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Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control

Abdoulie Jallow[1,2,3] | **Huali Xie**[1,2,3] | **Xiaoqian Tang**[1,2,3] | **Zhang Qi**[1,2,3,4] | **Peiwu Li**[1,2,3,4]

[1] Oil Crops Research Institute, Chinese Academy of Agricultural Science (CAAS), Xudong, Wuhan, Hubei 430062, China

[2] Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, 2nd Xudong Road, Wuhan, Hubei 430062, China

[3] Key Laboratory of Detection for Mycotoxins, Ministry of Agriculture, Xudong, Wuhan, Hubei 430062, China

[4] Laboratory of Quality and Safety Risk Assessment for Oilseeds Products, Ministry of Agriculture, Xudong, Wuhan, Hubei 430062, China

Correspondence
Zhang Qi, Oil Crops Research Institute, Chinese Academy of Agricultural Science (CAAS), Xudong 2nd Rd, Wuhan, Hubei 430062, China.
Email: zhangqi01@caas.cn

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Abstract

Aflatoxins represent a global public health and economic concern as they are responsible for significant adverse health and economic issues affecting consumers and farmers worldwide. Produced by fungal species from the *Aspergillus* genus, aflatoxins are a toxic, mutagenic, and carcinogenic group of fungal metabolites that routinely contaminate food and agricultural products. Climate and diet are essential factors in the aflatoxin contamination of food and subsequent human exposure process. Countri
es with warmer climates and staple foods that are aflatoxin-susceptible shoulder a substantial portion of the global aflatoxins burden. Enactment of regulations, prevention of pre- and postharvest contamination, decontamination, and detoxification have been used to prevent human dietary exposure to aflatoxin. Exploiting their chemical and structural properties, means are devised to detect and quantify aflatoxin presence in foods. Herein, recent developments in several important aspects impacting aflatoxin contamination of the food supply, including: fungal producers of the toxin, occurrence in food, worldwide regulations, detection methods, preventive strategies, and removal and degradation methods were reviewed and presented. In conclusion, aflatoxin continues to be a major food safety problem, especially in developing countries where regulatory limits do not exist or are not adequately enforced. Finally, knowledge gaps and current challenges in each discussed aspect were identified, and new solutions were proposed.

KEYWORDS
aflatoxin, agricultural products, control, food safety, occurrence

1 | INTRODUCTION

Aflatoxins are a group of structurally related toxic, mutagenic, and carcinogenic mycotoxins that contaminate large numbers of food and agricultural products with a special affinity to cereals and nuts. Mycotoxins are chemical substances produced by certain species of fungi as secondary metabolites in the field and during the storage of agricultural products (Shephard, 2009). Among the many analogs and derivatives of aflatoxins that have been identified, the

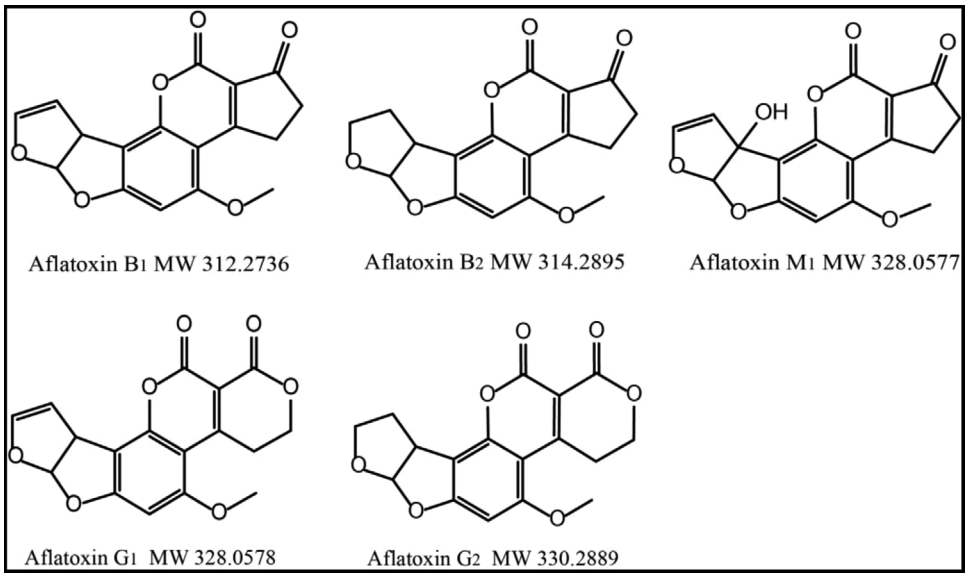

FIGURE 1 Chemical structures of class B1, B2, G1, G2, and M1 aflatoxin

B-series (aflatoxins B1 and B2), the G-series (aflatoxins G1 and G2), and M-series (aflatoxin M1; Figure 1) are of the most relevance from a food safety point of view (Afsah-Hejri et al., 2013).

Depending on environmental factors (temperature, humidity, and rainfall) and farm management practices (cropping, harvesting, and storage conditions), fungal proliferation and subsequent mycotoxin excretion could happen at any stage of the crop production chain (Waliyar et al., 2015). Plant immunocompromising factors such as drought stress, injury, pest infestation, and poor fertilization are also known enablers of aflatoxin production in agricultural products (Bhat & Vasanthi, 2003). Aflatoxins are ubiquitous and occur in various food crops, including cereals, nuts, dairy products, among other food and agricultural products. A recent review by Eskola et al. (2019) suggests that about 60% to 80% of the global food crops are contaminated with mycotoxins. This estimation pushed back the widely cited 25% estimation attributed to the Food and Agricultural Organization (FAO) of the United Nations. Nonetheless, these figures are staggering; a large proportion of the world's population is faced with the risks associated with exposure to aflatoxin and a host of other mycotoxins. A recent rise in global temperatures has also presented another scenario; aflatoxins are increasingly detected in some parts of Italy and South Europe in quantities not seen before (Moretti et al., 2019).

Biblical and other written records of human illness and death related to food spoilage suggest mycotoxins have haunted humans for an extended period, perhaps since the beginning of human engagement in crop production for food (Pitt & Miller, 2017). However, scientific research into aflatoxin did not start until 1960 when a sudden death of more than 100,000 young turkeys in English poultry farms due to an unknown condition termed "turkey X disease" caught the attention of scientists (Stoloff, 1976). Multidisciplinary scientific research attributed these deaths to feed contaminated with a toxin produced by the fungus *Aspergillus flavus*, thus the name "aflatoxin" (Nesbitt et al., 1962). Research resulted in a sufficient understanding of the toxin enabling the development of better analytical methods and a better understanding of its public health impact (Pitt & Miller, 2017).

Humans may be exposed to aflatoxins through the consumption of aflatoxin-contaminated foods or the ingestion of foods produced by animals previously exposed to aflatoxins (Leong et al., 2012). Chronic dietary exposure to aflatoxins poses severe health complications in humans and animals (Williams et al., 2004). Aflatoxin B1, due to its toxic, mutagenic, immunotoxic, teratogenic, and carcinogenic effect on humans and animals, is classified as a group 1 carcinogen in the International Agency for Research on Cancer (IARC) classification of carcinogenic substances (Ostry et al., 2017). As potent carcinogen, aflatoxin B1 may affect organs like the liver and kidneys (Alvarez et al., 2020; Li et al., 2018). It is also reported to suppress humans' immune systems, rendering them vulnerable to infectious diseases like HIV and AIDS (Jiang et al., 2008; Jolly et al., 2013). Prolonged exposure to aflatoxin has also been linked to congenital disabilities and stunting in children (Smith et al., 2015).

Additionally, acute aflatoxin exposure can be life-threatening. Exposure to high levels of aflatoxin within a short period is found to cause aflatoxicosis (Williams et al., 2004). Recently, multiple outbreaks of acute aflatoxin exposure have been reported, particularly from regions with tropical climates, such as Kenya and Tanzania (Awuor et al., 2017; Kamala et al., 2018).

The implications of aflatoxin contamination of agricultural products go beyond public health issues; it equally carries trade and economic ramifications for both developed and developing countries. Maize farmers in the United States incur an annual loss of $160 million due to aflatoxin-related issues (Wu, 2015). These figures are higher in developing countries, especially sub-Saharan Africa, where losses amount to $450 million, representing 38% of the global agricultural losses due to aflatoxin (Gbashi et al., 2018).

Furthermore, aflatoxin is responsible for a significant decline in agricultural trade between developed and developing countries (Wu, 2015). Attempts to quantify the health and economic burden of aflatoxin contamination of food crops have been made by Liu and Wu (2010) at a global scale, and country and regional studies are published by Matumba et al. (2019).

Different mitigation and control measures are being applied to prevent or minimize human and animal exposure to aflatoxin. These include regulation enactment (FAO, 2004), prevention of pre- and postharvest contamination of agricultural products, or their reduction to acceptable levels in already contaminated products through removal, degradation, or decontamination. Basic measures such as Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) as aflatoxin preventive measures have proven effective when combined with proper postharvest handling practices (Hell & Mutegi, 2011). Scientific advances have also permitted the use of sophisticated biological, chemical, and physical measures for the prevention and decontamination of already contaminated agricultural products (Lizárraga-Paulín et al., 2013).

Since its discovery, aflatoxin has been extensively studied, creating a good body of knowledge. This review compiles the most significant recent developments in the research and understanding of aflatoxin from a scientific perspective, from fungal producers of aflatoxin, agricultural products, and food contaminated by aflatoxin, worldwide regulations, current detection, prevention, removal, to degradation methods. The review also identified research gaps, discuss futures challenges, and propose new research efforts to control aflatoxin contamination of food.

2 | FUNGAL PRODUCERS OF AFLATOXIN

Aflatoxin production and contamination of crops is a long biochemical process that initiates with the *Aspergillus* fungi producers of aflatoxin's invasion and subsequent toxin production in infected crops (Abrar et al., 2013). The biosynthesis of aflatoxin consists of a sequence of about 13 enzymatic reactions starting with a fatty acid synthase-hexanoate. About 30 genes are involved in the fungal production of aflatoxins (Yu, 2012). Fungal invasion, growth, and aflatoxin production in crops is principally determined by environmental factors, the type of crops, and other ecological make-up of an environment (Negash, 2018). Not all *Aspergillus* species produce aflatoxin, and not all species invade all types of crops. Therefore, the levels and severity of aflatoxin contamination of agricultural products are, to a certain extent, determined by the fungal ecology of the production field (Cotty & Mellon, 2006). A considerable amount of research has been done to identify those fungal spices capable of excreting aflatoxin. Unfortunately, certain species have been wrongly identified and assigned as aflatoxin producers (Sohrabi & Taghizadeh, 2018). Although modern molecular tools have remedied this to a large extend, there is still a considerable amount of details to learn and elucidate as to why certain members of the *Aspergillus* genera produce aflatoxin. Members of the *Aspergillus* genera are genetically diverse (Kjærbølling et al., 2020). Studies on the *Aspergillus* secondary metabolism gene clusters revealed that the degree of similarity between clusters from different species could determine the potential similarities in the metabolites that they excrete. Clusters with 90% to 100% similarity normally code for the same secondary metabolite (De Vries et al., 2017).

As detailed in (Table 1), currently available literature indicates that aflatoxins are excreted by around 24 species of the *Aspergillus* genus belonging to three sections: *Flavi, Nidulantes*, and *Ochraceorosei* (Varga et al., 2015):

2.1 | Section *Flavi*

This section includes 33 species, the majority of which are toxigenic (produce aflatoxin). Prominent toxigenic members of the section are *A. flavus* and *A. parasiticus*. It was generally accepted that *A. flavus* only produces B-series aflatoxins; however, a recent study has reported Korean strains that excrete G-type aflatoxins (Frisvad et al., 2019). *A. flavus* is a ubiquitous soil-borne fungus that resides on nutrient-rich sources like organic remains, grains, and other food sources (Klich, 2002, 2007). Its optimal growth conditions are temperatures between 28 and 37 °C with the ability to survive 12° above or below that. *A. flavus* is not host-specific; it invades different types of food crops (Makhlouf et al., 2019).

A. parasiticus, on the other hand, is more host-specific with a high affinity for peanuts (Kumar et al., 2017). Almost all known strains of *A. parasicticus* are toxigenic and produce other metabolites, including kojic acid and

TABLE 1 Fungal producers of aflatoxin

Section	Species	Types of toxins produced	Crops and foods it infects	References
Section *Flavi*	*A. flavus*	B1, B2, G1,G1	Nuts, cereals, and several other commodities	Frisvad et al. (2005)
	A. novoparasiticus	B1, B2, G1, G1	Maize	Viaro et al. (2017)
	A. mottae	B1, B2, G1, G2	Cereals	Moral et al. (2020)
	A. parasiticus	B1, B2, G1, G2	Peanut, maize	Frisvad et al. (2019)
	A. nomius,	B1, B2, G1, G2	Wheat and other substrates	Frisvad et al. (2005)
	A. sergii	B1, B2, G1, G2	Cereals, oilseeds	(Benkerroum, 2020)
	A. pseudotamarii	B1, B2	Cereals	Yoko et al. (2001)
	A. pseudocaelatus	B1, B2, G1, G2	Maize	Viaro et al. (2017)
	A. transmontanensis	B1, B2, G1, G2	Cereals	Benkerroum (2020)
	A. luteovirescens (formerly *bombycis*)	B1, B2, G1, G2	Cereals	Frisvad et al. (2019)
	A. parvisclerotigenus	B1, B2, G1, G2	Peanut	Frisvad et al. (2019)
	A. minisclerotigenes,	B1, B2, G1, G2	Peanut	Moral et al. (2020)
	A. arachidicola	B1, B2, G1, G2	Maize, Arachis glabrata	Viaro et al. (2017)
	A. austwickii	B1, B2, G1, G2,	Cereals	Moral et al. (2020)
	A. aflatoxiformans	B1, B2, G1, G2	Cereals	Moral et al. (2020)
	A. pipericola	B1, B2, G1, G2	Cereals	Benkerroum (2020)
	A. cerealis	B1, B2, G1, G2	Cereals	Benkerroum (2020)
	A. Togoensis	B1, B2	cereals	Benkerroum (2020)
Section *Nidulante*	*A. astellatus*	B1	Cereals and other substrates	Benkerroum (2020)
	A. miraensis	B1	Cereals	Benkerroum (2020)
	A. olivicola	B1	Cereals	Benkerroum (2020)
	A. venezuelensis	B1	Cereals	Benkerroum (2020)
Section *Ochraceorosei*	*A. rambellii*.	B1	Cereals	Varga et al. (2009)
	A. ochraceoroseus	B1	Cereals	Varga et al. (2009)

aspergillic acid (Al-Hmoud et al., 2012). It produces both B- and G-series of aflatoxin (Martins et al., 2017). *A. toxicarius*, another member of the section and closely related to *A. parasiticus*, produces both B- and G-type aflatoxins (Murakami, 1971; Pildain et al., 2008). *A. pseudotamarii* and *A. togoensis* are other species of the section that only produce aflatoxins B1 and B2 (Pildain et al., 2008) while there are 14 different species that both produce aflatoxins B1, B2, G1, and G2 (Frisvad et al., 2019).

2.2 | Section *Nidulante*

This section consists of few toxigenic species: *A. astellatus*, *A. olivicola*, *A. venezuelensis*, and *A. miraensis* (Frisvad et al., 2004; Frisvad & Samson, 2004; Zalar et al., 2008). Members of this section also produce other metabolites apart from aflatoxin (Chen et al., 2016). For instance, *A. astellatus* produces aflatoxin B1, arugosins, asperthecin, shamixanthone, sterigmatocystin, terrain, and variecoxanthones (Frisvad et al., 2004), while *A. venezuelensis* produces aflatoxin B1, sterigmatocystin, terrein, and compounds with chromophores of the shamixanthone, emerin, and desertorin type (Frisvad & Samson, 2004). Another toxigenic species, *A. olivicola* produces B1, sterigmatocystin, and terrain (Zalar et al., 2008).

These three species require different growth conditions. *A. olivicola* grows well at 37 °C in contrast *to A. astellatus* and *A. venezuelensis* that have shown little to zero growth in the same conditions. Members of this section are ubiquitous and are known to play an active participatory role in decomposition processes (Chen et al., 2016; Domsch et al., 2007). They are mostly found in indoor environments. Members of the section are known to be infectious to humans (Henriet et al., 2012).

2.3 | Section *Ochraceorosei*

Established by Frisvad et al. (2005), the section comprises two toxigenic species: *A. ochraceoroseus* and *A. rambellii* excreting aflatoxin B1 and sterigmatocystin with 3-*O*-methylsterigmatocystin reported. The ability to produce aflatoxin and sterigmatocystin simultaneously is a feature unique to members of this section (Varga et al., 2009). Based on physiological and morphological resemblance, *A. ochraceoroseus* was initially placed under *Aspergillus* section *Circumdati* however, the characterization and phylogenetic analyses based on DNA sequence analysis of the aflatoxin biosynthetic genes of *A. ochraceoroseus* and aspergilli from sections *Circumdati, Flavi, Nidulantes*, and *Versicolores* revealed that the taxonomic status of *A. ochraceoroseus* was closer to the subgenus *Nidulantes* than to *Circumdati* (Cary et al., 2005). Isolated in Ivory Coast, members of this section include species incapable of growing at 37 °C, producing yellow ellipsoidal conidia, biseriate conidial heads and long, smooth conidiophore stipes (Cary et al., 2009). Compared to other toxigenic species, *A. rambellii* is more productive in producing aflatoxin B1 than any other species, while *A. ochraceoroseus* produce more aflatoxin B1 than *E. venezuelensis* and *E. astellata*, but less than members of section *Flavi* (Frisvad et al., 2005).

3 | AGRICULTURAL PRODUCTS AND FOODS CONTAMINATED BY AFLATOXINS

Mycotoxigenic molds are widespread throughout the world; thus, mycotoxins, including aflatoxin, contaminate a variety of crops and foods worldwide, especially those grown in tropical regions. As mentioned earlier, the level of aflatoxin contamination is determined by a myriad of factors, including crop types and environmental factors (Reddy et al., 2010). Although aflatoxin's occurrence is widespread and affects many food crops, certain crops are more susceptible than others.

Crop susceptibility to fungus invasion and subsequent toxin production is determined by a combination of environmental and crop intrinsic factors, including nutritional content, moisture content, pH, among others (Smith et al., 2016). As reviewed and outlined by Tai et al. (2020), the role of environmental factors on crop susceptibility to aflatoxin contamination is adequately understood. However, it is essential to note that these factors do not work in isolation; they interact with plant intrinsic features to enable fungal invasion and toxin production. Nutritional composition plays an important role in crop susceptibility to aflatoxin contamination. For instance, Liu et al. (2016) observed low levels *of A. flavus* invasion and aflatoxin production in defatted peanut, soybean, corn, wheat, corn germ, and corn endosperm substrates, but aflatoxin levels significantly increased when corn-oil was added to these same substrates. They also observed that low concentrations of soluble sugars (stachyose, raffinose, sucrose, fructose, maltose, and glucose) have no effects on aflatoxin B1 production by *A. flavus*. However, when these concentrations were increased to 3% and 6%, aflatoxin B1 production was significantly enhanced suggesting a positive correlation. In another study, Rajasekaran et al. (2017) observed that total lipid content positively influences the formation of aflatoxin B1 in cotton seeds during growth. Additionally, Casquete et al. (2017) demonstrated that substrate's pH, temperature, and water activity significantly influence growth and aflatoxin production by *A. flavus* in cheese. Moreover, Majeed et al. (2017), in their work, revealed that tocopherols content negatively correlates with aflatoxin formation in maize. Furthermore, Singh and Sinha (2013) demonstrated a positive correlation between aflatoxin B1

formation in paddy rice and total starch and amylopectin content.

The influence of water activity (a_w) in the accumulation of aflatoxin in agricultural products has been studied and reported on numerous occasions (Hassan & Aziz, 1998; Mannaa & Kim, 2017; Sahar et al., 2015; Trenk & Hartman, 1970). Generally, low water activity is required to minimize postharvest accumulation of aflatoxin in cereals. According to a study by Lahouar et al. (2016), aflatoxin B1 occurrence in stored sorghum could be prevented if grain water activity is maintained at ≤0.9. Equally, a water activity of 0.83 has been reported to prevent aflatoxin accumulation in peanuts (Dorner, 2008).

As previously mentioned, these factors are interdependent and interact with each other to enable aflatoxin production by the fungal producers of the toxin. Ribeiro et al. (2006) studied the influence of water activity, temperature, and incubation time on mycotoxins production on barley rootlets. They observed that at 0.95a_w and 25 °C, *A. flavus* produced more aflatoxin B1 than other levels during 7, 14, and 21 days of incubation, however, at 0.80a_w and 25 °C, aflatoxin B1 accumulation reaches maximum levels in 14 days of incubation. The authors further noted that an increase in a_w levels reduced the aflatoxin B1 production at 30 °C. Therefore, aflatoxin occurrence in crops is determined by the interaction between environmental and crop intrinsic factors.

Levels of crop contamination will, to a large extent, determine the levels of human exposure to aflatoxin. For instance, several surveys in Africa (Table 2) report high levels of contamination in staple crops (Gnonlonfin et al., 2013); this is consistent with the observed high levels of human dietary exposure (Table 3) in these countries.

3.1 | Occurrence of aflatoxins in cereals and cereal products

Cereals are the staple foods for a large portion of the world's population (Awika, 2011). Wheat provides up to 14.1% and 24.3% of the total calorie intake in America and Asia, respectively, while rice alone provides up to 28.5% of total calorie intake in Asia (Andrade & Caldas, 2015). Africans get 30% of their daily energy from wheat and maize (Andrade & Caldas, 2015). Cereals also form a large proportion of infant formulas worldwide (Nicklas et al., 2020). Additionally, cereals also make a significant proportion of animal feed in all parts of the world (Alvarado et al., 2017). Unfortunately, cereals and cereal-based products are prone to aflatoxin contamination.

Due to its worldwide production and consumption, maize (*Zea mays L*) is one of the primary human-exposure vehicles to aflatoxin. Several aflatoxicosis outbreaks originate from contaminated maize (Muthomi et al., 2009). For this reason, maize-related dietary data forms a vital component of many aflatoxin risk and exposure assessments. Maize-induced human and animal aflatoxin-exposure remains a significant food safety concern, as maize is a staple food in regions where climatic conditions are favorable for fungal growth and aflatoxin production. There are multiple reports documenting aflatoxin contamination of maize and maize-based products from almost all parts of the world (Lee & Ryu, 2017).

Aflatoxin occurrence in maize starts in the field where the kernels are infected with the fungi producers of aflatoxin and continue to accumulate as the products progress along the value chain. For instance, in a study to analyze aflatoxin occurrence in the maize supply chain in Congo, Kamika, and Tekere (2016) observed that aflatoxin incidence rate increased as the maize progressed through the value chain, from 32% during preharvest to 100% at retail level of the 52 samples. They observed that aflatoxin levels also increased from 3.1 μg/kg to 300 times higher than the maximum limit of 10 μg/kg for total aflatoxin set by Codex Alimentarius. In a similar study Liverpool-Tasie et al. (2019) investigated the co-occurrence of aflatoxin and fuminisin in the maize value-chain in Southwest Nigeria. Of 140 samples analyzed, 52% were contaminated with aflatoxin at levels beyond the Nigerian Regulatory limit. Surveys in Burundi, Togo, and Kenya (Hanvi et al., 2019; Nabwire et al., 2020; Udomkun et al., 2018) reported similar contamination levels. As detailed in Table 2, these high incidence rates and levels are not uncommon in sub-Saharan Africa, where one of the most severe mycotoxin-poisoning incidents in the last decade occurred (Chemining'wa et al., 2009). It is not a coincidence that the highest aflatoxin-exposure levels are reported (Table 3) from this part of the world.

Several studies in Latin America have also reported maize contamination with aflatoxin. In a survey conducted between 2003 and 2015 in Costa Rica, 1285 maize and maize-based product samples (intended for human food and animal feed) were collected and screened for aflatoxin contamination. Of these samples, the highest levels of aflatoxin were detected in maize (38.6%) and maize products (27.8%; Granados-Chinchilla et al., 2017). In another survey conducted in Mexico, where maize is a staple crop, 171 samples of maize products were analyzed for aflatoxin B1, 18% and 26% of the samples exceeded the Mexican and EU regulatory limits, respectively (Zuki-Orozco et al., 2018). Previous studies of similar products in different parts of Mexico also reported aflatoxin contamination levels beyond the Mexican regulatory limit of 12 ug/kg (Castillo-Urueta et al., 2011; Espinosa et al., 1995). In Brazil, of 148 maize samples screened for mycotoxin contamination; aflatoxins B1 and G1 were detected in 25.6% (38) and 7.4% (11) of the

TABLE 2 Recent surveys of food and agricultural products contaminated with aflatoxin

Food matrices	Class of aflatoxin	Incidence rate % (sample size)	Detection range	Country	References
Maize	AFTotal	37.7 (326)	<LOD to 341 μg/kg	Ghana	Agbetiameh et al. (2018)
Maize	AFB1, AFB2	24.6 (61)	0.02 to 0.19 μg/kg	Egypt	Abdallah et al. (2019)
Maize	AFTotal	25.8 (256)	0 to 3760 μg/kg	Uganda	Sserumaga et al. (2020)
Maize	AFTotal	100 (150)	20 to 91.04 μg/kg	Ethiopia	Chauhan et al. (2016)
Maize	AFTotal	88.4 (112)	0 to 3000 μg/kg	Niger and Benin	Bakoye et al. (2017)
Maize	AFB1	76 (70)	75.9 μg/kg	Togo	Hanvi et al. (2020)
Maize	AFB1, AFG1	26, 11 (148)	0.5 to 49.9 μg/kg	Brazil	Oliveira et al. (2017)
Maize	AFTotal	40 (270)	–	Argentina	Castellari et al. (2015)
Maize	AFB1, AFB2, AFG1	15, 15, 5 (20)	1.9 to 458.2 μg/kg	Colombia	Diaz et al. (2015)
Maize	AFTotal	64.6 (82)	1 to 17 μg/kg	Peru	Coloma et al. (2019)
Maize	AFTotal	75 (140)	–	Italy	Camardo Leggieri et al. (2015)
Maize	AFTotal	48.2 (56)	LOD to 9.14 μg/kg	Serbia	Torović (2018)
Maize	AFTotal	4 (1055)	7.96 to 163.62 μg/kg	Turkey	Artik et al. (2016)
Maize	AFB1	2.3 (44)	0 to 148.4 μg/kg	China	Xing et al. (2017)
Maize	AFTotal	50 (72)	0 to 40 μg/kg	Pakistan	Manzoor et al. (2018)
Maize	AFB1, AFB2	1 (507)	5.2 μg/kg	South Korea	Kim et al. (2017)
Maize	AFB1	33.71 (2370)	LOD to 34.8 μg/kg	Vietnam	Lee et al. (2017)
Rice	AFB1	100 (40)	0.29 to 2.92 μg/kg	Iran	(Eslami et al., 2015)
Rice	AFB1	73.3 (2047)	1.17 to 6.91 μg/kg	Pakistan	Asghar et al. (2016)
Rice	AFTotal	2.3 (87)	21.581 to 22.989 μg/kg	India	Mukherjee et al. (2019)
Rice	AFB1	12.5 (24)	100 to 200 μg/kg	Egypt	Moharram et al. (2019)
Rice	AFTotal	36.9 (38)	00 to 20.2 μg/kg	Nigeria	Rofiat et al. (2015)
Sorghum	AFB1, AFB2, AFG1, AFG2	10.81, 5.41, 18.92, 32.43 (37)	–	Kenya	Kange et al. (2015)
Sorghum malt	AFB1, AFB2, AFG1	44, 9, 17 (45)	0.61 to 28.3, 0.14 to 2.35, 0.39 to 6.95 μg/kg	Namibia	Nafuka et al. (2019)
Sorghum	AFB1	100 (90)	<LOD to 33.10 μg/kg	Ethiopia	Taye et al. (2016)
Sorghum	AFTotal	28.6 (146)	0.96 to 21.74 μg/kg),	Nigeria	Apeh et al. (2016)
Sorghum malt	AFB1	25 (50)	46.33 to 254.73 μg/kg	Burkina Faso	Bationo et al. (2015)
Sorghum	AFB1	0.7 (275)	1 to 14 μg/kg	Uruguay	Del Palacio et al. (2016)
Sorghum	AFB1	71.42 (15)	0.005 to 0.02 μg/kg	India	Jayashree and Wesely (2019)

(Continues)

TABLE 2 (Continued)

Food matrices	Class of aflatoxin	Incidence rate % (sample size)	Detection range	Country	References
Wheat and wheat crackers	AFB1	5.6 (178)	0.03 to 0.12 μg/kg	China	Zhao et al. (2018)
Wheat flour	AFTotal	80 (180)	0.01 to 0.5 μg/kg	Iran	Jahanbakhsh et al. (2019)
Wheat	AFB1	33.33 (36)	<LOD to 49.79 μg/kg	Egypt	Hathout et al. (2020)
Wheat	AFTotal	2 (141)	0.21 to 0.44 μg/kg	Turkey	Turksoy and Kabak (2020)
Wheat (pizza dough)	AFB1, AFB2	23, 32 (60)	1.03 to 9.50 μg/kg, 0.34 to 0.67 μg/kg	Spain	Quiles et al. (2016)
Pearl Millet	AFB1	8.6 (76 commercial samples)36.6 (144 farm samples)	Max. 117 μg/kgMax. 1046 μg/kg	Tunisia	Houissa et al. (2019)
Pearl Millet	AFB1	64 (205)	<1.0 to 1658.2 μg/kg	Kenya	Sirma et al. (2016)
Peanuts	AFB1	80 (20)	LOD to 21.34 μg/kg	Pakistan	Masood et al. (2015)
Peanuts	AFTotal	0.15 (2494)	0.06 to 1602.5 μg/kg	China	Wu et al. (2016)
Peanuts	AFTotal	25 (1089)	LOD to 432.0 μg/kg	Taiwan	Lien et al. (2019)
Peanuts	AFTotal	10 (119)	0.3 to 100 μg/kg	Brazil	Martins et al. (2017)
Soybean paste	AFTotal	24.4 (45)	0.88 to 16.17 μg/kg	Korea	Jeong et al. (2019)
Peanut	AFTotal	25 (8)	186.6 to 375.1 μg/kg	Haiti	Aristil et al. (2020)
Peanut and Peanut cake	AFTotal	32 (160 peanut)68 (50 peanut cake)	<LOD to 2368 μg/kg < 20 to 158 μg/kg	Ethiopia	Mohammed et al. (2016)
Peanut	AFB1	57 (49)	LOD to 193 μg/kg	Algeria	Ait Mimoune et al. (2018)
	AFTotal	39 (84)	LOD to 2076 μg/kg	Nigeria	Oyedele et al. (2017)
Peanut	AFTotal	84 (102)	0.2 to 2177.2 μg/kg	Turkey	Lavkor et al. (2019)
Walnut	AFB1	7.5 (40)	0 to 8.2 μg/kg	Iran	Habibipour et al. (2016)
Hazelnuts and dried figs	AFTotal	6.5 (170 hazelnuts)12.3 (130 dried figs)	0.09 to 11.3 μg/kg0.1 to 28.2 μg/kg	Turkey	Kabak (2016)
Cashew nuts	AFTotal	42 (12)	0 to 122.35 μg/kg	Brazil	Kujbida et al. (2019)
Roasted cashew nuts	AFTotal	100 (27)	0.1 to 6.8 μg/kg	Nigeria	Adetunji et al. (2018)
Sesame seeds	AFB1	77.6 (30)	LOD to 14.49 μg/kg	Greece	Kollia et al. (2016)

(Continues)

TABLE 2 (Continued)

Food matrices	Class of aflatoxin	Incidence rate % (sample size)	Detection range	Country	References
Milk and milk products	AFM1	100 (678 raw milk) 100 (438 heat treated milk)100 (322 milk products)	≤0.025 to 1 μg/kg	Serbia	Tomašević et al. (2015)
Raw milk	AFM1	42.4 (3635)	<0.0066 to 0.4081 μg/kg	Macedonia	Dimitrieska-Stojković et al. (2016)
Raw and UHT milk[a]	AFM1	2.8 (826 raw milk)2.6 (69 UHT milk)	0.005 to 0.05 μg/L0.005 to 0.050 μg/L	Kosovo	Rama et al. (2016)
Raw milk	AFM1	12.3 (416 cow milk)7.2 (388 buffalo milk)	0.004 to 0.052 μg/kg0.004 to 0.031 μg/kg	Italy	De Roma et al. (2017)
Milk and milk products[a]	AFM1	35.8 (53)	0.0035 to 0.1005 μg/L	Malaysia	Nadira et al. (2017)
Raw milk[a]	AFM1	4.64 (1207)	LOD to 0.06 μg/L	China	Li, Min, et al. (2018)
Raw milk[a]	AFM1	70 (960)	0.3 to 1.0 μg/L	Pakistan	Akbar et al. (2019)
Breast milk[a]	AFM1	89 (112)	0.00301 to 0.03424 μg/L	Mexico	Cantú-Cornelio et al. (2016)
Milk and cheese[a]	AFM1	95.7 (70 milk)37.2 (70cheese)	0.019 to 0.629 μg/L0.031 to 0.276 μg/L	Costa Rica	Chavarría et al. (2015)
Raw milk	AFM1	100 (96)	0.0154 to 4.563 μg/kg	Kenya	Kuboka et al. (2019)
Infant formula[a]	AFM1	20 (55)	0.040 to 0.450 μg/L	Mexico	Quevedo-Garza et al. (2020)
Infant formula[a]	AFM1	88 (84)	LOD to 0.0481 μg/L	Lebanon	Elaridi et al. (2019)
Infant formula	AFB1	83.9 (248)	0 to 672.9 μg/kg	Burkina Faso	Ware et al. (2017)
Spices	AFTotal	19 (94)8 (38)	2.2 to 1118.3 μg/kg	Lebanon	El Darra et al. (2019)
Spices	AFTotal	85.4 (55 red chilli)76 (42 black pepper)	0 to 219.6 μg/kg0 to 219.6 μg/kg	India	Jeswal and Kumar (2015b)
Spices	AFTotal	85 (34)	0.01 to 9.34 μg/kg	Malaysia	Ali et al. (2015)
Coffee beans	AFTotal	100 (25 roasted)100 (25 green)	14.255 to 23.231 μg/kg 14.694 to 27.176 μg/kg	Yemen	Humaid et al. (2019)
Tea	AFTotal	78.3 (94)	0.11 to 16.17 μg/kg	Pakistan	Ismail et al. (2020)
Tea	AFTotal	58.9 (1290	1.2 to 116.2 μg/kg	Morocco	Mannani et al. (2020)
Cocoa beans	AFTotal	16.3 (123)	0.35 to 30 μg/kg	Brazil	Pires et al. (2019)

Abbreviations: AFB1, aflatoxin B1; AFB2, aflatoxin B2; AFG1, aflatoxin G1; AFG2, aflatoxin G2; AFM1, aflatoxin M1; AFTotal, total aflatoxins (AFB1, AFB2, AFG1, AFG2).
[a]Liquid products.

TABLE 3 Recent aflatoxin exposure assessments

Country	Population segment	Products considered	Class of toxin assessed	Observed levels	References
Bangladesh	General	Human urine	AFM1	31 to 348 pg/mL	Ali et al. (2016)
Thailand	General	Rice	AFB1	0.12 to 0.80 μg/kg/bw/day	Panrapee et al. (2016)
China	General	Different food matrices	AFB1	0.48 to 0.94 ng/kg bw/day	Zhang et al. (2020)
Nepal	Children	Plasma	AFB1-Lys	3.62 pg/mg albumin	Mitchell et al. (2017)
Ethiopia	Children	Human urine	AFTotal	LOD to 0.070 ng/mL	Ayelign et al. (2017)
Nigeria	Children	Plasma	AFB1	0.2 to 59.2 pg/mg	Mcmillan et al. (2018)
Senegal	Adults	Plasma	AFB1	5.5 to 588.2 pg/mg albumin	Watson et al. (2015)
Kenya	Children	Different food matrices	AFTotal	21.3 ng/kg bodyweight per day	Kiarie et al. (2016)
Tanzania	General population	Plasma	AFB1-Lys	5.1 pg/mg albumin (mean)	Chen et al. (2018)
Uganda	General	Serum	AFB-Lys	0.40 to 168 pg/mg albumin	Kang et al. (2015)
Italy	General	Milk	AFM1	0.025 to 0.328 ng/kg(bw)/day	Serraino et al. (2019)
Portugal	children	Breakfast cereals	AFTotal	0.012 ng/kg bw/day	Assuncao et al. (2015)
Spain	Children	cereals	AFB1	0.17 to 0.37 ng/kg bw/day	Herrera et al. (2019)
Serbia	General	Milk and dairy	AFM1	62 to 0.074 ng/kg bw/day	Djekic et al. (2020)
Chile	General	Total diet	AfB1	0.005 ng/kg bw/day	Foerster et al. (2020)
Brazil	General	Peanut	AFTotal	1.28 μg/kg bw/day	Santos et al. (2018)
Guatemala	General	Serum	AFB1-alb	0.2 to 814.8 pg/mg albumin	Smith et al. (2017)
Jordan	Children	Infant formula	AFM1	1.557 to 1.551 ng/kg bw/day	Awaisheh et al. (2019)

Abbreviations: AFB1, aflatoxin B1; AFB1-alb, aflatoxin B1-albumin; AFB1-Lys, aflatoxin B1-lysine; AFM1, aflatoxin M1; AFTotal, total aflatoxins (B1 + B2 + G1 + G2).

samples, respectively. Of these positive samples, 1% was beyond the Brazilian regulatory limits of 20 ug/kg (Oliveira et al., 2017). Similar levels have been previously reported in Brazil (Amaral et al., 2006; Souza et al., 2013).

A review by Sun et al. (2017) indicated that maize aflatoxin-contamination in China varies from region to region. Still, overall incidence rates ranged from 36.96% to 91.84%, with a mean contamination range of 1.15 to 107.93 ug/kg. However, in a more recent assessment, Yang et al. (2019) reported a lower incidence level (0.87%) of aflatoxin B1 in maize. Variance in contamination levels could be due to different reasons, including different climatic conditions within the same country. Different incidence rates and levels have been reported from other countries within Asia (Table 2).

Until recently, aflatoxin contamination of food was not a food safety concern in Europe; however, recent fluctuations in climate patterns have changed this situation (Battilani et al., 2016). In a survey in Serbia, 180 maize samples were analyzed for aflatoxin contamination. Aflatoxins B1, B2, G1, and G2 were detected in 57.2%, 13.9%, 5.6%, and 2.8% of maize samples in the concentration ranges of 1.3 to 88.8, 0.60 to 2.8, 1.8 to 28.5, and 2.1 to 7.5 μg/kg, respectively. Of these, 32.2% and 21.1% of the samples presented contamination levels beyond the regulatory limits for aflatoxin B1 and total aflatoxins, respectively. In another survey in France, 6% of 114 maize field samples and 15% of 81 of maize silo samples were found aflatoxin-positive (Bailly et al., 2018). Moreover, an exposure and risk assessment of aflatoxins intake through consumption of maize products in the adult populations of Serbia, Croatia and Greece, concluded that average aflatoxin intake through the consumption of maize or maize-based products was between 0.44 and 5.59 ng/kg bw/day (Udovicki et al., 2019).

In the United States, occurrence of aflatoxin in food is generally considered low. However, from 2004 to 2013, there were 18 reports of food and feed recalls due to aflatoxin contamination, although most of these recalls were related to dog feed (Mitchell et al., 2016). In a more recent survey, Zhang et al. (2018) did not detect aflatoxin in any of the 215 samples of infant foods and breakfast cereals in the US retail market screened for the presence of mycotoxins. Additionally, according to the Corn Harvest Quality Report 2018 to 2019 of the US Grains Council, of 181 maize samples screened for aflatoxin, 98% have no detectable levels. This is similar to the previous 2 years when only 0.6% (1) of the 181 samples showed an aflatoxin level higher than the Food and Drug Administration (FDA) regulatory limit of 20 ppb (parts per billion; US Grain Council, 2019).

Rice (*Oryza sativa* L.), one of the most consumed cereals globally, is another food crop susceptible to aflatoxin contamination (Millán & Martinez, 2003). Rice is mainly produced in environmental conditions suitable for fungal growth and aflatoxin production. Hence, its contamination with aflatoxin starts in the field where grains are infected with fungal producers of aflatoxin (Sales & Yoshizawa, 2005). This aggravates during postharvest sun-drying, leaving the grains' moisture content much higher than the required 14% (Reddy et al., 2009). Prietto et al. (2015), in their study, observed that stationary drying of newly harvested rice resulted in higher levels of aflatoxin contamination. Therefore, postharvest drying and moisture content reduction in grains is critical for preventing aflatoxin-contamination in rice. Generally, drying within 24 h of harvest to reduce the moisture content down to14% or below inhibits the growth and aflatoxin production by *Aspergillus* producers of the toxin (Masood et al., 2018).

In China, Lai et al. (2015) investigated the presence of aflatoxin in 370 rice samples collected from six different regions of the country. Of these samples, 63.5% were aflatoxin B1 positive, of which 1.4% contained levels beyond the EU regulatory limits. In a mini-survey in Saudi Arabia, samples of imported rice from India, Pakistan, the United States, Egypt, and Australia were analyzed for the presence of aflatoxin. Apart from the US parboiled rice, at least one class of aflatoxin was detected in all the 75 samples in levels ranging from 0.014 to 0.123 µg/kg for aflatoxin B1 and 0.052 to 2.58 µg/kg for total aflatoxins (Al-Zoreky & Saleh, 2019).

Furthermore, in a study carried out in Brazil, 187 rice samples from the field, processing and retail markets from, wetland and dryland, were analyzed for fungi belonging to *Aspergillus* section *Flavi* and the presence of aflatoxins. Five fungal species were identified: *A. flavus, A. caelatus, A. novoparasiticus, A. arachidicola*, *and A. pseudocaelatus*, and 14% of the samples were aflatoxin-positive with two samples exceeding the Brazilian regulatory limit of 5 ug/kg (Katsurayama et al., 2018). In another study conducted in Guyana, 186 samples of rice were tested for aflatoxin, of these, 16 and 3 samples had detectable levels of aflatoxin in concentrations higher than the US and EU regulatory limits, respectively (Morrison, 2016). Iqbal et al. (2016) screened 208 samples of rice and rice-based products collected from central areas of Punjab, Pakistan. Results obtained indicated that 35% of the samples were contaminated with aflatoxin, of which 19% and 24% of the samples were above the EU regulatory limit for aflatoxin B1 and total aflatoxins, respectively. The authors observed that brown rice has a higher mean level of aflatoxin B1 and total aflatoxins. This is in accordance with previous observations (Choi et al., 2015; Mousa et al., 2013), suggesting that brown rice is more prone to aflatoxin contamination than other types of rice.

Although aflatoxin levels in rice are lower than other staple cereals like maize, it is a staple food in many parts of the world. Therefore, it could represent a significant route

of human exposure to aflatoxin (Elzupir et al., 2015). A similar observation was made in a global assessment of aflatoxin's human exposure through cereal consumption (Andrade & Caldas, 2015).

Sorghum, wheat, barley are other cereals reported to be contaminated with aflatoxin. In a study in Kenya, 164 sorghum samples were screened for the presence of aflatoxin B1 of which 60% were found positive and 11% of the samples with levels beyond the Kenyan regulatory limit of 5 ppb for sorghum (Sirma et al., 2016). In another survey by Hanvi et al. (2019), of 12 samples of sorghum collected from Togolese markets, 25% were contaminated with aflatoxin with a contamination range of 6 to 16 μg/kg. Like other cereals, fungal proliferation and aflatoxin production in sorghum can happen both in the field and during storage if the conditions are adequate (Kange et al., 2015). A report by Ssepuuya et al. (2018) studied mycotoxins' occurrence in sorghum and its contribution to human dietary exposure in Burkina Faso, Ethiopia, Mali, and Sudan revealed that sorghum could be a significant contributor to aflatoxin-dietary exposure among sub-Saharan Africans. In India, 1606 sorghum samples collected across 4 years were screened for aflatoxin contamination. The authors concluded that aflatoxin contamination of sorghum in India is not a big concern as only 35 samples of the 1606 samples analyzed contained aflatoxin B1 at levels beyond the Indian regulatory limit of 20 ug/kg (Ratnavathi et al., 2016).

In a survey in Lebanon, of two sets of wheat samples collected from different warehouses, 23.3% and 25.3% were found to be contaminated with aflatoxin B1 at levels beyond the 2 μg/kg regulatory limit (Joubrane et al., 2020). Turksoy and Kabak (2020) analyzed 144 wheat samples from different parts of Turkey for the presence of aflatoxin and ochratoxin A. Aflatoxin was detected in 2% of the 141 samples in levels ranging from 0.21 to 0.44 μg/kg. Furthermore, of 36 wheat samples collected from different parts of Egypt, about 33% were aflatoxin B1 positive, 16% of which were beyond the EU regulatory limit of 2 μg/kg (Hathout et al., 2020). Generally, compared to other cereals, wheat is less prone to aflatoxin contamination (Armorini et al., 2015).

Mycotoxins occurrence in cereal food crops is a common issue regardless of the geographic or climatic conditions. Fungal toxin production happens both in cereals in the field and stored grains (Filazi & Sireli, 2013).

3.2 | Occurrence of aflatoxins in oilseeds and oilseed products

Oilseeds crops, including peanut, sunflower, soybeans, canola, rapeseed, flaxseed, mustard seed, sesame, cottonseed, and their products, are susceptible to aflatoxin contamination (Filazi & Sireli, 2013). Other nuts, including almonds, pistachios, walnuts, chestnuts, apricots, Brazil nuts, are equally prone to aflatoxin contamination (Diella et al., 2018). Among oils crops contaminated with aflatoxin, peanuts are the most susceptible. The invasion of peanuts plants by toxigenic fungus and the posterior contamination of the nuts with aflatoxin is a serious food safety concern in peanut-producing regions worldwide (Waliyar et al., 2015). Peanuts are a significant contributor to aflatoxin's dietary exposure among consumers in West Africa, where it is both a cash and staple crop (N'dede et al., 2012). The type of soils, environmental and farm conditions under which peanut is typically grown, favors toxigenic fungal proliferation and aflatoxin production in peanuts (Bankole & Adebanjo, 2003).

As previously mentioned, surveys of aflatoxin-contamination of peanuts in Africa and other parts of the world revealed that peanuts and their products are highly prone to aflatoxin-contamination (Chauhan, 2017). A decade-long survey of aflatoxin contamination in Gambian peanuts found that 42% of 1168 analyzed samples were contaminated with levels higher than the Codex recommended limit of 15 ppb. Contamination levels ranged from 8.55 to 112 ppb (Jallow et al., 2019). Like several other aflatoxin-susceptible crops, the invasion and aflatoxin production in peanuts starts in the field and continues as the products progress toward the consumers (Soni et al., 2020). A study in Mali reveals that aflatoxin accumulation in peanuts occurs at all stages of production; however, it is more pronounced during postharvest storage (Waliyar et al., 2015). Occurrence and factors associated with aflatoxin contamination of raw peanuts from Lusaka district's markets, Zambia was investigated by Bumbangi et al. (2016). Of the 92 samples analyzed, 51 were positive with levels ranging from 0.014 to 48.67 ppb, 6.5% of which were above the codex limits for aflatoxins in peanuts. Another study in the same country surveyed the presence of aflatoxin in imported and nationally produced peanut butter from 2012 to 2014. Of the samples collected from 2012 to 2014, 73%, 80%, and 53% were contaminated with levels ranging from 20 to 1000 μg/kg, respectively. Some of the products were imported from countries within the East African region (Njoroge et al., 2016).

Contrary to initial suppositions that vegetable oils are not prone to aflatoxin contamination (Mahoney & Molyneux, 2010), several studies have reported the presence of aflatoxin in peanut oil. In Guangdong, China, from 2016 to 2017, 427 samples of peanut oil were screened for aflatoxin content. Aflatoxin B1 was detected in 22.5% of the samples in levels ranging from 15.4 to 49.9 μg/kg in 2016 and in 15.1% of the samples in a range of 8.8 to 22.2 μg/kg in 2017. Another study in Haiti reported a carry-

over of 0.5% of initial aflatoxin in peanut to the extracted oil (Schwartzbord & Brown, 2015). The same authors reported that 14%, 97%, and 30% of 21 raw peanut samples, 32 peanut butter samples, and 30 maize samples, were contaminated with aflatoxins, respectively. In Malaysia, aflatoxins occurrence in raw peanut samples collected from retailers and manufacturers was investigated by Norlia et al. (2018). Of the screened samples, 38% and 22% of the samples exceeded the Malaysian Regulation limit. Aflatoxin contamination of peanuts in Malaysia has been recently reviewed (Nor-Khaizura et al., 2019).

3.3 | Spices and herbal products affected by aflatoxin

Spices and herbs are widely used food products with an estimated global production of $3 billion. Black pepper, capsicums, cumin, cinnamon, nutmeg, ginger, turmeric, saffron, coriander, cloves, dill, mint, thyme, and curry powder are the most widely used in the food and culinary industry spices worldwide as organoleptic enhancers, preservatives, and medicines in some cultures. Sadly, spices and herbs are reported to be a source of human-aflatoxin exposure (Kabak & Dobson, 2017). Although studies have reported black pepper plants to have some inhibitory powers against the growth of toxigenic strains of *A. flavus* and *A. parasiticus*, and toxin production (Ibrahim et al., 2017), several studies have reported aflatoxin presence in black pepper. In a survey conducted in Iran, aflatoxin was detected in 5 of 40 black pepper samples (12.5%) in levels ranging from 0.88 to 1.45 μg/kg. In the same study,100% of 36 red pepper samples were contaminated with aflatoxin ranging from 4.22 to 28.6 μg/kg (Barani et al., 2016). In another survey conducted in India, aflatoxin was detected in 78.1% of 55 black pepper samples contaminated with total aflatoxins with average amount of 320 ppb (Jeswal & Kumar, 2015a).

Chilli (*capsicum*) is another widely consumed spicy product that is susceptible to aflatoxin contamination. The climatic conditions of the major producers, handling, and chili processing procedures make it vulnerable to fungal invasion and subsequent aflatoxin contamination. Like other crops, chilli contamination with aflatoxin can occur both during pre-and postharvest stages (Duman, 2010). Reports from different parts of the world have documented aflatoxin presence in chili in levels higher than regulatory limits (Ezekiel et al., 2019; Golge et al., 2013; Klieber, 2001).

Evidence of aflatoxin contamination of other spicy products including cinnamon and cassia, cloves, coriander, cumin, ginger, nutmeg, saffron, turmeric, black cumin, dill, mentha, thyme, curry powder, among others, have been detailed by Kabak, and Dobson (2017). Additionally, aflatoxin present in samples of Chinese Traditional Medicines and other herbal medicines are reported in various published literature. A summary of research data accumulated from 2000 to 2018 indicates that 2979 batches of Chinese traditional herbs from 66 varieties prone to aflatoxin contamination were tested, of which 697 batches tested positive for aflatoxin with levels ranging from 0.02 to 1268.8 μg/kg (Qin et al., 2020). Another study by Zhao et al. (2016) reported that 14 of 22 samples were contaminated with at least one type of aflatoxin at concentrations ranging from 0.2 to 7.5 μg/kg. A similar study in South Korea reported 58 of 700 samples of herbal medicines were aflatoxin positive, with 6 and 10 samples exceeding the Korean regulatory limits of 10 and 15 μg/kg for aflatoxin B1 and total aflatoxins, respectively (Shim et al., 2012).

3.4 | Coffee and tea

Despite previous assumptions that caffeine can inhibit fungal toxin production, aflatoxin has been isolated in both ground and coffee beans. In a study to assess human exposure to mycotoxins through coffee consumption, samples collected from nine different countries were analyzed. Although sporadic and lower (maximum level of 1.2 μg/kg) in quantity compared to other mycotoxins, aflatoxin was detected in some samples (Bessaire, et al., 2019). In a study in Spain, aflatoxin was detected in 53% of 169 studied coffee samples. Of these, no sample exceeded 2 μg/kg of aflatoxin B1, but 15% of samples had a concentration of total aflatoxins beyond 5 μg/kg (García-Moraleja et al., 2015). In Pakistan, 30 coffee samples were analyzed for aflatoxin and heavy metals. Of these samples, 50% and 20% exceeded the EU limit for aflatoxin B1 and total aflatoxin, respectively. Contamination levels ranged from < LOD (limit of detection) to 25.75 μg/kg and < LOD to 13.33 μg/kg for total aflatoxins and aflatoxin B1 (Azam et al., 2020).

Studies have revealed that aflatoxin levels in roasted coffee are often low (Al-Ghouti et al., 2020; Bessaire, Perrin, et al., 2019). This is attributed to the thermal treatment coffee beans are subjected to during the roasting process. A study by Micco et al. (1992) found that roasting reduces aflatoxin levels in coffee beans. Different reduction levels have been reported. A recent study by Humaid et al. (2019) observed that roasting of aflatoxin-contaminated green coffee beans resulted in a 20% reduction in initial aflatoxin concentration levels. In a previous study, Soliman (2002) reported that roasting reduced about 42.2% to 55.9% of aflatoxin content in coffee beans depending on the type and roasting parameters. This same study also concluded that caffeine content in coffee beans reduces potential fungi growth and aflatoxin production.

As one of the most consumed beverages globally, tea is often associated with healthy food choices due to its high content of health-promoting phytochemicals like polyphenols (Khan & Mukhtar, 2013). Until recently, scientific research on tea has mostly focused on its health benefits, paying little attention to its safety. However, new data from different parts of the world suggest that mycotoxin-contamination of tea should be a cause of concern (Sedova et al., 2018). Aflatoxin occurrence in tea has been reported from different parts of the world (Sedova et al., 2018). For instance, of 44 commercial tea samples analyzed for the presence of mycotoxins, aflatoxin B2 (14.4 to 32.2 μg/L) and aflatoxin G2 were detected in 14% and 18% of the samples, respectively (Pallares et al., 2017). In another study conducted in Iran, 60 tea samples were tested for aflatoxin content. The results showed that 40% of the samples presented detectable levels of aflatoxin but within the regulatory limit of ≥10 μg/kg (Pakshir et al., 2020). Few studies have looked into the carryover of aflatoxin from contaminated raw tea leaves to the beverage. Ismail et al. (2020) observed that more than half of the aflatoxin content in naturally contaminated raw leaves is transferred to the beverage during processing. Another study reported a 28% to 33% carryover in artificially contaminated tea samples (Viswanath et al., 2012).

3.5 | Alcoholic beverages

The presence of aflatoxin in wine is not frequent. However, traces of aflatoxin have been occasionally isolated in both traditional and industrially made beer (Scott, 2008). Di Stefano et al. (2015) reported a 57.9% incidence rate of aflatoxin G1 in 57 market samples of Sicilian red wines. One of the samples contaminated with aflatoxin G1 contained 0.13 and 0.15 μg/L of aflatoxin G1 and aflatoxin B1, respectively.

In a survey, a selection of 1000 beer samples, of which 60% were traditionally brewed (craft beers) collected from 47 different countries, were screened for the presence of aflatoxin B1 and other mycotoxins. Of these samples, five were confirmed to contain aflatoxin B1 in levels ranging from 0.1 to 1.2 μg/L. Three of the aflatoxin B1-positive samples also contained aflatoxin B2 in concentrations ranging from 0.1 to 0.2 μg/L. Aflatoxin M1 was detected in one of the samples (Peters et al., 2017). The authors further noted that all the aflatoxin-contaminated samples were from Africa. Furthermore, in a survey conducted in China, 101 market samples of beer were analyzed for the presence of aflatoxin B1 and sterigmatocystin. Interestingly, none of the samples, presented detectable aflatoxins levels (Zhao et al., 2017).

Reports have indicated that locally brewed traditional alcoholic beverages could be a source of aflatoxin exposure among Africans primarily due to the high contamination levels in the raw materials (Lulamba et al., 2019). A survey evaluated the aflatoxin levels in industrially brewed local and imported beers collected from an Ethiopian local market. Of 12 domestic alcoholic beer brands sampled, 11 were aflatoxin-positive with a range of total aflatoxins between 1.23 and 12.47 μg/L. None of the imported brands had detectable aflatoxin. The authors observed that aflatoxin-related knowledge was low among local manufacturers (Nigussie et al., 2018). In another survey in Nigeria, 90 samples of imported and locally brewed beers were screened for aflatoxins. Aflatoxin B1 levels ranging from 3.43 to 38 ug/L was detected in 17.9% of the locally made beers and 16.7% of the imported beer (Salami Oluwafemi et al., 2019). In another survey in South Africa, aflatoxin B1 was detected in only 2 of 32 beer samples in values ranging from 5.8 to 7.0 μg/L, which are higher than the South African national regulatory limit for aflatoxin B1 (5 μg/kg) but within the permissible levels of total aflatoxins (10 μg/kg) in South African foods (Adekoya et al., 2018).

Carryover of aflatoxins from contaminated raw materials into beer has been previously studied (Chu et al., 1975). In more recent works, a laboratory-scale study evaluated the fate of mycotoxins during brewing of beer and wine. The authors observed that brewing reduced aflatoxin levels to below 20% of the initial contamination levels (Inoue et al., 2013b). A similar study evaluated aflatoxin's fate during beer and wine fermentation and observed that aflatoxin B1 levels remained unaffected by the fermentation used for beer production but were reduced to 30% of their initial concentration in wine fermentation (Inoue et al., 2013a).

3.6 | Aflatoxin contamination of foods of animal origin

Aflatoxin contamination of food is not unique to plant-based products. Numerous reports have documented the presence of aflatoxins in foods of animal origin. Their detection in dairy products, eggs, and edible animal products triggered the formulation of regulations to control their presence in animal feed, the route via which animals are exposed to aflatoxins (Fink-Gremmels & Van Der Merwe, 2019). Aflatoxin has also been detected in human milk among lactating mothers in aflatoxin hotspots of the world. A Survey in Nigeria by Oluwafemi (2012) concluded that about 14% of 121 samples of human breast milk was contaminated with aflatoxin M1 at levels between 2 and 187 ng/L.

Twelve hours upon the consumption of aflatoxin B1-contaminated feed, milking animals start to excrete aflatoxin M1 in their milk (Applebaum et al., 1982). Aflatoxin B1 is transformed in the liver by the hepatic microsomal

cytochrome P450 into aflatoxin M1 in the lactating animals through the hydroxylation of the fourth carbon in the aflatoxin B1 molecule (Nabney et al., 1967).

The amount of aflatoxin B1 converted to aflatoxin M1 varies depending on several factors, including milk-yield and lactating period (Veldman et al., 1992). Britzi et al. (2013) reported a carryover of 1% to 2% in low-milk-yielding cows and up to about 6% in high-milk-yielding cows. A more recent study by Churchill (2017) reported a 6.5% carry-over in high-milk-yielding dairy cows. In ewes and goats, estimated levels of 1.5% and 0.8% per kg milk, respectively, have been reported (Walte et al., 2016). Generally, carryover of aflatoxin B1 from the feed to milk varies among animal species and rates are higher during the early stages of lactation (Prandini et al., 2009).

As a possible food safety risk to consumers, aflatoxin M1 is categorized as a group 2B human carcinogen by the IARC (Ostry et al., 2017). Hence, its presence in foods and agricultural products is regulated in many countries (Iqbal et al., 2015).

Numerous studies in different countries have reported different occurrence levels of aflatoxin M1 in different categories of milk and dairy products. A study conducted in Lebanon surveyed the aflatoxin contamination in 868 samples of raw milk, pasteurized ultra-high temperature (UHT) milk, and dairy products. The study revealed an occurrence rate of 58.8%, 90.9%, and 66% in raw milk, pasteurized UHT milk and dairy products, respectively, in levels ranging from 0.011 to 0.440, 0.013 to 0.219, and 0.015 to 7.350 μg/L, respectively. The authors noted that 28%, 54.5%, and 45.5% of the contaminated raw milk, pasteurized and dairy products were, respectively, above the EU regulatory limits for aflatoxin M1 (Daou et al., 2020). In Indonesia, another survey screened 20 samples of fresh, 16 pasteurized milk, and 16 recombined milk products for aflatoxin M1. Of these samples, 92.5% were contaminated in a range of 24 to 570 ng/L. The highest average concentration of aflatoxin M1 was detected in the pasteurized milk at 244 ng/L, followed by fresh milk at 219 ng/L. The lowest was observed in the recombined milk samples (131 ng/L; Sumantri et al., 2019). A study in South China found aflatoxin M1 in 62.5% of 136 raw buffalo milk and 74.4% of 86 dairy products samples in concentrations ranging from 4 to 243 ng/kg and 4 to 235 ng/kg, respectively (Guo et al., 2019). The authors observed that aflatoxin M1 incidence rate and concentration levels were higher in cheese than other products. A more recent survey around the same area in China observed an 80.4% incidence rate of aflatoxin M1 in 734 milk samples, comprising raw milk (133 samples), pasteurized milk (410), extended shelf-life milk (93), and UHT milk (98) with a mean concentration ranging from 5.1 to 104.4 ng/L (Xiong et al., 2020). Furthermore, in a study in Italy, 31,702 milk samples were tested for aflatoxin M1. Of these samples, 63 (0.20%) raw milk samples contained aflatoxin M1 higher than 50 ng/kg (Serraino et al., 2019).

Aflatoxin M1 incidence rates and levels in milk and dairy products, generally vary and hardly follow a regular pattern as evidenced in the cited literature and those detailed in Table 2. This variability could be due to multiple factors including processing procedures, storage, types of product, geographical and seasonal effects (Barukčić et al., 2018; Campagnollo et al., 2016; Peña-Rodas et al., 2018; Tomašević et al., 2015). A 2-year survey in Egypt observed a significant ($p \leq .001$) variation in aflatoxin M1 levels in milk. Milk samples collected in winter presented higher incidence and contamination levels than those collected in summer (Ismaiel et al., 2020). In a similar study in El Salvador, Peña-Rodas et al. (2018) registered more aflatoxin M1-positive samples during a drought year than a nondrought year. Their study revealed a 16.5% higher incidence rate during the drought year compared to the nondrought year. Contrastingly, in a survey conducted in Brazil, 40 samples of milk collected from subtropical and temperate regions of the country were screened for the presence of aflatoxin M1, of these samples 87.50% contained detectable levels of aflatoxin M1 with a mean concentration of 16.66 ng/L. However, the authors did not observe any significant differences in the levels of aflatoxin M1 between the two climate zones in both summer and winter (Venâncio et al., 2019).

Aflatoxin M1 is known to be stable under different conditions and processing parameters (Iha et al., 2013). However, certain levels of reduction have been reported as a result of processing. For instance, researchers subjected aflatoxin M1-contaminated milk to pasteurization (95 °C for 5 min) and observed 18% and 16% reduction of in milk contaminated with 1.5 and 3.5 μg/kg of aflatoxin M1, respectively (Şanli et al., 2012). Another study reported a 7.62% reduction in aflatoxin M1 with similar processing parameters (Bakirci, 2001). Deveci (2007) reported that pasteurization (72 °C for 2 min) resulted in about 12% and 9% reduction in aflatoxin M1 in milk contaminated with 1.5 μg/kg, and 3.5 μg/L aflatoxin M1, respectively. Purchase et al. (1972) observed that in general, aflatoxin M1 reduction in milk positively correlates with the amount of heat applied in the processing. Contrastingly, Jasurent et al. (1990) observed that pasteurization (95 °C for 3 min) had no significant effect on aflatoxin M1 content in milk. Other studies by Govaris et al. (2001) and Awasthi et al. (2012) reached similar conclusions.

In terms of processing, Iha et al. (2013) observed that aflatoxin M1 in milk was reduced by 3.2% when processed into cheese and by 6% when processed into yogurt. Some researchers reported higher reduction levels by lowering the dairy product's pH (Iqbal et al., 2015). For instance, Kuboka et al. (2019) reported a 73.6% reduction in

aflatoxin M1 in yogurt after lowering its pH from 4.4 to 4.0 through fermentation. Differences in reduction levels could be attributed to various factors including, the time-temperature combination of the process, initial aflatoxin M1 load of the raw milk, and the type of analytical method used to assess the end product (Rustom, 1997).

Occurrence and levels of aflatoxin M1 in dairy products are heavily dependent on animal feed (Der Fels-Klerx & Camenzuli, 2016). Therefore, it is of food safety relevance that aflatoxin levels in animal feeds are monitored and kept at minimum levels. Moreover, while milk and dairy products have not been reported as significant contributors to the overall aflatoxin exposure levels, they form a large part of children and infant diets that may be more sensitive to high exposure levels. The same could be said about the presence of aflatoxins in human breast milk. Consumption of aflatoxin-contaminated foods poses a risk to the lactating mothers and the more vulnerable breastfeeding children and infants. Therefore, attention needs to be paid to the contamination levels in milk and other dairy products. Additionally, as observed by Van Der Fels-Klerx et al. (2019), changes in global climate patterns will impact aflatoxin levels in animal feed, which will lead to fluctuations in aflatoxin levels in animal-derived foods. Therefore, it is crucial that the aflatoxins levels in foods of animal origin are monitored.

3.7 | Recent aflatoxin-exposure assessments

Evaluation of dietary exposure to aflatoxin and the risk this poses to consumers has been the objective of numerous recent studies (Table 2). In general, two approaches are used to estimate human-aflatoxin exposure. The first and the most widely used method involves integrating aflatoxin levels in food samples with food consumption data (Udovicki et al., 2019). These results are further standardized by dividing them by average human body weight (60 kg for adults) and expressed as nanogram/kilogram body weight/day (ng/kg bw/day; Li et al., 2018). Dietary data are typically obtained through dietary intake surveys like 24-h recalls and food frequency questionnaires. The European Food Safety Authority (EFSA) has developed detailed guidelines for dietary data collection and processing for risk and exposure assessment purposes (European Food Safety Authority, 2009).

Using the method described above, Kabak (2016) assessed aflatoxin exposure through aflatoxin-contaminated hazelnuts and dried figs among the Turkish. The author indicated that on a daily basis, Turkish consumers are exposed to 0.014 to 0.018 ng/kg bw at the lower bound and 0.016 to 0.023 ng/kg bw at the upper bound of aflatoxin B1 from the consumption of hazelnuts. Dry figs contribute a mean aflatoxin B1 intake of 0.004 ng/kg bw/day at the lower bound and 0.005 ng/kg bw/day at the upper bound. In another work Kooprasertying et al. (2016) used the same method to assess the exposure and risk posed by aflatoxin-contaminated peanuts to Thai consumers. The authors estimated the average daily intake of aflatoxin at 0.49, 0.40, and 2.13 ng/kg bw/day for raw, roasted, and ground peanuts, respectively, and an estimated potential risk of liver cancer of 0.01 to 0.12 cancer/year/100,000 persons. Additionally, a 1.34 ng/kg bw/day aflatoxin exposure from the consumption of Omena (*Rastrineobola argentea*) among Kenyans has been reported by Marijani et al. (2020). Another study in Tunisia reported that daily intake of aflatoxin B1 from pearl millet consumption is 3.89 ng/kg bw/per day. The authors concluded that millet could be a significant route of aflatoxin exposure among Tunisia consumers (Lasram et al., 2020). Furthermore, it is reported that consumers in Caldas Colombia are exposed to 0.732 and 3.093 ng/kg bw/day of aflatoxin B1 from the consumption of "arepas" (maize tortillas) and rice, respectively (Martinez-Miranda et al., 2019). In Spain Herrera et al. (2019) reported an aflatoxin B1 exposure level ranging from 0.17 to 0.37 ng/kg bw/day when they assessed aflatoxin exposure among infants through the consumption of cereal-based baby foods.

Although this method is widely used and has proven effective in measuring human-aflatoxin exposure, it has some limitations that may compromise the accuracy of the collected data. As pointed out by Gong et al. (2016), the typical heterogeneous distribution of aflatoxin in food samples combined with the subjectivity and bias-proneness of dietary intake surveys may result in over or underestimation of aflatoxin exposure levels among consumers. Additionally, management of contamination levels below the LOD of analytical methods and effect of food processing on aflatoxin levels could threaten the exactitude of estimations made through this method (Marin et al., 2013).

An alternative approach considered a direct and more accurate way of measuring human-aflatoxin exposure is by measuring aflatoxin biomarkers in human biological fluids (Al-Jaal, Jaganjac, et al., 2019). Aflatoxin biomarkers, including the aflatoxin-N7-guanine adduct excreted in urine and aflatoxin M1 excreted in breast milk are used to determine the short term exposure to aflatoxin B1 and aflatoxin–albumin adduct in plasma or serum is used for chronic exposure assessment, (Routledge & Gong, 2011).

A study in Portugal analyzed urine samples for the presence of aflatoxin biomarkers and found that 13% contained aflatoxin B1, 16% contained aflatoxin B2, 1% had aflatoxin G1, 2% contained aflatoxin G2, while 19% of the samples contained aflatoxin. Daily intake of aflatoxin was

estimated to be 13.43 ng/kg bw/day resulting in 0.13 extra cases of hepatocellular carcinoma (Martins et al., 2020). In the Gambia, where peanut is a staple food, Hernandez-Vargas et al. (2015) assessed aflatoxin exposure in pregnant women using an ELISA method to measure aflatoxin–albumin (AF–alb) adducts in plasma. AF–alb levels ranging from 3.9 to 458.4 pg/mg albumin were detected in all the samples. The authors further noted that aflatoxin exposure in the mothers was associated with DNA methylation in their infants.

Being a carcinogenic substance, there is no established tolerable daily intake (TDI) for aflatoxin B1 (EFSA Panel on Contaminants in the Food Chain et al., 2020). Daily exposure to levels as low as 1 ng/kg is considered dangerous and a threat to human health (Al-Jaal et al., 2019). As such, taking into account the as low as reasonably achievable (ALARA) approach applied when managing aflatoxin-related risks in food, aflatoxin-exposure levels (Table 3) remain high in numerous countries in the world, especially in countries in sub-Saharan Africa and some parts of Asia. Staple diets made up of aflatoxin-prone foods and climatic conditions suitable for fungal proliferation and aflatoxin biosynthesis are the major contributors to these high levels. However, socioeconomic factors, including subsistence farming, low awareness, and the inability to establish and enforce regulations, also play significant roles.

4 | WORLDWIDE REGULATIONS OF AFLATOXIN IN FOOD

Since their discovery, aflatoxins have remained a persistent food safety issue and continue to pose risks to human and animal health. The US FDA considers them unavoidable contaminants in food (Wood, 1992). Therefore, countries, regions, and international agencies enacted regulations to minimize the levels of aflatoxin in food and feed (Williams et al., 2004).

Development and institutionalization of food safety regulations require a thorough consideration of both scientific and socioeconomic factors. Scientific factors, including the availability of toxicological and human exposure data, sampling, and analytical capacity, are indispensables for the development of aflatoxin and other food safety-related regulations. Socioeconomic factors such as food security, existing regulations in trade partners' countries, level of development of a country or region, and the type of food system in a country are equally important factors taken into account when formulating regulations to protect consumers against contaminated foods (FAO, 2004).

Aflatoxin regulatory limits are formulated using risk assessment models. They are often developed by specialized national and multilateral agencies, like the FAO/WHO Joint Expert Committee on Food Additives of the United Nations (JECFA), EFSA, The Ministry of Health of the People's Republic of China, and the FDA in the United States (Van Egmond et al., 2007; Zhang et al., 2018). Difference in countries' risk perception, data, approaches, and risk assessment models create disparities between countries in terms of aflatoxin regulatory limits.

Developed countries with better scientific and technical know-how often tend to adopt lower regulatory limits (more stringent) than those set by the global food safety regulatory body-WHO/FAO joint Codex Alimentarius Commission. In some instances, these disparities have brought up trade disputes between importing and exporting countries, with importing countries often accused of using food safety regulations to disguise trade barriers. Fortunately, The World Trade Organization's Sanitary and Phyto-sanitary (SPS) Agreements recognized the limits set by Codex Alimentarius Commission as the standards upon which international trade dispute settlements will be based. According to the SPS Agreements, Countries could impose more stringent limits, provided that these are based on logical scientific reasoning reached through a risk assessment (Roberts & Unnevehr, 2005).

Some countries and regions have moved to harmonize their aflatoxin-related food safety regulations to facilitate trade and avoid trade conflicts. Australia and Zealand, The EU, and Mercosur (Argentina, Brazil, Uruguay, Paraguay, and Venezuela) are notable examples of these kinds of initiatives (King et al., 2017).

Notwithstanding, setting up and enforcing regulations requires a considerable amount of resources and efforts. Some countries have been more successful than others in doing so. For instance, The EU possesses arguably the best food safety (including aflatoxins) surveillance and information sharing system in the Rapid Alert System for Food and Feed (RASFF). The system enables the simple, rapid collection, sharing, and storage of food safety-related data (Parisi et al., 2016). This allows fast decision-making among relevant institutions to prevent the entry of aflatoxin-contaminated food products in the EU. Data from the system (Figure 2) are often cited to point out aflatoxin-related indicators like economic losses incurred by exporting countries attributable to aflatoxin, remotely evaluate aflatoxin-contamination levels in exporting countries and even evaluate exporting countries' regulation enforcement effectiveness. A high number of alerts for products from a particular country may indicate one of these issues.

Globally about 120 countries have enacted regulatory limits on allowable aflatoxin levels in human food and animal feed (Bui-Klimke et al., 2014). Some countries set limits for the four most prominent types of aflatoxins in food: B1, B2, G1, and G2. For example, the US and Kenyan

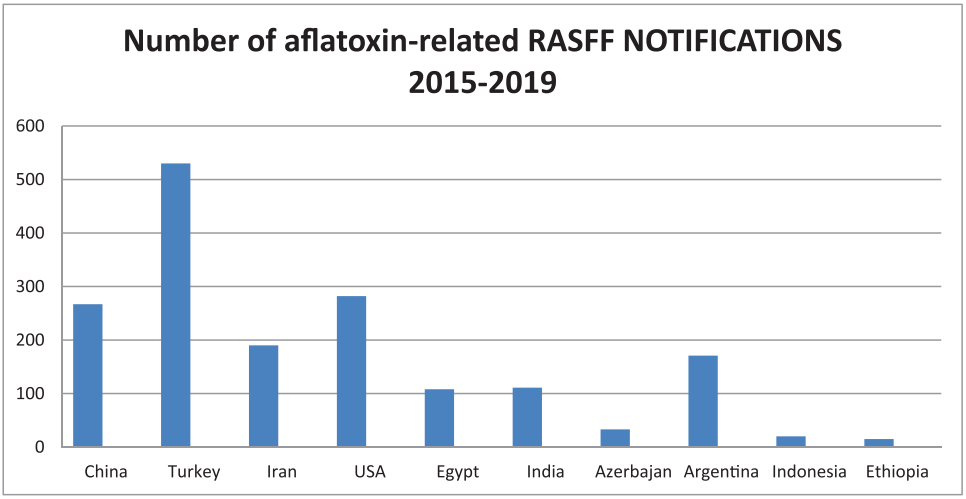

FIGURE 2 Number of aflatoxin-related Rapid Alert System for Food and Feed (RASFF) notifications of the 10 countries with the highest number of notifications from 2015 to 2019

Note: Data were adopted from the RASFF annual reports of 2015 (European Union, 2015), 2016 (European Union, 2016), 2017 (European Union, 2017), 2018 (European Union, 2018), and 2019 (European Union, 2019)

regulations stipulate a maximum limit of 20 and 10 ppb for the sum of the four types of aflatoxins (total aflatoxins), respectively. In contrast, the EU that has different limits for different aflatoxin–food combinations has a maximum level of 2 and 4 ppb for aflatoxin B1 and total aflatoxins in maize and peanuts, respectively (European Commission, 2006). Additionally, many countries have adopted maximum limits for milk and dairy products (aflatoxins M1 and M2; Lalah et al., 2019).

In Latin America, several countries, including nonmembers of the Mercosur group, have enacted regulations to prevent aflatoxins in food and feed (Miranda et al., 2013). In Asia, almost all the states have written regulations for aflatoxin, mostly for cereals, nuts, and their products (Anukul et al., 2013). Members of the Gulf Cooperation Council (the United Arab Emirates, Saudi Arabia, Qatar, Oman, Kuwait, Bahrain) have also jointly adopted aflatoxin-related regulations (Al-Jaal, Salama, et al., 2019).

Notably, despite the high occurrence and exposure levels often reported from Africa, only a few African countries have aflatoxin regulations (Matumba et al., 2017); among them are Nigeria, Kenya, Ivory Coast, Zimbabwe, Senegal, Mauritius, Algeria, South Africa, Malawi, Egypt, Morocco, and Tunisia (Chauhan, 2017; Lahouar et al., 2018). Socioeconomic issues, such as food scarcity, lack of proper infrastructure, expertise, and technical know-how are among the many reasons few African countries have aflatoxins regulations, and those with regulations barely enforce them (Shephard & Gelderblom, 2014).

Regulation development and enforcement is an essential piece in the overall institutional setup against human-exposure to aflatoxin. When effectively implemented and enforced they serve as the last line of defense against human-aflatoxins exposure. Therefore, they should be developed through robust risk assessments, sound, and representative data obtained from unbiased sources or means.

5 | CURRENT AFLATOXIN DETECTION METHODS

As previously mentioned, about 120 countries have established regulatory limits to protect consumers against aflatoxins' harmful effects. To monitor and enforce these regulatory limits, fast, accurate, and reliable means of detecting and quantifying aflatoxins in foodstuffs are required. As such, multiple detection methods have been developed (Krska et al., 2008). Detection methods need to be sensitive, accurate, reproducible, and easy-to-use. Aflatoxin detection procedures are multistage. They involve sampling, extraction, purification, enrichment, analysis, and post-analysis data interpretation (Wolf & Schweigert, 2018). Due to the uneven distribution of aflatoxin in agricultural products, sampling is essential in its detection in food products (Whitaker et al., 1974; Whitaker et al., 1994). However, sampling has been identified as a significant source of error in the process of aflatoxin detection in food samples. Common mistakes related to sampling are either good lot (a lot that has overall content within the required limit) will fail and will be rejected, or bad lots will be tested negative and accepted. Proper and adequate

sampling reduces results variability and the number of misclassified lots. Therefore, sampling is an attention-worthy step in the overall detection process, regardless of the type of detection method used (Reiter et al., 2009).

In the sample preparation of grains or nuts or any particulate product for mycotoxin detection, it is essential that the entire representative sample be comminuted before laboratory samples are collected for analysis (Whitaker, 2003). For effective detection and quantification of aflatoxin in food samples, chromatography and immunoassay-based methods require the toxin to be extracted from the sample and cleaned up to minimize the effects of the matrix on the final analytical results (Krska et al., 2008). There are different extraction and clean-up methods used for aflatoxin detection. The choice of extraction and clean-up method to a large extent depends on the type of matrix, the detection techniques to be used, and the level of contamination of the food samples. Currently, the following extraction and clean-up techniques are widely used: liquid–liquid extraction (LLE), liquid–solid extraction (LSE), Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), ultrasonic extraction, pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), solid-phase extraction (SPE), immunoaffinity chromatography (IAC), microwave-assisted extraction (MAE), solid-phase microextraction (SPME), matrix solid-phase dispersion (MSPD), and Mycosep multifunctional clean-up (MFC; De Saeger, 2011; Miklós et al., 2020; Reiter et al., 2009; Sirhan et al., 2014).

Broadly, aflatoxin detection methods could be categorized into three groups: chromatographic, immunochemical, and spectroscopic. Each of these methods has its advantages and drawbacks (Table 4). Additionally, novel, portable, faster, and reliable emerging techniques are being developed for the on-site determination of aflatoxins in food products (Wolf & Schweigert, 2018).

5.1 | Chromatographic methods

Chromatography analysis is one of the oldest and most widely used aflatoxin detection methods. The most common aflatoxin detection techniques based on this method include gas chromatography (GC), liquid chromatography (LC), high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and high-performance thin-layer chromatography (HP-TLC). The overall idea of aflatoxins' chromatography detection is based on dividing a sample solute between two phases of the stationary and mobile phase. The mobile phase (often a fluid) passes through the stationary bed (liquid or solid; Wacoo et al., 2014a).

GC was first used to detect aflatoxin B1 in 1981; it was further developed and adopted for the detection of both B1, B2, G1, G2 classes of aflatoxin (Goto et al., 1990). GC as an aflatoxin detection technique is fading away principally due to the availability of cheaper and less labor-intensive alternatives (Liang et al., 2005). It has been previously used to determine aflatoxin in different food matrices (Goto et al., 1988; Trucksess et al., 1984).

Chromatography techniques are the reference methods for the determination of aflatoxin in food samples. HPLC is one of the most used laboratory-based methods for detecting and quantifying organic compounds (Li et al., 2011). Currently, HPLC connected to a fluorescent detector (FLD), ultraviolet (UV) detector, photodiode array detector (PDA), mass spectrometer (MS), single mass spectrometry, or tandem MS (MS/MS) are employed for the determination of aflatoxin and other contaminants in food samples (Valenta, 1998). HPLC-FLD is currently one of the most widely used techniques in determining aflatoxin in food products, principally due to its sensitivity. Based on the natural fluorescence exhibited by aflatoxin, FLD is more sensitive and specific than other detectors (Zhang & Banerjee, 2020). Although in some matrices, the natural fluorescence of aflatoxins B1 and G1 need to be enhanced through postcolumn derivatization to increase sensitivity (Kok et al., 1986). This is in most cases done using trifluoroacetic acid and potassium bromide (KBr) as reagents (Miklós et al., 2020).

Several methods based on this technique have recently been reported. Kim–Soo and Chung (2016) analyzed milk, yogurt, and cheese samples for aflatoxin M1 using an IAC clean-up with reversed-phase HPLC separation coupled to an FLD detector. They obtained an LOD of 0.003 μg/kg in milk, 0.07 μg/kg in yogurt, and 0.05 μg/kg in cheese, and an overall recovery rate of 83%. In another work, Muñoz-Solano and González-Peñas (2020) reported developing an LC-FLD-based method suitable for the determination of aflatoxins in animal feed. The technique's analytical characteristics were an LOD of 2 μg/kg for aflatoxins B1 and G1 and 0.64 μg/kg for aflatoxins B2 and G2 and a recovery rate of 73.6% and 88.0% for all toxins.

As discussed above, UV detectors are less widely used than FLD; however, they are still used by researchers to determine aflatoxin in various food samples. For instance, an HPLC-UV-based technique was used by Amirkhizi et al. (2015) to detect aflatoxin B1 in eggs and chicken liver matrices. The LOD and limit of quantitation (LOQ) were 0.08 and 0.28 μg/kg, respectively. In another study, an SPE method and a reverse-phase HPLC coupled to a UV detector was used by Kulkarni et al. (2015) to quantify aflatoxin in dry coconut samples. Moreover, an HPLC-PDA-based method was developed by Mochamad and Hermanto (2017) for the determination of aflatoxin B1 in

TABLE 4 Current aflatoxins detection methods, their advantages, and drawbacks

Class of detection method	Methods	Advantages	Drawbacks	References
Chromatographic-based methods	HPLC	Offers accuracy, reliability, and high sensitivity.	Extensive sample treatment, tedious pre- and postcolumn derivation processes to improve sensitivity	Mahfuz et al. (2018)
	TLC	Capable of detecting multiple metabolites in a single test and offer good level of sensitivity.	Proneness to error; require skilled operator, extensive sample treatments, and expensive equipment	Mahfuz et al. (2018)
	HPTLC	Sensitive, limited errors		
	GC	Suitable for multi-toxin detection situations.	Nonlinearity of calibration curves, drifting responses, effects from previous samples, and high variation in terms of precision.	Mahfuz et al. (2018)
	LC	Highly sensitive and versatile.	Slow compared to other methods	Mahfuz et al. (2018)
	LC-MS/MS	Offers sensitivity, reliability and does not require the immununo-affinity clean-up columns	Expensive, cumbersome sample preparation, and requires a highly trained operator	Ouakhssase et al. (2019)
	UHPLC-MS/MS	Suitable for multicontaminant detection, sensitive reliable and less solvent consumption and fast analysis.	Require trained personnel, expensive high matrix effects	Rathod et al. (2019)

(Continues)

TABLE 4 (Continued)

Class of detection method	Methods	Advantages	Drawbacks	References
Immunochemical methodsImmunochemical methods	ELISA	It offers simplicity, cheap, rapid, and multiple samples can be tested at the same.	Cross-reactivity, matrices-dependent and time-consuming clean-up	Pal et al. (2004)
	Lateral flow immune-assay	Offers rapid, straightforward in-the-field analysis and adaptable for multi-analyte detection	Expensive as compared to other immunochemical methods, result and data management and interpretation, cross-reactivity	Ho and Wauchope (2002)
	Radioimmunoassay	It offers advantages including high sensitivity, low LODs, minimal matrix effect	Safety concerns related to the radioactive elements use in the assay, false-positive possibility, requires pure anti-bodies, and there are concerns as to the disposal of waste material	Matabaro et al. (2017)
	Biosensors	Excellent sensitivity, low LOD, portable and suitable for on-site testing.	High rates of false positives, performance affected by matrices conditions	Larou et al. (2013)
Spectrometric-based methods	Fourier-transform near-infrared (FT-NIR) spectrometry	Fast, environment-friendly, and requires little skills to operators	Time-consuming calibration is required	Durmuş et al. (2017)
	Laser-induced fluorescence (LIF) screening method	This method is suitable for samples with low contamination levels.	The high cost of laser materials limits its usage	Alcaide-Molina et al. (2009)
	Black-light test	Suitable for screening purposes	High possibility of false positives, high dependency on sample size and freshness of the samples	Glória et al. (1998)
	Ion mobility spectrometry (IMS)	These methods offer rapidity, simplicity, and sensitivity		Sheibani et al. (2008)
	Machine vision (HIS based)	The method offers simplicity and rapidness	Problems include results interpretations	Ataş et al. (2012)

animal feed supplements. The LOD, LOQ, and recovery rate of the method were 3.5×10^{-6}, 1.06×10^{-5} μg/mL, and 88% to 98%, respectively.

Building on the knowledge and understanding of HPLC accumulated over the years led to the development of ultra-HPLC (UHPLC)—a more efficient HPLC-based separation technique that overcomes the limitations of ordinary HPLC (Nováková et al., 2017). The ability to use small particle-packed columns with small diameter of the stationary phase is an advantage UHPLC has over the ordinary HPLC method, as it positively affects both system efficacy and duration of analysis (Huertas-Pérez et al., 2018; Nováková et al., 2006). Additionally, UHPLC is cheaper to run as it consumes less solvents than the ordinary HPLC (Chawla & Ranjan, 2016).

UHPLC has been applied in the detection of mycotoxins in different food samples. For instance, Huertas-Pérez et al. (2018) detected aflatoxins B1, B2, G1, and G2 in rice samples using a UHPLC coupled to chemical postcolumn derivatization and fluorescence detection. According to the authors, the method has limits of detection and quantification below the maximum limits established by the EU regulation for aflatoxins in rice. Recently, Kumar et al. (2020) reported a method for analysis of aflatoxins in animal feeds by UHPLC with fluorescence detection. According to the authors, in pigeon pea husk feed, the method reached an LOQ of 0.5 ng/g for each aflatoxin with recoveries of aflatoxins B1, B2, G1, and G2 as 71.5%, 75.6%, 82.4%, and 78.2%, respectively.

LC-MS/MS has recently gained popularity due to its sensitivity, selectivity, and suitability for multi-toxins detection in food matrices (Woo et al., 2019). Additionally, unlike HPLC, LC-MS/MS does not require derivatization for fluorescent enhancement (Rahmani et al., 2009). A recent international collaborative study by 23 entities evaluated the performance characteristics of a LC-MS/MS procedure for the simultaneous determination of 12 mycotoxins, including aflatoxins B1, B2, G1, G2, and M1 in spices, nuts, milk powder, dried fruits, cereals, and baby food. Relative standard deviations of repeatability and reproducibility and trueness values for each of the analyzed samples confirmed the suitability of the method for analyzing regulated mycotoxins in food samples, including those intended for infants and younger children (Bessaire, Mujahid, et al., 2019). Al-Taher et al. (2017) reported overall recoveries of 81% to 130% in rice, 70% to 119% in barley, 87% to 123% in oat, and 82% to 127% in mixed-grain cereals and a relative standard deviation of < 20% for all analytes in infant cereals using an LC-MS/MS-based technique for the detection of aflatoxins B1, B2, G1, G2, and other mycotoxins. Furthermore, Deng et al. (2020) reported LOD and LOQ ranging 0.1 to 2.0 and 0.3 to 5.0 μg/kg, respectively, using a LC-MS/MS for the detection of mycotoxins including aflatoxins in dried seafood samples.

LC-MS/MS is limited by ion suppression or enhancement due to matrix effects (Li et al., 2011) therefore, to enhance sensitivity and selectivity, MS/MS coupled with UHPLC to determine aflatoxin in food samples has been reported. Liang et al. (2019) developed a UHPLC-MS/MS-based method for the simultaneous determination of several mycotoxins including aflatoxins B1, B1, G1, and G2 in chestnut samples. The technique achieved a LOD and LOQ ranging from 0.02 to 1 and 0.1 to 2 μg/kg, respectively, and recovery rates ranging from 74.2 to 109.5%, with relative standard deviations below 15%. Kos et al. (2016) compared ELISA, HPLC-FLD, and HPLC-MS/MS methods for determining aflatoxin M1 in milk Samples and concludes that both methods were suitable for determining aflatoxin M1 in milk samples.

TLC is one of the oldest chromatographic techniques used to determine aflatoxin in food samples (Wacoo et al., 2014b). It has been used to quantify aflatoxin in different food matrices (Trucksess et al., 1984). TLC's sensitivity combined with its simplicity, cost-effectiveness, and capacity to detect multiple toxins in a single test made it a widely used technique for the screening and quantification of aflatoxin, especially in developing countries (Marutoiu et al., 2004; Miklós et al., 2020; Stroka & Anklam, 2000). Aiko and Mehta (2016) used TLC and HPLC techniques to detect aflatoxin B1 and citrinin in medicinal herbs and spices in India. Additionally, Qu et al. (2018) combined TLC with surface-enhanced Raman spectroscopy for the rapid sensing of aflatoxin B1, recording a detection limit of 1.5×10^{-6}, 1.1×10^{-5}, 1.2×10^{-6}, and 6.0×10^{-7} M for aflatoxins B1, B2, G1, and G2, respectively. However, vulnerability to fluorescence interferences, lack of precision due to accumulated errors during sample application, plate development, and plate interpretation are typical TLC problems.

Efforts to overcome these drawbacks led to high-performance TLC (HPTLC), an upgraded version of the ordinary TLC. HPTLC is more efficient than the conventional TLC method, as it is more selective, accurate, and less prone to error as the sample handling is automated. The main differences between the two techniques are the differences in the stationary phases' particle size sensitivity and data processing methods (Gurav & Medhe, 2018). Using HPTLC, Matsiko et al. (2017) determined aflatoxin in cassava and maize flours, recording a LOD of 0.15, 0.2, 0.2, and 0.5 ppb for aflatoxins B1, B2, G1, and G2, respectively.

As mentioned in Table 4, although they generally offer sensitivity, reliability, and accuracy, chromatography techniques have some significant drawbacks. Chromatography methods generally require a highly trained operator, are

cumbersome, expensive, and are not suitable for on-site use (Maragos, 2004).

5.2 | Immunochemical methods

Immunochemical detection techniques have been used as an alternative to chromatographic detection methods for over a decade now. Aflatoxin determination techniques based on these methods offer simplicity, rapidity, easy-to-use, sensitivity, high sample throughput, and straight forward analysis, often without the pre-analytical steps required in the chromatographic methods (Goryacheva et al., 2007). These methods are principled on the ability of a specific antibody to recognize the three-dimensional structure of a particular mycotoxin (Zheng et al., 2006).

Multiple detection techniques based on this technology have been developed over the years. Enzyme-linked immunosorbent assays (ELISA), lateral flow immunoassays (LFIAs), immunosensors, colorimetric and luminescent sensors, and Surface plasmon resonance sensors are among the techniques based on this technology (Goryacheva et al., 2007; Zheng et al., 2006).

Developed in the 1960s by Rosalyn Yalow and Solomon Berson, ELISA is one of the most widely used immunochemical method for the detection of mycotoxins in food samples (Agriopoulou et al., 2020). The basic operational principle of ELISA is that a range of antibodies is printed on a microplate or column. When an analyte is passed through this plate or column, it is recognized by the antibodies printed on the plate or column to form a complex that then interacts with a chromogenic substrate and creates a readable signal (Bakırdere et al., 2012). There are different formats of ELISA (Aydin, 2015), however, the indirect competitive format is the most widely used for mycotoxin detection (Nolan et al., 2019).

One of the most cherished features of ELISA is its sensitivity as it is one of the most sensitive immunoassays used in the determination of aflatoxin in food samples. Recently different detection ranges have been reported. Peng et al. (2016) developed a monoclonal antibody-based indirect competitive ELISA for the determination of the aflatoxin M1 in milk. The assay exhibit recovery rates ranging from 85.3% to 107.6% and a positive correlation ($r > .99$) when compared to results obtained with HPLC-MS/MS. The LOD and LOQ were 27.5 and 35 ng/L, respectively. In another work reported by Chu et al. (2015) aflatoxin B1 was detected in lotus seeds using an indirect ELISA assay. The LOD obtained was 0.128 μg/L and a good correlation ($R^2 > .978.$) was observed when results obtained with the ELISA method were compared with those obtained with an ultra-fast LC-MS/MS.

Different detection ranges and assay efficiencies have been reported. The analytical capacity of immunoassays is determined by various factors, but principally by the antibody–antigen interaction (Zhang, Garcia-D'angeli, et al., 2014). Other important factors include the type of labels and the concentration of the assay immunogens (Cox et al., 2019). As detailed in Table 4, ELISA is highly sensitive, selective and cost effective, however, false positives or negatives, cross-reactivity and antibody instability are drawbacks that could limit it usage (Sakamoto et al., 2018).

LFIA also referred to as immunochromatographic strip is another immunochemical method widely used for the detection of aflatoxin in food. LFIAs gained attention largely due to their simplicity, portability, and their multiplexibility. Based on the affinity between specific antibodies and an antigen, LFIA are a paper-based test strips made up of a sample pad, a nitrocellulose membrane and an absorbent pad all fixed in a backing card (Bahadır & Sezgintürk, 2016). When a sample is dropped on the sample pad, it moves across the strip via capillary force to react with the immune-reagents immobilized on the membrane and forms a readable complex (Koczula & Gallotta, 2016). There are direct and indirect LFIAs formats, however, the indirect format is the approach used for the detection of small molecules like aflatoxins (Kaiser et al., 2018).

Like other immunoassays, antibodies are the backbone of the LFIAs, their specificity and sensitivity determine the analytical features of the immunoassay. Another important component of the LFIAs is the label. Labeling material are required to be stable under different conditions, detectable over a wide dynamic range and easy to conjugate (Koczula & Gallotta, 2016). Different labeling materials are use in the development of LFIAs including quantum dots (Hu et al., 2017), lanthanide nanoparticle (Salminen et al., 2019), carbon nanoparticles (Zhang et al., 2017), and gold nanoparticles (Chen et al., 2016). The use of gold nanoparticle-specific antibody conjugates in colorimetric LFIA is currently a widely used approach (Urusov et al., 2014). This is principally due to the favorable physiochemical characteristics of gold nanoparticles, including high surface area, affinity with biomolecules like antibodies, among other signal-enhancement convenient features (Koczula & Gallotta, 2016).

Several LFIAs have been reported recently. For instance, Santos et al. (2017) described a LFIA suitable for the detection of aflatoxin in soybean-based foods. The method detected aflatoxin at levels as low as 0.5 ug/kg within 10 min. Versatility is an added advantage of LFIAs as the same strip can be multiplexed for the simultaneous detection of multiple mycotoxins in a single test (Li et al., 2018). Chen et al. (2016) developed a gold nanoparticle-based multiplex LFIA for the simultaneous determination

of aflatoxin B1, zearalenone, and ochratoxin A in corn, rice, and peanut samples. The assay achieved a LOD of 0.10 to 0.13 μg/kg for aflatoxin B1 and a mean recovery rate ranging from 86.2% to 114.5%. Other authors have also reported the simultaneous determination of different mycotoxins using LFIA (Di Nardo et al., 2019; Han et al., 2019). Multi-mycotoxins detection is of particular importance as it lowers cost and could be useful in a scenario where food products need to be screened for different contaminants. A complete review of immunochemical methods used in the detection of aflatoxins has been compiled by Matabaro et al. (2017).

Radioimmunoassay (RIA) is another type of immunochemical method use for the determination of aflatoxin in food (Ayoub et al., 2016). However, the use of RIA is limited principally due to the complexity of the assay, safety concerns in dealing with radioactive materials and extensive incubation periods (Kim et al., 2015). Fluorescence polarization immunoassay is another type of immunochemical method used for the determination of aflatoxin in food products (Zhang et al., 2019).

As detailed in Table 4, immunochemical methods offer numerous advantages and possibilities in the determination and quantification of aflatoxin in food. Suitability for on-site use is indeed an important feature of these methods. However, development of assays that could tolerate different environmental conditions, reduction of matrix effects, and improvement of immunoassay-specialized data management software are still challenges that need to be overcome.

5.3 | Spectroscopic methods

Spectroscopic methods such as near-infrared (NIR), Raman, fluorescence, and hyperspectral imaging (HSI) techniques have been used for the nondestructive evaluation of quality and safety attributes of food and agricultural products for a while now (Boyaci et al., 2015). Some of these techniques are being applied to detect mycotoxins in foods (Wu et al., 2018). Spectroscopic detection methods represent the most widely nondestructive aflatoxin determination methods. They offer a variety of advantages over the previously discussed techniques. They are nondestructive, as such, minimum to no sample preparation is required; they offer the possibility to locate and eliminate contaminated foodstuffs within a lot by simple, rapid, and nondestructive means (De Saeger, 2011). Based on the behavior of light (absorption, emission, and scattering) when interacting with a specimen over a broad wavelength range, spectroscopic techniques have been used in screening and detecting aflatoxin in a variety of food matrixes (Min & Cho, 2015). Equally, some techniques based on this idea have demonstrated good fungal detection ability (Tao et al., 2018). Durmuş et al. (2017) obtained a classification accuracy of 100% using a Fourier-transform NIR reflectance spectroscopy method to group figs into aflatoxin-contaminated and uncontaminated. Durmuş et al. (2015) reported similar results using the same technique in the same matrices. In another study, Chu et al. (2017) obtained classification accuracies of 83.75% and 82.50% for calibration and validation set, respectively, using wave infrared HSI to detect aflatoxin B1 in maize kernels. The authors observed that the classification accuracy of kernels reached 95.56%, 96.15% for low level (< 20 ppb), and 82.35%, 75.00% for high levels (> 100 ppb). Chaitra, and Suresh (2016) obtained and evaluated imaging techniques such as thermal imaging, fluorescence imaging, and color imaging for their suitability for aflatoxin detection in peanut. Results showed that color imaging was more effective in screening peanuts for aflatoxin content; the method obtained an accuracy of 100% and 90.62% depending on the statistical tool used for data processing. The method has also been used for on-processing line sorting and identification of aflatoxin-contaminated nuts. Liu et al. (2019) obtained a 99% specificity and 75% sensitivity for aflatoxin B1 using a laser-based in-line sorting technology in a peanut processing factory. Of 80 trials, the technique with 99% accuracy detected aflatoxin B1 below 10 μg/kg at an average operational speed of 3.2 tons/h. In another work using an on-line laser induced fluorescence spectroscopy system coupled with three collection probes Wu, and Xu (2020) obtained more than 91% accuracy classifying single variety of pistachios contaminated with low concentration of aflatoxin B1.

Although techniques based on this technology offer many possibilities in aflatoxin detection, especially for large-scale production line screening, they are mostly limited to screening as they are still limited in calibration. Their functionality is overly matrix-dependent and are unsuitable for multi-toxins detection.

In response to the co-occurrence of different mycotoxins in food samples, a lot of scientific research has been dedicated to the development of highly sensitive and multi-toxin-capable detection methods. However, a recent study by Sa'ed (2019) indicates that aflatoxin-related scientific research is shifting toward the development of rapid on-site analytical methods. Some of these rapid detection techniques are still in their infancy and come with considerable drawbacks. Therefore, optimizing them to minimize these drawbacks and increase their field usability in developing countries could be helpful in tackling the aflatoxin problem. They will also be useful in screening grains and nuts to determine the necessity for a further laboratory test, saving cost and time in the entire process.

6 | CURRENT AFLATOXIN-CONTAMINATION PREVENTION METHODS

GAPs (timely planting, timely harvesting, use of resistant crop varieties, crop rotation, irrigation, insect control, and proper soil management techniques), good storage (at low humidity and low temperatures) combined with GMPs (sorting of raw materials, washing, dehulling), and Hazard Analysis and Critical Control Points (HACCP) implementation are proven basic effective aflatoxin preventive measures. Additionally, biological, chemical, and plant-breeding techniques are currently being applied to this end (Mahato et al., 2019).

6.1 | Biological preventive measures

Several microorganisms including fungi, bacteria, and yeast possess exploitable features to the benefit of the fight against aflatoxins. To this end, many organisms have been screened for their suitability as aflatoxin bio-control agents. Research on this particular area is currently attracting a lot of interest. Three mechanisms of action are being studied: the antagonistic method (a particular species will out-compete the toxigenic strains for survival in the field), the growth inhibition method (a particular microorganism will prevent the growth and eventual colonization of the toxigenic strains) and inhibition of the fungal–aflatoxin production (Mwakinyali et al., 2019).

Although a good number of microbial species have demonstrated their ability to counter the growth and aflatoxins excretion in toxigenic *aspergillus* species, currently, the most successful bio-control measure is the use of atoxigenic species of *Aspergillus flavus* strains to out-compete toxigenic strains in the field. The idea is to alter a particular area's fungal community, outnumbering the toxigenic fungi strains with atoxigenic strains (Mehl et al., 2012). This method is being successfully commercialized in West Africa (Aflasafe) and the United States (Aflaguard; Gasperini et al., 2019; Weaver & Abbas, 2019). Additionally, in Italy, research is in its final stages to produce aflatoxin bio-control products under the commercial name AF-X1™ (Mauro et al., 2018). A study in Serbia by Savić et al. (2020) reported a 73% reduction in on-field aflatoxin contamination of maize using similar approaches. Many factors, including formulation, inoculation rate, and application time of the bio-control product on the field, are determinant factors in this method's success (Jane et al., 2012). Reasonable contamination reduction rates are reported from areas where this method is applied. For instance, considerable reduction in contamination levels were observed in peanuts and maize fields in Nigeria and Senegal where biocontrol products were applied (Bandyopadhyay et al., 2019; Senghor et al., 2020). However, there are still environmental concerns and some "unknowns" as to this method. As pointed out by Chang et al. (2012), there are concerns that the repeated application of atoxigenic strains may result in an ecological imbalance, and these strains may end up affecting unintended targets.

Additionally, there are concerns that due to the diversity and genetic complexity of *Aspergillus* species, genetic mutations may occur in the atoxigenic *Aspergillus* strains enabling them to develop aflatoxin production capacity (Ren et al., 2020). Therefore, it is recommended that research focus on these concerns but also explore the use of other types of microorganisms to avoid the overdependence on one kind of bio-control agent to enable large-scale and long-term use of this method to prevent aflatoxins-contamination of foods.

Another fungal species that has shown potentials in inhibiting aflatoxin production in *Aspergillus* species is *Trichoderma;* a mycoparasitic fungus, which has in various studies demonstrated the ability to inhibit aflatoxin production (Braun et al., 2018). A laboratory study by Gachomo and Kotchoni (2008) identified two strains of *T. harzianum* and two isolates of *T. viride*, that are capable of suppressing peanut molds' growth and significantly crippling their aflatoxins excretion capabilities. It was also observed that, the degree to which *Trichoderma* species suppressed the growth of peanut molds correlated to their extracellular enzymatic activities.

Research on the potential use of bacterial species to antagonize or inhibit toxin production in toxigenic *Aspergillus* species is active and positive results have been reported at least from laboratory studies (Dorner, 2004). *Bacillus* spp., *Streptomyces* spp., *Pseudomonas* spp., among other bacterial species have demonstrated inhibitory powers against aflatoxin producers (Azeem et al., 2019; Caceres et al., 2018; Schallmey et al., 2004; Shams-Ghahfarokhi et al., 2013; Siahmoshteh et al., 2017; Silva et al., 2015). *Bacillus* spp. is among the most studied microorganisms for bio-control. In fact, according to a recent review by Ren et al. (2020), up to 21% of the published research on microbial control of aflatoxin is related to this microorganism. This could be due to many reasons, including *Bacillus's* competitive and colonization ability, production of lipopeptides and antibiotics, and other features that allow it to outcompete and suppress competitors (Shafi et al., 2017). Siahmoshteh et al. (2017) studied the efficacy of *Bacillus subtilis* and *Bacillus amyloliquefaciens* to prevent the growth and aflatoxin production by *Aspergillus parasiticus* in pistachio samples. Both strains demonstrated the ability to suppress fungal growth and significantly reduce aflatoxin

production. Some of these studies using this organism reported a significant level in inhibiting toxin production in toxigenic *Aspergillus* strains. For instance, Siahmoshteh et al. (2018) reported that *Bacillus subtilis* was able to suppress 92% growth and inhibited aflatoxin production in toxigenic strains of *A. parasiticus*.

Furthermore, macro-molecular organics, organic acids, anti-bodies, and enzymes produced by certain species of *Bacillus*, *Lactobacillus, Streptomyces*, and yeast strains have in laboratory trails shown potentials to inhibit the production of aflatoxins by toxigenic species (Ren et al., 2020).

Moreover, plant extracts are also being screened for their potential use in the control of aflatoxin contamination of agricultural products (Saleem et al., 2017). Bioactive plant compounds like carvacrol, cinnamaldehyde, eugenol, limonene, terpineol, thymol, and turmerone are reported to be effective in suppressing fungal growth and aflatoxin production. Their mechanism against toxigenic fungi includes tampering with the cell membrane, suppressing the ability of the fungus to secretes enzymes involved in the synthesis of cell wall components, weakening its ergosterol metabolism, inducing ultrastructural changes in cell compartments, inhibiting cytoplasmic and mitochondrial proteins and altering the osmotic and the redox balance in fungi (Loi et al., 2020). A recent study by Wang et al. (2019) demonstrated the inhibition power of complex essential oils (cinnamaldehyde, citral, eugenol, and menthol) against toxigenic fungi strains.

Bio-control, without doubt, has shown promising potentials in the fight against aflatoxins-contamination of food. However, there are still knowledge gaps that need to be bridged to realize these possibilities. As noted by Ren et al. (2020), due to the complexities associated with growing these organisms in the field, the majority of the success achieved so far is limited to laboratory studies; therefore, on-field trials must be conducted to enhance understanding of the interaction between the biocontrol agents and environmental factors. It is equally essential to increase and diversify bio-control agents and application methods. New and creative means like bio-active packaging could be other avenues to explore.

Moreover, it is essential to note that before commercial production of any bio-control product, cost considerations should be made; the cost of producing a bio-control product should not outweigh its benefits (Dorner, 2004). The same could be said about the use of plant extracts to counter aflatoxins in food; many of the studies done so far are in vitro. Therefore, there is little knowledge of how plant bioactive compounds will react in an in vivo scenario and how crops' defensive mechanisms will react to these compounds. Additionally, they could be limited in application due to their instability and volatility (Loi et al., 2020).

6.2 | Crop breeding methods

Although there is still no commercially available aflatoxin resistance cultivars, real progress has been registered in the understanding of the host genes responsible for resistance against aflatoxin in peanut and maize (Fountain et al., 2016; Soni et al., 2020). A recent study by Sharma et al. (2018) using biotechnological tools offers a glimpse of hope in the pursue of aflatoxin-resistant peanut. Intense research to identify mycotoxins resistance traits in maize is underway. Scientists from different institutions around the world are using various means including molecular (proteomics, genomics, transcriptomics), and breeding techniques like quantitative trait loci (QTLs), Genome-Wide Association Study (GWAS) techniques, among other means to identify resistant traits in maize (Pandey et al., 2019; Warburton & Williams, 2014). There is hope that inheritable resistance in these crops could be achieved shortly.

6.3 | Predictive modeling of aflatoxin occurrence

Predictive models fed with environmental data such as temperature, humidity, rainfall are currently being employed to predict aflatoxins contamination of crops in the field and during storage. These models have been successfully used to predict the aflatoxin contamination of food crops in Australia and Europe (Ojiambo et al., 2018).

The development of mycotoxin predictive models is a difficult task as the conditions that favors fungal growth may not necessarily mean mycotoxin production, and the presence of the fungal producers of aflatoxin may not necessarily mean a food product contain aflatoxin (Garcia et al., 2009). Therefore, different modeling approaches have been used to predict aflatoxin occurrence in food and agricultural products. Kaminiaris et al. (2020) recently developed a mechanistic weather-driven model to predict *Aspergillus flavus* growth and the aflatoxin B1 contamination of pistachios. Internal validation of the model indicated that 75% of the predictions were correct and the external validation with an independent 3-year dataset shows a 95.6% correct prediction rate. A different approach was used by Jiang et al. (2019) who developed a probabilistic model based on logistic regression and versicolorin A levels to estimate the risk of aflatoxin contamination in stored corn. The model obtained a 96.4% and 93.3% precision in internal and external model validations, respectively.

These kinds of models are preventive in that they provide farmers and decisionmakers the right information in terms of planting, harvesting time, and storage conditions to prevent or limit aflatoxin contamination of crops in the field and stored harvests. However, as pointed by Battilani

and Leggieri (2015) predictive models can never be error-free and therefore their use should be combined with other aflatoxin management methods.

6.4 | Aflatoxin-related awareness creation

Creating awareness among stakeholders (farmer, consumers, policymakers, etc.) does not often receive much attention compared to other aflatoxins mitigation and control measures despite many reports documenting the generally low awareness as to the risks posed by aflatoxins in many developing countries (Udomkun et al., 2018). For instance, A study by Ayo et al. (2018) found that aflatoxin awareness was deficient among uneducated and socially unexposed farmers in Tanzania. Similar low awareness levels are reported from Ethiopia and Uganda (Guchi, 2015; Nakavuma et al., 2020). Studies in Vietnam and Nigeria also reported similar low aflatoxin-related awareness among consumers (Adekoya et al., 2017; Lee et al., 2017). Interestingly, this low awareness among stakeholders regarding mycotoxins' occurrence in agricultural products, is not confined to developing countries. A study by Sanders et al. (2015) revealed that Belgians are more aware of bacterial-related food contamination than mold contamination. In the same study, it was observed that, 39.3% of 140 people working in the agricultural sector did not know whether toxic plants, bacteria, molds, or viruses are producers of mycotoxins.

An increase in the understanding of the issues surrounding aflatoxins-contamination of food, its health, and economic effects, preventive and control measures will go a long way in alleviating the menace. For example, a public information campaign on aflatoxin contamination of maize grains in market stores in Benin, Ghana, and Togo resulted in better handling of maize and reduced aflatoxin contamination levels (James et al., 2007). A more recent study by Anitha et al. (2019) observed that despite adverse weather conditions mean aflatoxin levels in grains were reduced from 83.6 to 55.8 ppb as a result of training farmers on aflatoxin-related issues.

Raising aflatoxin-related awareness among consumers indeed can be a useful tool in preventing human exposure to aflatoxins, however, as an extremely "scientific" topic; care must be taken to avoid misunderstanding and unnecessary panic among consumers. For example, in Ghana and Ethiopia, misleading aflatoxin-related news headlines resulted in panic among consumers, warranting governments' and the scientific community interventions (Stepman, 2018). Therefore, aflatoxin-related risk communicators must ensure that the right information is adequately delivered to stakeholders.

7 | CURRENT AFLATOXIN REMOVAL AND DEGRADATION METHODS

Effective pre- and postharvest aflatoxins-contamination preventive measures are the first line of defense against the hazards associated with aflatoxin-contamination of food. When correctly implemented, these measures prevent or minimize the level of contamination in harvested food crops (Tian & Chun, 2017). However, due to the difficulties associated with preventing aflatoxin contamination of food, Multiple physical, biological, and chemical means have been employed to degrade, detoxify or remove aflatoxin from already-contaminated agricultural products (Wang et al., 2019). Each of these methods uses different mechanisms to degrade or remove aflatoxins, and each comes with certain advantages and drawbacks.

7.1 | Biological methods

Biological methods are believed to be less aggressive, more specific, environment-friendly, and cost-effective compared to other methods of detoxifying aflatoxin. They involve the use of microorganisms and their products to remove aflatoxin through surface adsorption, degradation into nontoxic compounds, and inhibition of their bioavailability by binding (Tian & Chun, 2017).

Several microbial spices are reported to alter the chemical structure of aflatoxin into a nontoxic substance rendering it harmless to human and animal consumers. A study by Harkai et al. (2016) using *Streptomyces* sp. achieved 88% degradation and total elimination of genotoxicity in aflatoxin B1 without forming a new toxin. Similar achievements were reported by Wang et al. (2018) when they used a *Bacillus licheniformis* (BL010) strain to degrade aflatoxin into a nontoxic substance. Strains of another bacterial species, *Actinomycetales*, are reported to be effective in degrading aflatoxin (Lapalikar et al., 2012). A study to elucidate the aflatoxin biodegradation mechanism using three different strains of *Actinomycetes*, Eshelli et al. (2015) concluded that each strain has a different way of degrading aflatoxin. The authors observed that pH and temperature were essential parameters in the process. *Lactobacillus*, a bacterium used in the fermentation and preservation of food, have been reported in numerous scientific literature to remove aflatoxin from contaminated mediums (Moghaddam et al., 2019). Due to its nonpathogenic nature combined with its aflatoxin-removal ability, the possibility to select strains with probiotic characteristics to remove aflatoxin from food is being actively explored by researchers (Elsanhoty et al., 2014; Silva et al., 2015).

Furthermore, some yeast strains through cell-wall adhesion have shown aflatoxin detoxification prowess. A study by Dogi et al. (2017) suggested that yeast could be another candidate for a probiotic against aflatoxin contamination as it has an excellent binding ability and is not new to the food industry. The use of yeast and *Lactobacillus* strains as binders has been extensively studied and continues to attract scientific attention. However, contradicting results have been reported as its effectiveness against aflatoxin, for instance, Blanco et al. (1993) reported that aflatoxin M1 remained unchanged in *lactobacillus*-fermented yogurt, whereas Van Egmond et al. (1977) reported a slight increase in aflatoxin M1 levels in fermented yogurt using the microorganism for fermentation. In contrast, recent studies have reported high reduction rates. For instance, Shigute and Washe (2018) reported 57.33% and 54.04% aflatoxin M1 reduction in natural and lactic acid bacteria (LAB) inoculums-initiated fermentation, respectively, in 5 days of incubation. Kuharić et al. (2018) described a method that yields a 95% reduction in aflatoxin levels without altering the organoleptic characteristics of milk using LAB. According to the authors, the method consists of refrigeration at 4 °C with heat-treated *L. plantarum* KM and then centrifugation and filtering. Apart from this study, few studies have looked into how microorganisms as aflatoxins decontaminants will impact the organoleptic and even the nutritional features of food products.

These reported differences in reduction levels could be attributed to the numerous factors that affect the microbial binding process, including product type, strains of microorganism, pH, incubation period, levels of contamination, and the binding organisms' condition (viable or inactivated). Temperature and inoculum size are equally important factors in the microbial-binding process (Nguyen et al., 2020). It is essential to note the binding between aflatoxin and microorganisms is reversible. A 27.8% to 94.4% reversibility has been observed depending on the strain (Moghaddam et al., 2019). The binding mechanism is not entirely clarified, it is believed that the process involves aflatoxin molecules attaching to the microbial cell walls (Kuharić et al., 2018). This reversibility leads to questions about how the acidic conditions of the human gastrointestinal tract and the presence of bile will affect the bond between aflatoxin and the microorganisms. A study by Huang et al. (2017) indicates digestive tract conditions affect the binding stability of viable lactobacilli strains to aflatoxin; however, it is not significantly affected when heat-killed cells of the same strains are used. Another study reported by Ben et al. (2015) suggest that the bind could withstand the conditions of the gastrointestinal tract.

Interestingly, fungi are being used to degrade aflatoxin. Fungal species, including *A. niger, Eurotium herbariorum*, a *Rhizopus* sp., and atoxigenic species of *A. flavus*, can convert aflatoxin B1 to aflatoxicol (a less toxic substance) by reducing its cyclopentenone carbonyl (Wu et al., 2009). Other fungal species belonging to phylum *basidiomycota*, white-rot fungi such as *Peniophora, Pleurotus Ostretus*, and *Trametes versicolor* are reported to produce oxidative enzymes capable of degrading aflatoxin (Alberts et al., 2009). High degradation levels have been observed from certain fungi species. For instance, Jackson and Pryor (2017) reported a 94% degradation of aflatoxin B1 in naturally contaminated maize using the fungal strain white-rot fungus *Pleurotus ostreatus* (oyster mushroom) as a degrading agent. A study by Zhang et al. (2014) suggests that pH, temperature, and metal ions were essential factors in the fungal degradation of aflatoxins.

Enzymes of different origins have demonstrated to be effective aflatoxin degraders in different conditions and mediums (Xu et al., 2017). *A Laccase* enzyme recovered from *Trametes versicolor* is reported to degrade 87.34% of aflatoxins in a 72-hr incubation (Alberts et al., 2009). In another recent study, Song et al. (2019) demonstrated that an enzyme isolated from *Pseudomonas aeruginosa* degraded aflatoxin B1. As outline in a reviewed by Lyagin and Efremenko (2019), indeed, there are multiple reports supporting enzymes as possible candidates in the degradation of aflatoxins in agricultural products as they are considered mild, safe, and precise. However, there are little data about the degradation mechanism and toxicology of the new products formed from the degradation of aflatoxins by enzymes (Ji et al., 2016).

Other natural substances, including phytochemicals, have also been screened for their ability to detoxify aflatoxins. Friedman and Rasooly (2013) reported that organic citric acid degraded 96.7% of AFB1 in maize with an initial concentration of 93 ng/g. *Trachyspermum ammi* (ajowan), an annual plant from the parsley family, has been reported to detoxify aflatoxin. In an experiment using aqueous extracts of the seeds of *Trachyspermum ammi* Hajare et al. (2005) achieved an 80% reduction in total aflatoxins. In a similar study, dialyzed extracts of the seeds of this same plant degraded about 90% of aflatoxin G1. In the same study, it was observed that the aflatoxin degradation prowess of *Trachyspermum ammi* reduced considerably when its temperature rose to 100°C (Velazhahan et al., 2010). It is believed that alteration of the lactone ring in the chemical make-up of aflatoxin is the mechanism through which these products degrade aflatoxin (Velazhahan et al., 2010).

Biodegradation, due to the absence of elevated temperatures, pressure, and the application of chemicals, is considered the best among the aflatoxin degradation method for food safety and quality reasons. However, for its practical use, some hurdles, including the reversibility

observed in microbial adsorption, incomplete degradation, and extended incubation periods need be overcome. Additionally, some aspects of the entire biodegradation process need clarification. As noted by Ahlberg et al. (2019), in using microorganisms as aflatoxin binders in human food, there are still ethical and safety concerns that need to be addressed. According to the authors, experimental setups and data interpretations from studies on the effectiveness and safety of binding still need to be critically reviewed. Moreover, as pointed out earlier, there are very little data and information as to the safety and bioactivity of the bio-degraded aflatoxin. Another concern is that microorganisms, when introduced to agricultural products as biocontrol agents, would feed on the food to meet their growth needs, thereby multiplying their numbers in the food and possibly excreting undesirable metabolites. A good number of the active plant extracts are generally considered safe for human consumption; however, their practical applicability as aflatoxin-degrading agents in food may be constrained by the fact they can alter the organoleptic characteristics of food products when in direct contact with it (Loi et al., 2020). Although, some authors suggest that this could be overcome by using plant extracts in their vaporized form (Mateo et al., 2017).

7.2 | Chemical methods

Various chemical products have been tested for their aflatoxin degradation and detoxification ability. These including oxidizing agents, reducing agents, acids, bases, among other chemical products (Yang, 2019). In terms of bases used in the detoxification of aflatoxin, ammonia treatment is an extensively studied aflatoxin degradation technique often reporting high degradation rates (Weng et al., 1994). Because aflatoxins are unstable under alkaline conditions, the mechanism of ammonia and other bases against aflatoxin is opening the lactone ring in the toxin's chemical built-up reducing it to a less toxic substance (Moerck et al., 1980). The degradation rate is mainly dependent on certain intrinsic and extrinsic factors of the substrate. For instance, a study by Weng et al. (1994) concluded that during ammonia treatment of contaminated maize samples, aflatoxin degradation rates increased with moisture content and temperature of the medium. Pressure and the type of substrate are also determinant factors in the efficacy of ammonia against aflatoxin (Cucullu et al., 1976). Other bases that have demonstrated various degrees of success in the degradation of aflatoxin include sodium hydroxide and calcium hydroxide (Čolović et al., 2019). Another alkaline-based treatment process is nixtamalization, a food processing technique of Mexican origin; it is a known aflatoxin-decontamination technique. It involves heating of cereal grains in abundant limewater ($CaOH_2$) and then steeped for 8 to 16 h before the solution is decanted. The grain is thoroughly washed to leave the grain ready for milling to obtain the maize dough for making the tortillas (Méndez-Albores et al., 2004).

Ozone, an oxidizing agent, has been postulated as another aflatoxin-degrading agent. A good number of published literatures reported high rates of aflatoxin degradation with ozone. It is recognized as generally recognized as safe (GRAS) by the FDA (FDA, 2001). The mode of action involves its reaction with the C8 to C9 double bond of the furan ring of aflatoxin through electrophilic addition, resulting in the formation of primary ozonides and subsequence reformation of derivatives such as aldehydes, ketones, and organic acids (Proctor et al., 2004). In a study reported by Porto et al. (2019), a corn grits product was treated with gaseous ozone resulting in a 57% reduction in the levels of aflatoxin. A separate study to determine the factors that affect ozone detoxification of aflatoxin El-Desouky et al. (2012) reported that the efficacy of ozone increases with the level of contamination and the amount of time the product is subjected to the ozone. Another oxidizing agent that has been used in the degradation of aflatoxin in food is hydrogen peroxide. It has been reported to have degraded aflatoxin in various matrices, including milk, figs, corn, peanut products, and other substrates with time being an essential factor in the degradation process (Fountain et al., 2015; Karlovsky et al., 2016). Additionally, certain chemical food additives including sodium bisulfite ($NaHSO_3$) sodium hydrosulfite ($Na_2S_2O_4$) and sodium metabisulfite ($Na_2S_2O_5$) have been reported to be effective in degrading aflatoxin into nontoxic products (Temba et al., 2016).

The use of chemical treatments to decontaminate aflatoxin-contaminated agricultural products has been successfully applied in different settings, process parameters and food products, and has proven effective and, to some extent, regarded as safe. However, some concerns in terms of food safety, food quality, and environmental issues remain unsolved. Not all the approaches herein mentioned are approved for application in human food production. According to the FAO for any detoxification measure to be considered fit for use on human food it is required: to inactivate, destroy, or remove aflatoxin; not produce nor leave toxic/carcinogenic/mutagenic residues on the treated substrate; retain the nutrient, sensory or other quality attributes of the food product and must be capable of eliminating fungal and their remnants spores or mycelium that could proliferate and produce new toxins (Shi, 2016). The use of chemical reagents raises the concern of residues remnants that could be toxic and unsafe for human and animal consumption. Furthermore, current research has extensively focused on only degrading

aflatoxin paying little attention to the degraded products resulting in little knowledge as to the toxicology and safety of the substances formed when aflatoxin is chemically degraded. Research data from such studies could be useful in evaluating the safety and effectiveness of degradation methods.

7.3 | Physical decontamination, removal, and degradation of aflatoxins

Sorting, segregation, sieving, washing, dehulling, floating, milling, heat treatment, and other physical means are used to decontaminate aflatoxin-contaminated agricultural products. Though tedious and ineffective for extensive scale application, hand sorting and segregation of grains base on their physical features have been reported to be effective in reducing aflatoxin in agricultural products. Damaged and broken grains in a lot carry a more considerable amount of mycotoxins; therefore, their removal reduces the overall contamination in the lot (Dickens & Whitaker, 1975). Matumba et al. (2015) studied the effectiveness of hand-sorting, flotation, and dehulling on the decontamination of mycotoxin-contaminated white maize and observed that hand-sorting is more effective as around 94% reduction was observed in the aflatoxin levels of hand-sorted maize samples. The authors observed that floatation has the least effect on the levels of aflatoxins. Another study by Xu et al. (2017) revealed a 96.7% reduction in aflatoxin levels in contaminated peanuts when hand-sorted. Zivoli et al. (2016) reported similar reduction levels using hand-sorting in apricot kernels.

For practical reasons and perhaps due to increased production volumes, grain and nuts sorting has come from handpicking through air-floating, mechanized sorting based on grain size and color to sensor-based optical sorting. Currently, UV light illumination is being used to segregate aflatoxin-affected products (Karlovsky et al., 2016; Leslie & Logrieco, 2014; Stasiewicz et al., 2017). Cheng et al. (2019) used UV to NIR spectroscopy to segregate aflatoxin-contaminated single corn kernels.

Certain food processing Unit Operations such as wet and dry milling, dehulling, and thermal treatments (extrusion, roasting, and cooking), decrease aflatoxin levels in processed foods (Kaushik, 2015). In milling, the toxin is redistributed to the process by-products, including the germs and bran (Park, 2002). Zhong et al. (2015) observed that 60 s of milling reduced the concentration of aflatoxin B1 fivefold and completely removed all aflatoxin B2 in rice, but significantly increased aflatoxin levels in the bran. Brera et al. (2006) studied the effect of industrial milling processes on the distribution of aflatoxin and zearalenone in milled fractions of two corn lots. They observed a four-time reduction factor in aflatoxin levels in the end product of the processed maize and a significant increase in the by-products like germs and bran. It is essential to mention that conventional cooking temperatures have minimal effects on aflatoxin levels as it decomposes at temperatures ranging from 237 to 306 °C (Rustom, 1997). Therefore, it is not advisable to entirely depend on cooking to prevent human exposure to aflatoxins (Kabak, 2009).

Blanching is another physical method known to be effective in removing aflatoxin in contaminated nuts (Dorner, 2008). Mahoney et al. (2020) observed that blanching reduced total aflatoxins in naturally contaminated almonds by 13% to 76%, depending on the quality of the almond and the process time-temperature combination. In another study Roby and Samah (2019) indicated that blanching reduced 30% of the total aflatoxins in artificially contaminated tiger nuts. Dorner (2008) suggested that combining blanching with color-aided sorting is an effective strategy in removing aflatoxin from contaminated peanuts. He reasoned that blanching removes the seed coat from the kernels enhancing the identification of discolorations associated with aflatoxin contamination in the kernel tissue. Similarly, Anyebuno et al. (2018) indicated that manual sorting of blanched peanuts kernels, offers a practical possibility in reducing aflatoxin levels to below regulatory limits. It has also been observed that the combination of roasting, blanching and sorting can reduce aflatoxin accumulation in stored peanuts (Darko et al., 2018).

Irradiation, a widely accepted and extensively studied food processing technology, is reported to be effective in aflatoxin decontamination. Sometimes referred to as "cold pasteurization," it involves subjecting prepackaged or bulk foodstuffs to ionizing energy (Calado et al., 2014). The effectiveness of irradiation in degrading aflatoxin is conditioned by certain factors, including the initial levels of aflatoxin in the matrix, the irradiation dose, physical state, and type of matrix (Calado et al., 2014). High oily substrates reduce the irradiation process's effectiveness (Ghanem et al., 2008). More sophisticated and novel physical measures, including microwave heating (Mobeen et al., 2011) and cold plasma (Gavahian & Cullen, 2020), among other technologies have shown promising results in the decontamination of aflatoxins in a variety of food samples.

Physical methods like sorting, handpicking, floating are useful but only suitable for small-scale applications, and processes like wet-milling may redistribute the toxins to process-waste products that may be used as animal feed, which may eventually lead the toxins back to human food. Another critical issue to consider is the use of high temperatures in the physical degradation of aflatoxins may lead to nutritional loss and alteration of food products' organoleptic characteristics.

8 | CONCLUSION

This review details the fungi producers of aflatoxins, agricultural and food products prone to aflatoxins contamination, aflatoxins regulations around the world, current aflatoxins detection methods, and current aflatoxins preventive and curative measures. Despite the amount of aflatoxin-related scientific information gathered from 1960 to date, aflatoxin-contamination of food remains a significant food safety challenge globally. To no small extent, the preventive and mitigation efforts applied so far have proven insufficient, as evidenced by the high exposure levels in a considerable number of countries. Total elimination of aflatoxins in the global food chains will be close to impossible, as its production by toxigenic molds is dependent mainly on environmental factors that are beyond human control.

Effective implementation of GAPs, GMPs, HACCP, proper storage, informed stakeholders and the ability to predict on-field and in-store contamination of food products could serve as first line of defense against human exposure to aflatoxins. On a long-term basis, it is essential that on-field contamination of crops is minimized either through the deployment of bio-control measures or the use of resistant crop varieties. As logic dictates, preventive measures should be preferred over aflatoxin curative measures as there are still open knowledge gaps that need to be bridged. Regulations, when effectively enforced, are the last line before the food gets to the consumers. Greater portion of countries in the world have enacted aflatoxins-related regulations. Despite being an aflatoxins hotspot, the majority of African countries are without aflatoxins regulations. Precise, accurate, affordable and simple aflatoxin detection means are required not only to enforce regulations but to gauge the effectiveness of aflatoxins control measures. Current chromatographic, immunochemical, and spectroscopic methods used to determine aflatoxins levels are effective, but new or improved methods to enhance the speed, detection, and accuracy of aflatoxin analysis would lead to safer food and agricultural products.

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AUTHOR CONTRIBUTIONS

The idea was initiated and drafted by Abdoulie Jallow, who prepared the initial draft and final version. Huali Xie and Xiaoqian Tang enriched the literature; Zhang Qi and Peiwu Li proofread and edited the manuscript. All the involved authors approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Abdoulie Jallow https://orcid.org/0000-0002-4097-8666

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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