



## Dietary Exposure of Infants and Young Children to Aflatoxins and Fumonisin in the East African Region: A Review

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### Abstract

Proper supplementary nutrition is essential for nurturing and promoting the growth and development of Infants and Young Children (IYC). Poor food quality and safety during this critical period can result in malnutrition. Complementary foods (CF) commonly provided to IYC in East African countries often contain ingredients highly susceptible to mycotoxin contamination, particularly aflatoxins (AFs) and fumonisins (FBs). This narrative review sought to explore the contamination of infant diets with AFs and FBs, as well as the exposure of IYC to these toxins. The review covers the types of CF used in infant diets, their susceptibility to AFs and FBs contamination, associated dietary exposure, and detection methods, along with regulatory and mitigation strategies in the East Africa region. Studies revealed widespread contamination of staple crops with AFs and FBs, highlighting that IYC primarily encounter these toxins through the consumption of cereal-based CF, often supplemented with legumes and oily seeds. Maize and groundnuts emerge as the predominant ingredients in CF. Despite established regulatory limits for these toxins in food intended for the general population, no specific limits exist for IYC, who are particularly vulnerable due to their high consumption of the susceptible crops and relatively small body size. Limited enforcement of existing regulations, unfortunately, allows the problem to persist in these low-resource countries. Focusing on East Africa, this review synthesizes findings from scientific studies to assess the dietary exposure of children to AFs and FBs through CFs. The findings underscore the need for further research on the magnitude and effects of AFs and FBs exposure, coupled with awareness campaigns to promote the demand for clean and safe CF within the East African Community countries.



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## Introduction

As Infants and Young Children (IYC) grow, complementary foods (CFs) play a crucial role in meeting their nutritional needs when breast milk alone is no longer sufficient.<sup>1</sup> According to Oladiran and Emmambux (2022), it is widely recognized that CF must offer sufficient energy, protein, vitamins, minerals, and other essential micronutrients to cater to the nutritional needs of children.<sup>2,3</sup> Adequate and proper CF are crucial for the growth and cognitive development of IYC.<sup>4,5</sup> Recent assessments by the World Health Organization (WHO)<sup>6</sup> indicate that globally, less than 25% of infants aged 6 to 23 months adhere to the recommended dietary diversity guidelines, and only a minuscule proportion receives diets that fulfill the necessary nutritional adequacy. These findings underscore a concerning deficiency in infant nutrition at a global level.

Common CF in the East African region traditionally consists of cereal-based diets.<sup>7-10</sup> Maize and groundnuts serve as the primary ingredients of CF but are susceptible to contamination with mycotoxins, especially aflatoxins (AFs) and fumonisins (FBs). AFs and FBs are secondary metabolites of *Aspergillus*<sup>11,12</sup> and *Fusarium*,<sup>13,14</sup> with four types of AFs outlined by the International Agency for Research on Cancer (2002), specifically AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub>. AFB<sub>1</sub>, the most potent variant, is responsible for approximately 75% of the overall food contamination attributed to AFs.<sup>15</sup>

The consumption of mycotoxin-contaminated foods can result in acute or chronic exposure. Chronic dietary exposure to AFs is linked to immunosuppression, liver cancer, and growth impairment. Simultaneously, ingesting higher doses can lead to acute aflatoxicosis, manifesting as severe illness and death due to damage to the liver, kidneys, and reproductive organs.<sup>16,17</sup> FBs are associated with oesophageal cancer in adult populations and neural tube defects in infants whose mothers were exposed to high toxin levels through maize-based foods during the first trimester of pregnancy.<sup>17-20</sup> Despite the severe health effects and consequences of dietary exposure to AFs and FBs, mothers and caregivers in rural communities of low-income countries often lack knowledge about nutrition and mycotoxin contamination of common CF,<sup>21-23</sup> thereby presenting a potential health risk during complementary feeding. Recent studies

by Lesuuda *et al.* (2021) and Ayo *et al.* (2018) have demonstrated a lack of awareness regarding mycotoxin contamination of foods in Kenya and Tanzania. The combined impact of traditional feeding practices and food product contamination with mycotoxins has resulted in persistent dietary exposure of IYC to AFs and FBs.

This review delves into the contamination levels of AFs and FBs in CFs consumed by infant and young children in the East African region, with a focus on assessing their potential dietary exposure to these mycotoxins. It also reviews the detection capacities for these mycotoxins to establish their strengths and weaknesses and provides an overview of practical mitigation efforts that can minimize the risks of IYC exposure to these toxins within five out of thirteen East African Countries. The information presented here contributes to understanding the magnitude of contamination in conjunction with IYC habitual diets and dietary exposure to these toxins.

## Searching Process

The study relies on research articles examining dietary exposure to AFs and FBs within the context of appropriate complementary feeding practices across five East African countries. This paper aims to provide a comprehensive overview of the impact of mycotoxins on children. The research involves a review of relevant literature, including scholarly manuscripts and articles that provide up-to-date insights, practical findings, and theoretical perspectives on the effects of AF and FB in the region.

## Infant Diets in the East African Region

This review focuses on a subset of countries in the East African region: Tanzania, Kenya, Uganda, Rwanda, and Burundi, chosen from a total of 13 countries, which includes Burundi, Comoros, Djibouti, Ethiopia, Eritrea, Kenya, Rwanda, Seychelles, Somalia, South Sudan, Sudan, Tanzania, and Uganda. The selection of these focus countries was guided by regional economic communities, as cited in the works of Nyakabwa-Atwoki (2020), Onyango *et al.* (2019), and Zewdie (2019). These particular countries share commonalities such as their participation in the Common Market, similarities in food production methods, and child feeding practices.<sup>26-28</sup> Notably, maize, which is susceptible to contamination by AFs

and FBs, represents a substantial portion of their food supply.<sup>29-31</sup> Furthermore, these five nations also share advantageous tropical conditions that facilitate

the production of mycotoxins, thereby posing significant chronic health risks (Refer to Table 1).

**Table 1: Common ingredients of CFs in the East African region**

Country	Food/ingredient	Age of infants (months)	Reference(s)
Kenya	Cereals, legumes, vegetables, fruits	6-23	117
	Grains, roots and tubers, milk, beans, eggs, chicken, goat and green grams	6-23	118
	Rice, groundnuts, cassava, maize, sorghum	4-6	64,119
	Maize	6-23	120
	Rice porridge	6-12	121
	Sweet potato puree	6-12	122
	Peanut butter	6-12	123
	Maize porridge	6-12	124
Rwanda	Maize thin porridge mixed with groundnut and soya flour	6-23	125
	Soybean	6-23	126
	Porridge - Maize and sorghum	5-30	127,128
	Porridge - maize, sorghum, soya beans, and wheat	6-12	129
	Maize	6-23	130
Burundi	Milk, rice, potatoes, lentils, meat, eggs	6-24	131
	Infant formula, glucose, water	6-24	132
Tanzania	Mashed bananas	6-12	133
	Mashed bananas, beef soup, or sour milk	6-24	134-137
	Maize, sorghum, pearl millet, rice, and groundnuts	6-12	138
	Cereals	6-24	139
	Finger millet porridge	6-12	140
	Cassava Porridge	6-12	141
Uganda	Maize, groundnuts, sorghum.	< 36	142,143
	Beans, mushrooms, ghee	6-23	144
	Porridge (maize, millet, pasted green vegetable leaves, beans, and groundnut flour)	6-23	145
	Plantain porridge	6-12	141

According to Kimanya *et al.* (2009), the consumption of maize by infants in rural Tanzania is relatively high. Results from this study show 89% consumed maize, ranging from 2.37 to 158 g/person/day at a mean of 43±28 g/person/day. This consumption is moderately high compared to the recommended daily maize intake as complementary food that should not exceed 20g for a child under one year of age.<sup>30</sup> In Uganda, maize is also the staple food for most of the population, including children.<sup>31</sup> Moreover, it is reported that 94.4% of common foods given to children aged 6–23 months in Kitui, Kenya, consisted of maize. Another study found

that the most frequently introduced first solid food to children in Wakiso District, Kenya is maize.<sup>32</sup> Similarly, maize is commonly used in complementary feeding in Uganda.<sup>33</sup>

Groundnuts are legumes commonly used to enrich cereals for CF.<sup>34</sup> Reports confirm the utilization of this ingredient in Uganda.<sup>35,36</sup> Nassanga *et al.* (2018) describe groundnut flour as typical CF food in the Acoli sub-region of Uganda. In Tanzania, maize is usually pre-blended with groundnuts to a composite porridge flour locally known as lishe.<sup>37</sup> In a study conducted in the Rutsiro district

in Northwest Rwanda, mothers reported feeding maize thin porridge mixed with groundnut.<sup>38</sup> Another study, which assessed the types of CF and child-feeding practices in the Dodoma region, Tanzania revealed that maize, sorghum, and finger millet were the commonly used cereals in children's diets.<sup>39</sup> Other legumes, including beans or bean soup, are given to babies as part of family meals and are occasionally used to improve the protein content of complementary porridge.<sup>40</sup> Bean soup has also been part of CF in Ugandan babies.<sup>32</sup> As a significant source of affordable protein and oil, soybeans have also been reported in Rwanda and Burundi.<sup>41</sup> Mashed bananas have been used in CF in a few areas, like the Kagera region of Tanzania, with slightly diverse food sources.<sup>42</sup> Likewise, mashed bananas, beef soup, or sour milk are reported to be used in the Northern and Southern Highlands of Tanzania.<sup>39,43,44</sup> Boiled and mashed bananas have been used in Uganda and some parts of Rwanda.<sup>38,45,46</sup> On the other hand, Irish potatoes have been mentioned in all these five countries.<sup>38,47-50</sup>

Fruits, green vegetables, and animal food products are limited, as mentioned.<sup>51</sup> Similarly, there has been minimal consumption of porridge made from sorghum and millet.<sup>52-55</sup> In general, sole or multiple cereal-based, sometimes enriched with legumes, nuts, or oily seeds such as groundnuts, are the

leading CF across the East African region. While some parts of these countries have access to relatively diverse foods, a majority rely on traditional staple cereals in which maize and groundnut account for the most significant proportion. However, these cereals are susceptible to AFs and FBs.

### The Presence of AFs and FBs in Commonly Complementary Foods within East Africa

Despite the known health effects of mycotoxins, reports on their contamination of food crops and the subsequent exposure of IYC are relatively limited in East and sub-Saharan Africa (Table 2). Some studies specifically sampled CF are reported here, While others focused on the primary ingredients used in CF, which are likely to be fed to children. For instance, Kamala *et al.*'s. (2016) assessment of maize samples from the northern highlands, south-western highlands, and eastern lowland zones of Tanzania, such as Kilosa, Hanang, and Rungwe revealed that 45% and 85% of the samples were contaminated with AFs and FBs, respectively, with 33% co-occurrence of both toxins. The contamination of CF and their ingredients tends to increase during storage.<sup>56-58</sup> For instance, in the Kongwa district of central Tanzania, an increase in mean levels of AFs was reported in stored maize from 13.12 µg/kg on day one to 14.75 µg/kg and 19.39 µg/kg at day 90 and 180, respectively.<sup>58</sup>

**Table 2: Contamination of complementary foods with AFs and FBs in the East African region**

Mycotoxin	Produce	Method(s)	Contamination range/mean -µg/kg	Maximum total limit (µg/kg)	Country	Reference(s)
Aflatoxins (µg/kg)	Shelled maize and groundnuts	ELISA	38.3 - 271	10	Tanzania	142
	Maize	TLC	2- 82	10	Kenya	148
	Groundnuts	ELISA	0 – 2688	10	Kenya	144
	Maize	ELISA	0-4800	10	Kenya	145
	Groundnuts		0-7500			
	Groundnuts	HPLC	1.1 – 428.55	10	Tanzania	151
	Blended flour		2.29 –18.97			
	Maize	HPLC	1.0-1081	10	Tanzania	6
	Maize	HPLC	0.1 – 269	10	Tanzania	152
	Maize	ELISA	0.7 – 88.6	10	Uganda	62
	Maize	ELISA	0 –34.5	10	Kenya	101
	Groundnuts	ELISA	1.63 –591.1	10	Kenya	153
Maize	ELISA	0-15.62	5 AFB <sub>1</sub>	Rwanda	146	

	Groundnuts	ELISA	0 – 126.6	10	Rwanda	67
	Groundnuts	ELISA	2.2 – 2,410	10	Burundi	68
	Maize	ELISA	2.5 –350	10	Burundi	68
	Maize	ELISA	12.7 – 123.5	10	Uganda	147
	Maize	ELISA	0.33–69.47	10	Tanzania	94
	Maize	HPLC	0.3 – 2,128	10	Tanzania	156
	Maize flour	HPLC	0.24-1.39	10	Tanzania	81
	Maize	ELISA	2 – 150	10	Rwanda	60
	Soybeans	ELISA	1-11.2	10	Rwanda	126
	maize, groundnuts, sorghum, pearl millet, rice	HPLC	0.27 – 317	10	Tanzania	100
	Milk, Maize, sorghum	ELISA	0 – 194.4	10	Kenya	148
Fumonisin (ppm)	Maize	ELISA	0 - 18 ,184	2	Tanzania	145
	Maize	ELISA	0–18,184	2	Tanzania	145
	Maize	HPLC	44–16203	2	Tanzania	6
	Maize	HPLC	49 – 18 273	2	Tanzania	152
	Maize	ELISA	0.27 –10	2	Uganda	63
	Maize	ELISA	15-2,918	2	Burundi	68
	Maize	ELISA	48–1224	2	Tanzania	94

TLC = Thin Layer Chromatography, ELISA = Enzyme-linked Immunosorbent Assays, HPLC = High-Performance Liquid Chromatography

A recent study assessed total AFs in stored maize, maize flour, and stiff porridge consumed in the Dodoma Region. Samples of corn ( $n = 52$ ), corn flour ( $n = 50$ ), and stiff porridge ( $n = 34$ ) from various schools in the Dodoma Region revealed that AFs could be found in 69.2%, 72%, and 29.4% of the samples of preserved corn, corn flour, and stiff porridge, respectively, at concentrations of 1.6-86.6  $\mu\text{g}/\text{kg}$ , 1.5-60.1  $\mu\text{g}/\text{kg}$ , and 1.8-55.1  $\mu\text{g}/\text{kg}$ .<sup>59</sup> Furthermore, 17 of the 52 maize samples showed AF concentrations above the maximum tolerated limit (MTL) of 10  $\mu\text{g}/\text{kg}$ , and around 69% of the samples had overall AF contamination.

The contamination of CF and their ingredients with AFs in Uganda is lower than in Tanzania. Research on AF levels in the market and household maize, common ingredients in CF in Kampala, established that 20% of the maize from six markets was contaminated with AFs ranging from 0.7 to 88.6  $\mu\text{g}/\text{kg}$  with a mean contamination level of  $7.6 \pm 2.3 \mu\text{g}/\text{kg}$ .<sup>60</sup> The same study in Uganda revealed widespread AF contamination in household samples, with 74% containing AFs, with a mean level of  $22.2 \pm 4.6 \mu\text{g}/\text{kg}$ . Notably, one sample reached a staggering 268  $\mu\text{g}/\text{kg}$ , exceeding the East African

region limit of 10  $\mu\text{g}/\text{kg}$  by over 26 times, although this contaminated ingredient was not specifically intended for CF. Furthermore, the occurrence of FBs appears heavily influenced by altitude, favouring *Fusarium* growth in high-altitude areas. A study comparing FB contamination in maize samples across Uganda three agroecological zones (high altitude, mid-altitude moist, and mid-altitude dry) underscored this trend. High-altitude areas exhibited significantly higher FB levels, ranging from a concerning 850 to 10,000  $\mu\text{g}/\text{kg}$  with an average of 4,930  $\mu\text{g}/\text{kg}$ , which is more than double the EAC limit of 2,000  $\mu\text{g}/\text{kg}$ . In contrast, the mid and high altitudes showed substantially lower concentrations, ranging from 0.47 to 8.3 and 0.85 to 10  $\mu\text{g}/\text{kg}$ , respectively.<sup>61</sup>

A study to ascertain the level of AF contamination in peanut butter and groundnuts in Nairobi and Nyanza, Kenya involved obtaining eighty-two samples comprising raw and roasted groundnuts and peanut butter from the market of these two areas. AF levels in all samples ranged from 0 to 2377.1  $\mu\text{g}/\text{kg}$ .<sup>62</sup> Another study by Menza *et al.* (2015), which assessed the contamination of groundnuts with AFs in Busia and Kisii Central districts in western Kenya revealed that 97.06% of the groundnut samples from

Busia were contaminated with AFs in the range of 0.1 to 268 µg/kg, while all groundnut samples from Kisii were contaminated with AFs in the range of 1.63 to 591.1 µg/kg. Although groundnuts are not CF, they are commonly used in East African countries as CF ingredients, and thus, they are likely to be consumed by infants and exposed to AFs.

Mirroring the situation in Uganda, maize samples from Rwandan markets, often used as ingredients, exhibit concerning levels of AF contamination. A study quantifying AFB<sub>1</sub> at two-time points in 684 maize flour samples in Kigali retail markets, intended for both family and CF, revealed substantial variations. In some markets, mean levels reached a concerning 24.7 + 23.7 µg/kg, with a maximum of 98.6 µg/kg. Conversely, other markets displayed lower mean levels of 8.0 + 5.6 µg/kg. A second analysis confirmed this trend, with mean levels ranging from 10.2 ± 8.4 µg/kg to 25.7 + 25.9 µg/kg and a maximum of 116.9 µg/kg.<sup>63,64</sup> Further investigation into CF ingredients (maize, groundnut, and cassava flour), across four Rwandan provinces revealed widespread mycotoxin contamination. Maize, a key ingredient, showed contamination in 89 of the samples, with AFs and FBs reaching 16.8 µg/kg and 48.1 µg/kg, respectively. Groundnut samples, another potential ingredient, exhibited a 100% contamination rate with AFs reaching 126.6 µg/kg. Notably, cassava flowers, while less prone to contamination, still showed AFs in 33% of samples, with concentrations reaching 2.7 µg/kg, while FBs were either non-detectable or present at low levels.<sup>65</sup>

Burundi, like other East African nations, exhibits a widespread presence of AFs and FBs in CF ingredients. A May – July 2016 market survey of 244 sorghum, maize, and groundnuts samples revealed that 218 (88.5%) were contaminated with AFs, ranging from 1.3 to 2,410 µg/kg levels and an average of 117 µg/kg.<sup>68</sup> Sorghum displayed the highest range (2.5-490 µg/kg), followed by maize (2.5-350 µg/kg) and groundnuts (2.2-2,410 µg/kg). Sorghum displayed the highest range (2.5 and 490 µg/kg), followed by maize (2.5 to 350 µg/kg), and groundnuts (2.2 to 2,410 µg/kg). Notably, these concentrations significantly exceed those documented in Tanzania, specifically in the Rombo district. There, maize and maize-based CF contained AFs in the range of 0.11 to 386 µg/kg.<sup>67</sup> Consistent with other East African countries, FBs were also

prevalent in Burundian CF ingredients, particularly in maize (15-2,918 µg/kg) and sorghum (3-374 µg/kg).<sup>66</sup> This widespread contamination of CF with AFs and FBs across the region, as evidenced by the reviewed literature, is likely driven by various factors, as discussed in this subsection.<sup>68</sup>

### **Factors Promoting AFs and FBs Contamination of Common CF within the EAC**

Numerous factors contribute to an elevated risk of AFs and FBs in the East African regions, encompassing climate variations, recurrent droughts, pest invasions, inappropriate farming and storage methods, socioeconomic status, limited awareness, detection methodologies, deficient regulatory oversight, and persistent food insecurity. Many food commodities in the EAC, including those used as CF, can be contaminated with mycotoxins at pre- or post-harvest.<sup>69-73</sup> Food contamination with mycotoxins in the EAC is primarily exacerbated by the tropical and sub-tropical climates that strongly favour the growth of *A. flavus*. For example, regions situated within the latitudinal range of 40°N to 40°S, including East Africa, are conducive to mold proliferation and elevate the potential for AF exposure.<sup>74</sup> This phenomenon occurs because elevated temperatures support the production of *A. flavus* conidia, their dispersion, and an increased kernel infection rate, all collectively contributing to the accumulation of AFs in various food products.<sup>75</sup> This predominantly occurs in lower elevations, which typically experience higher temperatures and humidity, in contrast to higher altitudes characterized by lower temperatures and humidity levels.<sup>73</sup>

Delayed harvesting is a common practice in different parts of the East Africa region that further exposes crops to fungal growth and contamination with AFs and FBs.<sup>76</sup> A previous study by Kaaya *et al.*<sup>77</sup> recorded a seven-time increase in AFs when maize harvesting was delayed for a month in Uganda. The duration of storage can also influence the contamination of primary ingredients of CF with AFs and FBs. For instance, a substantial correlation was established between the quantities of AFs detected in stored maize following extended periods in agroecological regions characterized by dry climates.<sup>78</sup> AFs can be generated within agricultural commodities intended for complementary feeding<sup>79, 80</sup> during storage. In Dodoma, Tanzania, Aron *et al.*;2017 and Ngoma *et al.*;2016 that various post-harvest practices



significantly associated with AFs in complimentary composite flours included storage of cereals for over 12 months. Besides, numerous small-scale farmers frequently subject maize and other cereal grains to suboptimal storage conditions for prolonged periods before their utilization or sale, thus inadvertently fostering an environment conducive to fungal proliferation and AF contamination.<sup>76,79</sup> Again, when crops are not appropriately dried or kept in poorly ventilated stores with high relative humidity, as has been established in peanuts in markets and farms in Uganda.<sup>77</sup> According to Villers,<sup>81</sup> aflatoxigenic fungi thrive when the relative humidity of food commodities in storage surpasses the critical level that supports fungal growth. This is because temperature and water activity significantly affect *Aspergillus* growth and gene expression for the biosynthesis of AFs.<sup>82</sup>

On occasion, cereals are gathered with increased moisture levels and subsequently dried to lower their moisture content prior to storage. This delayed drying process elevates the potential for mold growth and the formation of AFs and FBs. Similarly, most farmers are reported not to be sorting or dehulling cereals like maize meant for infant feeding, which increases the risk of contamination with mycotoxins like AFs and FBs. Ngoma *et al.*,<sup>2016</sup> for instance, established that a majority (81.3%) of 364 study respondents in some districts in central Tanzania needed to be dehulling the crops used in preparing CF for children aged between 6 and 23 months. Makori *et al.* (2019) say this is a common contributor to the risk of contamination and exposure to AFs.

Several foodstuffs that constitute primary ingredients for CF are often dried on bare ground, predisposing them to contamination with AFs and FBs. The study by<sup>76,79</sup> in the central parts of Tanzania also reported a positive association between drying of maize on bare ground and mycotoxin contamination, which was accelerated when harvested maize was dried without husks because then the maize grains are in direct contact with soil, which is a source of molds. Substandard storage practices are also often associated with the contamination of common ingredients of CF.

The contamination of CF with AFs after harvest is possible under conditions that promote the growth of aflatoxigenic fungi during various stages, including harvesting, storage, milling, and distribution at both

the market and household levels.<sup>83</sup> *Aspergillus flavus* exhibits relatively higher moisture needs compared to other types of fungi. Consequently, the risk of grain contamination increases when the seed moisture content is elevated. For example, the presence of AFs in harvested maize is affected by elevated temperatures and moisture levels, typically ranging from 12 to 40°C and 3-18%, respectively.<sup>84</sup> Building upon Coppock *et al.*;<sup>2018</sup> findings, a confluence of factors can exacerbate aflatoxin and fumonisin contamination in East African crops. Delayed harvests due to wet conditions, combined with sufficient heat for aflatoxigenic fungi growth, create a perfect storm for toxin production and contamination. Additionally, any damage incurred by kernels or nuts during harvesting, cleaning, and handling processes can compromise their integrity, thereby creating entry points for fungal invasion and subsequent contamination.<sup>83</sup> However, it is crucial to recognise that inadequate household handling and storage facilities often emerge as even more potent factors, warranting special attention. Section 5 discusses analytical techniques for detecting AFs and FBs in food in the East African region. However, despite the prevalent mycotoxin contamination of essential crops in this area, the ability to conduct thorough analyses faces limitations due to inadequate infrastructure, skillsets, and the absence of rapid detection tools capable of onsite toxin identification before the harvest, sale, purchase, or consumption of vulnerable crops.

#### **Analytical Methods for AFs and FBs in Foods in the EAC**

High-Performance Liquid Chromatograph (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA) are the standard analytical methods used for detecting and quantifying AFs and FBs in foods, including CF in the East African region. For instance, 72 recently used ELISA to quantify AFs in sorghum, millet, finger millet, groundnuts, composite flour (lishe), and FBs in maize, with comparable results. Kibwana *et al.*;<sup>2013</sup> also used ELISA to assess the levels of AFs in stored maize, maize flours, and stiff porridge in schools in Dodoma, Tanzania. Clavel and Brabet (2013) assessed groundnut samples from Busia and Homabay districts in Kenya using ELISA and established levels of AFs ranging from 0 – 2688 µg/kg and 0-7525 µg/kg, respectively. According to Mollay *et al.*,<sup>2022</sup> HPLC was used to detect AFs in maize, sorghum, pearl millet, rice, and groundnuts

in the samples collected in the Kongwa district of Dodoma region. Similarly, Makori *et al.* (2019) used HPLC to analyze AF in CF samples collected in Chamwino Dodoma.

Despite its established efficacy in detecting food aflatoxins (AFs), Enzyme-Linked Immunosorbent Assay (ELISA) faces inherent limitations for field-based applications. While popular among scientists<sup>3</sup> for its reliability and simplicity in sample preparation, ELISA's dependence on laboratory settings and highly skilled personnel renders it impractical for field testing. Due to its popularity, attempts have been made to improve the sensitivity of ELISA for detecting mycotoxins.<sup>6</sup> Recognising this, research has shifted towards integrating specific molecular recognition elements, like immunochemical, directly with portable transduction systems to facilitate rapid, non-laboratory mycotoxin detection. HPLC, on the other hand, serves as a robust and globally

accepted quantitative method for food analysis and mycotoxins detection. Its impressive sensitivity and versatility, allowing simultaneous detection of over 50 analytes at  $\mu\text{g}/\text{kg}$  levels, solidify its position as the industry standard for mycotoxin testing.<sup>88,89</sup> The integration of the Immunoaffinity column (IAC) into HPLC further enhances its effectiveness by minimizing its mycotoxin loss and eliminating interfering substances through specific antigen-antibody interactions.<sup>90</sup> This combined approach has proven particularly accurate and valuable for evaluating AFs in CF, enabling informed strategy development and status assessments.<sup>91,92</sup> However, HPLC's advantages come at a cost. The technique's demanding sample preparation steps, requiring laboratory supplies and organic solvents, can be laborious and expensive. Consequently, its application is limited to small sample sizes and well-funded studies.

**Table 3: Exposure of infants and young children to aflatoxins and fumonisins in the East African region**

Country	Food ingredient	Analytical method	Mycotoxin	Range of Exposure (ng/kg. bw/day)	Reference
Kenya	Maize	HPLC & ELISA	AFB1 and AFM1	0.011 - 0.49	149
Kenya	Maize	HPLC	Total AFs	2.14 - 411	150
Kenya - Busii	Groundnuts	ELISA	Total AFs	0 - 2688	119
Kenya -Homa Bay	Groundnuts	ELISA	Total AFs	0 - 7525	119
Rwanda	Maize		Total AFs	0 - 100.9	160
Tanzania	Groundnut flour	HPLC	AFB <sub>1</sub>	0.1 - 23,172.81	156
Tanzania	Maize	ELISA	Total AFs	0.14 - 120	94
Tanzania	Maize	ELISA	FBs	0.005 - 0.88	94
Tanzania	Maize, sorghum, pearl millet, rice, and groundnuts	HPLC	AFs	0.33 - 1168	161
Tanzania	Maize-based flour	HPLC	AFs	1 - 786	69
Burundi	Maize, groundnuts	ELISA	AFs	1.3 - 2,410	68
Kenya	Milk, maize, sorghum	ELISA	AFs	0 - 21.3	102
Uganda	Maize-based	ELISA	FBs	0.40 - 168	162

These methods were reviewed to present how the toxins are quantified in different countries for regional comparison. The suitability of a method depends on its safety, effectiveness, ease of use, affordability, speed, and ruggedness. Although progress has been made in the region toward detecting AFs and FBs

in ingredients used in CF, more efforts are needed to enhance the mycotoxin analysis specifically for CF and infant diets at all levels. Also, common methods have been discussed to connect with regulations enforcing food safety within the region. Mycotoxin regulation is necessary to reduce food



contamination.<sup>93</sup> (Table 4), Implementing regulations in low-resource settings with pronounced food insecurity, limited testing technologies, and facilities can be challenging.

### **Documentation of IYC Exposure to AFs and FBs in the East African Region**

Numerous research studies have illustrated the persistent dietary exposure of IYC infants and in EAC mycotoxins over extended periods. For instance, investigators have documented elevated AF exposure levels in Tanzania attributable to diets heavily reliant on maize-based foods.<sup>92-94</sup> In Rombo district located in Northern Tanzania, the estimated dietary intake of AFs and FBs in 143 infants was found to be 0.14 to 120 ng kg<sup>-1</sup> body weight (bw) per day and 0.005 to 0.88 µgkg<sup>-1</sup> bw per day, respectively.<sup>95</sup> Infants and young children are considered particularly susceptible to exposure to AFs and FBs due to their reduced body weight and accelerated metabolic rate.<sup>96</sup> Children in the East African region are exposed to AFs and FBs mainly through CF (Table 3). While IYC can be exposed to AFs through various means, including in utero transmission from the mother through the placenta,<sup>97</sup> breastfeeding,<sup>98</sup> or direct ingestion of contaminated foods,<sup>99,100</sup> it is essential to note that CF remains the primary source of AFs and FBs exposure among IYC. An upsurge in harm to children's health, particularly their immune and digestive systems, has been linked to staple CFs contaminated with AFs. It impairs their physical development, causing stunting, wasting, and underweight conditions. At the same time, it hinders their learning and development. It is crucial to grasp the extent of exposure, its impact, and methods for diminishing IYC's susceptibility to AFs and FBs in the region.

Makori *et al.* 2019 conducted a study in the Dodoma region of Tanzania, where they examined CF and exposure to AFB<sub>1</sub> among a group of 101 children aged 6-23 months. Their investigation unveiled a broad range of exposure levels from 0.1 to 23,172.81 ng/kg bw/day. Another study on 18 to 24-month-old children in Rombo District, Tanzania, reported exposure to AFs at 1 to 786 µg/kg bw/day and FBs at 0.38 to 8.87 µg/kg bw/day.<sup>67</sup> Moreover, a research investigation conducted in Kongwa, Tanzania, examined the exposure risk among children aged 6 to 12 months and documented a range of AFB<sub>1</sub>

exposure levels from 0.33 to 1168 ng/kg bw/day, with a median value of 23.08 ng/kg bw/day.<sup>101</sup>

On the other hand, a study done in Kisumu, Kenya, reported that the AF exposure of children aged 4 -6 months ranged from 4.43 to 110.4 ng/kg bw/day from the consumption of CF, including groundnuts, cassava, maize, and sorghum.<sup>102</sup> Another cross-section study in two low-income areas of Kenya revealed that 98% of the food samples collected tested positive for AFs, resulting in an average exposure of 21.3 µg/kg bw/day.<sup>103</sup> Likewise, one of the cohort studies in Mukono district, Uganda, found an association between maternal AFs exposure of median maternal AFB-Lys level 5.83 pg/mg albumin (range: 0.71–95.60 pg/mg albumin, interquartile range: 3.53–9.62 pg/mg albumin during pregnancy and adverse birth outcomes. The study reported adverse birth outcomes, notably lower birth weight and smaller head circumference.<sup>104</sup> In EAC, Tanzania reported high AF exposure compared to other EAC countries, although most said AF exposure exceeded the exposure of concern of 0.017 ng/kg bw/day.<sup>67</sup> Even with this wide exposure range, enforcing mycotoxins regulation in these countries is still challenging.

### **Mycotoxin Regulation and Mitigation Strategies In The East African Region**

In the East African region, the vulnerability of infants to AFs and FBs through the consumption of contaminated maize remains a pressing concern, despite the established regulations. This issue is exacerbated by the decentralized nature of small-scale maize farming, where regulatory bodies must navigate the challenges presented by widely dispersed farmers who produce and distribute maize to the broader population.<sup>105</sup> The regulatory mechanisms have also focused on post-harvest interventions and rarely on pre-harvest and harvesting conditions. However, such field practices and technologies can also be regulated to minimize the fungal colonization of crops.<sup>106,107</sup> Moreover, discrepancies in implementing regulations have been reported. For instance, in the EAC, no specific standards are set for infant diets, which increases the risks of child exposure to mycotoxins and their subsequent health effects.<sup>108</sup> Owing to the immense health impacts of AFs and FBs on IYC, stringent regulations for food intended for IYC and additional

mitigation strategies should be implemented to reduce dietary exposure to this vulnerable group.

Even though standards exist for food and food products for the general population, inspection for mycotoxin contamination is mainly performed on food products aimed at the export market. In contrast, locally traded foods, especially those meant for use as CF, are often not tested.<sup>109</sup> Unless awareness creation and demand for local clean food are highly prioritized among consumers, implementing the regulation for local foods will take a long time to realize. Given that food can be contaminated in the field during harvest, transportation, marketing, and storage 110 customized strategies to mitigate mycotoxin contamination throughout the value chain support adherence to the regulations. These strategies include hand sorting (before storage and use), drying maize on mat/raised platforms, proper sun drying, applying storage insecticides, and dehulling before milling.<sup>7</sup> The application of sorting as a common strategy in reducing fungal growth and toxin production in food products has also been documented in Burundi.<sup>66</sup> Other studies have shown that sorting maize may not necessarily reduce AFs but can significantly reduce FBs.<sup>111,112</sup> Some low-cost grain sorting technologies have recently been developed to reduce mycotoxin contamination in maize and groundnut.<sup>112</sup> All these have been observed to be weak in these countries, and suggestions to control the moisture, temperature, and humidity in harvested and stored grains are also a core mitigation strategy to reduce fungal growth and grain contamination have been made.<sup>57</sup>

Enhancing skills through training in suitable pre- and post-harvest practices has been identified as a viable approach for mitigating AFs and FBs at the household level.<sup>111</sup> Additionally, in a cluster randomized controlled trial conducted by Kamala *et al.*,<sup>2018</sup> it was demonstrated that manual sorting and dehulling led to a substantial reduction in AFs and FBs levels in maize, subsequently lowering the dietary exposure of Tanzanian infants. The same research also found that drying maize on elevated platforms, plastic sheets, or canvas surfaces effectively reduced AFs compared to drying maize directly on bare ground. Another mitigation study after the 2004 outbreak of AFs in Kenya, where the Ministry of Public Health and Sanitation focused on preventive strategies to reduce dietary

contamination of AFs in homegrown maize by changing the standard limits for total AFs from 20 to 10 µg/kg.<sup>113</sup> However, lowering acceptable limits without stringent measures to ensure clean food production and monitoring locally consumed foods remains challenging.

Generally, widespread poverty and hunger in the East African region are some of the barriers reported to hinder the enforcement of mycotoxin regulation and mitigation efforts.<sup>114,115</sup> The regulation and mitigation strategies for mycotoxins in the East African region are insufficient, and those in place are still inadequately implemented. Therefore, intensified efforts, including Hazard Analysis Critical Control Point (HACCP) in the production process and land preparation crop rotation, planting and intercropping, and the application of botanical extracts and fungal biocontrol agents considered the most critical pre-harvest practices for controlling mycotoxin production are urgent and necessary in this regard.<sup>116</sup>

### Conclusions and future direction

Compelling evidence from this review illuminates the widespread dietary contamination and exposure of IYC to AFs and FBs in the East African region. Despite notable advancements in mycotoxin research and the rollout of mitigation interventions, tangible evidence of a decline in this public health threat remains elusive. A multifaceted approach that integrates robust regulatory enforcement, community engagement, and frequent monitoring appears crucial in achieving this goal. This includes targeted interventions focused on improving food handling practices throughout the food chain, paired with frequent monitoring and awareness campaigns to cultivate a consistent demand for clean food within the community. This combined approach can effectively help reduce the problem. Similarly, enforcing mitigation strategies encompassing adopting correct pre- and post-harvest practices and appropriate handling of foods at the household level, can significantly contribute to addressing the issue. This holistic approach aims to reduce contamination and limit the exposure of IYC to AFs and FBs via CF in resource-constrained settings across the East African region.

### Conflict of Interest

The authors declare that there is no conflict of interest.

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**Authors Contribution**

RAK; participated in the framing of the idea, performed literature search and review, and drafted

the manuscript. NK and FN conceptualized and framed the focus of the manuscript, and critically reviewed multiple versions of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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