

*Full Length Research Paper*

# Occurrence of fumonisins and deoxynivalenol in stored maize used in industrial productions in Zaria Nigeria

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**Fifty three samples of four varieties of maize were obtained from 18 storage facilities in Zaria and its environs, all within Kaduna State, Nigeria and plated directly on potato dextrose agar (PDA). Samples which yielded *Fusarium* species were further tested for the presence of toxins. Seven samples yielded fumonisins alone, while both fumonisins and deoxynivalenol (DON) were found in three samples corresponding to 18.87% toxin contamination rate. *Fusarium verticilloides* was found to be the most toxigenic species occurring in eight toxin-contaminated samples while *Fusarium proliferatum* occurred only once. Three samples had fumonisin content above the 5 ppm threshold value while the other samples did not exceed the 1 ppm upper limit for DON. *Fusarium* contamination of grains used in industrial productions exists and thus there is the need for regulations guiding the storage and industrial utilisation of maize to minimize the risk of mycotoxicoses in humans and animals.**

**Key words:** Fumonisin, deoxynivalenol, stored maize, *Fusarium*, Zaria, mycotoxin, contamination, industrial productions.

## INTRODUCTION

Maize (*Zea mays*) is a very versatile cereal and is used in the production of food, feed, adhesives, oil, syrups, flakes, alcoholic and non-alcoholic drinks, starch and ethanol among others while locally it is used to make pap, nokia, tuwo, pop-corn, etc. In addition, many feed mills in Zaria utilize maize as the principal raw material. Nigeria is the 10<sup>th</sup> largest producer of maize globally; producing about 98.8% of its domestic maize needs locally (USDA, 2010). These grains are usually stored for some time before use industrially. During storage, maize is subjected to various hazards including attack by fungi. Previous investigation has shown that stored maize was readily attacked by various fungi and *Fusarium* was one of the most important fungal genera isolated in the maize samples (Ameh et al., 2008). Furthermore, Ameh et al. (2008) reported the presence of various species of *Fusarium* in twenty samples corresponding to 37.73% infection rate, while the moisture content values of the grains ranged from 5.3 to 13% which is well below the

minimum range of 18 to 20% required for the growth of *Fusarium verticilloides* (Munkvold and Desjardins, 1997). *Fusarium* species such as *Fusarium proliferatum* and *F. verticilloides* have been reported to be prolific producers of toxins (Marasas, 2001) which are associated with various health conditions in humans and animals.

For instance, deoxynivalenol (DON) a type B trichothecene causes anorexia and weight loss in livestock, while zearalenone an oestrogenic mycotoxin that causes reproductive disorders in farm animals is also hepatotoxic in humans (Zinedine et al., 2007). Fumonisin on the other hand have been associated with the development of oesophageal cancer (Chu and Li, 1994; Shephard et al., 2000), liver and kidney cancer in humans (Howard et al., 2001) equine leukoencephalomalacia and porcine pulmonary oedema syndrome (Marasas et al., 1988). As a result of the widespread problem of mycotoxin contamination of maize, the Joint Food and Agricultural Organisation and World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA) allocated a Provisional Maximum Tolerable Daily Intake for humans of 2 µg/g for fumonisins (WHO, 2002). With respect to

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**Table 1.** *Fusarium* species isolated from toxin-contaminated maize samples.

Maize sample	<i>Fusarium</i> species
WC1	<i>Fusarium verticilloides</i>
WC10	<i>Fusarium proliferatum</i>
WC29	<i>Fusarium graminearum</i> , <i>F. verticilloides</i>
WC30	<i>F. verticilloides</i>
YC 1	<i>F. verticilloides</i> , <i>F. proliferatum</i>
YC3	<i>F. verticilloides</i>
YC8	<i>F. versicolor</i>
YC11	<i>F. verticilloides</i> , <i>F. sporotrichoides</i> , <i>F. graminearum</i>
YC16	<i>F. versicolor</i>
YC21	<i>F. verticilloides</i> , <i>F. acuminatum</i>

animals, the total fumonisin maximal tolerable levels recommended by United States Food and Drug Agency in maize-based feeds are 5 µg/g for feed meant for horses, 5 µg/g for rabbits, 60 µg/g for ruminants and 100 µg/g for poultry (FDA, 2000). For DON, the FDA recommends levels below 1ppm for human consumption and 5 ppm for animals. In the European Union, the limit for the presence of DON in foods is 1.25 ppm (Konvalina et al., 2011). In the light of the potential risk of consumption of contaminated maize as food or feed, this work was carried out to estimate the extent (if any) of fumonisin contamination in maize used industrially in food and feed production in Zaria, Nigeria.

## MATERIALS AND METHODS

### Sampling, isolation and identification of *Fusarium* sp.

#### Sampling

Samples were collected from 18 stores belonging to six companies during the dry season. The grains were usually stored in jute bags and were sampled using locally fabricated steel 50 cm grain probes with tapered ends which easily penetrated the sacks length-wise. Several samples were taken per bag and several bags per store were sampled and all samples mixed together to form the representative sample for that store. In all, 53 maize samples were collected this way. The samples were then stored in paper bags, and were labelled as WC (white corn) and YC (yellow corn) and numbered serially for example, WC1 and WC2, etc. They were then conveyed to the laboratory where they were stored in the refrigerator pending the experiments.

#### Media used

Malt Extract Agar (MEA) was used as the primary isolation medium while Potato Dextrose Agar (PDA) was used to prepare the pure cultures of the isolates.

#### Isolation of *Fusarium* species

Five grains were selected per sample and surface sterilized in 1% sodium hypochlorite solution for 30 s. The grains were then rinsed

in sterile distilled water thrice and transferred to MEA. The MEA plates were incubated at ambient temperatures with approximately 12 h rotations of light and darkness and the fungal growth observed morphologically and microscopically according to the methods of Nelson et al. (1983).

#### Detection of toxins

The samples that yielded *Fusarium* spp were tested according to the presence of fumonisins and deoxynivalenol using Agri-Screen® ELISA-based screening kits, produced by Neogen Corporation, U.K. according to the procedures described in the manufacturer's instructions. The tests categorized the toxin contamination of the grains into levels that were either above or below 1 ppm for DON and 5 ppm for fumonisins.

## RESULTS AND DISCUSSION

YC11 yielded three *Fusarium* samples while samples with WC29, YC1 and YC21 showed infection by two different species of *Fusarium* (Table 1). The most frequently isolated species was *Fusarium verticillioides* occurring in seven maize samples.

Out of the fifty three samples tested, ten samples (18.87%) showed evidence of fumonisins (FB1+FB2+FB3) production while three samples tested positive for DON (5.66%) and three (5.66%) yielded both toxins (Table 2). WC29, YC8 and YC11 which yielded DON also contained fumonisins. These samples were contaminated with *F. graminearum* which is a prolific producer of DON (Dorn et al., 2009), however all the samples contained quantities of DON below the recommended maximum of 1 ppm. The findings in this study contrast with those reported by Castella et al. (1999) who observed a high value of 79.5% fumonisin contamination in maize products, and Reddy and Salleh (2011) who detected fumonisins in 100% of the maize sampled. Of the grains sampled, 5.66% had > 5 ppm fumonisins while seven others (13.21%) had < 5 ppm. It is also possible that the actual fumonisin content in even the samples categorized as < 5 ppm of fumonisin exceeded the 2 ppm bodyweight/day Tolerable Daily

**Table 2.** Level of toxin contamination in maize samples.

Serial No.	Sample*	DON		Fumonisin	
		< 1 ppm	>1 ppm	<5 ppm	>5 ppm
1	WC1	-	-	+	-
2	WC10	-	-	+	-
3	WC29	+	-	-	+
4	WC30	-	-	+	-
5	YC1	-	-	+	-
6	YC3	-	-	-	+
7	YC8	+	-	-	-
8	YC11	+	-	-	+
9	YC16	-	-	+	-
10	YC21	-	-	+	-

\*, These are the samples which yielded *Fusarium* spp. and were then further tested for mycotoxin contamination.

Intake recommended by the JECFA. This means that those samples are potentially hazardous to human consumers. The contamination with these toxins may be ascribed to agricultural practices such as threshing which may cause grain breakage and favour the spread and proliferation of *Fusarium* and its toxins.

Another reason may be the fact that in this region, there are sometimes isolated showers late in the season which may favour toxin production in maize. The presence of toxins in the maize samples despite the low moisture content of 5.3 to 13% suggests that the toxins were probably formed in the field when the moisture content was higher, or immediately after harvest. This is particularly possible because *Fusarium* is primarily a field fungus (Christensen and Kaufmann, 1965) and begins to produce its mycotoxins on the grains in the field under favourable conditions. Also, the climate of Zaria is a typical Sudano-Sahel climate which is typified by hot days and cold nights, a condition which has been reported to be ideal for the production of zearalenone a *Fusarium* mycotoxin (Bennett et al., 1980; Shephard et al., 1996). There are currently no known standards set by the National Agency for Food Drug Administration and Control (NAFDAC) and very little information is available on this type of contamination of foods in Zaria and Nigeria as a whole. This is a disturbing trend especially as these samples were obtained from the warehouses of brewers and feed millers who often purchase the best portion of the annual agricultural produce. The actual figures in grain lots available to the subsistence consumer may be significantly higher.

### Recommendations

Further work should be done to determine the exact quantities of the various classes of fumonisins, particularly Fumonisin FB1, which is of the greatest economic importance.

### Conclusion

Most of the contaminated samples were below the benchmark figures. The results obtained show that there exists a danger of *Fusarium* mycotoxin contamination in the grains used in industrial productions. NAFDAC thus needs to set up benchmarks for the regulation of mycotoxin contamination of maize consumed locally or used in food industries. There is also need for inspection services to be put in place to ensure compliance with the set standards as little, if any information exists about the regulation of *Fusarium* toxins in Nigeria. Further work needs to be carried out for the quantification of the mycotoxins. This will provide exact contamination figures and help safeguard the population from consumption of possible carcinogens in their staples.

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