ナイジェリアにおけるゼアラレノンとT-2トキシンによるとうもろこし汚染とヒトへの暴露

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著者名	Afolabi,C.G. Ekpo,E.J.A. Bandyopadhyay,R.
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Maize contamination by zearalenone and T-2 toxin and human exposure in Nigeria

Clement G. AFOLABI[1, *], Ephraim J. A. EKPO[2], Ranajit BANDYOPADHYAY[1]

[1]International Institute of Tropical Agriculture, Oyo Road, Ibadan, Nigeria
[2]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria

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Abstract

Maize is one of the most important staple cereal grains grown and consumed in Nigeria. One hundred and four maize samples destined for human consumption were collected from thirteen popular different markets during a survey of three agro-ecological zones (AEZ) and levels of zearalenone (ZEA) and T-2 toxin (T-2) were determined by enzyme-linked immunosorbent assay (ELISA). Levels of ZEA contamination of maize samples ranged from < 50 μg/kg to 196 μg/kg. Although, ZEA was detected in 37% of the total maize samples, only 3.8% of these maize samples exceeded the EU maximum recommended limit for ZEA in unprocessed cereals of 100 μg/kg. Also, T-2 toxin was detected in 36% of the total maize samples, and the range of contamination was < 7.5 to 29 μg/kg. At present, there is no EU maximum tolerable limit for this toxin in unprocessed cereals. Eighteen samples constituting 17% of the total samples were found to be contaminated with the two toxins. On the distribution of these toxins on AEZ, the Derived Savanna (DS) zone had more positive samples of 50 and 38% for ZEA and T-2 respectively than the other two AEZ, though more samples were collected from Southern Guinea Savanna (SGS).

Introduction

Maize is an economically important crop grown all over the world. In Nigeria, maize is used in various ways than any other cereals as human food, feed grain, fodder crop and many industrial purposes. The various ways through which maize is utilized, underscore its importance to the Nigerian economy. However, maize production is bedevilled by a number of constraints amongst which are soil fertility, drought, pest and diseases. The diseases affect all the plants parts including the maize ear causing quality and quantity deterioration through mycotoxin contaminations.

Mycotoxins are chemical pollutants of biological origin produced by fungi. Zearalenone(ZEA) is one of the several known mycotoxins and some of its derivatives are produced by several *Fusarium* species that invade corn, wheat, and other cereals[11]. It has been found in a wide variety of plants and soils, with potential negative health effects on animal husbandry and humans. ZEA has been reported to cause premature puberty

Corresponding Author
*Department of Crop Protection, Federal University of Agriculture, PMB 2240 Abeokuta, Nigeria. Tel: +234 8060335246. E-mail: afolabicg@funaab.edu.ng
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in children of 7 and 8 years[16]. It has also been implicated in serious reproductive and toxicological problems in farm animals[10] fed with contaminated mouldy maize, causing hyper-estrogenic syndrome[13] and consequently a large economic loss.

T-2 toxin is a member of a large group of fungal metabolites with the same basic chemical structure, called trichothecene mycotoxins. Warm and moist weather conditions favour plant infection with *Fusarium* species, while improper storage and handling of grain with high moisture content can lead to T-2 toxin contamination[18]. Among all grains tested so far, corn, wheat, barley, oat, and rye are most frequently contaminated with this mycotoxin[7].

Recently, there is an increasing concern of the hazards of *Fusarium* mycotoxins to animal and human health in many countries. In Nigeria, the public is generally unawares of mycotoxins contamination of most grains and there are no guidelines yet to regulate its presence in food and feed. There was paucity of information on the levels of contamination of maize by these two mycotoxins from Nigeria. The present work provides for the first time a comprehensive summary of the concentrations of ZEA and T-2 of maize from thirteen markets covering three AEZ of the country. Previous studies have established the mycotoxins contamination of Nigeria feeds and foods by ZEA, ochratoxin, aflatoxin, fumonisin (FB) on rice[15], trichothecenes on maize[2], FB on maize[3, 5] but did not elucidate the occurrence of ZEA and T-2 toxins on maize. We therefore need to continuously inspect maize and other domestic feeds for contamination with *Fusarium* and other mycotoxins. Therefore, this study was undertaken to determine the incidence and levels of contamination of ZEA and T- 2 toxins on market maize samples meant for human consumption in Nigeria and to determine the exposure of Nigerian people to these mycotoxins.

Materials and Methods

Sample Collection A survey was carried out in maize producing areas in three AEZ of Nigeria; Humid Forest (HF), Derived Savannah (DS) and Southern Guinea Savannah (SGS), to evaluate incidence of ZEA and T- 2 toxin in maize kernels in Nigeria. The HF is characterized by two growing seasons, starting from April to November with an annual rainfall of 1,500 mm and 2,000 mm, average annual temperature of 24.5 to 27.5 °C and mean relative humidity of 78 to 100%. Derived Savannah has two growing seasons, starting from May to early November with an annual rainfall range of 1 , 200 mm to 1 , 700 mm, average annual relative humidity ranged from 66 to 78% with mean temperatures of 26-27 °C, while SGS has a single wet starting from May to October with an annual rainfall of 1 , 000 mm to 1 , 200 mm, average annual temperature of 25.5-26.5 °C and relative humidity of 57 to 66%. One hundred and four (104) maize samples were collected from 52 vendors from 13 major agricultural markets located in the 3 agro-ecological zones (Fig. 1). These markets were selected for the survey because of their strategic locations, economic potentials and the population of the inhabitants. Eight maize vendors randomly selected from each market per town were individually interviewed on source (s), length and type of storage, and management practices on their stock. Eight maize samples meant for human consumption were randomly collected from different sellers in each location making a total of 104 samples. Maize samples of about 1.5 to 2.0 kg grain were collected from each seller; hand mixed and sub-divided into two subsamples and was kept in a well labelled sampling bag. The samples were returned to the laboratory and stored at 4 °C in a cold room before mycotoxins analyses.

Mycological analyses Forty maize kernels selected arbitrarily from each sample were surface disinfested

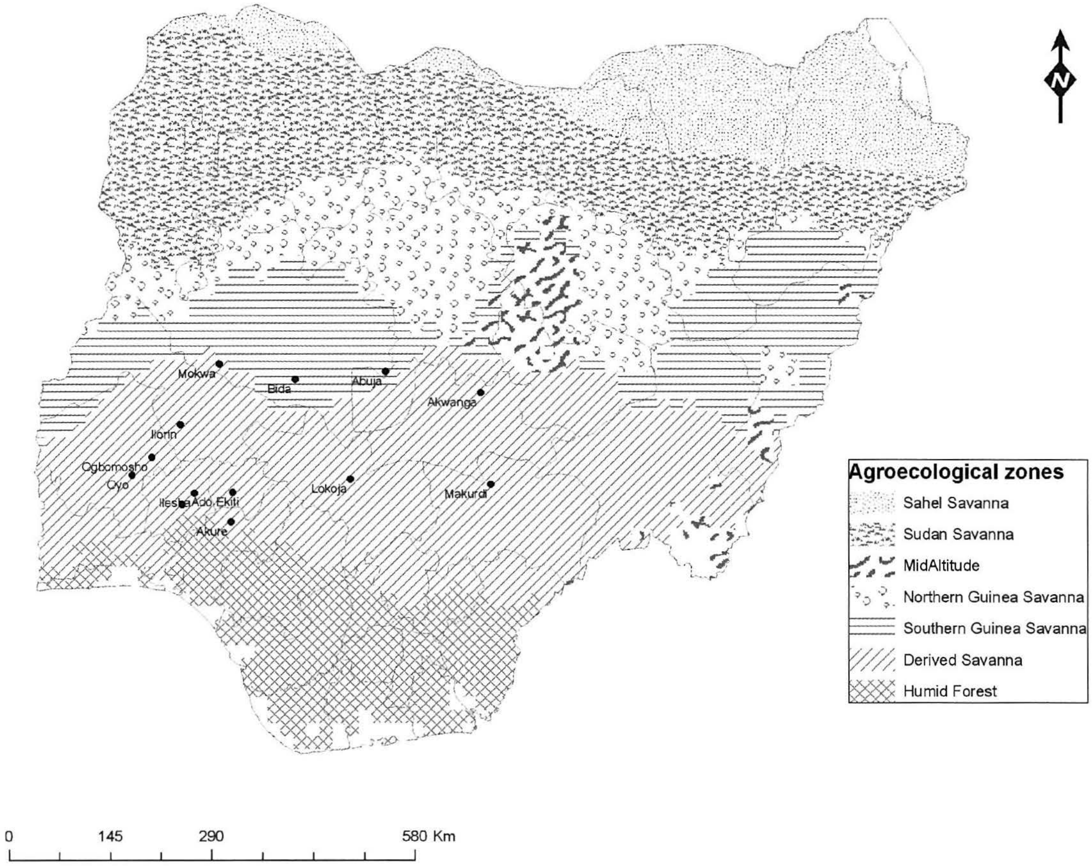

Figure 1. Map of Nigeria showing the sample collection sites and the agroecological zones

by immersing them for 30 s in 1% NaOCl and then rinsed twice in sterile distilled water and were then transferred to a semi-selective medium for *Fusarium*, peptone-pentachloronitrobenzene agar. The plates were incubated for 6 days under fluorescent lights on a 12 h day/night schedule at 22 to 24 °C. Single spores from *Fusarium* colonies were transferred to carnation leaf agar for identification on the basis of standard morphological criteria[14].

Sample preparation A 200 g maize grain from each sample was ground in a Romer grinding/sub sampling mill (Romer Series 11™ Mill; Romer Labs, inc. Union, MO, USA). Each ground sample was passed through a 20-mesh sieve, from which 20-g of subsample was used for toxin analysis. ZEA was extracted with 100 ml of 70% methanol, while T-2 toxin was extracted with 100 ml of 50% methanol using a high-speed blender (Waring Commercial Blender) for 3 minutes. The extract was filtered through a Whatman® number 1 filter paper (Whatman International Ltd Maidstone England) and 20 ml aliquot was collected for further analysis. A competitive direct enzyme-linked immunosorbent assay (CD-ELISA) in a microwell plate format (Veratox for ZEA and T-2 toxin, Neogen Corporation, Lansing, MI, USA) was used to quantify these toxins.

Analytical Methods In brief, each sample was diluted by adding 100 μl of extract to a sample dilution bottle containing 7.9 ml of 90:10 water-methanol, mixed by swirling the bottle and processed according to the kit manufacturer's instruction. Standard solutions containing known amounts of each toxin served as control.

The optical density for each sample was measured at 650 nm with a DYNATECH MR 250-microwell reader (Dynatech Laboratories Inc., Chantilly, Va.) and plotted against a standard curve generated with each microtiter plate from the toxins solutions of known concentration to determine the amount of toxin present. Samples with more toxin than in most concentrated standard were diluted appropriately with the extraction solvents and reassayed. The results obtained were multiplied by the dilution factor. The minimum detection limits were 50 μg/kg and 7.5 μg/kg for ZEA and T-2 toxin respectively. The recovery of ZEA and T-2 toxin averaged 88 and 87% respectively using an uncontaminated maize grain spiked with controls provided with the kits. The amount of variation between subsamples was < 15%.

Results and Discussion

The mycological results showed that *Fusarium verticillioides, F. proliferatum, F. andiyazi, F. semitectum, F. sporothrichioides* and *F. lateritium* were among the *Fusarium* species isolated from the maize kernels. However, *F. verticillioides* was the most dominant among the *Fusarium* species on all maize samples collected and confirms previous reports[2, 3, 5] of the predominance of the fungus on maize in Nigeria.

ZEA was detected in 37% of the maize samples. Maximum, mean and the median values were 196 μg/kg, 53 μg/kg and < 50 μg/kg respectively (Table 1). Only 3.8% of the maize samples exceeded the EU recommended value for ZEA in unprocessed cereals of 100 μg/kg. This result is similar to the report of Abd[1] who found low incidence (24 out of 135 samples) of ZEA in commercial cereal samples at concentration of 5-50 μg/kg in Egypt and concentration of 55 μg/kg in 1985 and 6.3 μg/kg in 1991 in Argentina[19], but was in contrast to other reports of high incidences and concentration of 1.45 mg/kg and 1.75 mg/kg in dried maize and freshly harvested infected maize ear respectively in France[4] and Italy[6].

T-2 was detected in 36% of the total maize samples. 29 μg/kg, 13 μg/kg and 12 μg/kg were the maximum, mean and median values for T-2 toxin respectively (Table 1). The level of T-2 contamination in this study was lower compared to results from other working groups. Tseng et al.[20] reported T-2 contamination at the levels of 78 to 650 μg/kg on 7.8 % maize samples in Taiwan, 65 % of maize samples in New Zealand had total toxin that was less than 1 mg/kg[12], while 210 μg/kg toxin level was the maximum level of detection in maize sampled in Croatia[17].

On AEZ basis (Table 1), the highest number of ZEA positive maize samples was from DS (50%), while samples from the HF (19%) were the least contaminated although both zones had less amount of maize samples compared with SGS with fifty-six samples and 43% positive samples. Likewise, both DS (38%) and HF (40%) had higher number of T-2 toxin positive samples than SGS (32%). However, the range of T-2 toxin contamination was similar in the three AEZ. The incidence of mycotoxins positive samples found in these zones could be directly attributed to the weather conditions prevalent in these zones during the growing seasons and is in agreement with previous reports which suggest that warm and moist weather conditions favour plant infection with *Fusarium* species[3], although, handling during storage under high moisture content could also increase the level of toxin content. Also, the low levels of mycotoxin concentration could be attributed to sorting and winnowing performed on the bulk samples by the vendors which reduced greatly visibly mouldy, discoloured or damaged grain incidence on the samples meant for sale. Vendors sort and winnow maize before being displayed to attract higher prices for clean and good looking samples, than those with mixture of discoloured and clean grains. This in itself would have reduced the level of incidence and

Table1. Zearalenone and T-2 toxin contamination of maize samples from different markets in Nigeria

	Market	N[a]	% Positive Samples	Zearalenone (μg/kg)			Samples above 100 μg/kg	% Positive Samples	T-2 toxin (μg/kg)		
				Max	Mean	Median			Max	Mean	Median
DS[b]	A	8	50	45	31	27	0	75	29	19	21
	B	8	50	196	137	144	3	0	0	0	0
Total		16	50								
Range of Contamination					< 50-196					< 14-28	
SGS[c]	A	8	50	42	33	31	0	0	0	0	0
	B	8	50	47	23	20	0	100	23	13	10
	C	8	50	40	30	29	0	50	17	9	10
	D	8	50	37	25	27	0	0	0	0	0
	E	8	0	0	0	0	0	25	25	13	13
	F	8	50	72	50	49	0	50	25	14	14
	G	8	50	95	64	71	0	0	0	0	0
Total		56	43								
Range of Contamination					< 50-95					<7.5-29	
HF[d]	A	8	0	0	0	0	0	50	23	14	12
	B	8	25	96	78	78	0	0	0	0	0
	C	8	50	153	68	55	1	100	13	51	5
	D	8	0	0	0	0	0	0	0	0	0
Total		32	19								
Range of Contamination					<50-153					< 7.5-23	

[a]N = Number of samples collected, [b]DS = Derived savannah, [c]SGS = Southern Guinea Savannah, [d]HF = Humid Forest

concentration of any toxin in the samples meant for human consumption. However, previous studies on mycotoxins contamination of maize samples confirmed fumonisin and *Fusarium verticillioides* as the most important toxin and *Fusarium* species respectively in Nigeria[3, 5]. Our present result also support this previous assertion that fumonisin is the most important toxin on Nigeria maize because of the low levels of these toxins. All samples contaminated with ZEA were positive for *F. semitectum* and all samples that contained T-2 toxin were positive for *F. sporotrichioides*.

Eighteen samples constituting 17% of the total samples were found to be contaminated with the two toxins with possible contamination by the major toxin (fumonisin) on Nigeria maize. The co-occurrence of these toxins in the samples is not a new report, but our concern is the potential health risk associated with the consumption of such maize grains since most of these samples contain toxin levels greater than maximum tolerable oral intake via food per day. Although, the implication of co-occurrence of these toxins on human health is unknown but concern has been expressed about the interactive effects of multiple toxins as a result of synergistic, additive or antagonistic effects in host organisms.

On human exposure to this toxin FAO[9] reported that Nigerians consume an average 138 kg of cereals annually and the European Commission[8] established a Tolerable Daily Intake (TDI) to ZEA of 0.2 μg/kg body weight/day. Considering that the average concentration of ZEA in this study is 52.5 μg/kg, Probably Average Daily Intake (PDI) is 0.33 μg/kg body weight/day (Table 2). Therefore, the estimate average consumption of ZEA was high in the analyzed maize sample. This may contribute significantly to health risk

Table 2. Derived Zearalenone and T-2 toxin exposure of Nigerians for maize consumption

Mycotoxin	Mean ($\mu g kg^{-1}$)*	Mycotoxin exposure		
		μg person^{-1}year^{-1}**	μg person^{-1}day^{-1}	$\mu g kg^{-1}$ bw^{-1} day^{-1}***
Zearalenone	52.5	7,245	19.85	0.33
T-2 toxin	11.9	1,642	4.50	0.08

*Mean of mycotoxin incidence based on analyzed field samples
**Obtained by multiplying the average cereal consumption per year (i.e. 138 kg person^{-1}year^{-1}) by the corresponding mycotoxin in maize
***Determined for a person with average body weight of 60 kg

of the people due to chronic exposure to ZEA in maize based diets especially in children where it has been reported to cause premature puberty in children aged 7 and 8[16].

On T-2 toxin, TDI for T-2 and HT-2 toxin is 0.06 μg/kg body weight/day[8]. The average concentration of T-2 in the analyzed samples was 11.9 μg/kg, and the Probable Average Daily Intake is 0.075 μg/kg body weight/day (Table 2) for an adult population with an average body weight of 60 kg. This shows the estimate average exposure to T-2 was high in the analyzed samples which can lead to alimentary toxic aleukia thereby causing a great risk to consumers' health.

In conclusion, although, the incidence of the toxins analyzed in this study has been found to be fairly low; however, some samples had toxins above acceptable limits. Thus, it is important to explore ways of reducing the infection of maize by mycotoxin-producing fungi in cereals, thereby, reducing the incidence of secondary metabolites and the hazards they may cause human and animal.

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Clement G. Afolabi[1], Ephraim J. A. Ekpo[2], Ranajit Bandyopadhyay[1]（[1]International Institute of Tropical Agriculture, Oyo Road, Ibadan, Nigeria, [2]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria）

とうもろこしはナイジェリアで栽培，消費されている最も重要な穀物のひとつである．3つの農業生態学的地帯（AZE）における13の市場から食用のとうもろこし104検体を集め，ELISA法によってゼアラレノン（ZEA）とT-2トキシン（T-2）レベルを測定した．とうもろこしのZEA汚染は50 μg/kg未満から196 μg/kgまでの範囲であった．ZEAは37%のとうもろこしから検出されたが，この中の3.8%だけがEUにおける未加工穀物100 μg/kgに対するZEAの上限値を超えた．T-2は36%のとうもろこしから検出された．汚染の幅は7.5 μg/kg未満から29 μg/kgであった．現在EUではT-2に対する規制値を設けていない．全検体の17%にあたる18検体から両トキシンが検出された．AEZ別にこれらのトキシン分布を見てみると，南ギニアサバンナ地帯（SGS）において最も多くのサンプルを収集したにもかかわらず，派生サバンナ地帯（DS）では他の二つのAEZに比べて，ZEAで50%，T-2で38%多く陽性検体が検出された．

キーワード：市場；ゼアラレノン；とうもろこし；ナイジェリア；マイコトキシン；T-2トキシン