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Detection of Aflatoxins from Foreign and Locally Made Beer

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Abstract: *Aflatoxin (AF) levels and mycoflora contamination levels were determined in beer samples sold in the market and bars in Enugu metropolis. The study was carried out on 90 beer samples which consist of 12 foreign and 78 local beer, and comprised of 39 can beers and 51 bottled beers. A total of 16 (17.8%) of the beer samples were contaminated by fungal agents, with a preponderance of Aspergillus flavus, Penicllium Spp and Trichoderma viridae each with a prevalence of 2 (2.2%). Other organisms include Saccharomyces spp, Geotrichum candidum, Cladosporum spp, Rhizopus spp and Tricophyton schoenlenii each, with 1(1.1%) prevalence. Five samples showed no evidence of identifiable fungal growth (Mycelia sterilia). Of the beer samples contaminated, 6(6.67%) and 10(11.11%) were from canned and bottled respectively, which was not statistically different (p>0.05). Aflatoxin B1 levels up to 38ug/l was detected in Star beer sample, and the least detectable AFB1 (3.43ug/l) was from a Guinness beer sample. Only one sample (Guinness beer) yielded AFB2 of 2.2ug/l. Fourteen (17.9%) local beer samples were contaminated by fungal agents when compared to foreign beer with 2(16.7%).There was however no statistical difference between the two (p>0.005). The need for strict regulations of Agricultural activities and quality control of brewing industries in Nigeria is hereby emphasized considering the public health consequences on the number of consumers of beer and other related products.*

Key words: *Aflatoxins, detection, beer.*

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I. Introduction

Aflatoxins are poisonous, carcinogenic by-products of the growth of the moulds *Aspergillus flavus* and *Aspergillums parasiticus* are the most studied and widely known mycotoxins. There are four major groups of alfatoxins: B1, B2 G1 and G2 plus two additional metabolic products M1 and M2 that are of significances as direct contaminations of foods and feeds. In Nigeria today, aflatoxin contamination of food resources is a major problem due principally to the prevalent weather and relative humidity, temperature and unsanitary condition which favor the growth of *Aspergillus flavus.* From the foregoing, it would appear that the aflatoxins problem is an endemic one in our region and so deserves closer research emphasis with a view to curtailing its adverse health effects on the populace. (Diener *et al.*,1996).

Alfatoxins are of economic and health importance because of their ability to contaminate human food and animal feeds, particularly cereals, nuts and oil seeds (Arim *et al.*,1995). Cheese, almonds, figs and spices have also been associated with aflatoxins according to Saad *et al.* (2005). The aflatoxins problem has been reported to be more serious in tropical and subtropical regions of the world where climatic conditions of temperature and relative humidity favour the growth of *Aspergillus flavus* and *Aspergillus parasiticus.*

According to Nelson and Max (2005), beers are known to be the world's oldest and most widely consumed alcoholic beverage and the tea. It is produced by the brewing and fermentation of starches, mainly derived from cereal grain most commonly malted barley, although wheat, maize (corn), and rice are widely used (Arnold *et al*., 2005). Most bear is flavoured with hops, which adds bitterness and acts as a natural preservative, though other flavourings such as herbs of fruits may occasionally be included. In Nigeria today, aflatoxin contamination of food resources is a major problem due principally to the prevalent weather and relative humidity, temperature and unsanitary conditions which favour the growth of *Aspergillus flavus.* Compounding this problem is the crude agricultural practices of uniformed farmers which often result in high level of physical damages to the grains. Malted Nigerian and Kenyan grains have been reported to have high levels of aflatoxins (Kenji *et al.,* 2000).

The malted grains are mainly used for the manufacture of traditional beer (pito) and sometimes used for the manufacture of local beer brands (Gulder, Star, Guinness Stout, Harp, Heineken and Legend), as it is usually

mixed with barley to save foreign exchange. The brew is consumed by most of the communities in Nigeria, hence if the brew produced contains aflatoxins and it is ingested, it poses a great health risk to the consumers. A survey of traditional by Ekpenyong *et al.*, (1984) on brewers' products in the Jos metropolis has showed high incidence of aflatoxin B1 contamination, which was also detected in the blood of apparently healthy blood donor's by Onyemelukwe *et al.,* (2010). Samples of traditional opaque sweet beverage (Thobwa) and beer prepared from sorghum, malts and grains were collected from the southern region of Malawi during the humid month of January. The results of the analysis for total aflatoxins using aflatest VICAM fluorometry procedure showed that 15% and 43% samples of the sorghum malt for beer brewing had a significantly ($P<0.01$) higher total aflatoxins contents (average 408 + 68 µg/kg) (SEM) than any other type of sample. The average aflatoxins content in the beer was 22.23µg/L which is higher than the permissible maximum level in ready to eat foods set by codex Alimentarium commission (10µg/L) according to Kenji *et al.,* (2000).

Aflatoxins are known to be portent toxins as well as carcinogens. In Nigeria, a number of deaths have been ascribed to the consumption of local brews. In most of these cases the brew has been said to contain high alcohol levels. Little or no attention has been given to the possible contribution of high level of aflatoxins in the brews to the general health of brew consumers.

JUSTIFICATION

Aflatoxins are of economic and health importance because of their ability to contaminate human food and animal feeds, particularly cereal nuts and oil seed (Arim *et al.,* 1995). Compounding this problem is the crude agricultural practices to the grains. Malted Nigerian and Kenyan grains have been reported to have high levels of aflatoxins. Many studies in Nigeria have reported local grains to have aflatoxin levels of between 25 – 77ppn. According to Cotty (1994), corn – based snacks (corn cake and corn roll snacks) have aflatoxin level of 15 – 1070 ppb and 10 – 160 ppb respectively. Hence, this study is aimed at ascertaining the level of aflatoxins and mycoflora in foreign and locally made beer.

AIM AND OBJECTIVES

1. To detect aflatoxins from foreign and locally made beer both qualitatively and quantitatively.
2. To detect any *Aspergillus flavus* group and microbial contamination in the beers.
3. To monitor differences in beer containers and beer brands in terms of their contaminations.
4. To make valid recommendation/inferences on the basis of the findings.

II. Methodology

A. SAMPLE COLLECTION

Beer samples used for the study, consists of six (6) local brands and two (2) foreign brands purchased from sellers in the market and bars in Enugu state, South - Eastern Nigeria. A total ninety (90) samples made up of 39 can beers and 51 bottled beers were analyzed. From the thirty – nine (39) can beers obtained from the market, six (6) were foreign and 33 were local beers. The fifty –one (51) bottled beers on the other hand, consist of six (6) foreign and forty – five (45) local beers. The samples were brought in their containers and transported in their sealed containers to the laboratory and analysed within 24hrs. Legend and Smirnoff ice beer (can) were not in the market during the study.

B. PROCESSING OF SAMPLES

Culture

The ninety (90) samples were cultured into freshly prepared Sabouraud Dextrose Agar (oxoid) supplemented with chloramphenicol (S+C). The beer containers surfaces were cleaned with cotton wool soaked in sodium hypochlorite, surface sterilized by flaming and then carefully opened near the flame. About 10ml of the beer were carefully tipped into the SAB slants (S+C), allowing a full coverage of the slant surface and excess drained off aseptically into Lysol. Culture tubes were incubated between 16-21 days to get good growth at room temperature.

C. IDENTIFICATION OF ISOLATES

Colonial morphology is done macroscopically by examining each culture tube under good source of light and the description noted include; Rate of growth, colour of the growth, texture of the growth, topography/elevation, reverse side or colour of the underside. Lactophenol cotton blue mount was carried out on the fungal cultures to identify the exact fungi based on the conida and hyphal arrangements. Arrangements, surface appearances, sizes, shapes, colour and distribution of fruiting bodies/spores and hyphae were studied and fungi identity derived with reference to mycological atlas.

D. DETECTION OF AFLATOXINS

Each culture filtrate (10ml) was extracted by using equal volumes of chloroform twice. Fifty aliquots of each extract were then applied to thin layer chromatography plates (TLC). Analytical methods were according to Mishra and Daradhiyars (1991).

E. QUANTITATIVE ESTIMATION OF THE AFLATOXINS

Quantitative estimation of the aflatoxins detected was done following the methods of Nabeny and Nesbitt (1965). The quantity of the aflatoxins was determined by optical density measurement at 363nm using a Bausch and Lomb spectrophotometer (BL spectronic2000). Analysis for aflatoxins was carried out in the foundation for health (NGO) research center, Enugu.

III. Results

Table 1: Shows the distribution of fungal growth according to types of beer containers.

ORGANISMS ISOLATED FROM BEERS	NUMBER	PERCENTAGE	TYPES OF BEER CONTAINERS	
			CAN	BOTTTLE
Aspergillus flavus	2	2.2	0	2
Saccharomyces spp	1	1.1	1	0
Penicillium spp	2	2.2	0	2
Geotrichum candidum	1	1.1	1	0
Trichoderma viridae	2	2.2	0	2
Cladosporuim spp	1	1.1	1	0
Rhizopus spp	1	1.1	0	1
Trichophyton schoenlenii	1	1.1	1	0
Mycelia sterilia	5	5.5	2	3
Total	16	17.8	6(6.6%)	10(11.11%)

From the total of 90 beer samples analyzed (Table1), 16 (17.8%) isolates of fungi were encountered which consists of 6 (6.6%) positive growth from can beer samples and 10 (11.11%) positive growth from the bottled beer, which difference was not significant ($p>0.05$). Out of the total 16 fungal isolates encountered in the study, 2 (2.2%) each, were *A. flavus, Trichoderma viridae and Penicillium spp* respectively. The rest yielded 1(1.1%) each isolates of *Saccharomyces spp, Geotrichum candidum, Cladosporium spp, Rhizopus spp* and *Trichophyton schoenlenii*. Meanwhile 5 (5.5%) of the culture were regarded as *mycelia sterilia*, showing no significant development of fruiting bodies for complete identification. Only hyphal elements were detectable in the culture by standard mycological techniques.

TABLE 2: Shows the distribution of the aflatoxins detected in the various beer samples analysed.

NAME OF BEER	NO POSITIVE FOR *A. FLAVUS*	TYPES & LEVEL OF AFLATOXINS PRODUCED (ug/l)				
		AFB_1	AFB_2	AFG_2	AFG_1	AFG_2
$Guinness_1$	+ve	13.32	-	-	-	-
$Star_1$	+ve	38	-	-	-	-
	NO POSITIVE FOR OTHER ORGANISMS.					
$Gulder_1$	+	6.12	-		-	-
$Guinness_2$	+	-	-		-	-
$Star_2$	+	-	-		-	-
$Gulder_2$	+	-	-		-	-
$Harp_1$	+	7.81	-		-	-
$Star_2$	+	-	-		-	-
$Star_3$	+	-	-		-	-
Smirnoff ice	+	-	-		-	-
$Guinness_3$	+	3.43	2.2		-	-
$Gulder_3$	+	-	-		-	-
$Harp_2$	+	-	-		-	-
Becks	+	-	-		-	-
Heineken	+	-	-		-	-
$Harp_3$	+	-	-		-	-

Amongst the beer samples showing fungal growth, only 5(5.5%) yielded demonstrable levels of aflaoxins, ranging from 2.2 – 38ug/l. (Table 2). The distribution of the aflatoxin detected showed that one Guinness and Star beer, each, with the growth of *A.flavus*, had 13.32 and 38 ug/1 AFB1 respectively. Strikingly, one sample without complete identifiable fungi (*mycelia sterilia*) yielded AFB1 level of 6.12ug/1 in Gulder.

Other beer sample with positive fungal isolate and aflatoxins detection include: Harp beer sample yielded *Saccharomyces cervisae*, with an AFB1 level of 7.81 ug/l; Guinness yielded *Rhizopus spp* with an AFB1 level of 3.43ug/1 and AFB2 level of 2.2 ug/l. Notably, no aflatoxins was detected in any of the two foreign brands beer samples analysed though, one (Smirnoff ice) beer sample yielded fungal growth of *Penicillium spp*, one (becks) beer, yielded *mycelia sterilia*. However, none was detected for can beer sample of which difference was statistically not significant ($p>0.05$).

Table 3: Shows the distribution of beers yielding positive fungal growth.

BRAND OF BEER	FOREIGN NO SAMPLE	BEER NO POSITIVE	LOCAL NO SAMPLE	BEER NO POSITIVE
Becks	6	1(16.7%)	0	-
Smirnoff ice	6	1(16.7%)	0	-
Legend	-	-	6	0(0%)
Heineken	-	-	15	1(6.7%)
Harp	-	-	15	3(20%)
Guinness stout	-	-	15	3(20%)
Star	-	-	15	4(26.7%)
Gulder	-	-	12	3(25.7%)
Total	**12**	**2(16.7%)**	**78**	**14(17.9%)**

Table 3 shows the distribution of beers yielding positive fungal growth. From the total of 90 beer samples analysed, 14 (17.9%) out of 78 local beer were positive for fungi growth while 2 (16.7%) out of 12 foreign beer were positive for fungal growth. The highest fungal growth in the local beer sample was recorded Star beer with 4 (26.7%) followed closely by Harp, Guinness stout and Gulder beer with fungal growth of 3 (20%) each. Heineken yielded only 1(6.7%) positive fungal growth while amongst the foreign samples, both, becks and Smirnoff ice yielded 1 (16.7%) positive fungal growth each.

Discussion

The results of this study showed that some of the beer samples analysed was contaminated with moulds of which *mycelia sterillia* was a dominant amongst the 9 group encountered. Others include *A. flavus, Pencillium spp* and *Trichoderma viridae*. It thus appear from previous works worldwide, that out of the many genera of moulds involved in food contamination, *Aspergillus* and *Penicillium spp* ranked highest amongst others (Adams and Moss,1996). As reported by Adebojo and Idowu (1996), surveys conducted worldwide showed that these 2 genera of fungi have consistently been prominent in food samples and animal feeds from Egypt, Nigeria and Argentina respectively. Meanwhile, Lacey (1989) reported that the spoilage of food and stored products of pre and post harvest involves a wide range of fungi contamination which differs greatly in their ecological determinants.

The large variety of fungi isolated from the current study is not surprising in view of the crude agricultural practice (during harvest) in Nigeria, thereby rendering many of these grains grossly damaged by primitive tools, coupled with poor drying and storage facilities. Also many of these grains are spread in the open on bare floor of the roadsides, rooftops in an environment with high level of humidity in the country. The pre-harvest attack of food items in the field by insects and birds is also a common feature in this part of the world. Sequel to this, the possible fungal contamination of these commodities is common. This is because, an entomological assessment of insect infestation, *Aspergillus flavus* infection, aflatoxin contamination in stored maize and their relationship carried out by Wright (2010) showed strong correlation to high densities of weevils (live or dead) and other secondary species. In Nigeria today, a good fraction of raw materials for beer production is locally sourced, through purchase of grains from farmers in order to save money being spent on purchasing foreign barley. These highly contaminated grains such as Sorghum and maize can therefore be a potent source of contamination through introduction of aflatoxigenic fungi into beer. Studies conducted in other parts of the world, have also shown a similar trend especially in African Countries. Thus, in South Africa, maize and barley, both of which are used for producing locally brewed alcoholic beer, are frequently contaminated by

mycotoxin moulds. The study was undertaken to investigate whether these toxins are present in raw grains and the traditional beers imbibed by the local black African population. It was established that the raw ingredients (sorghum, sorghum malt grains, maize grits), commercially produced traditional beers (utshwala and utfulamfua) were contaminated by bacteria and fungi (both yeast and moulds). The contaminating moulds identified were *Aspergillus flavus, A. alliaceus, A. clavatus, Penicillium spp, Rhizopus spp* and *Mucor spp.* The contaminated samples were analyzed for aflatoxin B1, B2, G1 and G2, zearalenone, cilrinin, deoxynivalenol, and ochretoxins A using a multi-mycotoxin thin-layer chromatography screening method and the toxins were quantified by high performance liquid chromatography. Infected Sorghum malt grain samples contained the toxin zearalenone. No mycotoxin producing fungi were present in the fermented beers however, two out of the six commercial beer samples contained aflatoxins (200 and 400mg/l). Thirteen (45%) out of 29 samples of the home brewed beers had zearalenone range of 2.6-426 mg/l and chratoxin A of 3-2340mg/l (Lawrence *et al.*, 1988). Also in Canada, between March 1998 and March 2002, 304 samples of domestic (Canadian) and imported beers from 36 countries were picked up for the determination of Aflatoxin B1, B2, G1 and G2. Twelve of the samples were positive with aflatoxins greater than the limit of quantitation (LOQ) [Aflatoxin B1, 4.4ng 1 (-1); Aflatoxin B2, 3.4ng 1 (-1); Aflatoxin G1, 11.2ng 1(-1); and Aflatoxin G2, 6.2ng (-1)]. Five from Mexico, two samples from Spain and one from Portugal contained aflatoxin B1 and B2. The remaining samples contained less than the limit of quantitation (LOQ) for B1, B2, G1 and G2 (Lawrence *et al.*, 1988).

Recoveries averaged 90-104%, 94%, 84-87% and 89% for aflatoxins B1, B2, G1 and G2 respectively, at levels of 9.7-133ng B1, 46ng, B2, 35-140ng G1 and 41ng G2/l. Detection limits were 19.20ng/l for aflatoxins B1 and G1 and 15.16ng/l for aflatoxins B2 and G2. The majority of samples from the United States and Mexican beer was at 49ng B1/l when determined at 360nm excitation, but reanalysis result of 23 of the samples using 340nm indicated that an additional 4 Mexican samples and one Brazilian sample contained aflatoxin B1 at low levels (<10ng/1) (Lawrence *et al.*, 1988). Therefore, the contaminations of the beers in this study up to 38µg/l may not be surprising in view of the ubiquitous nature of *A. flavus* and other organisms in the grains used locally for beer brewing, and coupled with the environment as a whole.

Foreign beers were less contaminated by both the fungal agents and *A. flavus* compared with the local beers. This may not be too surprising knowing that the warm humid environment typically in Africa/Nigeria favour the proliferation of *A. flavus* and other moulds (Lacey 1989). In the previous studies conducted in several parts of Nigeria, it was established that stored maize recorded high mycoflora and aflatoxin levels ranging up to 600ppb from fungal contamination by *A. flavus, penicillium spp, Trichoderma spp, Fusarium, Candida spp,* and very high rate isolation of other moulds (Onyemelukwe *et al.*, 2010; Cotty, 1991; Okonkwo and Obiona, 1981; Apeh *et al., 2016*).

Thus, it is therefore very visible that locally made beers are bound to be more contaminated than foreign brands. The *A. flavus* may not thrive well in the cold or humid environment when compared with this environment. In this study it was also observed that 10 (11.11%) bottled beers were more contaminated than 6 (6.6%) canned beer. This might be as a result of inadequate or incomplete sterilization of the bottles, since they are recycled bottles for re-use in Nigeria, bearing in mind that the bottles were collected from different locations after consumption where they can easily be contaminated fungi spores, contact with carrying insects and through domestic use by people to store materials that could promote fungal growth in them. However, cans are disposed off after use, giving no chance for contaminations. Therefore, of the 5 aflatoxin positive samples encountered, all were bottled samples and no canned samples were recorded.

IV. Conclusion

From this study, it could be concluded that local beers in Nigeria are liable to natural contamination by aflatoxins (AF) which may differ according to the grains variety used for the production of the beer. Star beer which is a local beer yielded higher average aflatoxins (AF) levels than the rest of the local beers and also was more contaminated with fungi. The bottled containers preponderance was observed both in the mycoflora and aflatoxin levels. This therefore raises concern about their impact on human health and safety.

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