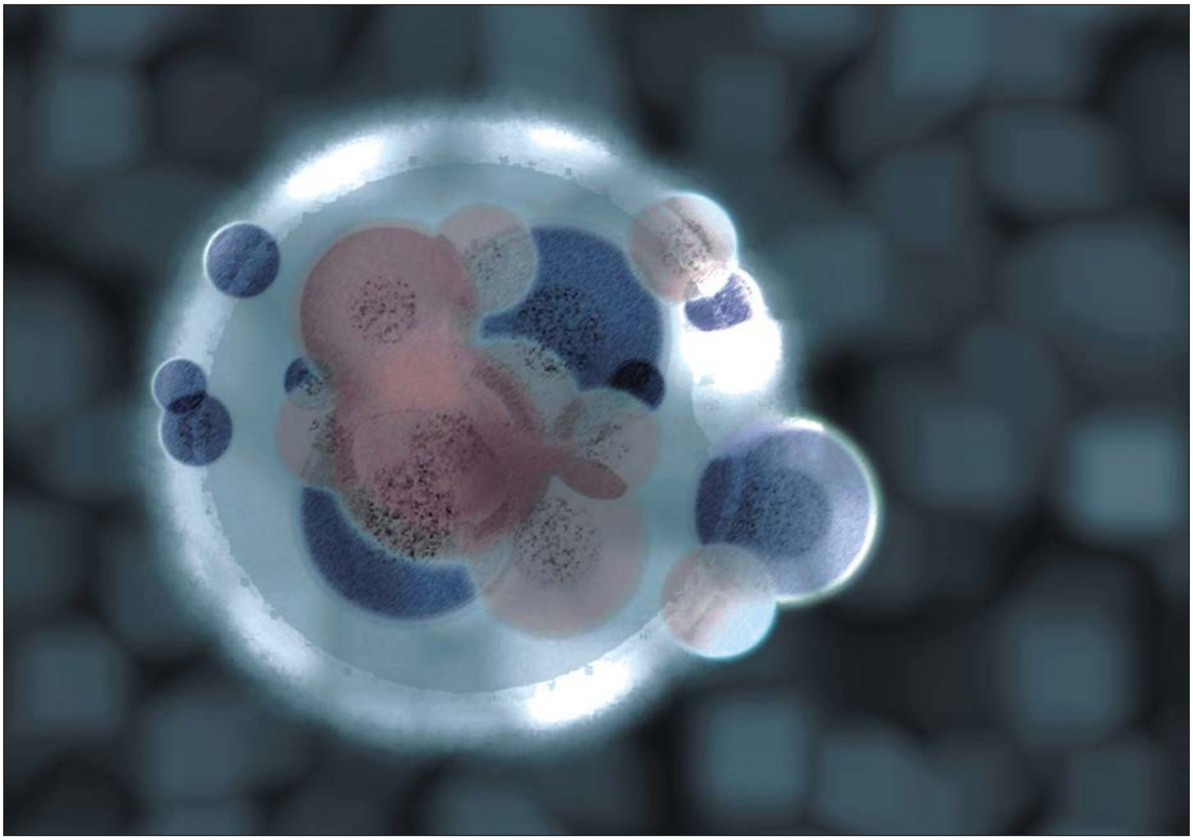

Journal of Biological Sciences

ISSN 1727-3048

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2016.44.48

Short Communication
Aflatoxin Contamination of Selected Staple Foods Sold for Human Consumption in Kampala Markets, Uganda

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Abstract
Aflatoxins contaminate approximately 25% of agricultural products worldwide and have negative public health implications. Little is known on the level of aflatoxins in foodstuffs in Kampala markets and yet open markets in sub-Saharan Africa have been implicated to have high risk of aflatoxin contamination. A cross-sectional survey was conducted to quantify levels of aflatoxin contamination in major staple foodstuffs in five major markets in Kampala in the month of June, 2014. From each market, 4 incremental samples of approximately 250 g of each food type were picked at random from different stalls to make up a sample of 1000 g. Overall, 20 samples of staple foodstuffs sampled in Kampala markets were analyzed for aflatoxin contamination. Foodstuffs included groundnuts, groundnut paste, cassava flour and maize grains. Total aflatoxins were identified and quantitated using the VICAM AflaTest fluorometric method (VICAM LP, 34 Maple Street, Milford, MA 01757 USA). Aflatoxins were detectable in 16/20 (80%) of the samples and 8/20 (40%) of the samples exceeded FDA/WHO regulatory limit of 20 μg kg^{-1}. All the groundnut paste samples tested 5/5 (100%) had aflatoxin concentration levels more than the 20 μg kg^{-1}. None of the maize grain samples analyzed exceeded the permissible level of 20 μg kg^{-1}. Laboratory analyses indicate that groundnuts and groundnut paste had extremely high alarming aflatoxin levels of up to 940 and 720 μg kg^{-1}, respectively. There is high aflatoxin contamination in groundnuts and groundnut paste. Therefore, interventions should focus on strengthening health inspections, creating awareness and routine monitoring of aflatoxin levels in staple foods in Kampala markets.

Key words: Aflatoxin, contamination, staple foods, *Aspergillus flavus*, cassava

Received: September 07, 2015 Accepted: November 14, 2015 Published: January 15, 2016

Citation: Jimmy Osuret, Geoffrey Musinguzi, Trasias Mukama, Abdullah Ali Halage, Archileo Kaaya Natigo, John C. Ssempebwa and Jia-Sheng Wang, 2016. Aflatoxin contamination of selected staple foods sold for human consumption in Kampala markets, Uganda. J. Biol. Sci., 16: 44-48.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aflatoxins (AFs) are fungal metabolites mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* that contaminate foods such as groundnuts, maize, millet (Smela *et al*., 2001; Asiki *et al*., 2014). Aflatoxins and fumonisins are the most important mycotoxins today (Wu *et al*., 2014) and they contaminate about 25% of agricultural products worldwide (Yard *et al*., 2013; Williams *et al*., 2004). Some findings suggest that occurrence of AFs in foods is as a result of direct contamination and their ability to survive food processing (Okello *et al*., 2010). Aflatoxins contamination of staple food in Uganda has also been attributed to poor crop planting and harvesting practices (DANYA International, 2015; Klich, 2007). Field and storage contamination in the seeds of a number of crops usually occur when conditions are favorable to allow growth of the fungus *Aspergillus flavus* e.g., high moisture levels, humid conditions, drought and temperatures of about 29°C (Klich, 2007; Gnonlonfin *et al*., 2013).

Ingestion of foods contaminated with AFs causes acute and chronic effects such as liver damage, bile duct proliferation, edema, lethargy, impair the function of macrophages, affect production of lymphokines, reduce antibody response to vaccines, all of which result in sickness or death (Smela *et al*., 2001; Yard *et al*., 2013; William *et al*., 2004). This has public health implications and this problem is wide spread across Africa (Yard *et al*., 2013; William *et al*., 2004). Previous estimates show that approximately 4.5 billion people in developing countries are at risk of exposure to AFs (William *et al*., 2004). Exposure to AFs in developing countries has been implicated in impairing growth and development in children (William *et al*., 2004; Gong *et al*., 2002). This has prompted the need for governments to address health, agricultural and trade issues related to AF contamination of staple food supply (Wu *et al*., 2014).

Foods such as maize, groundnuts and cassava are the major staple foods cultivated in Uganda because of their value as a source of protein, carbohydrates, high energy (Okello *et al*., 2010) and these make up approximately 40% of rural and urban diets (DANYA International, 2015). Staple foods such as ground nuts, maize and cassava are largely cultivated in the Northern and Eastern regions of Uganda with low rainfall and are consumed both in Uganda and neighboring countries like South Sudan (Okello *et al*., 2010).

Previous studies have detected AF-albumin adducts in sera of adults and children in Uganda and other African countries (Asiki *et al*., 2014; Turner *et al*., 2008) and this has heightened the need for identifying foodstuffs implicated to have highest risk of AFs in order to reduce contamination. Foods in retail stores and open markets have been implicated to have the highest risk of AF contamination (DANYA International, 2015) and yet little is known about the levels of AFs concentrations in foodstuffs sold in markets of Kampala. In particular, this study focused on evaluating the occurrence of AF contamination in maize grains, cassava flour, ground nuts and groundnut paste samples from selected markets in Kampala. The findings will partly provide baseline information to develop practical and sustainable interventions.

MATERIALS AND METHODS

Study design: The study was a cross-sectional survey. Food samples of maize grains, groundnuts, cassava flour and groundnut paste were collected in the month of June, 2014 from 5 major markets in Kampala.

Study area: The study was conducted in Kampala district located in the central region of Uganda. Kampala has 5 divisions having urban, semi urban and rural communities with an estimated population of 1.72 million (Uganda Bureau of Statistics, 2012). The population is engaged in various economic and social activities including; business/trade, small scale industrial manufacturing among others (Uganda Bureau of Statistics, 2012). Kampala has both informal and formal markets registered under Kampala Capital City Authority (Vogelius, 2013). Most of the market traders are women operating in fixed stalls and shops where goods and services are offered ranging from the sale of foodstuffs to clothes (Vogelius, 2013). Vendors without permanent space normally hawk their products or operate in makeshift stalls.

Sampling procedures: Five markets (Kalerwe, Nateete, St. Balikudembe, Nakasero and Nakawa) with the highest number of stalls in Kampala were selected from which foodstuff samples of maize grains, groundnuts, cassava flour and groundnut paste were bought. From each market, 4 incremental samples of approximately 250 g of each food type were picked at random from the same supply sources at different stalls throughout the sampling area to make up a sample of 1000 g. The incremental samples were packed in sterile paper bags after purchase. A total of 20 samples consisting of maize grains, groundnuts, cassava flour and groundnut paste were collected from all the different markets and then transported to Makerere University, School of Food Science, nutrition and bio-engineering laboratories for aflatoxin analysis. The incremental samples of each foodstuff were then immediately and carefully mixed using quartering method to obtain a homogenized sample. The homogenized samples were then packaged immediately in labeled water proof polyethylene bags to prevent moisture and any other form of contamination.

The samples were then wrapped in water proof polyethylene bags and tightly sealed and stored in the freezer at a temperature of -20°C in order to arrest other microbial growth and reactions and prevent further postharvest accumulation of molds growth and AF production until analysis.

Method for determination of AF level: The procedure of aflatest quick fluorometry for maize and groundnuts were used to test for AFs using AflaTest® Fluorometer (VICAM L. P, USA). The maize/groundnut kernels or cassava (where appropriate) were ground using the Waring Blender with stainless steel container (VICAM Product No. 20202, USA). From each sample, 50 g of flour were weighed, mixed with 5 g sodium chloride and placed in the blender jar. A methanol: water solution (80:20, v/v) was added to the flour and blended at high speed for 1 min. The blended mixture was filtered using a fluted filter paper and the filtrate collected in a clean vessel. The filtrate (10 mL) was pipetted into a clean vessel and diluted with 40 mL of distilled water, mixed thoroughly and filtered through glass microfibre into a clean glass syringe. From the syringe, 2 mL of the filtered dilute extract (2 mL = 0.2 g sample equivalent) was passed through Aflatest-P affinity column at a rate of 1-2 drops per second and the column rinsed with distilled water. The affinity column was eluted with 1 mL HPLC grade methanol at a rate of 1-2 drops per second and the eluate collected in a glass cuvette. Aflatest developer solution (1 mL) was added to the eluate, mixed thoroughly and the cuvette was placed in the AflaTest fluorometer that was earlier calibrated to read total AF. Aflatoxin levels (μg kg^{-1}) in the samples were detected and recorded after 60 sec.

RESULTS

Aflatoxins were detected in 16/20 (80%) of the samples. Basing on the WHO/FDA regulatory limit of 20 μg kg^{-1} (Kaaya and Kyamuhangire, 2006), none of the maize grain samples exceeded the permissible level of 20 μg kg^{-1} while 8/20 (40%) of the samples of other foodstuffs exceeded the permissible level of AF adopted in most countries (20 μg kg^{-1}); these samples corresponded to cassava flour, groundnuts and groundnut paste. It is observed that all the groundnut paste samples tested (5/5, 100%) had AF levels above the permissible level. In addition, 2/5 (40%) samples of groundnuts and 1/5 (20%) of cassava samples had AF levels higher than the permissible level. Overall analyses indicate that groundnuts had the highest levels of AFs, up to 940 μg kg^{-1} followed by groundnut paste with 720 μg kg^{-1}.

Figure 1 shows variation in AF levels by foodstuff type in the different markets. Aflatoxins were found in samples of

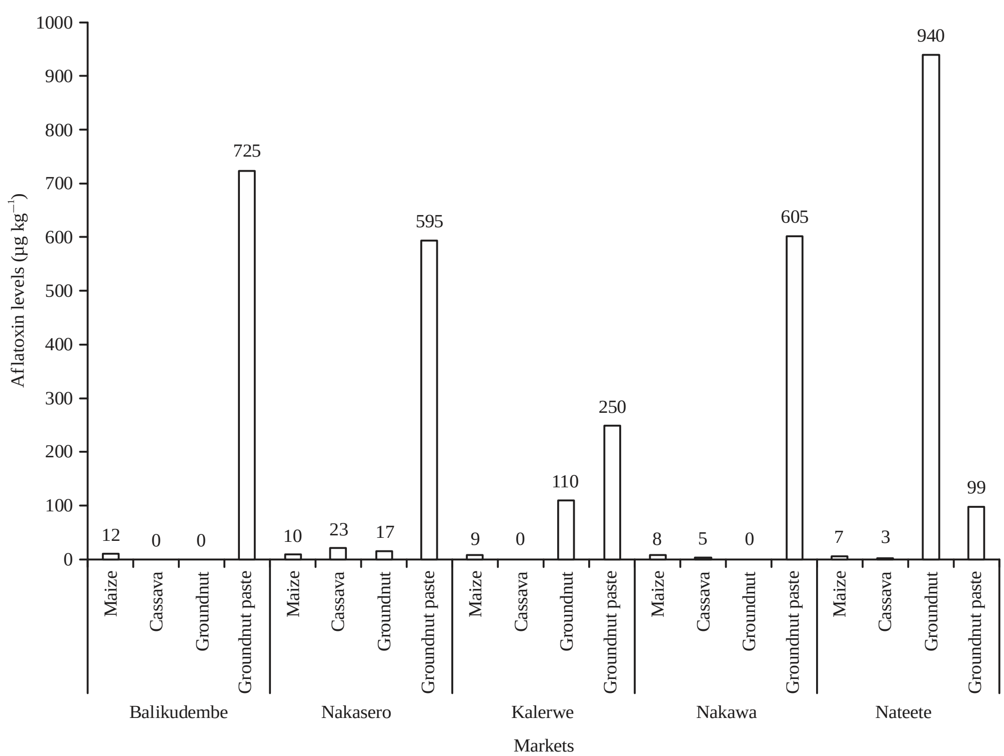

Fig. 1: Aflatoxin levels of staple food stuffs in 5 major markets in Kampala

groundnut paste from all markets and the concentrations were extremely high with values of up to 725 μg kg^{-1}. High levels of AF were also isolated in groundnuts from Kalerwe and Nateete markets at 110 and 940 μg kg^{-1}, respectively.

DISCUSSION

The contamination and detection of AFs in 80% of the samples is comparable to those earlier reported in studies conducted in sub-Saharan Africa and other developing countries (Mupunga *et al*., 2014; Kamika *et al*., 2014). The possible explanation for this could be partly due to hot and humid conditions that favour fungal growth during storage (Mupunga *et al*., 2014; Bigirwa *et al*., 2006). Presence of AF in many of the samples could also be due to contamination as a result of environmental factors such as poor transportation, poor storage practices and poor phytosanitary regulation. Therefore, understanding the underlying drivers for AF contamination in markets of Kampala is important for targeted control interventions.

Although, all the maize samples tested positive for AF contamination, none of them exceeded the permissible level of 20 μg kg^{-1}. This finding might be explained by the fact that the moisture content of the maize grains sampled was within safe storage levels (Bigirwa *et al*., 2006; Kaaya and Kyamuhangire, 2006). This could also be due to the fact that market vendors sort out poor maize grains manually or use sieves. Much as these maize grain samples were with the permissible levels, they might not necessarily be safer for human consumption since there is a possibility of other forms of mycotoxins occurring in maize grains which were not quantified and detected (Bigirwa *et al*., 2006). There is need to further test and determine other types of mycotoxins that might be naturally occurring in the maize grains. In West Africa, maize grain has been documented to have extremely high levels of AFs exceeding 100 μg kg^{-1} in Benin and Ghana (Hell *et al*., 2000; Kpodo *et al*., 1996).

Having 40% of the samples (cassava flour, groundnut and groundnut paste) exceeding the permissible level of AF adopted in Uganda (20 μg kg^{-1}) could have serious health implications if consumed. This could be due to longer storage periods of food staffs in shops/stalls under favourable conditions of temperature and humidity which allowed for Aspergillus growth and AF production over time (Hell *et al*., 2000). Findings on the occurrence of AFs in the cassava flour samples are comparable to that of a study conducted in various markets of Tanzania and the Democratic Republic of Congo which reported high levels of AFs in samples stored for 4 months (Manjula *et al*., 2009).

The results also indicate that AF is frequently found on groundnuts and groundnut paste on sale in markets around Kampala, Uganda. These findings on AF levels are comparable to those earlier reported in studies conducted in Uganda and other developing countries which found AFs to occur on groundnuts with levels exceeding the permissible 20 μg kg^{-1} (Kaaya and Kyamuhangire, 2006; Elzupir *et al*., 2011). This has implications for interventions on AF control and management to target these specific food items since groundnut and groundnut paste are staple foodstuffs consumed by many poor people in Kampala and so potentially more risky to their health than maize and cassava flour.

Higher levels of AF were also identified in groundnuts from Kalerwe and Nateete markets because these have informal structures with many temporary stalls having poor storage conditions and poor practices such as longer storage of food stuffs that favour AF contamination and fungi growth compared with the other markets that have well planned permanent structures.

This study's limitation is the small sample size and so further research on staple food should be carried out with a much larger sample size exploring the relationship between AF levels and storage practices and conditions in markets of Kampala. Nevertheless, this study provides information about the occurrence of AF contamination in maize grains, cassava flour, groundnuts and groundnut paste samples from selected markets in Kampala, Uganda and confirms necessity for prioritizing interventions to reduce contamination in higher risk staple foods.

CONCLUSION

The study revealed high AF contamination in most staple foods especially groundnut and groundnut paste which could have health consequences when consumed. So the local authority need to carry out health inspections, routine monitoring of AF levels in order to prevent the sale of contaminated foodstuffs. Awareness interventions about mycotoxins among market vendor need to be carried out.

ACKNOWLEDGMENT

This study was supported by the research grants from the U.S. National Institute of Health (Grant numbers: 1R24TW009489 and 1R24TW009556).

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