage moisture contents of the grains ranged from 10-13% in Pennisetum americanum, 12-15% in Sorghum bicolor, 12-18% in Triticum aestivum to 16-23% in Zea mays.

Discussion

The microbial count in the samples which ranged from 104 to 106 for both TAPC and Fungal count cannot be considered as a challenge or treat because it is a raw food which will be processed by heat before consumption. Heat treatment is a control measure and could be a critical control point that will reduce the contaminants to insignificant level before consumption. Bacillus species are spore formers and common environmental contaminants, which could explain their presence in the grains. The presence of coliforms specifically faecal coliforms is an indication of contamination of some of the grains by faecal matter. This could be from the food vendors or from the environment in which grains are often spread to dry or displayed for sales. The low moisture contents of the grains could account for low contamination of the grains by bacteria except for bacillus which are known to produce resistant spores and Klebsiellae which are known to be hardy organisms. The presence of fungi in all the samples could be attributed to the normal flora of the plants. Fungi are spore bearers and common environmental contaminants that could have contaminated the grains from the field or during storage and display for sales.

The microbial contamination of a product has been reported to be dependent on the environment it passed through and to the sanitary quality observed during processing (WHO, 2008; Oranusi and Braide, 2012). Fungi are known to tolerate low water activity levels; this could also explain why they could survive in the grains with low moisture contents.

Fumonisins was detected in eight maize and two wheat samples at concentrations higher than accepted limits in food materials (FDA, 2001), this could be associated with the presence of Fusarium spp which are known to produce this mycotoxin. Fusarium spp are known to contaminate grains in the farm or in storage and under favourable conditions elaborate Fumonisins (Eugenia et al., 2014; Shephard et al., 2005).

The presence of DON in all the wheat samples in this research could be as a result of contamination of samples with Fusarium spp which are known to produce DON. Kushiro (2008) observed that Deoxynivalenol mycotoxin (DON) is one of several mycotoxins produced by certain Fusarium species (Fusarium graminearum, Fusarium culmorum, Fusarium proliferatum), that frequently infect corn, wheat, oats, barley, rice, and other grains in the field or during storage. This makes it one of the most encountered mycotoxins. Pestka and Smolinski (2000); Herrman et al. (2002) reported the presence of DON in food and feeds. They observed that

mycotoxin produced can withstand high temperatures of about 170◦C–370◦C; the danger resulting from this is that the toxin still remains in foods and feeds after basic culinary treatment.

Conclusion

Although the levels of microbial counts of the samples are considered to be at levels that will not pose any treat to consumer because the samples are raw materials that must be processed before consumption. The detection of Fusarium spp and consequent Fumonisin and DON at concentrations ≥ 4.0 mg/kg and 1.25 mg/kg respectively is a cause for concern since these mycotoxins can withstand processing temperature. Education of farmers and food vendors on the need for proper management of grains in the farm and during storage to prevent mould contamination is imperative. Consumers/ public enlightenment on the need to purchase good quality food items and application of GMP and HACCP in food production will be a step in the right direction.

Table 1 Mean microbial count cfu/g and assay for Fumonisin and DON mg/kg

Outlet/ Sample		Fungal count	Total aerobic Plat count	Coliform count	Fumonisin ≥4.0 mg/kg	DON ≥1.25 mg/kg
Outlet 1						
1)	Sorghum	5.0×10^4	3.0×10^6	$1.2x10^2$	-	-
2)	Maize	3.7×10^5	2.7×10^5	1.0×10^2	+	-
3)	Millet	3.6×10^5	2.0×10^4	NG	-	-
4)	Wheat	5.0×10^5	2.4×10^6	NG	-	+
Outlet 2						
5)	Sorghum	8.0×10^5	5.6×10^6	NG	-	-
6)	Maize	4.6×10^5	5.0×10^5	NG	+	-
7)	Millet	3.8×10^5	3.7×10^6	NG	-	-
8)	Wheat	5.3×10^5	2.4×10^6	$1.1x10^1$	-	+
Outlet 3						
9)	Sorghum	3.0×10^4	3.4×10^6	$1.3x10^2$	-	-
10)	Maize	3.5×10^5	3.5×10^5	NG	+	-
11)	Millet	1.5×10^5	3.2×10^5	NG	-	-
12)	Wheat	4.0×10^4	9.0×10^4	NG	-	+
Outlet 4						
13)	Sorghum	1.5×10^5	3.2×10^6	$2.0x10^3$	-	-
14)	Maize	3.3×10^5	7.0×10^4	NG	+	-
15)	Millet	5.0×10^5	4.0×10^6	NG	-	-
16)	Wheat	5.0×10^5	8.3×10^5	NG	-	+
Outlet 5						
17)	Sorghum	2.0×10^5	4.8×10^6	NG	-	-
18)	Maize	1.1×10^5	3.3×10^6	NG	+	-
19)	Millet	2.0×10^5	6.5×10^5	NG	-	-
20)	Wheat	4.5×10^5	5.4×10^5	NG	-	+

Outlet 6						
21)	Sorghum	1.0×10^5	3.4×10^6	NG	-	-
22)	Maize	1.6×10^5	2.4×10^5	NG	+	-
23)	Millet	2.8×10^6	4.5×10^6	< 10	-	-
24)	Wheat	6.3×10^4	6.4×10^5	NG	-	+
Outlet 7						
25)	Sorghum	2.6×10^5	7.9×10^4	NG	-	-
26)	Maize	2.9×10^5	2.4×10^5	NG	+	-
27)	Millet	1.0×10^4	2.1×10^6	$3.5x10^1$	-	-
28)	Wheat	1.6×10^5	8.0×10^6	NG	+	+
Outlet 8						
29)	Sorghum	4.0×10^5	8.4×10^6	NG	-	-
30)	Maize	4.7×10^4	3.8×10^5	NG	-	-
31)	Millet	2.0×10^4	2.6×10^6	NG	-	-
32)	Wheat	6.0×10^6	2.3×10^6	NG	-	+
Outlet 9						
33)	Sorghum	2.0×10^5	6.7×10^5	NG	-	-
34)	Maize	4.5×10^5	7.0×10^5	NG	+	-
35)	Millet	1.0×10^6	4.6×10^6	NG	-	-
36)	Wheat	5.0×10^4	3.5×10^6	$2.3x10^2$	+	+
Outlet 10						
37)	Sorghum	4.0×10^5	4.5×10^6	$2.9x10^2$	-	-
38)	Maize	2.5×10^4	5.2×10^5	NG	-	-
39)	Millet	1.2×10^4	2.2×10^6	NG	-	-
40)	Wheat	5.8×10^5	8.0×10^5	NG	-	+

KEY: NG: No Growth; -- = Not detected

Table 2 Microbial isolates from different grain samples

Grain sample	Microbial isolates
Maize	*Aspergillus niger*, *Fusarium* species, *Mucor mucedo,* *Penicillium* species Klebsiellae, *E. coli*, Bacillus spp
Millet	*Aspergillus* species, *Fusarium* species, Penicillium species, *Pseudomonas*, Bacillus spp.
Wheat	*Aspergillus* species, *Fusarium* species, Bacillus spp, *Pseudomonas*, Klebsiellae
Sorghum	*Aspergillus* species, *Fusarium* species, Bacillus spp, Klebsiellae

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