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Seasonal Determination Of Aflatoxin Level In Maize Sold In Some Retail Outlets In Zaria

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Abstract: ***A seasonal study on the prevalence of aflatoxin in maize sampled from some retail outlets in Zaria, Nigeria, was undertaken using Enzyme linked immune-sorbent assay (ELISA) method. A total of 150 samples were collected during wet and dry seasons. (75 samples each for wet and dyy season).Fungal count of 4.58±0.24 (log10cfug-1) in the dry season while the wet season count was 5.6±0.36 (log10cfµg-1). The predominant mycoflora on maize grain were Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus. Penicillum sp. Fusarium sp. and Rhizopus sp. The aflatoxin level obtained showed that yannika recorded the highest aflatoxin level of 2.2(ppb) and 2.8 (ppb) during wet and dry period respectively. Handling practices by local processors that involved simultaneous storage and milling processes of maize grain in the area could be the major cause of the higher mould and aflatoxin contamination.***

Keywords: Aflatoxin, Maize, Season.

I. INTRODUCTION

Maize (Zea may) is the most important cereal in the world after wheat and rice with regards to cultivation areas and total production (Purseglove, 1992; Osagie and Eka, 1998). Maize is a stable food taken in various forms (flour or whole) with acceptability cutting across socio-economic strata in Nigeria. The population involed is thus large. The manner of storage is worrisome, as the maize are stoked in sacks and heaped in stores either at home or in the market places. (Nigeria stored products research institute 1982). This is capable of creating congenial moisture content in the maize which can highly favour growth of aflatoxin. maize serve as an excellent substrate to mould growth and mycotoxin contamination (Bouraima et al.,1993). Therefore investigation of the food for any possible contamination by the causal agents and aflatoxin is highly imperative.

Aflatoxins are groups of mycotoxins mainly produced by the fungal Aspergillus flavus and Aspergillus parasiticus, with Aspergillus flavus being the most common producer (Bradburn et al.,1993). Aflatoxin contamination has been associated with abiotic factors such as draught and high temperature and biotic factor such as insects damage (Mcmillian et al., 1985, Payne, 1992).

Among 18 DIFFERENT TYPES OF AFLATOXIN IDENTIFIED, the major ones are aflatoxin B1, B2, G1 and G2. Aspergillus flavus typically produces aflatoxin blue fluorescence (AFB1 and AFB2) whereas Aspergillus parasiticus produces aflatoxin green fluorescence (AFG1 and AFG2) as well as AFB1 and AFB2. Four other aflatoxins M1, M2, B2A and G2A may be produced in minor amounts. Several reports have shown that aflatoxin is a potent carcinogenic immunosuppressive agent which causes liver cancer in both animal and humans (Castegnaro and McGregor, 1988). Ingestion of higher doses of aflatoxin can result in acute aflatoxicosis which manifests in hepatoxicity (Fung and Clark 2004). It has also been implicated as the cause of Rey's syndrome and chronic hepatitis. Symptom of toxicity in animals range from death to chronic disease, reproductive interferences immune suppression, decreased milk and egg production (Fung and Clark 2004). Animals are exposed to aflatoxin by consumption of feeds conterminated by aflatoxin producing fungal strains during growth, harvest and storage.

II. MATERIALS AND METHODS

SAMPLE COLLECTION

A total of one hundred and fifty samples was collected during dry and wet seasons from five retail outlets in zaria.the outlets were yannika kasuwa mata, sabo gari samaru and tudun wada. A period of fifteen weeks was used for the collection of samples for wet season and another fifteen weeks was used for dry season. Making a total of one hundred and fifty samples for all the outlets. The selection of the markets outlets were based on availability of the samples, the number of retailers the population served and market size. Samples were collected in clean polythene bags and taken to the laboratory at the department of microbiology Ahmadu bello university zaria for analysis.

Determination of fungal counts in maize grain during wet and dry season.

Ten gram (10g) of maize grain were weighed and transfer into conical flask containing 90ml of 0.1%w/v buffered peptone water to give the stocksolution.one mililitre (1ml) was transferred from the stock solution into a test tube containing 9ml of 0.1% peptone water to give 1:10 dillution was achieved, then 0.1ml from the 1:1000 was transferred aseptically to the surface of solidified potatoe dextrose agar plate. An L-shaped rod was used to spread it on the medium and then incubated at room temperature (30c±2) for 3-5 days. The fungal colonies were counted and counts expressed as colony forming units per gram (cfu/g) using this formular:

Fungal counts = average/mean colony count × dillution factor÷inoculum volume (colony forming unit /gram)

MICROSCOPIC IDENTIFICATION OF FUNGAL ISOLATES

A drop of lactophenol cotton blue was placed on a clean slide using a pointed needle, a portion of the mycelium from the fungal cultures was placed on the drop of lactophenol cotton blue and teased. A cover slip was then gently placed and observed using × 10 and × 40 objective lens. The fungal isolates were identified based on cultural characteristic and microscopic morphology according to the manuals of Barnett and Hunter, (1972) and Ellis, (2006).

QUANTITATION OF AFLATOXIN LEVELS

The helical total aflatoxin assay kit (model CAT NO.941 AFL.01M-96) was utilized for detection of aflatoxin level in maize grain.

Extraction solution (70% methanol) was prepared by adding 30ml of distilled water to 70ml of methanol. Then 20g of maize sampe was added to 100ml of extraction solvent in the ratio of 1:5(w/v) samples to extraction solvent then the sealed container was manually shaken for 2minutes and allowed to settle.it was then filtered through Whatman 1 filter paper and the filterate was dispense into microtitre wells and measured optically by a micro litre plate reader with an absorbance wave length of 450nm (OD 450) The optical densities of each microwell was read and recorded.

III. RESULTS

OUTLETS	DRY SEASON	WET SEASON
Yannika	4.58±0.24a	5.59±0.36b
Kasuwa mata	4.51±0.25a	5.58±0.38b
Sabo	4.45±0.08	5.58±0.05b
Samaru	4.42±0.25a	5.57±0.05b
Tudun wada	4.41±0.78	5.47±0.05b

Values are log10 of mean standard deviation of duplicate samples. Mean from the same row with varying superscript differ significantly ($p<0.05$) n=total number tested.

Table 1: Fungal counts (log10 cfμg-1) of maize grain sold in some retail outlets in Zaria during wet and dry season

Species	Wet season	Dry season	Mean n=150
Aspergillus flavus	25.5±0.5	35.26±8.06	30.32±6.90
Aspergillus parasiticus	47.68±4.05	32.11±9.25	39.89±1.10
Aspergillus niger	14.6±6.27	15.26±	14.93±0.47
Penicillum sp	*1.99±0.01*	*17.37±1.52*	*9.68±10.9*
Fusarium sp	*8.94±0.91*	*0*	*4.47±6.32*
Rhizopus sp	*1.32±*	*0*	*0.66±0.93*

Value are mean ± standard deviation of duplicate samples n=total number tested 0= (no growth of specie).

Table 2: Frequency of Occurrence (%) of Fungal Isolate on Maize Grain Sold In Some Retail Outlets In Zaria During Wet And Dry Seasons

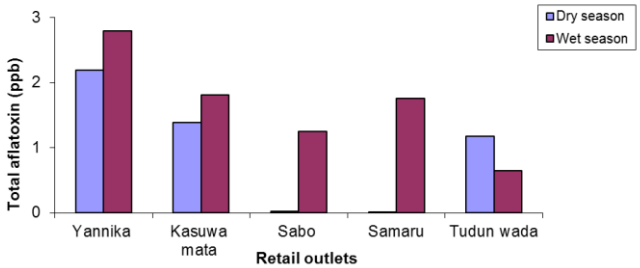

Figure 4.1 Mean total aflatoxin on maize grain sold in some retail outlets in Zaria during dry and wet seasons

Figure 1: Total Number Of Positive Samples With Aflatoxin In Maize Grain From Five Retail Outlets In Zaria

IV. DISCUSSION

The total fungal count is presented in Table 1. A significant high fungal count was observed in all the maize sampled in the wet season compared to the dry season. The mould contamination level of the samples exceeded the standard regulatory limits of (10^3-10^4 $cfug^{-1}$) set by the food and agricultural organization (FAO) for cereals (FAO, 1991). High total fungal count observed in the wet season had been reported previously Rocha et al., 2009.the finding was attributed to relatively high humidity in the wet season which encourage germination and colonization of these fungi Tanboon-ek et al.,1986).similar studies had also reported higher total counts than values in this study. Muthomi et al., (2009).had also shown variation in fungi contamination based on agro-economical zones.

Table 2 shows frequency of occurrence of fungal isolates on maize grain. The study show that Aspergillus was the most dorminant genus on maize and a significant frequency of occurrence of Aspergillus parasiticus and Aspergillus flavus during wet and dry season was observed. This is in agreement

with Abdullahi and Muhammed (2002). Who showed that Aspergillus flavus and Aspergillus parasiticus have the highest occurrence on maize and cereal grain from different parts of the world (WHO 2006, Kumar et al., 2008). Similarly high frequency of occurrence of Aspergillus flavus had also been reported in maize kernel (Youssef, 2009).

Figure 1 Data from this study indicated that a significant quantity 39 (26%) was contaminated with aflatoxin for both season. There was an obviously higher aflatoxin levels in Yannika and Kasuwa mata outlets with mean values of 2.7ppb and 1.6ppb respectively, this spread of aflatoxin contamination on maize grain is in agreement with previous report on maize grain (Hennigen and Dick 1995; Bhat et al.,1997; Gloria et al.,1997; Ali et al.,1998; Machinsky et al., 2001; Vargas et al., 2001). In a similar study in Kenya Bouraima et al., (1993). found aflatoxin B1 level up to 14µg/kg in stored maize .Udoh et al., (2000) reported 33% aflatoxin contamination in maize sampled from different ecological zones of Nigeria. Hell et al., (2000a) All the maize samples collected from silos and ware houses in Ghana contained aflatoxins at levels ranging from 20-355µg/kg (Kpobo,1996).

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