Aflatoxins in Uganda: An Encyclopedic Review of the Etiology, Epidemiology, Detection, Quantification, Exposure Assessment, Reduction and Control

Timothy Omara[1,2,3], Winfred Nassazi[1,3], Tom Omute[4], Aburu Awath,[5,6] Fortunate Laker[3,7], Raymond Kalukusu, [3,7] Bashir Musau[3,7], Brenda Victoria Nakabuye[7,8], Sarah Kagoya[3,9], George Otim[3], and Eddie Adupa[3,10]

[1] Department of Chemistry and Biochemistry, School of Biological and Physical Sciences, Moi University, Uasin Gishu County, Kesses, P.O. Box 3900-30100, Academic Highway, Eldoret, Kenya
[2] Department of Quality Control and Quality Assurance, Product Development Directory, AgroWays Uganda Limited, Plot 34-60 Kyabazinga Way, P.O. Box 1924, Jinja, Uganda.
[3] Department of Chemistry, Faculty of Science, Kyambogo University, P.O. Box 1, Kampala, Uganda
[4] Department of Biochemistry, Faculty of Health Sciences, Lira University, P.O. Box 1035, Lira, Uganda
[5] Standards Department, Uganda National Bureau of Standards, Plot 2-12 Bypass link, Bweyogerere Industrial and Business park, P.O. *Box* 6329, Kampala, Uganda
[6] Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bioengineering, College of Agricultural and Environmental Sciences, Makerere University, P.O.Box 7062, Kampala, Uganda
[7] Department of Quality Control and Quality Assurance, Leading Distillers Uganda Limited, Plot 3382/83, Buloba, P.O.Box 12369, Kampala, Uganda
[8] Department of Food Processing Technology, Faculty of Science, Kyambogo University, P.O.Box 1, Kampala, Uganda
[9] Department of Quality Control and Quality Assurance, Product Development Directory, Sweets and Confectioneries Section, Kakira Sugar Limited, Jinja-Iganga Highway, P.O. Box 121, Jinja, Uganda
[10] Department of Quality Control and Quality Assurance, Abacus Parenteral Drugs Limited, Block 191, Plot 114, Kinga, Mukono, P.O. Box 31376, Kampala, Uganda

Corresponding Author:
Timothy Omara [1,2,3]
Africa Center of Excellence II in Phytochemicals, Textile and Renewable Energy, Moi University, Uasin Gishu County, Kesses, Eldoret-Kenya
Email address: prof.timo2018@gmail.com/timothy.omara@agroways.ug

Abstract

Uganda is predominantly an agricultural country where farming employ more than 60% of the population. Aflatoxins remain a scourge in the country, unprecedentedly reducing the value of agricultural foods and in high enough exposure levels, implicated for hepatocellular carcinoma, stunted growth in children and untimely deaths. This review synthetizes the country's major findings in relation to the mycotoxin's etiology, epidemiology, detection, quantification, exposure assessment, control and reduction in different matrices. It also highlights some of the management strategies for aflatoxin control that could be adopted in Uganda. Review results indicate that aflatoxins in Uganda is majorly produced by *Aspergillus flavus* and *A. parasiticus* and have been reported in maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), sesame (*Sesamum indicum*),

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beans (*Phaseolus vulgaris* L.), sunflower (*Helianthus annus*), millet (*Eleusine coracana)*, a bovine milk-based product, peanuts (*Arachis hypogaea* L.) and cassava (*Manihot esculenta*) with the highest content reported in cassava, beans and peanuts. The causes and proliferation of aflatoxigenic contamination of Ugandan foods have been largely due to poor pre-, peri- and post-harvest activities, poor government legislation, lack of awareness and low levels of education among farmers, agri-entreprenuers and consumers on the plague. Aflatoxin B_1 is the most prevalent aflatoxin in Uganda. There is still limited research on aflatoxins in Uganda because the surveillance, reduction and control carry prohibitive costs. A few exposure assessments have been done especially in human sera and dependence on a single or a related set of foods with little diet diversity has exacerbated the risk of exposure to aflatoxins in Uganda because most of the staple foods are aflatoxin-prone. On the detection, control and reduction, these are still marginal, though some devoted scholars have devised and validated a sensitive portable device for on-site aflatoxin detection in maize as well as shown that starter cultures used for making some cereal-based beverages have the potential to bind aflatoxins. More effort should be geared towards awareness creation through training of farmers and traders in the cereal value chain as well as developing capacity to monitor aflatoxins. Vaccination against Hepatitis B and Hepatitis A should be emphasized to reduce the risk of development of liver cancer among the populace.

Introduction

1.1 Brief historical perspective

Aflatoxin is a portmanteau combining "a" for the *Aspergillus* genus, "fla" for the species *flavus*, and *toxin* for poison [1-3]. The discovery of aflatoxins traces back to 1960 in which a severe outbreak of turkey "X" disease was recorded in England with more than 100,000 turkeys, 20,000 ducklings, pheasants, chicks and partridge poults were reported to have died from the calamitous incident [4]. The cause was chromatographically declared to be due to a series of fluorescent compounds in a peanut meal imported from South America (Brazil) that was served to the poults [5]. Later, the disease syndrome was reported in domesticated animals outside the Great Britain. The causative mold, *Aspergillus flavus,* was finally isolated from a meal later related with a hepatic problem in ducklings in Uganda [6]. The early history of the Turkey ''X'' disease outbreak in Great Britain was described in sufficient details by Blount [4, 7] and the toxicity recorded in various animal species were recapitulated by Allcroft [8].

1.2 Structure and properties of aflatoxins

Aflatoxins (AFs) are highly oxygenated polysubstituted coumarins with structures that differ only very slightly. At least 18 different types of AFs have been chemically characterized, with the six major ones being aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2) [9], aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) (**Table 1**). The B-aflatoxins, typically pentanone derivatives, exhibit strong blue fluorescence under ultraviolet light while the G-series (six-membered lactones) fluoresce yellow-green on thin layer chromatography plates, thus the B and G designations [10, 11]. AFB_2 and AFG_2 are dihydroxy derivatives of AFB_1 and AFG_1, and

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the latter AFs (AFG_1, AFB_2 and AFG_2) are not usually reported in the absence of AFB_1 [12]. The M series are toxic metabolic derivatives of the B series that exhibit blue-violet fluorescence and have been reported in milk of animals fed with aflatoxin-contaminated feed [13, 14], hence the designation M [10, 15-18]. The subscripts 1 and 2 in AFs nomenclature are designations for major and minor respectively. The minor AFs (M-series and lower members) have received description as mammalian biotransformation products of the major metabolites [19].

Aflatoxins are produced mainly by *Aspergillus flavus, A. parasiticus, A. nomius* and *A. tamarii* [20-23] which are universally soil-borne fungi responsible for decomposition of plant materials. About 20 *Aspergillus* species have been reported to produce AFs [24], though the exploration of more novel and potential aflatoxigenic fungi continues [25-29]. Most species produce B-type AFs via the polyketide pathway as difuranocoumarin derivatives, although species related to *A. parasiticus* and *A. nomius* are usually able to additionally produce G aflatoxins. Other species with reported potential for production of both B and G AFs include *A. toxicarius, A. bombycis, A. parvisclerotigenus, A. minisclerotigenes,* and *A. arachidicola* [30]. Four other aflatoxins: AFM_1, AFM_2, AFB_{2A}, AFG_{2A} which may be produced in minor amounts have been isolated from cultures of *A. flavus* and *A. parasiticus*. Closely related compounds namely: $AFGM_1$, parasiticol and aflatoxicol are also produced by *A. flavus* [14].

Chemically, AFs are unique highly substituted coumarins containing a fused dihydrofurofuran moiety [31]. The B-series are characterized by fusion of a cyclopentenone ring to the lactone ring of the coumarin moiety, whereas the G series contain a fused lactone ring [32]. AFB_1 and AFG_1 possess an unsaturated bond at the 8, 9 position on the terminal furan ring, and some studies illustrated that oxiranation at this chemical position is pivotal for their toxicological potency. AFB_2 and AFG_2 are comparatively less toxic, unless they are first oxidized to AFB_1 and AFG_1 *in vivo* [31]. AFs are soluble in polar protic solvents [13].

1.3 Toxicological properties of aflatoxins

In kingdom animalia, AFs are reported to be multiplicatively carcinogenic, genotoxic, tremorgenic, haemorrhagic, dermatitic, mutagenic, teratogenic and immunosuppressive [9]. They display potency of toxicity, carcinogenicity and mutagenicity in the order : $AFB_1 > AFM_1 > AFG_1 > AFB_2 > AFM_2 > AFG_2$ as exemplified by their lethal dose that causes the death of 50% of subjects (LD_{50} values) being 0.1–50 mg/kg body weight for most animal species and < 1.0 mg/kg body weight for susceptible species [33-35] (**Table 2**). The order also reflects the role played by the epoxidation of the 8,9-double bond, and the greater potency associated with the cyclopentenone ring of the B-series. Trial tests on animal species and mammalian cells have unveiled toxicities of AFG_1, AFB_2 and AFG_2 as approximately 50%, 20% and 10% that of AFB_1 [36]. Susceptibility though, varies with breed, species, age, dose, length of exposure and nutritional status (**Table 2**).

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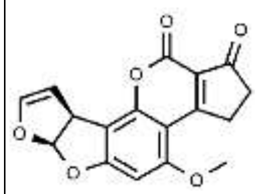
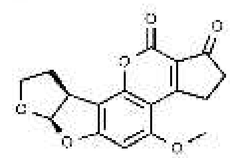
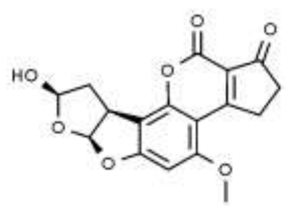
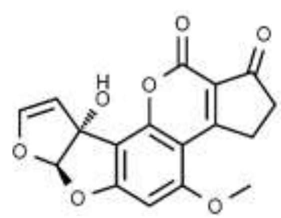
Table 1. Types and chemical structure of common aflatoxins

Difuranocoumarins	**Aflatoxin**	**Chemical structure and molecular formula**	**Molecular weight (Kg/mol)**	**Metabolites**
Difurocoumarocyclopentenone series	AFB_1	 $C_{17}H_{12}O_6$	312.274	
	AFB_2	 $C_{17}H_{14}O_6$	314.2895	
	AFB_{2A}	 $C_{17}H_{14}O_7$	330.2889	
	AFM_1	 $C_{17}H_{12}O_7$	328.273	Metabolite of AFB_1 in humans and animals and comes from a mother's milk. It is belived to be associated with the casein fraction of milk

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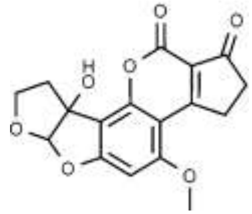
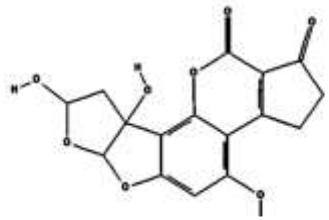
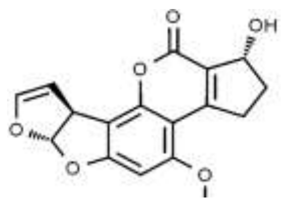
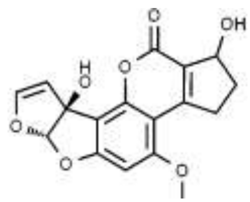
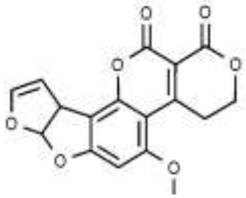
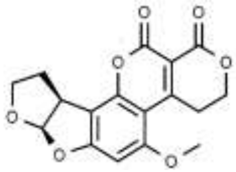
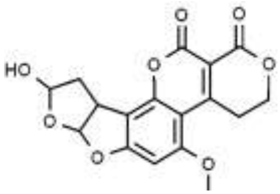
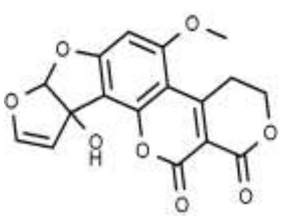
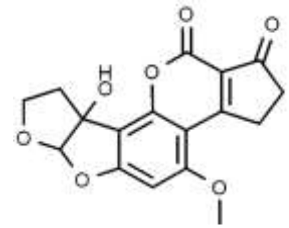
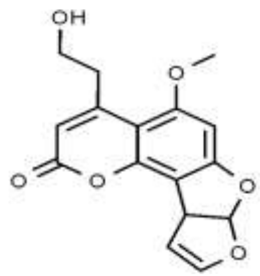
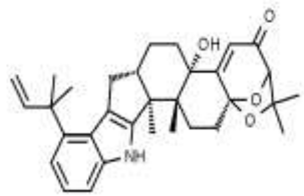
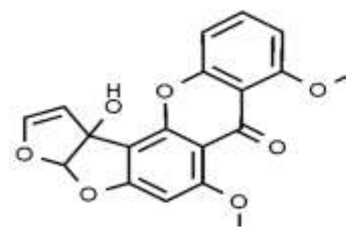
	AFM2	 $C_{17}H_{14}O_7$	330.2889	Metabolite of aflatoxin B_2 in milk of cattle fed on AF contaminated foods
	AFM2A	 $C_{17}H_{14}O_8$	346.069	Metabolite of AFM_2
	Aflatoxicol (AFL)	 $C_{17}H_{14}O_6$	314.289	Metabolite of AFB_1
	Aflatoxicol M_1	 $C_{17}H_{14}O_7$	330.2889	Metabolite of AFM_1

Difurocoumarolactone series	AFG_1	 $C_{17}H_{12}O_7$	328.273	
	AFG_2	 $C_{17}H_{14}O_7$	330.289	
	AFG_{2A}	 $C_{17}H_{14}O_8$	346.2883	Metabolite of AFG2
	$AFGM_1$	 $C_{17}H_{12}$	344.272	

	$AFGM_2$	 $C_{17}H_{14}O_7$	330.2889	Metabolite of AFG2
	$AFGM_{2A}$			Metabolite of AFGM2
	AFB_3/ Parasiticol	 $C_{16}H_{14}O_6$	302.279	
	Aflatrem	 $C_{32}H_{39}NO_4$	501.656	
	Aspertoxin	 $C_{19}H_{14}O_7$	354.310	

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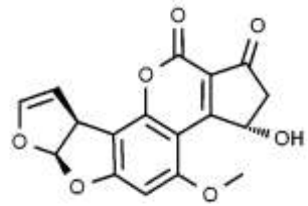
	AFQ_1	 $C_{17}H_{12}O_7$	328.273	Major metabolite of AFB_1 in *in-vitro* liver preparations of other higher vertebrates

Source: Modified after [18][37].

Table 2. Median lethal dose (LD_{50}) values for aflatoxin B1 administered as a single dose to different animal species

Animal	**LD_{50} value (mg/Kg body weight)**	**Animal**	**Sex**	**Age/Size**	**Oral LD_{50} value (mg/Kg body weight)**
Rainbow trout	0.6	Golden hamster	Male	30 days	10.2
Dog	1.0	Rat	Male	21 days	5.5
Mouse	9.0	Rat	Female	21 days	7.4
Duckling	0.3-0.6	Duckling	Male	1 day	0.37
Chicken	6.3	Dog	Male/Female	Adult	0.5
Monkey	2.2	Rat	Male/Female	1 day	1.0
Sheep	2.0	Pig	Unspecified	6-7 kg	0.6
Rat	5.5-17.5	Chicken embryo	Unknown	N/A	0.025
Guinea pig	1.4-2.0	Rat	Male	0.001 kg	17.5
Turkey poultry	0.5				

Source: Agag [36], Ciegler [37], Robens and Richard [38]; 1 mg/kg = 1000 µg/kg, N/A-Not applicable

Aflatoxin B_1 has been listed as a human class 1 carcinogen [39, 40] and the most potent carcinogen known [41, 42] that may play a part in the etiology of human liver cancer. This is due to its demonstrated ability to bind to nucleic acids (DNA and RNA) and proteins, forming adducts such as aflatoxin B_1-lysine with albumin [39, 43, 44]. The carcinogenicity of AFs have been shown to operate by a genotoxic mechanism involving metabolic activation to a genotoxic epoxide metabolite, formation of DNA adducts and modification of the TP53 gene where there is transversion of guanosine to thiamine leading to carcinogenicity [42]. AFs interact with the basic metabolic pathways of the cell, disrupting key enzyme processes including carbohydrate and lipid metabolism as well as protein synthesis. It is unfortunately reported that where AFs are detected in foods, AFB_1 usually exceeds half the total amount present, explaining the reason why compliance limits for AFs include AFB_1, and a number of analytical methods have been developed and validated to quantify its concentration [45]. Aflatoxin M_1, like AFB_1 is a classified group 2B probable human carcinogen [46].

Human exposure to AFs has documented deleterious health effects, including acute aflatoxicosis and, following chronic exposure, liver cancer with 8.19 cases reported per 100,000 inhabitants in Africa annually [43], about 3,700 of which is from Uganda [47]. In fact, the risk of developing liver cancer is reported to be high (50% more) in cases where the individuals are carriers of Hepatitis B and Hepatitis C surface antigens [48]. In addition, AFs impair protein synthesis, induce coagulation, weight gain and immunogenesis [38].

Food-borne AFs have been implicated for inducing infantile stunting [49, 50] probably by interfering with protein synthesis and the activity of micronutrients (vitamins: A, B_{12}, C, D and E, zinc, selenium, iron and calcium) [14]. Diminished feeding and weight loss have been reported in domesticated animals fed on AF contaminated feed [49], ensued by death. AFs also cause lower milk and egg production as well as immune suppression due to the reaction of AF with T-cells (perforin, perforin-expressing and granzyme A-expressing $CD8^+$ T cells) [51] and a decrease in vitamin K activities [38].

All these have economic impacts, extensible to the national economy, estimated at 128 billion annually for Uganda [52]. In 2013, more than 600,000 tons of maize worth Uganda shillings 10 billion destined for export to the neighbouring Kenya was rejected because they had AFs above regulatory limits [53].

2.0 Etiology of Aflatoxins in Uganda and the Commodities Contaminated

2.1 Etiology

In Uganda, AFs are produced predominantly by *A. flavus* and *A. parasiticus* [54]. *A. flavus* is ubiquitous and is reported to produce AFB_1 and AFB_2 along with other mycotoxins: cyclopiazonic, kojic and aspergillic acids [30]. *A. parasiticus* produces AFB_1, AFB_2, AFG_1 and AFG_2 accompanied by mycotoxic Kojic and aspergillic acids [30, 55, 56].

The climatic conditions in Uganda such as heavy rains, sudden droughts, high humidity, average temperature of 25^{o}C, occasional floods as well as poor pre-, peri and post-harvest handling of foods by farmers and traders in the food value chain (drying harvested food crops on bare grounds, drying on polyethene/polypropylene sheets, drying on papyrus mats, leaving crops to dry in the field for a long time thus predisposing them to pest damage as well as mechanical damage) have been implicated for the proliferation of AFs in Ugandan foods [54].

Other biophysical factors such as soil factors (substrate composition), crop species (host-plant susceptibility and genotype), fungal populations (strain specificity and variation, instability of toxigenic properties) as well as levels of education, awareness and gender are another probable set of direct factors contributing to AF contamination and prevalence in agricultural foods in Uganda as reported elsewhere [57, 58] . Other factors that may influence AF production include water

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activity, pH, atmosphere (concentration of oxygen and carbon dioxide), microbial competition, mold lineage, plant stress and use of fungicides or fertilizers.

A. flavus and A. parasiticus are semithermophilic and semixerophytic, thriving favourably between 12°C to 48°C and at lower water potentials [59]. Optimum growth occurs between 25°C to 42°C while they flourish at high temperatures and low water activity associated with droughts as in Uganda. These factors contribute to the epidemiology of the two *Aspergillus* fungi. Despite the optimum temperature for AF biosynthesis reported to be 28°C to 35°C, some studies indicate higher temperatures inhibit AF biosynthesis [60, 61]. Considering this observation, the conditions in Uganda favour *A. flavus* and *A. parasiticus* growth along with their aflatoxigenic contamination of foods.

In Uganda, AFB_1 is the most studied [62] while AFM_1 have received little attention [63]. Thus, most studies reported AFB_1 levels or did not distinguish between the different types [64-69]. Others, such as the validation survey of Wacoo *et al.* [70], Muzoora *et al.* [71], Baluka *et al.* [72] and Wacoo *et al.* [73] differentiated the AFs. By and large, the lack of this depth in most researches can be tailored to the overall priority of simply analysing the safety of foods and/or individuals. More so, there was limited facility to handle AF analysis as well as lack of funds to procure the analytical grade reagents [54]. Despite the documented differences in toxicity, all AFs are harmful and should be detected, quantified and rigorously controlled. Further, there is dire need for comprehensive and coherent data on potential mycotoxins [74].

2.2 Commodities contaminated

Aflatoxigenic contamination in Uganda have been reported in maize (*Zea mays* L.) [65, 68, 69, 75], sorghum (*Sorghum bicolor L.*), finger millet (*Eleusine coracana)* and their local products [64], peanuts (*Arachis hypogaea* L.) [63, 71, 72, 76], cassava (*Manihot esculenta*) [77], rice (*Oryza sativa)* [78], sunflower (*Helianthus annus*), sesame (*Sesamum indicum* L.) [69], animal feeds [79], bovine milk-based products [63]. Aflatoxin have also been detected in human sera [66, 67, 80]. Virtually, all grains, spices and other oil seeds can not be exempted [53].

2.21 Peanuts (*Arachis hypogaea* L.)

Peanuts (groundnuts) is the only cheap source of plant proteins, second in importance to beans *(Phaseolus vulgaris L.*) and majorly cultivated in Eastern and Northern Uganda but consumed countrywide [81]. It is consumed in various forms as raw, roasted, blanched, seeds, as peanut butter, crushed and mixed with traditional dishes as a sauce or as *ebinyewa* (paste/flour) [82].

Lopez and Crawford [76] reported on the AF content of peanuts sold for human consumption in Uganda. On average, 15% of the samples had more than 1.0 μg/kg AFB_1 while 2.5% contained more than 10 μg/kg of AFB_1. The contamination levels were at peak at the end of the rainy season, prior to the new harvest season. Further, Korobkin and Williams [83] reported the need for AF analysis of peanuts consumed by the community of West Nile, Uganda as investigation of primary liver carcinoma and groundnut growing regions of Arua showed some significant correlation between cancer cases (reported in the tumor registry of Kuluva Hospital, a small mission hospital in the area between 1951 to 1965) to the distribution of the peanut growing areas.

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Total AFs were reported in 80% of peanut and peanut paste samples traded in metropolitan Kampala with 40% of these having AF content exceeding FDA/WHO compliance limit of 20 µg/kg by Osuret *et al.* [84]. Unprecedented AF levels (940 µg/kg and 720 µg/kg) were reported in peanut paste and peanut seeds respectively. In a similar concerted study [63], up to 100% of peanut flour samples used in Southwestern Uganda culinary recipes were reported positive for total AFs with a mean of 11.5 ± 0.43 µg/kg (**Table 3**). Lack of awareness and knowledge of AF contamination control was reported to be the direct reasons for the high AF levels recorded.

Table 3. Total aflatoxin content of some selected foods in some Ankole districts of South Western Uganda

Matrix/food sample	Samples analysed	Aflatoxin positive samples		Average total aflatoxin content (µg/kg)
		< 4.0 µg/kg	> 4.0 µg/kg	
Peanut flour	n = 3	0	3	11.5 ± 0.43
Sorghum (flour & porridge)	n = 7 & n = 15	3 & 2	4 & 13	15.2 ± 0.20
Millet (flour & porridge)	n = 12 & n = 21	3 & 0	9 & 21	14.0 ± 1.22
Cassava flour	n = 18	7	11	16.0 ± 1.66
Eshabwe (porridge) sauce	n = 14	1	13	18.6 ± 2.40

Source : Kitya *et al.* [63].

The aforeacknowledged studies never correlated the AF contaminations with their causes. Subsequently, Kaaya *et al.* [85] reported in a correlative study that at farm level in villages, up to 60% of peanuts had detectable AFs (**Table 4**). Further, low levels of awareness, poor storage practices, and poor processing practices (drying, sorting and milling) were implicated for the heightened AF levels registered, stressing that aflatoxigenic contamination commences right from farms. Comparative analysis of market peanuts unveiled significantly higher AF contents in retailed samples than those wholesaled.

Table 4. Aflatoxin content of peanuts from farmers in some selected peanut growing districts of Uganda

Village (district)	Samples analyzed	Aflatoxin status				Aflatoxin concentration (µg/kg)
		Positive		Negative		
Kabulamuliro (*Mubende*)	n = 25	n = 20	80%	n = 5	20%	12.4 ± 5.31
Kiboyo (*Iganga*)	n = 20	n = 15	75%	n = 5	15%	10.5 ± 6.15
Bugodi (*Mayuge*)	n = 15	n = 9	60%	n = 6	40%	7.3 ± 4.98
Gayaza (*Mubende*)	n = 12	n = 8	67%	n = 4	33%	9.8 ± 4.32

Adapted from Kaaya *et al.* [85].

Muzoora *et al.* [71] screened 120 peanut samples sourced from Ugandan districts of Kampala, Mubende, Gulu, Pader, Mbarara, Masindi and Kaberamaido for AFs followed by competitive enzyme linked immunosorbent assay (ELISA) quantification. Their report indicated that 72% of the samples were AF-positive with 26% having AFB_1, AFB_2, AFG_1 and AFG_2 whereas AFB_1 and AFG_1 containing samples constituted 74% of the total samples. More urban samples (67.1%) were AF positive than rural samples (47.6%). ELISA gave 81% AF-positive samples, with milled groundnuts registering higher total AF (range: 0.31 to 1,1732 µg/kg; mean: 1,277.5 ± 382.2 µg/kg)

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compared to whole groundnut seeds (range: 1.6 to 516 μg/kg; mean: 84.7 ± 43.8 μg/kg). Up to 52% of the samples in the study registered total AF contents greater than FDA/WHO maximum compliance limit of 20 μg/kg for total AF in peanuts. There were typically no significant differences reported in the AF content of peanuts from the different regions. The study implicated milling of fungal contaminated peanuts by traders to shield evidence of spoilage from consumers and the skewed distribution of AF in the studied matrices for the reported relative differences in AF levels of milled and whole peanuts.

Partnership for Aflatoxin Control in Africa (PACA) report [86] indicate that peanuts in Uganda are mycotoxicologically unfit for human consumption. Kioga plains (Iganga and Soroti districts) in a survey had 20% of the peanuts with AF levels above 10 μg/kg while Tororo had 10% of the samples above the regulatory limit of 10 μg/kg. In addition, other agroecological zones had 10% peanut samples with AF contamination in levels above 10 μg/kg with exception of North Eastern which had none of the samples with detectable AFs. The report is substantiated by investigations of Baluka *et al.* [72] which reported that 34% of 55 peanut samples analyzed in a study contained AFs in concentrations greater than the East African and FDA/WHO compliance limits for AFs in peanuts.

From the foregoing reports, it can be noted that very high concentrations of AFs have been reported in peanuts in Uganda. This could be because as the pods grow in the soil, various aflatoxigenic fungi contaminate the shells, testa and seeds. Worse still, mechanical damage during harvest, drying and storage further increases the chances of fungal contamination and mycotoxin production. This is substantiated by a study which revealed that grains and oilseeds from maize, sorghum and sunflower produced in above the ground reproductive structures had relatively lower AF contamination compared to those produced in geocarpic structures of groundnut and bambara nut [87].

2.22 Cereals (maize, millet, sorghum, rice) and cereal-based products

The occurrence of mycotoxins and associated aflatoxigenic *A. flavus*/ *A. parasiticus* in staple Ugandan foods and their derivative poultry feeds were evaluated by Sebunya and Yourtee [72]. The 54 samples of maize, peanuts, soybean and poultry feed samples taken and precultured on *A. flavus*/*parasiticus* selective agar (AFPA) were analyzed for their fungal content on a coconut agar medium under ultraviolet light with a subsequent confirmatory scrutinization for AF production in a pure culture. 25 of the samples were analyzed for AFB_1, AFG_1, zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin and diacetoxyscirpenol. *A. flavus/parasiticus* were reported in 77% of maize, peanuts (36% human food; 83.3% animal feed) and 66.6% in poultry feed. No fungus was detected in soybeans whereas 8% (two) samples of the 25 mycotoxin-scrutinized samples had 20.0 μg/kg of AFB_1 (4 times the statutory limit of 5.0 μg/kg for AFB_1 in Ugandan foods).

Five baby food products locally produced in Uganda were bought from different shops and supermarkets at the stage of consumption and investigated for contamination by different toxigenic fungi and aflatoxins by Ismail *et al.* [80]. These foods, each with one or more cereal flour as an ingredient were cultured using dilution plate method and three selective isolation media

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(pentachloronitrobenzene rose Bengal yeast extract sucrose agar (PRYES), peptone-pentachloronitrobenzene agar (peptone-PCNB) and AFPA) and enumerated. PRYES plates revealed high level of contamination of the foods by *Penicillium*, with three species being nephrotoxigenic (*P. viridicatum, P. verrucosum* and *P. citrinum*). On the one hand, 9 species of *Fusarium* were recovered in high frequencies and counts on peptone-PCNB. Of these, *F. verticillioides* followed by *F. solani* were the most prevalent while *F. proliferatum* and *F. tricinctum* had more propagules. In addition, aflatoxigenic *Aspergilli* were isolated on AFPA from the majority of samples of all the products investigated in this study. *A. flavus*, *A. niger*, *Cladosporium* and yeasts were prevalent. Regarding total AFs, all samples analyzed were contaminated, though the levels detected (0-10 µg/kg and 10-20 µg/kg) were below or in the current tolerance level of 10 µg/kg and 20 µg/kg accepted in foodstuffs by Ugandan standards and WHO/FDA respectively. The contaminated foods constitute a health hazard to babies as they have a more restricted diet and generally consume more food on a body weight basis than adults. They concluded that the foods must be examined at regularly to assess their quality.

Lee *et al.* [67] reported that 11% of 55 maize samples collected in a survey were contaminated with AF in the range of 12.7-123.5mg/Kg, 9 % of which exceed the maximum regulatory limit. PACA [78] reported that sorghum from the different agroecological zones represented by Lira, Gulu, Amuria, Soroti and Tororo districts of Uganda recorded between 90 to 100% of the samples positive for AFs, with total AFs ranging from 4.0 to 265.5 µg/kg (mean from 11.5 to 170.1 µg/kg). Between 85% to 100% of the samples registered total AF greater than 4 µg/kg, while between 70% to 100% of the samples had total AF greater than 10 µg/kg. Between 65% to 100% of the samples had AF content greater than 20 µg/kg. Kitya *et al.* [25] further reported that millet and sorghum from Southern Uganda had mean total AF contents of 14.0 ± 1.22 µg/kg and 15.2 ± 0.2 µg/kg respectively (**Table 3**). A regional report cited in [88] indicate that maize grains in Uganda is the least contaminated in the East African Community (**Table 5**).

Table 5. Per capita food and aflatoxin contamination patterns in the East Africa region

Food	Country	Per capita food consumption (g/person/day)	Mean aflatoxin content (µg/kg)
Maize	Uganda	400	9.7
	Tanzania	69	49.7
	Kenya	405	131.7
Groundnuts	Uganda		25.1
(peanuts)	Tanzania		15.0
	Burundi	65	12.5
Cassava chips	Uganda		0.5
	Tanzania	214	0.9
Sorghum	Tanzania	40	3.0
Milk	Kenya	750 ml	0.8
	Tanzania	750 ml	0.9

Source: Adapted from the report by the East African Community's aflatoxin working group in April 2013 (Dar es Salaam, Tanzania (EAC/TF/405/2013) cited in a penultimate study [88].

The moisture and total aflatoxin content of 27 samples of fresh harvested maize from Mubende, Ibanda, Jinja, Mayuge, Buikwe, Hoima, Mpigi, Masindi and Bugiri districts of Uganda representing the agroecological zones: Lake Victoria crescent, Western Highlands, South East and

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Lake Albert Crescent were determined by Omara [65]. The moisture content ranged from 12.9% to 18.8% (mean : 13.9 ± 0.35% to 17.2 ± 1.55%) with the highest moisture recorded in maize from Ibanda. The highest mean AF content of 11.0 ± 3.01 µg/kg was recorded in maize from Hoima while the lowest AF content of 3.8 ± 1.30 µg/kg was reported in maize from Mpigi. All the samples had detectable AFs but none had AF content greater than 20 µg/kg. The lower levels of aflatoxin recorded in this study was attributed to the fact that the maize had not undergone post-harvest handling practices which are reported to increase AF content in maize [58]. The study concluded that maize in Uganda are pre-contaminated by AFs prior to harvest and recommended that farmers should plant maize varieties with established maturity periods to ensure timely harvesting.

2.23 Cassava (Manihot esculenta)

Cassava is one of the most important staple foods in Uganda grown majorly in Northern Uganda and Eastern Uganda [81]. The dynamics in cyanogen levels during the processing, the associated microflora, proteinaceous content, amino acid patterns and mycotoxin contamination of cassava products processed traditionally by the Alur people of West Nile were investigated by Essers *et al*. [89]. Cassava tuber processing was monitored at six rural households and replicated in an analytical laboratory setting, comparing it to sun-drying. Cassava flours from the rural households were analyzed for residual cyanogens, mutagenicity, cytotoxicity and AFs. Mean total cyanogen levels in flours collected were 20.3 ± 16.8 mg CN equivalents kg^{-1}dw in 1990 for 23 samples and 65.7 ± 56.78 mg CN equivalents kg^{-1}dw in 1992 for 21 samples. Mean cyanohydrins plus HCN levels were 9.1 ± 8.7 in the 1992 samples. Total cyanogen levels in the village monitored batches reduced significantly following heap-fermentation to 20.4 ± 14.0 from 436.3 ± 140.7 mg CN equivalents kg^{-1}dw cassava. Residual cyanogen levels were positively correlated with particle size of the resulting crumbs. Hence, heap-fermentation proved significantly more effective in reducing cyanogen levels than sun-drying alone, though it did not always result in innocuous levels of cyanogens. Dominant mycelial growth reported was from the fungi *Neurospora sitophila, Geotrichum candidum* and *Rhizopus oryzae*. No mutagenicity, cytotoxicity nor AFs were detected in the flours while protein quantity and quality were not significantly reduced. The authors further reported that since the removal of cyanogens was more efficient and no new obvious health risks were noted, heap-fermentation can be regarded as an improvement compared to sun-drying alone in areas where cassava varieties with higher cyanogen levels prevail, with optimization of the process not to compromise the final product safety [82].

Data available in open literature have reported AF contamination of cassava in Uganda at an average content of 0.5 µg/kg (**Table 5**). Osuret *et al.* [84] found 20% (1/5) samples of cassava sold in metropolitan Kampala to be aflatoxigenically contaminated in levels above WHO/ US EPA compliance limit of 20 µg/kg. In a similar concerted investigation, Kitya *et al.* [63] bewrayed that cassava chips in South Western Uganda are mycotoxicologically contaminated with mean total AF content of 16.0 ± 1.66 µg/kg. Kaaya and Eboku [77] reported *Rhizopus* (66.7%), *Mucor* (37%), *Penicillium* (22.2%), *Aspergillus* (20.4%) and *Fusarium* species (5.6%) as the fungi contaminating dry cassava chips in Eastern Uganda with up to 30% of the samples registered positive for AF

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(mean AF content was 0.51 µg/kg; AF range was 0.0 to 4.5 µg/kg). *A. flavus* regrettably was reported in 18.5% of the analyzed samples.

2.24 Animal products

Most of AFB_1 and AFB_2 ingested by mammals are eliminated through urine and faeces. A fraction of this is biotransformed in the liver and excreted in milk and urine as AFM_1 and AFM_2 respectively. AFM_1 is detectable in milk 12-24 hours after the first AFB_1 ingestion, reaching a high level after a few days. Thus, dietary exposure to AFs through consumption of milk from lactating animals fed on AF-contaminated feeds in Uganda is as high as microbial contamination of milk reported in Metropolitan Kampala [83]. In Western Uganda, Kitya *et al.* [63] reported that a bovine milk-based ghee sauce (*Eshabwe*) had a mean total AF content of 18.6 ± 2.4 µg/kg which was the highest of all the matrices tested for aflatoxin in the Ankole districts of Mbarara, Ntungamo, Rukungiri, Kasese and Kabale (**Table 3**). *Eshabwe* is a traditional Ankole delicacy prepared from unprocessed ghee, rock salt, boiled cold water and salt and is commonly prepared for special ceremonies as a condiment [90]. Given the fact that this sauce is almost prepared by every Ankole family, the study indicated that the high incidences of hepatocellular carcinoma could be correlated to the consumption of such aflatoxin-contaminated foods resulting from the traditional food processing techniques.

Upon ingestion of AFB_1, Cytochrome P450 enzymes (CYP) (including CYP1A2, CYP3A4 and CYP2A6) in the liver and other tissues convert AFB_1 to epoxides (AFB_1-8,9-exo-epoxide and AFB_1-8,9-endo-epoxide) and to AFM_1, AFP_1, AFQ_1 and its reduced form aflatoxicol. Of the epoxides, the AFB_1-8,9-exo-epoxide can form covalent bonds with DNA and serum albumin resulting in AFB_1-N7-guanine and lysine adducts respectively. Like AFB_1, AFM_1 can also be activated to form AFM_1-8,9-epoxide that binds to DNA resulting in AFM_1-N7-guanine adducts. These guanine and lysine adduct have been noted to appear in urine. The metabolites AFP_1, AFQ_1 and aflatoxicol are thought to be inactive and are excreted as such in urine, or in the form of glucuronyl conjugates from bile in faeces [91].

In Uganda, there is no report on the aflatoxin content of other products of animal origin such as meat and blood.

2.30 Co-occurrence of aflatoxins with other mycotoxins in Ugandan foods

Several mycotoxins can occur simultaneously in matrices [84]. The statutory and regional regulations in place for food and feed products are based entirely on AFs, failing to take into consideration possible combined toxic effects of different mycotoxins. Some studies in Uganda have reported the co-occurrence of AFs with some mycotoxins.

In an investigation by Sebunya and Yourtee [72] on 25 samples of foods analyzed for AFB_1, AFG_1, zearalenone, sterigmatocystin, ochratoxin A, citrinin, vomitoxin and diacetoxyscirpenol, two samples had 20 µg/kg of AFB_1. Zearalenone and vomitoxin were detected in 3 and 2 samples of maize respectively.

Following a WHO meeting on nodding syndrome in Kampala, Uganda in 2012, it was recommended that fungal contamination of foods should be investigated as a possible cause of the

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disease. Echodu *et al.* [66] assessed the relationship between consumption of mycotoxin-contaminated foods (sorghum, millet, sunflower, groundnut, sesame and maize) and the development of nodding syndrome in the affected Northern districts of Lamwo and Kitgum. Very high levels of total AFs and ochratoxins in millet, sorghum, maize and groundnuts in both households with and without children with nodding syndrome were registered. No significant association between concentrations of the mycotoxins and the presence of children with nodding syndrome in households was noted. Sorghum in this study had the highest total AF ranging from 0.00 to 68.2 μg/kg while the lowest AF was recorded in sesame (maximum AF of 4.5 μg/kg). In this study, the highest ochratoxins and vomitoxin/deoxynivalenol content were 7.647 μg/kg and 2.606 μg/kg reported in sorghum and maize from Lamit Tumangu village, Kitgum district respectively.

Baluka *et al.* [72] compared mycotoxins and selected trace metal content of peanuts sold in selected markets in Kampala, Uganda to those traditionally prepared in homes. Market processed peanut samples (n = 33) were purchased from four St. Balikuddembe, Nakawa, Kalerwe and Bukoto markets of Metropolitan Kampala, central Uganda. Control samples (n = 5) were unground peanuts bought from markets but processed in homes by traditional methods and others by metal grinding. Aflatoxins: B_1, B_2, G_1, G_2; Fumonisins; Deoxynivalenol, Nivalenol, Ochratoxin A, T2 toxin, Zearalenone, and Zearalenol and heavy metals: Arsenic, Boron, Barium, Cadmium, Chromium, Copper, mercury, Magnesium, Nickel, Lead and Zinc were analysed. AFs, particularly AFB_1, was reported as the predominant mycotoxins in the samples. There were significantly higher concentrations of AFs in market-processed than in home-processed samples. AF concentrations were in the range of 0–540 μg/kg for AFB_1, 0–141 μg/kg for AFB_2, 0–213 μg/kg for AFG_1, 0–36 μg/kg for AFG_2 and 0–849 μg/kg for total AFs. Aflatoxin B_1 was most abundant AF in concentrations greater than FDA/WHO limit of 20 μg/kg. The Cadmium and Lead content of the samples were below the method limit of detection of 0.25 ppm though one sample (2.6%) had arsenic concentration above the FDA maximum permitted concentration of 1.4 ppm. The concentrations of chromium and mercury in 100% of the samples were below the FDA acceptable limit of 1 and 0.5 ppm respectively. Roasting and duration of grinding had no appreciable effect on AFs and metalliferous content of the samples. The study recommended the need for food-borne toxicant monitoring of foods for human consumption in Ugandan public markets [72].

2.40 Geographical distribution of aflatoxins in Uganda

Brazil was the first hotspot of AFs recorded [92] before subsequent reports cited Uganda, Kenya, Senegal, Mozambique, Swaziland, Nigeria, China, Thailand and Philippines [93]. Arne Sherck-Hanssen [94] reported in 1970 a case report that implicated the death of a Ugandan to be linked with ingestion of aflatoxin-contaminated cassava. The 15-year-old boy was admitted to Mulago Hospital, Kampala, Uganda on June 4^{th}, 1967 with abdominal pains and swelling of the legs for a couple of days. The pulse rate was declared normal (100/50 mm Hg). Probing clinical analyses declared he was in heart failure. Upon administration of digestoxin and Mersalyl sodium, the boy passed on two days after admission. An autopsy recorded edema and congestion of the lungs with diffuse necrosis of the liver. Histology demonstrated centrolobular necrosis and subsequent

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aflatoxigenic investigation of a sample of the cassava eaten by the boy with his sister and brother (who also became ill but survived) indicated the cassava had 1,700 µg/kg of aflatoxin B_1 which is markedly lethal if ingested for over three weeks when compared with the acute toxicity dose of 220 µg/kg aflatoxin B_1 in African monkeys [95].

Uganda is divided into ten agro-ecological zones: Southern highlands, Southern dry lands, Lake Victoria crescent, Eastern, Mid Northern, Lake Albert crescent, West Nile, Western highlands, South East and Karamoja drylands [96]. AFs tend to be recorded at nearly equal concentrations in food samples from the different zones. This can be attributed to the similarity in the agronomic, pre-, peri- and post-harvest handling practices and the inter-regional marketing of foods in Uganda [86].

In one of the pioneering surveys, the AF content of 480 foods stored for consumption between harvests in Uganda between September 1966 to June 1967 (for nine solid months) were evaluated by Alpert *et al.* [97]. Up to 29.6% of these had detectable AF with 3.7% of the samples recording >1.0 µg/kg AF content. Beans had the highest aflatoxin content (72%) while the prevalence of aflatoxins in maize, peanuts and cassava were reported at 45%, 18% and 12% respectively. Rice in this study had no detectable aflatoxins. The high prevalence of aflatoxigenic contamination reportedly correlated with provinces with a high recorded hepatoma incidence, or moldy food consumption (**Table 6**). This led to the postulation that AF exposure may be a contributing factor for the elevated levels of hepatoma in Uganda [89].

Table 6. Aflatoxin content of some staple foods in Uganda

Sample/Matrix	Number of samples			Total aflatoxin (µg/kg)		
	Analyzed	AF positive	% AF positive	1-100	100-1000	>1000
Beans	64	46	71.9	30	11	5
Maize	49	22	44.9	13	9	0
Sorghum	69	26	37.7	19	5	5
Peanuts	152	27	17.8	11	8	8
Millet	55	9	16.4	9	0	0
Peas	19	3	15.8	3	0	0
Cassava	34	4	11.8	0	2	2
Rice	11	0	N/A	0	0	0
Other grains	11	2	18.2	0	1	1
Grain mixtures	16	3	18.7	2	0	0
Total	**480**	**142**		**87**	**37**	**18**

Adapted from [97], N/A-Not applicable

In the same study, the local cancer registry in the regions where samples were drawn were checked for the period 1964 to 1966. The study indicated that Karamoja region had the highest hepatoma frequency of 6.8 cases per 1,000 people per annum with a frequency of AF contamination at 44% (**Table 7**). Overall, hepatoma occurred at an average rate of 1.0 to 2.7 cases per 1,000 people per year [97].

No study has reported in open literature on the AF content of beer consumed by Ugandans, yet it is among the most consumed foods that perhaps uses all the major cereals: maize, sorghum and barley as well as cassava. Beers are practically products of mixed-culture fermentations, a

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process that continues upto consumption time. Thus, brewing is an ideal route for exposure to AFs as it offers favorable conditions for aflatoxigenic fungal growth [90] and creates an avenue for use of contaminated grains as the final consumers will not be able to physically detect as reported for peanut paste [71].

Table 7. Hepatoma incidence and frequency of aflatoxin contamination of some staple foods in Uganda

Region	Hepatoma cases/100,000 people per annum	Aflatoxigenic contamination				
		Analyzed samples	% of samples AF positive	Total aflatoxin (μg/kg)		
				1-100	100-1000	>1000
Toro	No data collected	29	79.3	10	31	38
Karamoja	15.0	105	43.8	24	15	5
Buganda	2.0-3.0	149	28.9	23	4	1
West Nile	2.7	26	23.1	19	4	0
Busoga	2.4	39	10.3	05	5	0
Acholi	2.7	26	15.4	15	0	0
Ankole	1.4	37	10.8	11	0	0
Rwanda immigrants	3.0	None collected	Not applicable	Not applicable	Not applicable	Not applicable

Modified from [97], regions have different tribes with different traditional practices and ways of handling foods.

3.0 Capacity for detection and quantification

Specific, sensitive and simple analytical methods for detection and quantification of AFs are prerequisites for their accurate detection and quantization given their presence in very meagre concentrations and their skewed nature of distribution in matrices [98]. The accuracy, precision, reproducibility and repetitiveness of analytical techniques for detection and quantification of the AF content of a commodity is largely influenced by the way each step in the analytical process from sampling to extraction, clean-up and quantification is perfected. One of the biggest challenges is that it is often hard to obtain representative samples for AF analysis for bulk lots of commodities. This is in part due to the fact that the aflatoxigenic molds do not grow uniformly in the matrices, giving a skewed distribution [98].

3.1 Methods of detection and quantification employed by AF investigations in Uganda

The methods for detection of AFs in agricultural foods have been reviewed in sufficient details by some Ugandan authors [13]. This also explains, in part, the fact that most AF investigations in Uganda following this review such as that of Muzoora *et al.* [71], Echodu *et al.* [69], Wacoo *et al.* [73] and Byakika *et al.*[64] employed selective and highly sensitive methods. **Table 8** summarizes some of the methods employed by aflatoxigenic investigations in Uganda.

Generally, aflatoxigenic analysis of samples employed laboratory-based high performance liquid chromatography (HPLC), thin layer chromatography (TLC), enzyme linked immunosorbent assays (ELISA), fluorescence spectrophotometry (FL) and liquid chromatography tandem mass spectrometry (LC-MS/MS) which are expensive, labour intensive and time consuming [13].

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Unlike reported before [54], Uganda have developed some appreciable capacity to detect and quantify specific AFs with laboratories at Makerere University, Chemiphar Uganda Limited, Uganda National Bureau of Standards, Uganda Industrial Research Institute and Directorate of Government Analytical laboratory. Unfortunately, all these laboratories are located in the country's capital (Kampala) making them inaccessible to other regions. At industrial level, agro-processing companies are monitoring total AFs in cereals using single step lateral flow immunoassays utilizing Reveal Q+ test strips that are developed and read on AccuSan Gold readers [52, 68].

Table 8. Some of the analytical methods employed by aflatoxigenic investigations in Uganda.

Method	**Sample (s)**	**Year**	**References**
Lateral flow immunochromatography	Maize grain	2019	Omara [68]
HPLC	Maize-based product (*Kwete*)	2019	Wacoo *et al.* [73]
ELISA	Sorghum, millet, *obushera*	2019	Byakika *et al.* [64]
ELISA, HPLC	Maize flour	2018	Wacoo *et al.* [70]
ELISA	Maize, sorghum, millet, sesame, peanuts	2018	Echodu *et al.* [69]
HPLC	Human sera	2018	Lauer *et al.* [92]
TLC, ELISA	Peanuts (seeds and paste)	2017	Muzoora *et al. [71]*
LC/MS/MS	Peanuts (seeds and paste)	2017	Baluka *et al.* [72]
FS	Peanuts (seeds and paste), cassava flour, maize grains	2016	Osuret *et al.* [84]
ELISA	Human sera	2015	Khang *et al.* [67]
ELISA	Human sera	2014	Asiki *et al.* [66]
ELISA	Cereal-based baby foods	2011	Ismail *et al.* [99]
FS	Cassava	2010	Kaaya and Eboku [77]
FS	Sorghum, millet, *Eshabwe,* peanut (seeds and paste), cassava chips	2010	Kitya *et al.* [63]
ELISA	Maize	2006	Bigirwa *et al.* [100]
FS	Peanuts	2006	Kaaya *et al.* [85]

Years cited represent the year the data were published with most data collected in over 2 months to 1 year.

Due to limited access to the aforelisted laboratory-based analytical methods, a rapid on-site AF portable immunosensor based on a glass-electroless-plated silver/cysteine platform for detection of total AF was constructed at Uganda Industrial Research Institute, plot 42A, Mukabya road, Nakawa, Kampala, Uganda by Wacoo and his teammates [101]. This electrochemical immunosensor device was subsequently validated in a penultimate study [70] which assessed the AF content of 60 maize flour samples in six principal markets and 72 samples from selected households in Metropolitan Kampala. The immunosensor was reportedly validated with a linear range of 0.7 ± 0.1 to 11.0 ± 0.3 μg/kg and limit of detection of 0.7 ± 0.0 μg/kg. Maize flours from the scrutinized markets of Usafi, Nakawa, St. Balikudembe (also called Owino), Nakasero, Kireka and Kalerwe had a mean total AF of 7.6 ± 2.3 μg/kg with approximately 20% of the samples having higher than 10 μg/kg statutory AF limit while 45% of household samples had total AF

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above compliance limit. The AF results from the immunosensor reportedly correlated with HPLC and ELISA results with correlation coefficients of 0.94 and 0.98 respectively [70].

Bright greenish-yellow fluorescence (BGYF) or the black light test, which can locate lots presumed to be contaminated with aflatoxin have not been reported in Uganda. This is a simple test for AF in maize where kernels are viewed under an Ultraviolet lamp at 365 nm for characteristic bright greenish-yellow fluorescence. This indicates a possible presence of aflatoxigenic fungi or the mycotoxin itself [102]. Regulatory bodies in Uganda should develop capacity to perform this simple detection test for surveillance surveys.

3.2 Exposure assessment

Humans are exposed to AFs through oral ingestion of contaminated plant products (such as peanuts) primarily as AFB_1 or animal products such as meat and milk from animals previously fed on AF-contaminated feed (in form of AFM_1) [14]. Farmers and other agricultural workers may also get exposed by inhaling dust generated during the handling and processing of contaminated crops and feeds.

Analytical detection and quantification of AFs in foods does not give the exact exposure levels as the quantities detected in raw foods are not necessarily equivalent to that ingested. Losses are possible, and therefore, epidemiological biomarkers on dietary exposure have been employed to assess the level of exposure. Biomarkers are more precise for assessing the degree of exposure to AFs, as they are non-subjective and can determine the internal and biologically effective doses. Aflatoxin biomarkers in use currently include the AF-N_7-guanine adducts excreted in urine (reflect the previous day's exposure), AFM_1 (primarily in breast milk, and reflects exposure over the previous 24 hours) and the aflatoxin-albumin adduct (AF-alb) in plasma or serum with half-life of about 2 months (this allows for assessment of chronic and routine exposure to AFs) [103]. Albumin, the only serum protein that binds AFB_1, forms a high level of adducts [104], while haemoglobin (Hb) binds AFB_1 in a very low yield [105]. Albumin extracted from human blood and urine avail a measure of the biologically effective dose of ingested AFB_1. Aflatoxins: B_1 and G_1 can be bound by albumin, and are metabolised to 8, 9-epoxide [106]. The AF-alb adduct levels are considered as AFB_1 amount ingested as AFG_1 are less prevalent in foods [36]. Thus, the AF-alb biomarker is the more commonly employed as it can be easily detected by ELISA (with results in pg AF-alb/mg albumin or in pg AF-lysine equivalent/mg alb) [107]. Quantification of aflatoxin-lysine adduct (AFB_1-lysine) in proteolytic digests of serum with HPLC-FS or LC-MS/MS have also been alternatively employed [108, 109].

In Uganda, Asiki *et al.* [66] reported human sera samples positive for AF-alb adducts in South-western Uganda. The AF-alb adduct ranged from 0 to 237.7 pg/mg alb among 100 adults (18–89 years) and 96 children (0–3 years) with 75% of the participants having AF-alb adduct levels above 7.1 pg/mg alb, 50% levels above 10.3 pg/mg alb while 25% had levels above 15.1 pg/mg alb. Overall, all the adults and the four children had detectable AF-alb adducts in the study. Respondents living close to trading centres had significantly ($p = 0.003$) higher levels of detectable AF–alb adducts compared to their counterparts living in villages. Respondents consuming *matooke* (banana) had half detectable AF–albumin adduct compared to those who did not consume it. This

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is because these respondents are more likely to consume other foods which are prone to AF contamination hence people consuming *matooke* are less likely to have detectable AF–albumin adduct.

A longitudinal exposure study by Kang *et al.* [67] assessed AF exposure in South-western Uganda, reporting that 90% (642/713 of the sera) samples drawn from the General Population Cohort were positive for AFB-Lys with a median level of 1.58pg/mg and albumin range of 0.40–168 pg/mg. AFB-lysine adducts from 1999–2003 in the Rakai Community Cohort Study, showed a detection rate of 92.5% (346/374) with a median of 1.18 pg/mg and a range of 0.40–122.5 pg/mg. Thus, it was deduced that AF exposure is high in the studied area and a similar finding is expected in other parts of Uganda. Further, a study done round the same time in the Northern part of Uganda [50] reported that there is a casual effect relationship between AF exposure and impaired growth in infants.

A cohort study by Lauer *et al.* [80] evaluated the association between maternal AF exposure during pregnancy and adverse birth outcomes, lower birth weight, in a sample of 220 mother–infant pairs in Mukono district, Uganda. Maternal aflatoxin exposure was assessed at 17.8 ± 3.5 pg/mg week gestation. Anthropometry and birth outcome characteristics were obtained within 48 hours of delivery. Median maternal AFB-Lys level was 5.83 pg/mg alb (range: 0.71–95.60 pg/mg alb, interquartile range: 3.53–9.62 pg/mg alb). Increase in maternal AFB-Lys levels were significantly associated with lower weight ($p = 0.040$), lower weight-for-age z-score ($p = 0.037$), smaller head circumference ($p = 0.035$), and lower head circumference-for-age z-score ($p = 0.023$) in infants at birth. The team concluded that there is a correlation between maternal AF exposure during pregnancy and adverse birth outcomes, particularly lower birth weight and smaller head circumference, though these warrant further probing studies.

3.3 Co-exposure assessment with other mycotoxins

The likelihood that mycotoxins may interact synergistically to induce amplified toxicity in animals is high because toxigenic fungi often occur simultaneously in the same batch of food/matrix and some fungi are capable of simultaneously producing several mycotoxins in a single given substrate. Unfortunately, there is no data in open literature in Uganda reporting on the assessment of co-exposure of AFs with other important mycotoxins such as fumonisins, ochratoxins, trichothecenes and zearalenone. The paucity of this data is partially due to the underdevelopment of valid biomarkers [110]. Mycotoxin-specific biomarkers for common mycotoxins such as fumonisins and deoxynivalenol have been developed only very recently [111, 112] and their utilization in epidemiological studies can be termed as nascent. Therefore, there is need for assessment of co-exposure to aflatoxins in Uganda with other mycotoxins.

4.0 Prevention and control (reduction)

4.1 international, regional and statutory efforts

Efforts have been put on AF control in Uganda through countrywide awareness creation [113-115]. This is being currently done by the Eastern Africa Grain Council (EAGC) in collaboration

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with Uganda National Bureau of Standards (UNBS) through the Eastern Africa Grain Institute with its headquarters at Muyenga-Kampala. Between 2015 and 2018, maize exporters, traders, farmer based organizations (FOBs) and warehouse handlers were trained on understanding the integrated East African maize standard (EAS 2:2013), food standardization, comparison of East African standards with international standards, standard maize sampling methods, maize grading and mycotoxins and the available methods for mycotoxin analysis [116].

Since its launch in 2006, EAGC has been leading the fight against AFs, working on a range of interventions to reduce the incidence, including assisting with the harmonization of AF control measures and improving the regulatory environment, running AF control training programs, providing moisture analyzers and tarpaulins to support farmers in drying and storing grains safely, sourcing for cheaper field based AF testing kits and methods for measuring aflatoxins, conducting field surveys, regular analysis and random sampling during harvesting at farm level to assess the prevalence and extent of contamination, working with East African Community to increase AF testing and surveillance in maize, participating in the development of the Partnership for Aflatoxin Control in Africa (PACA) strategy 2013-2022 as well as advising on the East African Community AF communication strategy [117].

National Agricultural Research Organization (NARO) in connection with Makerere University in 2010 developed a manual for management of AF in peanuts [81]. The manual gives a general overview of AFs (structures, health and economic effects), how to control AFs and some of the farming practices in Uganda that favours AF growth. It was particularly drafted to provide ample guidance on the best practices in limiting AF contamination in peanuts and to raise the value of groundnuts and its products

4.2 Scholarly efforts

Probing investigations of Wacoo and his team [73] revealed that probiotic enrichment of a local maize based traditional beverage (*kwete)* using starter culture with the probiotic *Lactobacillus rhamnosus* yoba 2012 and *Streptococcus thermophilus* C106 produced the beverage acceptable with consumers` acceptability score of greater/equal to 6 on a 9-point hedonic scale. The beverage remained stable for a month with reported *L. rhamnosus* counts of $>10^8$ cfu/g, pH 3.9 and 0.6% w/v titratable acidity. AF analysis indicated that the water-soluble fraction of the beverage following fermentation had more than 1000-fold reduction in AFB_1, AFB_2, AFG_1 and AFG_2 initially spiked in the ingredients. The efficiency of *L. rhamnosus* to bind AFB_1 was reported at 83.5% as determined by *vitro* fluorescence spectroscopy.

Mold and total AF content of cereal flours and *Obushera* (a local cereal based beverage) from markets in metropolitan Kampala were evaluated by Byakika *et al.* [64]. The capacity of lactic acid bacteria (LAB) starters from *obushera*; *L. plantarum* MNC 21, *Weisella confusa* MNC 20 and *L. lactis* MNC 24 to bind AFB_1 was evaluated against *L. rhamnosus* yoba 2012 (as a reference starter strain). The authors reported that mold counts in sorghum, millet and *Obushera* were between 0.0–2.4 log cfu/g, 2.0–6.5 log cfu/g and 2.0–5.5log cfu/g respectively. The mold counts in all the flours as reported exceeded the maximum food safety compliance limit of 4.0 log cfu/g of molds; 88.0% of *obushera* had counts within the maximum compliance limit of 1.3 log cfu/g.

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Aflatoxigenic results revealed that total AF content of investigated matrices (sorghum, millet and *obushera*) respectively in µg/kg were 22.3 ± 21.2, 9.9 ± 10.0 and 10.4 ± 6.1. The LAB bound 19.3–69.4% of AFB_1 in a 1000 µg/kg matrix, with binding efficiency in the order of *L. rhamnosus* yoba 2012 = *L. plantarum* MNC 21 > *W. confusa* MNC 20 = *L. lactis* MNC 24. The LAB-AFB_1 complex was reportedly stable to physiological saline washes, indicating that the LAB with AF-binding properties can be harnessed for controlled fermentation to reduce AF content of *obushera* [64]**.**

4.3 Suggested management strategies

The following control measures are suggested by this review for control of AFs.

4.31 Pre-harvest strategies

Crop varieties that are less susceptible to fungal growth should be bred and planted. This has been reported to be one of the best approaches for reducing effects of mycotoxin-producing fungal species [118]. Thus, local varieties of crops resistant to AF-producing fungi warrant investigation as some studies have unveiled that some local crop cultivars (maize) had lower AF levels than imported varieties [119]. In Uganda, Serenut 2 (a peanut variety) have been cited as a genetically more resistant variety to fungal growth and the production of AFs [82]. Drought, disease and pest tolerant/resistant crop varieties have been found to greatly reduce AF contamination. More so, host and parasite macro- and micromolecular trafficking that suggests the possibility to circumvent the AF problem by use of cross species RNA interference have been suggested. This equips particularly maize with molecules that shuts down AF biosynthesis upon infection with aflatoxigenic fungi, thwarting AF accumulation.

Timely harvesting of grains with the husks upon maturity in dry conditions and early removal of any damaged maize kernels or cobs is a feasible AF reduction strategy [119].

Visual sorting, winnowing, washing, crushing and dehulling have been found to contribute up to a 40–80% reduction in AF levels in grains [120, 121]. Sorting is highly recommended for reducing AF content in foods, peculiarly in peanuts [119, 122-124] and cassava. Though, sorting and giving children the moulded peanuts (called '*lake'* in Northern Uganda) or using them for making peanut paste should be discouraged. Sorting can be done using clean water; the damaged seeds/grains are buoyant while good ones sink and can be cooked directly. This is traditionally practiced in Northern Uganda with beans, peas and cow peas**.** Soaking and cooking in magadi soda, malting and roasting are other methods that have been used to reduce the levels of AFs in maize [121, 125-127]. Magadi soda is unknowingly used by rural community of Lango subregion, as a catalyst for fastening the cooking of beans, peas, white ants and sesame-based dishes (*alakena* and *agwaca),* vegetables and sometimes cassava.

Protection of crops from pest attack. This can be done using ash while in storage as is done in maize [128, 129] and plant essential oils such as *Eucalyptus saligna* that have reported bioinsecticidal activity [130].

Biocontrol strategies employing concoctions from plants such as *Ocimum gratissimum*, *Aframonium* species, *Zingiber officinalis* (ginger), *Xylopia aethiopica, Monodera myristica, Ocimum baslicum, Tetrepleura tetrapeta* and *Piper guineense* have been investigated and reported

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to inhibit *A. flavus* mycelial growth and proliferation. Essential oils of *Azadirachta indica* (neem) and *Morinda lucida* have been reported to retard aflatoxigenic *A. flavus* growth and its AF biosynthesis potential in inoculated maize grains [131]. Powder of *Aframomum danielli* (Zingiberaceae) can regulate moulds and insect infestation in maize and soybeans in storage for over a year under ambient conditions [132].

Competitive exclusion. *A. flavus* strains differ in AF production and this influences their crop contamination potential. Some strains, the toxigenic strains ('S' strains) produce a lot of AFs with numerous small sclerotia (< 400 µm). The 'L' strains are atoxigenic, produces low AF levels and a few large sclerotia that are > 400 µm [133]. There is always competitive exclusion when one strain competes to exclude another in the environment. Thus, a shift of strain profile from toxigenic to atoxigenic is a viable biological control strategy. Such atoxigenic strains of *A. flavus* have been combined as a bio-control product. This competitive exclusion strategy has yielded good results in some investigations with up to 96% reduction in AF levels [133].

A biopesticide, consisting of a rhizosphere-competent non-aflatoxigenic strain of *Aspergillus* with competitive saprophytic ability, may competitively exclude toxigenic strains from infecting the crop. Fluorescent pseudomonads and several strains of *Trichoderma* species inhabit the rhizosphere of many crop plants and have been identified as potentially promising biocontrol agents against *A. flavus.* Since the beginning of the 21st century, many *Trichoderma* (>250) and *Pseudomonas* (> 100) species have been isolated from peanut rhizosphere and evaluated for their antagonism towards *A. flavus* and their ability to reduce pre-harvest kernel infection of peanuts. Significant reduction of *A. flavus* populations and kernel infection occurred in both greenhouse and field experiments. Two *Trichoderma* isolates, Tv 47 and Tv 23, and two bacterial isolates *P. cepacia* (B 33) and *P. fluorescens* (Pf 2), were effective in reducing aflatoxin content in the kernels. Control of AF contamination have also been reported to be effective using non-aflatoxigenic biocontrol *A. flavus* strains that outcompetes the wild strain, reducing their concentration at the contaminated site [134]. However, the efficacy of these agents warrant establishment under Ugandan conditions so that affordable, readily available and effective formulations can be developed for use. Further, their integration with host plant resistance and agronomic management would provide an environmentally friendly option for the management of AF contamination in groundnuts.

4.32 Post-harvest management

The cost of prevention versus the cost of cure is not a new debate. However, some cure technologies for AFs are in place. One of the credited strategies is to reduce on the moisture content of grains before storage. Rapid and proper drying of crops to moisture level of 13% or below are recommended.

Clays such as Novasil has been demonstrated to bind AF in animal feeds [135] and reduce its content. An innovation for post-harvest AF elimination called the ''Toxin Scrub'' has been demonstrated by Grain and Toxins in Uganda but its usage has been delimited by its prohibitive cost [53]. The technology utilizes ozone, a strong oxidizer to eliminate nearly all the mycotoxins

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in the grain. This is supported by the fact that AFs are unstable to UV light in the presence of oxygen, to extremes of pH (<3, >10) and to oxidising agents such as sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone and sodium perborate [19]. AFs are also degraded by reaction with ammonia, various amines and sodium hypochlorite. Some compounds such as curcumin can alter the microsomal activation of AFB_1 and reduce the AFB_1 toxicity by increasing its detoxification.

Chemoprotection against AFs consumed by animals has also been reported. It utilizes compounds such as esterified glucomanoses and other yeast extracts that increases the animal's detoxification process or otherwise prevent the production of AF- epoxide, thereby reducing or blocking AFB_1-induced hepatocarcinogenesis. Oltipraz and chlorophyll are used to reduce the biologically effective dose and acts by binding AFs, thereby rendering them biologically unavailable to humans and animals.

4.4 Treatment

No scientifically proven and wholly specific antidote for ingested AFs have been reported. However, timely use of l-methionine (200 mg/kg) and sodium thiosulfate (50 mg/kg) after every 8 hours have reported therapeutic significance. Dietary intake of protein, vitamins, and antioxidants can be encouraged in case of aflatoxicosis [136].

5.0 Conclusion

Aflatoxin surveillance in Uganda is done through reactive approach. Ugandan foods are mycotoxicologically contaminated with aflatoxins and this has serious health implications. Limited studies have been done on aflatoxins in Uganda. No study in Uganda have assessed AFs in beers, imported rice such as basmat and sugarcane despite them being daily consumables. The Ugandan government through its ministries should develop capacity to detect, quantify, monitor and regulate AFs in foods produced and sold within the country and those exported/imported. There is need for more aflatoxin exposure assessments as well as co-exposure to aflatoxins with other mycotoxins.

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