Incidence of Mycoflora and Mycotoxin Contamination in Pupuru; a locally Fermented Cassava Flour Sold in Okitipupa, Ondo State, Nigeria

Adeyemo, I.A.* and Olaribigbe, O.
Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria.

ABSTRACT: This study estimated the mycoflora and mycotoxins (aflatoxin and fumonisin) contamination of Pupuru; a locally fermented cassava flour sold in four markets (Okitipupa, Ilutitun, Igodan and Ikoya) in Okitipupa, Ondo state, southwestern Nigeria. Sixty (60) samples gotten from the markets were found to be lightly contaminated with aflatoxin ($P<0.05$) and fumonisin ($p<0.05$) using high performance liquid chromatography (HPLC) and enzyme linked immunosorbent assay (ELISA) respectively, although one of the samples from Okitipupa market had an undetectable level of fumonisin (>REF). The aflatoxin concentration in the samples ranged from 0.00096 to 0.0081 ppm while the fumonisin concentrations in the samples were between 0.08 and 0.68 ppb. Fungi species isolated from samples are *Penicillium chrysogenum, Penicillium italicum, Aspergillus flavus, Fusarium moniliforme, Rhizopus stolonifer* and *Aspergillus niger.* It is recommended that proper care should be taken during handling and storage of cassava used in the production of Pupuru to prevent the infestation microorganisms so as to ultimately reduce mycotoxin levels in the Pupuru..
Keywords:. Mycotoxins, Aflatoxin, Fumonisin, Pupuru, Cassava

INTRODUCTION

Pupuru is a staple starchy food popular in Nigeria, especially in the south western part of the country; produced from fermented cassava. Dried cassava pulp is milled into flour, which is used for human food in a variety of recipes. In Okitipupa, cassava flour is used for making a semi solid paste popularly known as "pupuru" in Ikale, native language of Okitipupa population. This semi solid paste is eaten with various types of soup sometimes combined with different vegetables and fish or meat. It is eaten by both children and adults from all levels of the economy. Apart from "pupuru", cassava flour is also cooked as "lafun" or fufu (Bankole and Majekobaje, 2004). During cassava harvest, handling and storage; it is inevitably contaminated with various contaminants including molds which produce harmful mycotoxins. Despite its importance in the food systems, cassava production is declining due to pests, management factors and poor post-harvest handling techniques at farm level (Bassa *et al*., 2001). A major constraint is the occurrence of aflatoxins produced by *Aspergillus* spp. caused by poor processing and storage conditions. *Aspergillus flavus* growth leads to cassava products quality deterioration, unfit for trade (marketing), household and public consumption.

[1]*Corresponding author:
adeyemiyourfriend@gmail.com;
08035671847

There is paucity of research in the area of pupuru microbiology of the various cultivars of cassava. Tropical climate of some geographical areas of cassava production may contribute to fungal development of many species and subsequent toxinogenesis on such raw material (Gomez *et al., 2004*). Moreover, the processing conditions and storage premises are not always well adapted to protect cassava products from secondary contamination and/or fungal development. Considering the importance of this crop in developing countries including Nigeria and subsequent possible fungal toxin production, various studies have attempted to evaluate cassava products contamination with moulds and mycotoxins (Bankole and Majekobaje, 2004). A number of potentially mycotoxigenic fungi have been isolated from cassava products and mycotoxins contamination of cassava has been documented but the potential sanitary risk of such contamination was not fully assessed (Velluti *et al.,* 2000). The study focused on the evaluation, isolation and identification of mycoflora infection and detection of aflatoxin and fumonisin in 'pupuru'; a locally fermented cassava flour sold in selected markets in Okitipupa, Nigeria.

MATERIALS AND METHODS

Study Location

This study was carried out in Okitipupa, a town in Southern Ondo State, Nigeria between July and August 2017 by collecting samples from four markets (Igodan, Ilutitun, Ikoya and Okitipupa) within the study area.

The average annual rainfall in Okitipupa is 1900 mm and 2030 mm with the rainy season lasting from April to November. The vegetation of Okitipupa area is the lowland rainforest. The name Okitipupa originated from the elevation of the town and the colour of the soil of the town which is red. The red colour is referred to as 'pupa' in Yoruba language and its dialects. Okitipupa is native to Ikales, who are a sub-set of the larger Yoruba tribe.

Collection of samples

A total of sixty (60) samples of 'pupuru; flour were analysed. Five (5) samples from five (5) randomly selected vendors were gotten from four (4) different locations in Okitipupa metropolis namely: Okitipupa main market, Ilutitun market, Ikoya market and Igodan-lisa market. These samples were divided into three equal parts and stored in polythene bags. Twenty 'pupuru' samples each were transferred aseptically in polythene bags to; The National Agency for Food and Drugs Administration and control (NAFDAC) Central Laboratory, Oshodi, for fumonisin analysis; Central Research Laboratory, University of Lagos, Nigeria for aflatoxin analysis and Microbiology laboratory of Ondo state University of Science and Technology Okitipupa for microbiological analysis.

Isolation of Fungal Isolates

Nine milliliters (9 mls) of sterile distilled water was dispensed into 100 test tubes which were subsequently plugged with cotton wool and aluminum foil. These tubes were autoclaved at 121°C for 15 minutes, and allowed to cool. Serial dilution process was carried out to thin out the microbial population of the 'pupuru' samples to prevent overpopulation of the colonies on the agar plates.

One gram (1g) of each 'pupuru' sample was carefully weighed into adequately labeled test tubes which contained 9 ml of sterile distilled water. The samples were homogenized through thorough shaking of the plugged test tubes. From these test tubes, serial dilution was carried out on all the labeled samples up to 10^{-5} dilution factor. From the tubes labeled 10^{-5}, 1 ml of each suspension was aseptically transferred into sterile petri dishes. Pour plate method of inoculation was used for this procedure. After allowing the media to solidify, the plates were incubated at room temperature in the dark for four (4) days. After incubation, the organisms were enumerated and purified by successive streaking on fresh agar plates to get pure cultures. Pure culture slants were stored in the refrigerator at 4°C.

Characterization and Identification of Isolates

The fungal isolates were characterized based on their macroscopic and microscopic characteristics. The macroscopic properties observed include: size of colony, filamentous or colonial, surface colour of colony, nature of hyphae, presence or absence of spores and color of reverse side of the plate. Microscopically, the features observed were: nature of hyphal wall whether thin/thick or smooth/rough, color of hyphae, presence or absence of septa in the hyphae and nature of spore whether rough or smooth.

Quantification of Fumonisin

Five grams (5g) of the flour sample were weighed into a clean sample container and tightly sealed and labelled appropriately. Twenty five milliliters (25 ml) of 70:30 methanol-water extraction solvent was added to the container containing the weighed sample and the container was tightly sealed. An orbital shaker was used to vigorously shake the containers containing the samples at 250 rpm for 3 minutes and the samples were allowed to settle, the top layers of the extracts were filtered using a filter paper and a funnel, into a labelled test tube. Nine hundred and fifty micro-litres (950 µl) of distilled water were added into test tubes labelled with corresponding sample numbers and fifty micro-litres (50 µl) of the filtered extracts were transferred into the appropriately labelled test tubes containing distilled water. The appropriate numbers of blue /green bordered dilution strips were placed in a microwell strip holder. One dilution well was required for each standard, (that is 0.25, 1.0, 2.5, and 5.0 ppb) or sample. Equal numbers of antibody coated microwell strips were placed in a microwell strip holder.The required amount of conjugate from the green- capped bottle was placed in a separate container (reagent boat). Using an 8-channel pipette, two hundred microliters (200 µL) of conjugate was dispensed into each blue/green-bordered dilution well. Using a single channel pipette, hundred microliters (100 µL) of each standard and sample were transferred into the appropriate dilution wells containing 200 µL of conjugate.

A fresh pipette tip was used for each standard and sample. Using an 8-channel pipette with fresh tips for each 8- well strip, each well was mixed by carefully pipetting it up and down 3 times and immediately 100 µL of the contents from each dilution well was transferred into corresponding antibody coated microwells and incubated at room temperature for 10 minutes. After incubation the contents of the microwell strips were emptied into a waste container. The wells were washed by filling each microwell with distilled water, and then dumping the water from the microwell strips. This step was repeated four (4) times for a total of five (5) washes. Several layers of absorbent paper towels were laid on a flat surface and the microwell strips were tapped on the towels to expel as much residual water as possible after the fifth wash. The bottoms of the microwells were cleaned with a dry cloth. The required amount of substrate from the blue capped bottle was measured and dispensed into a separate container. Hundred microliters (100 µL) of the substrate was transferred into each microwell strip using an 8 channel pipette. And the wells were incubated at room temperature for 5 minutes. The required amount of stop solution from the red capped bottle was measured and dispensed into a separate container. Hundred microliters (100 µL) of the stop solution was transferred into each microwell strip using an 8 channel pipette. A colour change from blue to yellow was observed. The strips were read with a microwell reader using a 450 nm filter and a differential filter of 630 nm. Optical density (OD) readings for each microwell were recorded [20].

Quantification of Aflatoxin

The 4-step sample extraction was done using the QuEChERS method without further clean up. Two grams (2.0 g) of thoroughly homogenized sample was weighed into a 15 ml centrifuge tube and extracted with 10 ml of methanol/acetonitrile/water mixture 51:9:40 (%v/v) was added. One point five grams (1.5 g) of anhydrous magnesium sulphate ($MgSO_4$) and 0.5 g of sodium chloride (NaCl) were added into the mixture. The extract was centrifuged for 5 minutes at 4000 rpm and 1.0 ml of the upper organic layer was filtered through a 0.45 µm nylon syringe filter before high performance liquid chromatography (HPLC) analysis. The HPLC analysis was performed using an Agilent technologies 1200 series system and a fluorescence detector. A C-18 Zorbas XDB column (5 µm paticle size, 150 mm x 4.6 mm internal diameter) was used for chromatographic separation. The sample extracts were analyzed isocratically at 30^{o}C using a water/methanol/acetonitrile mixture (65:25:10 v/v/v) as the mobile phase. Flow rate was 1.0 ml/min and injection volume was maintained at 20 ml for both the sample and standard solutions [4].

Data Analysis

Descriptive statistics such as pie charts was used in the discussion of the results, in order to give a clear representation of the data analyzed. Analysis of Variance (ANOVA) at 5% ($p<0.05$) confidence interval for the total aflatoxin and fumonisin contents in samples from the four markets was also determined using Microsoft Excel 2010.

RESULTS

The maximum total Fumonisin contamination (0.0081ppb) was found in samples gotten from Ilutitun market with 33% contamination recorded while Ikoya, Igodan and Okitipupa markets had 28%, 25% and 14% respectively (Fig 1). For total aflatoxin contamination, samples gotten from both Ilutitun and Igodan markets had the highest concentrations (30%), while samples from Okitipupa and Ikoya had concentrations of 20% each (Fig 2).

From the statistical comparison it was observed that there was a significant difference ($P< 0.05$). ANOVA test carried out for the total aflatoxin (Table 5) and fumonisin (Table 6) content in all the markets surveyed showed that there was a significant difference in their concentrations. The variation in methods of processing of Pupuru, storage and geographical conditions might be the cause of variation of incidence of fungi and mycotoxins in these markets. However, all the samples from the selected markets had both aflatoxin and fumonisin concentrations lesser than the regulatory limit (10 ppb) for aflatoxin and (6 ppb) for fumonisin content set by the National Agency for Food and Drugs Administration and Control (NAFDAC) [1]. In Okitipupa, a sample had an undetectable concentration of fumonisin (>R EF) (Table 2), and 19 samples had undetectable levels of aflatoxin B1 (Table 3) the most common and dangerous form of aflatoxin.

The highest detected total aflatoxin content was 0.0081 ppm (Table 3) and the highest detected fumonisin content was 0.68 ppb (Table 2).

DISCUSSION

A wide range of fungal species were isolated from the locally processed cassava flour (pupuru). The fungal species *Aspergillus. flavus*, and *Penicillium. chrysogenum* that were isolated from 'pupuru' in this study have been implicated as major causal agents of rots in living, but dormant yam tubers, and cassava tubers (Bassa *et al.,* 2001). Being a soil fungus, direct contact between the tubers and soil could be the primary source of contamination by *A. flavus*. It has been reported that soil adhering to tubers contains many microorganisms that can infect the surface of freshly harvested tubers and root (Lewis *et al.,* 2005). On the other hand, the fungi may come from bruised and already contaminated tubers that are used to prepare the 'pupuru'. Fungal pathogens can enter the substrate through natural wounds in the tubers; the wounds can be caused by insects, nematodes and poor handling before, during and after harvest. Influence of climatic factors on the occurrence of toxigenic fungi such as *A. flavus* and *F. verticillioides* have been reported in Benin (Gong *et al.,* 2012). This is also evident in this work where 'pupuru' samples from across markets have varying occurrences of fungi isolates. Other studies have revealed their presence in cassava chips in Benin (Bassa *et al.,* 2001) in Nigeria (Bankole and Majekobaje, 2003) in Ghana (Lewis *et al.,* 2005) and in Congo and Tanzania (Gong *et al.,* 2012). The high concentration of Fumonisin concentration in Ilutitun can be attributed to the handling and processing methods of Pupuru samples in the area as various factors including climatic conditions, nature of the substrate and processing factors including: high relative humidity, high moisture content and high temperatures are similar across the study locations. Storage areas and materials for pupuru production are used for a long time without being cleaned, the condition that possibly might be the source of fungi inocula to newly processed cassava products thereby infecting it with higher content fumonisin than those from other study locations.

The diversified mycoflora showed by the isolation of different fungal genera indicates a competition for available nutrients in cassava pulp (Gong *et al.,* 2012). It has been previously pointed out that fungal interaction due to competition could lead to decreased mycotoxin levels (Udoh *et al.,* 2000). The low concentration of aflatoxins and total fumonisin in Pupuru produced in Okitipupa may be as a result of interactions between variables which were not fully taken in account in the present study. Anti-microbial and fungitoxic compounds such as scopoletin have been known to accumulate in roots and tubers as a result of post-harvest physiological deterioration. These compounds may affect the growth of some of the fungi and inhibit mycotoxin production (Fandohon *et al.,* 2005).

Environmental factors, genotype, management, and cultural practices have an impact on the growth of fungi and mycotoxin accumulation. Smoking of cassava chunk balls will probably reduce insect infestation, fungal infection, and aflatoxin accumulation by lowering the moisture content, and from the results gotten in this study (Table 2 and 3) the incidence of aflatoxin and fumonisin were indeed quite low (0.0081 ppb and 0.68 ppm) in the samples from all the selected markets. The effectiveness of smoke drying in reducing insect and fungal infection was confirmed by Udoh and colleagues (Udoh *et al.,*2000). The environment in which foodstuffs are displayed in the markets is not always hygienic and this is an avenue for contamination. Often, food vendors display the food samples in an open tray or bowl beside gutters or refuse heaps. This probably encourages fungal attack and subsequent production of toxins.

The knowledge of fumonisins as toxic compounds associated with harmful effects in animal or human health is relatively recent. Studies have related outbreaks of equine leucoencephalomalacia (ELEM), a fatal neurological disease in horses and equines with the consumption of maize contaminated by *F. moniliforme* (currently *F. verticillioides*). The International Agency for Research on Cancer (IARC) also indicated that fumonisins were associated with cancer-promoting activities in humans. However, the values in fumonisins levels connected to the samples dealt with in this research were not too high when contents often quantified in other food commodities such as maize, and other cereal-based foods are considered (Cucci *et al,*. 2007).

The implications of ingestion or absorption of mycotoxins on human health include immunosuppression, impaired growth, various cancers and death depending on the type, the period of exposure and the amount of toxins ingested (Udoh *et al.,*2000)). Moreover, a synergistic effect between mycotoxins exposure and some important diseases in the African continent such as malaria, kwashiorkor, hepatitis B and HIV/AIDS have been suggested (Williams *et al.,* 2004). The most toxic and dangerous mycotoxins are aflatoxins. Indeed, aflatoxin B1 (AFB1) is the most potent hepatic carcinogen known in mammals and has been classified by the International Agency for Cancer Research in the group I of molecules that are carcinogenic for both human and animals (IARC, 20002). Aflatoxin b1 (AFB1) also displays immunosuppressive properties (Meissonnier *et al., 2008)* and is involved in growth impairment observed in children (Gong *et al.,* 2002). Exposure to aflatoxin in Sub-Saharan Africa is very frequent. In some areas, 99% of tested children display aflatoxin residues in their blood (Gong *et al., 2004)* and this high exposure contributes to appearance of chronic hepatomegaly in children (Gong *et al.,* 2012). Contamination of food by very high levels of aflatoxin can also lead to fatal consequences such as the death of 125 people in Kenya (Probst *et al.,* 2007 ; Mestres *et al.,* 2004).

REFERENCES

Adebiyi O.A (2016), oral discussion during students industrial work experience scheme (SIWES) in mycotoxin laboratory, National Agency For Food and Drug Administration and Control, entral Laboratory Complex, Oshodi, Lagos Nigeria.

Bankole, S.A. and Adebanjo, A. (2003). Mycotoxins in food in West Africa: current situation and possibilities of controlling it. *African Journal of Biotechnology* 2: 254 – 263

Bankole S.A. and Mabekoje O.O., (2004). Mycoflora and occurrence of aflatoxin B1 in dried yam chips from markets in Ogun and Oyo States, Nigeria. *Mycopathologia* 157, 111-115

Bassa, S., Mestre C., Hell K., Vervia P. and Cardwell K., (2001). First report of aflatoxin in dried yam chips in Benin. *Plant Disease Journal*, 85: 1032.

Collaborative Study of Cassava in Africa (COSCA) Tanzania, (1996). Production prospects for Cassava in Tanzania (draft). COSCA Working paper No. 16. Collaborative Study of Cassava in Africa, International Institute of Tropical Agriculture (IITA) andMinistryofAgriculture,Tanzania.[http ://www.fao.org/docrep/009/a0154e/A01 54E09.htm]. 07/08/2017

Cucci, C., Mignani, A., Dall'Asta, C., Pela, R., and Dossena, A. (2007). A portable fluorometer for the rapid screening of M1 aflatoxin. *Sensors and Actuators B: Chemical,* 126(2):467–472.

Fandohan, P. B. Gnonlonfin, K. Hell, W.F. Marasas, and M.J. Wingfield. (2005). Natural occurrence of Fusarium and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. *International Journal of Food Microbiology*, 99, 173-183.

Gomez-Vasquez, R., Day, R., Buschmann, H., Randles, S., Beeching, J.R. and Cooper, R.M., (2004). Phenylpropanoids, phenylalanine amminono lyase and peroxidases in elicitor-challenged cassava (Manihot esculenta) suspension cells and leaves. *Annals of Botany*. 94, 87-97

Gong, Y., Hounsa, A., Egal, S., Turner, P.C., Sutcliffe, A.E., Hall, A.J., Cardwell, K. and Wild, C.P. (2004) 'Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa', *Environmental Health*, Vol. 112, No. 13, pp.1334–1338.

Gong, Y.Y., Cardwell, K.F. Hounsa, A., Egal, S., Turner, P.C., Hall, A.J. and Wild, C.P. (2002) 'Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: a cross-sectional study', *British Medical J*ournal, Vol. 325, No. 7354, pp.20–21.

Gong, Y.Y., Egal, S., Hounsa, A., Turner, P.C., Hall, A.J., Cardwell, K.F. and Wild, C.P. (2012) 'Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa; the critical role of weaning', *International Journal of Epidemiology*., 32, (4) , pp.556–562.

Hell, K., Cardwell, K.F., Setamou, M., Schulthess, F. (2000) Influence of insect infestation on aflatoxin contamination of stored maize in four agroecological regions in Benin . *African Entomology* (2).

IARC (2002). International Agency for Research on Cancer (IARC). Monographs 82, Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Fumonisin B1. Evaluation of carcinogenic risks to humans. Lyon France. pp. 301-366.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rodgers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., Misore, A., DeCock, K., Rubin,C. and the Kenya Aflatoxicosis Investigation Group. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives* 113(12): 1763-1767

Manjula, K., Hell, K., Fandohan, P., Abass, A. and Bandyopadhyay, R. (2009). Aflatoxin and fumonisin contamination of cassava products and maize grain from markets in Tanzania and Republic of Congo. *Toxin Reviews* 28: 63–69

Meissonnier, G.M., Pinton, P., Laffitte, J., Cossalter, A.M., Gong, Y.Y, Wild, C.P., Bertin, G., Galtier, P. and Oswald, I.P. (2008) 'Immunotoxicity of aflatoxin B1: Impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression', *Toxicology and Applied Pharmacology*, 231, (2), pp.142–149.

Mestres, C., Bassa, S., Fagbohoun, E., Nogo, M., Hell, K., Vernier, P., Champiat, D., Hounhouigan, J., and Cardwell, K.F., (2004). Yam chip food sub-sector: hazardous practices and presence of aflatoxins in Benin. *Journal of Stored Products Research*, 40: 575-585.

Probst, C., Njapau, H. and Cotty, P.J. (2007) 'Outbreak of an acute aflatoxicosis in Kenya in 2004: identification of the causal *agent'*, *Applied and Environmental Microbiology*, (8), 2762–2764.

Udoh J. M., Cardwell K. F. and Ikotun T. (2000). Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. *Journal of Stored Products Research*, 36 (2):187–201

Velluti, A., Marin, S., Bettucci, L., Ramos, A.J. and Sanchis, V., (2000). The effect of fungal competition of maize grain by *Fusarium moniliforme*, *Fusarium proliferatum* and *Fusarium graminearum* and on fumonisin B1 and zearalenone formation. *International Journal of Food Microbiology*, 59: 59-66

Westby, A. (2002) Cassava utilisation, storage and small-scale processing. In Hillocks, R., Tresh, J. and Bellotti, A. (Eds.): *Cassava: Biology, Production and Utilization*, CABI Publishing, Wallingford, UK. pp.281–300

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2004) 'Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions', *American Journal of Clinical Nutr*ition, Vol. 80, (5): 1106–1122.

TABLE 1: Sources and samples with identification tags

S.N	Samples	Source
1	1-5	Okitipupa
2	6-10	Igodan
3	11-15	Ikoya
4	16-20	Ilutitun

TABLE 2: Total fumonisin concentrations in pupuru samples

SAMPLE NUMBER	OPTICAL DENSITY (OD)	CONCENTRATION OF FUMONISIN (ppb)
1	0.732	0.30
2	0.699	0.31
3	0.755	0.26
4	0.906	>REF
5	0.834	0.11
6	0.803	0.17
7	0.710	0.35
8	O.703	0.36
9	0.668	0.44
10	0.606	0.38
11	0.767	0.23
12	0.754	0.26
13	0.717	0.33
14	0.651	0.48
15	0.599	0.61
16	0.574	0.68
17	O.605	0.59
18	O.622	0.55
19	0.707	0.33
20	0.852	0.08

Key: OD= Optical density ppb= parts per billion

Table 3: Total aflatoxin concentrations in pupuru samples

SN	G2(ppm)	G1(ppm)	B2(ppm)	B1(ppm)	Total aflatoxin (ppm)
1	0.00244	ND	0.00096	0.0012	0.0046
2	0.00105	0.00021	0.00032	ND	0.0016
3	0.00252	0.00011	0.00062	ND	0.0033
4	0.00212	ND	ND	ND	0.0212
5	0.00226	0.00015	ND	ND	0.0024
6	0.00258	ND	0.00119	0.0017	0.00547
7	0.00225	ND	0.00082	ND	0.00307
8	0.00115	0.00023	0.00067	0.0012	0.0033
9	0.000321	0.00043	0.00083	ND	0.0043
10	0.00253	0.00033	0.00071	0.0015	0.0051
11	ND	ND	0.00032	ND	0.00032
12	0.00105	0.00021	0.00041	ND	0.0017
13	0.00112	0.00032	0.00062	ND	0.0015
14	0.00244	0.00049	0.00087	ND	0.0038
15	0.00242	0.00068	0.00113	0.0020	0.0062
16	0.00265	0.00077	0.00172	0.0030	0.0081
17	ND	0.00051	0.00092	0.0017	0.0031
18	0.00255	0.00052	0.00085	ND	0.00392
19	0.00235	ND	0.00073	0.0015	0.00458
20	ND	ND	0.00096	ND	0.00096

Key: G1= Aflatoxin G1, G2= Aflatoxin G2, B1= Aflatoxin B1, B2= Aflatoxin B2, ppm= parts per million. ND= Not detected

Table 4: Morphological and microscopic characteristics of fungal isolates from analyzed pupuru samples

Isolate	Morphological characteristic	Microscopic characteristics
Penicillium chrysogenum	Sulcate, velutinous texture, with greyish green color on the obverse side, and white borders. The colony looked creamish yellow on the reverse side of the plate.	Short, smooth stripes with biverticillate penicilli and septate,hyaline hyphae and ellipsoidal conidia.
Penicillium italicum	Colonies are plane, heavy sporing and grey-green colored with white edges with a granular appearance.	The conidial apparatus consists of asymmetric penicillin bearing tangled chains of conidia, conidiophores are terverticillate. Septate, hyaline hyphae, smooth walled conidiophores and ellipsoidal conidia
Aspergillus flavus	Greenish yellow colony with a powdery texture.the reverse side of the plate is pale brown in colour.	Conidial head radiate hyaline. Conidiophore is rough walled and conidia are globose and finely roughened.
Fusarium moniliforme	Burgundy and pinkish macroconidia, with reverse side of the plate being whitish cream.	Macroconidia are hyaline, with two to several celled fusiform and sickle cell shaped, with an elongated apical cell and pedicellate basal cell.
Rhizopus stolonifer	Colonies grow rapidly and resemble cotton candy. Colonies darken with time and produces long unbranching sporangiophores.	Sporangiophores are simple and appear in clusters. Smooth walled, non septate hyphae. sporangia is globulase with flattening base.
Aspergillus niger	Purplish yellow colonies turning black with the formation of conidia.	Hyaline, septate hyphae, asexual conidiophores that is long and globose at the tip.

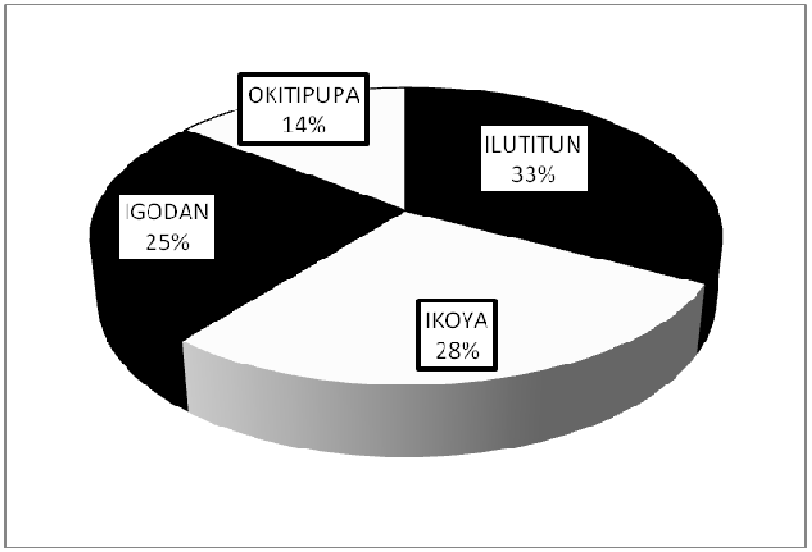

Figure 1: Average fumonisin content (ppb) detected in Pupuru samples from selected markets in okitipupa

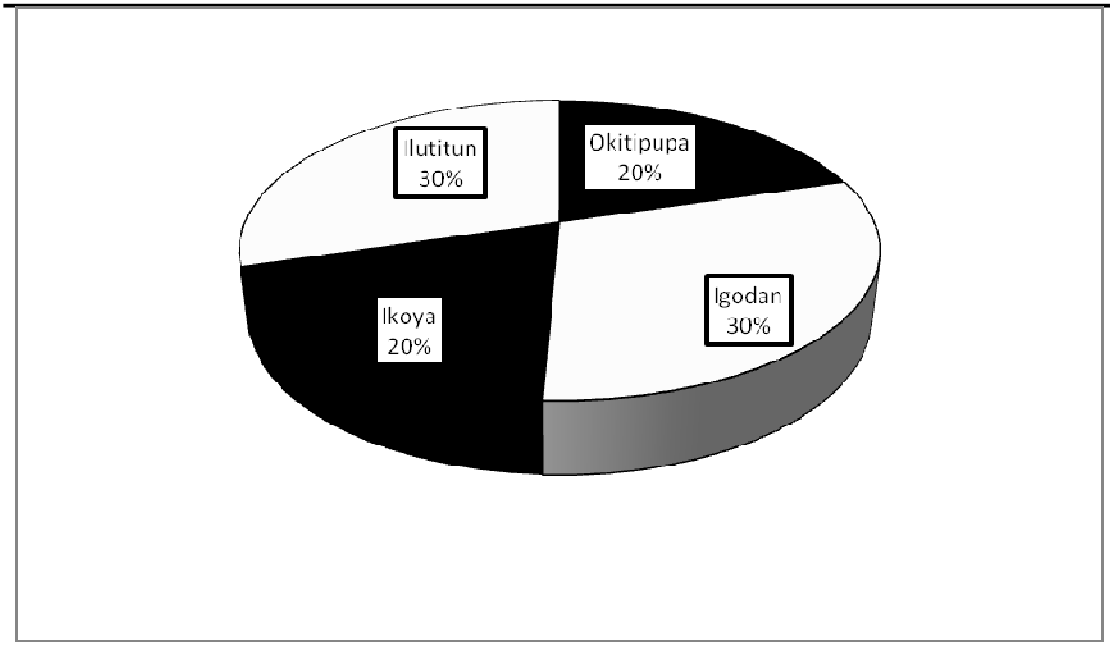

Figure 2: Average aflatoxin content (ppm) in Pupuru samples analyzed from selected markets in okitipupa