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INVITED REVIEW

THE AFLATOXIN OCCURRENCE OF FOOD COMMODITIES IN SRI LANKA: AN OVERVIEW OF PREVALENCE, DETECTION AND DECONTAMINATION TECHNIQUES

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ABSTRACT

Aflatoxins has been a topic of discussion in Sri Lanka over the recent past, due to many reported cases of aflatoxin prevalence in several food commodities. Most food spoilage and deterioration are attributed to fungal and mold attacks, whereas aflatoxins are a secondary metabolite produced by the fungal genus *Aspergillus* that dominates the food supply chain. Primarily four types of Aflatoxins; AFB1, AFG1, AFB2, and AFG2 were reported in food contamination and two types; AFM1 and AFM2 were reported to contaminate feedstuff and thereby present in milk and dairy products. Aflatoxins were given focused attention of this caliber due to their extreme toxicity which causes several diseases including aflatoxicosis in humans, livestock and domestic animals globally. The occurrence of these aflatoxins and a few outbreaks were reported in several food types worldwide including Vegetable oils, Nuts and Seeds, Cereals and Dairy products. In Sri Lanka, such occurrences have been reported since the early 1980s, to date. Considering the severity of toxicity, there have been several legislations and regulations introduced both locally and internationally. The Codex Alimentarius, FDA, and EU Legislation are some international regulatory bodies, while Sri Lanka has the regulations defined by the SLSI. According to the studies conducted in recent years, the reported occurrences of Aflatoxins in food and feedstuff were significantly reduced to the previous studies which could result from proper housekeeping practices, proper storage and adopting proper decontamination techniques. However, controlling and decontamination of the aflatoxins in food is a necessity to stay within the regulated maximum permissible levels by the regulatory bodies. Controlling Aflatoxin contamination may be either pre-harvest, which involves good agricultural practices, fungicide application, pest control and novel biocontrol techniques such as introducing nontoxigenic aspergillus strains to the crop produce for competing with toxigenic strains, or Post-harvest such as physical, chemical and biological decontamination methods.

Keywords: Aflatoxins, aflatoxin toxicity, aflatoxin occurrence, aflatoxin regulations, aflatoxin decontamination

INTRODUCTION

The majority of spoilage and deterioration in food and feed crops is caused by fungi and molds (Magan *et al.* 2004). Fungi thrive in tropical and subtropical conditions when temperature, humidity content, and improper post-harvest practices are in favor of their growth (Rawat 2015). Fungi can affect crop production at many stages, including in the field, harvest, transportation, and storage.

Aspergillus is the fungus that dominates the food supply chain in areas with wet and warm climates; producing aflatoxins, a toxic secondary metabolite, which are among the most significant mycotoxins in terms of their occurrence, toxicity, and impacts on human health and trade. They are particularly important as food spoilage organisms (Cotty

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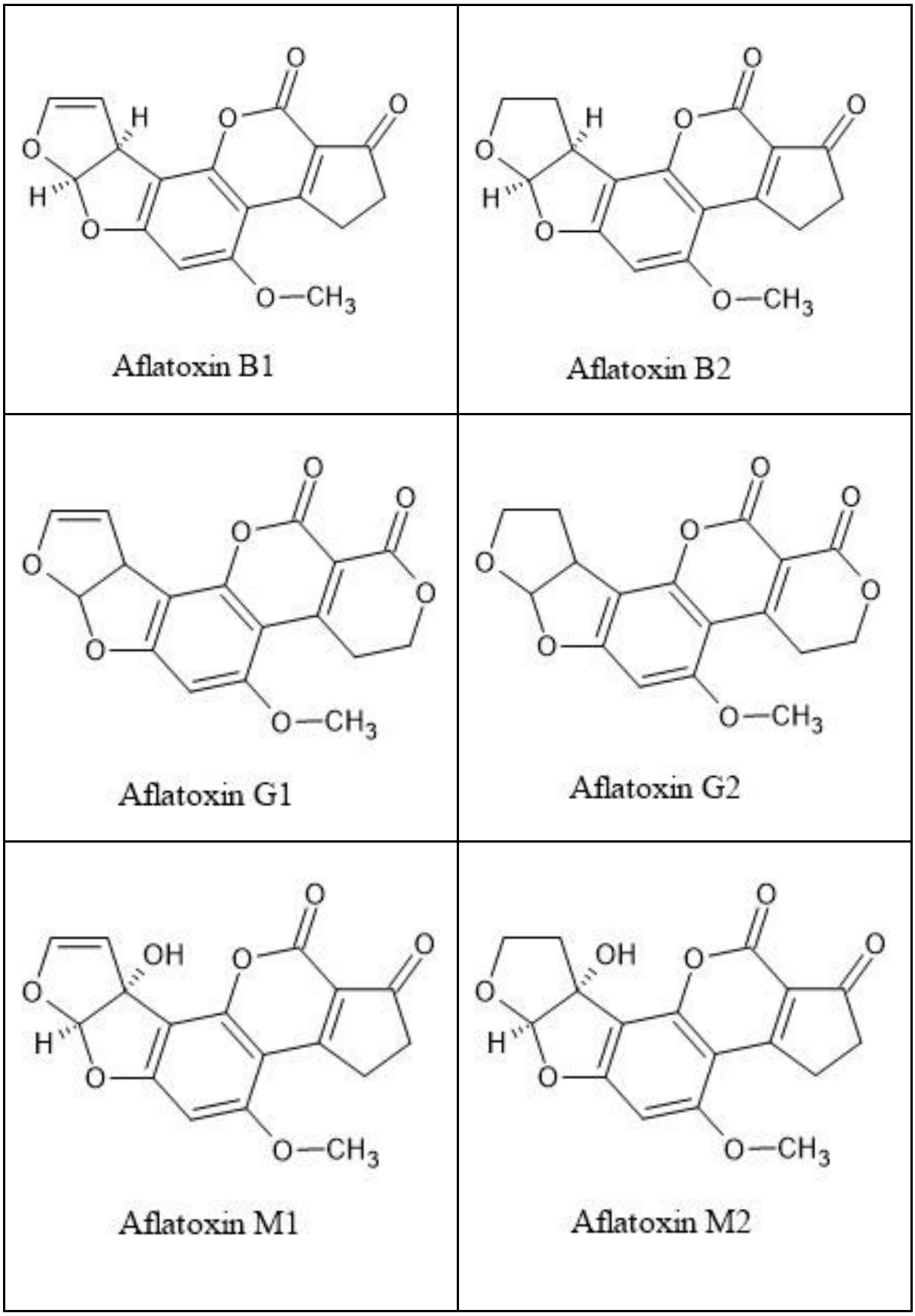

Figure 1: Chemical structure of different types of aflatoxin

and Jaime-Garcia 2007) Changes caused by Aspergillus species in food can be sensorial, nutritional, or qualitative in nature, such as rotting, pigmentation, discolouration and the development of unfavorable odors and flavors (Perrone *et al.* 2012).

While the *Aspergillus* genus has more than 200 species, only less than twenty species are known to produce aflatoxins. *Aspergillus flavus* and *Aspergillus parasiticus* are two species that produce toxic secondary metabolites; aflatoxins (Gourama and Bullerman 1995). Aflatoxins contaminate a variety of foods and stored grains, endangering the health of humans, livestock and domestic animals with various diseases such as aflatoxicosis worldwide.

The Food and Agriculture Organization (FAO) of the United Nations estimates that mycotoxins contaminate more than 25% of all agricultural products, with aflatoxins (AFs) being the most common and prevalent among them, primarily found in nuts, grains, seeds, and spices (Moretti *et al.* 2017).

Aflatoxin B1, B2, G1, and G2 (AFB1, AFB2, AFG1 and AFG2) are the four most common types of aflatoxin found naturally as dietary contaminants (Goldblatt 2012). Their structure consists of a bifuran ring fused to a coumarin nucleus with a pentenone ring or a six-membered lactone ring in G aflatoxins (Fig. 01). Aflatoxin M1 and M2 are the hydroxylated metabolites of aflatoxin B1 and B2 and they have been discovered in edible animal products such as meat and milk. Aflatoxin B1 is one of the most toxic and prevalent members of the aflatoxin family (Herzallah 2009; Sartori *et al.* 2015).

2.0 Toxicity of mycotoxigenic fungi in food and feed

Several species of filamentous fungi produce mycotoxins, which can contaminate food, feed, and raw materials (Richard 2007; Ashiq 2015; Jeswal and Kumar 2015). In addition, they cause mycotoxicosis in humans and animals. *Aspergillus*, *Penicillium*, and *Fusarium* are the most common genera of mycotoxigenic fungi, but *Trichoderma*, *Trichothecium*, and *Alternaria* are also important as food contaminants or plant pathogens, among others (Moss 1994; Richard 2007, Ashiq 2015).

The human body faces significant threats from mycotoxins, particularly AFs and ochratoxin A (OTA) which are produced by *Aspergillus* fungi sections Flavi, Ochraceorosei and Nidulantes. Aflatoxins are potent carcinogens that are associated with the hepatitis B virus and cause thousands of deaths each year, primarily in non-industrialized tropical countries. (Richard 2007; Shephard 2008).
The four major types of aflatoxins, AFG1, AFB1, AFG2 and AFB2 that are produced by the main two species of Aspergillus; *A. flavus* and *A. parasiticus* are known to contribute significantly to the contamination of agricultural commodities and have been classified by the International Agency for Research on Cancer as carcinogenic to humans (European Food Safety Authority (EFSA) 2020).

A. flavus, which is mostly found in soil, corn, cotton seeds, and tree nuts, produces only B group aflatoxins, whilst *A. parasiticus* in peanuts produces aflatoxin B and G. Aflatoxin M1 is produced in the liver of animals after ingestion of high levels of aflatoxin B1 through animal feed, and it is excreted in animal milk and urine. Aflatoxin M1 could enter the human body indirectly through the consumption of aflatoxin-contaminated milk and milk-based products. Aflatoxin B1 is the most powerful and dangerous and abundant toxin, and it is categorized under class 1 carcinogen in humans. The heterogeneity of AFB1 makes its risk more challenging (Gourama and Bullerman 1995; Razzaghi-Abyaneh *et al.* 2006; Milićević *et al.* 2010; Mahmoudi *et al.* 2014; Mollayusefian *et al.* 2021).

AFs interact with the cell's basic metabolic pathways, disrupting key enzyme processes such as carbohydrate and lipid metabolism, as well as protein synthesis. Moreover, aflatoxin toxicity may cause acute or chronic diseases in humans (Dawson 1991) and outbreaks of aflatoxin hepatitis in humans were reported in

India in 1975 and Kenya in 1982 (Bhat 1991). In malnourished children and Kwashiorkor victims, aflatoxin is also linked to hepatocellular damage, necrosis, cholestasis, hepatoma, acute hepatitis, haemorrhage, jaundice, and cirrhosis (Groupman *et al.* 1988). Moreover, a number of expert groups have examined the health effects of aflatoxins.

The Joint FAO of the United Nations/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (2017) projected potency values for AFB1 based on epidemiological data. The JECFA evaluated AFM1 separately from AFB1 due to its potential presence in milk and milk products from livestock-fed aflatoxins contaminated feed. The JECFA concluded that AFM1 should be presumed to induce liver cancer in rodents via a mechanism similar to AFB1 and that figures of AFB1 potency can be used to assess the risk due to AFM1 intake, including those for populations with a significant prevalence of hepatitis B virus carriers. Based on a comparative study in the Fischer rat conducted by Schrenk et al. (2020) the carcinogenic potency of AFM1 was estimated to be one-tenth that of AFB1.

3.0 Aflatoxin Occurrence in Food and Feed

Aflatoxins are found in a variety of food matrices, including spices, cereals, oils, fruits, vegetables, milk, meat, spices, and dried fruits (Martinez-Miranda *et al.* 2019). Aflatoxins pose a chronic risk to approximately 4.5 billion people, the majority of whom live in developing countries. According to Eskola *et al.* (2019), approximately 60% to 80% of global food crops are contaminated with mycotoxins, which even exceeds the FAO figure of 25%.

Impact of Climate Change on Aflatoxin Production

Climate change significantly impacts the quality and occurrence of aflatoxins in staple foods used in consumption. The change in climate simultaneously impacts the occurrence of complex communities of AF-producing fungi by altering the number of AF -producers to change its fungal community's structure (Mahato *et al.* 2019). *A. flavus* has highly evolved physiological mechanisms to acclimatize to adverse climatic conditions (Nesci *et al.* 2004; Magan 2007). Climate change alters the temperature and water activity (aw) in the environment which further influences the gene expression to produce AFs. The conditions of temperature and aw regulate the extent of fungal growth and AFs production (Schmidt-Heydt *et al.* 2009; Schmidt-Heydt *et al.* 2010). As per the finding of Moretti *et al.* (2019), AFB1 in cereals in the European Union as a result of climate change, for every 2 °C increase in temperature, there is an increase in AFs risk in the various regions of Spain, Italy, Greece, Portugal, Bulgaria, Albania, Cyprus and Turkey. It is estimated that the risk for AFs contamination in maize is likely to increase in Europe due to favorable climatic conditions for *A. flavus* in the next 30 years. Therefore, it is required to adopt proper detection techniques and control measures to combat the burning issues of AFs in food and feed.

3.1 Crops Affected by Aflatoxins

The most common foods for human consumption worldwide are cereals and cereal -based products (Temba *et al.* 2017). Due to changes in agricultural practices, rice and corn are most commonly contaminated by AFs under natural conditions. AFs are produced both before and after the harvest of a crop (Hesseltine 1974).

It is found that the crops are fungal contaminated particularly under certain conditions such as dry weather at the onset of crop maturity, higher degree of moisture content, and improper drying and storage conditions (Stepman 2018). Rice is more susceptible to AF contamination where the contamination is caused by the improper drying of rice grains leaving a high moisture content (>14%). These fungi consequently degrade the quality of the grains while also discoloring the grain and/or husk (Oyebamiji *et al.* 2021). Further, the groundnuts and beans are frequently used in many African diets known as highly susceptible to contamination by AFs in both field and storage conditions (Khan *et al.* 2021). Spices

can also be contaminated and afflicted with AFs due to improper storage and processing conditions. Furthermore, the presence of AFs in eggs collected from a poultry farm and in raw cow milk in Cameroon was reported by Tchana *et al.* (2010). Due to this, the affected crops contribute to the entry of AFs into the food chain, which is strongly dependent on climatic conditions. The occurrence of aflatoxin in food products varies geographically and the toxicity in particular food products depends on the frequency of consumption by different ethnic groups and nations.

Figure 2: Occurrence of aflatoxins in food crops

3.2 Occurrence of aflatoxin in Sri Lankan context

Due to reported and foreseeable health risks, the public in Sri Lanka is concerned about food safety hazards originating from primary production, contaminants, adulterants, and unsafe chemical use in commonly consumed food items. Aflatoxin contamination in food and feed receives the most attention, among all possible contaminations.

Aflatoxin contamination occurs in a wide range of regional crops and food commodities such as edible vegetable oils (Karunarathna *et al.* 2019), corn (Jayaratne *et al.* 2020), peanut (Dissanayake and Manage 2009), cow milk (Pathirana *et al.* 2010), rice (Bandara *et al.* 1991), spices (Rajasinghe *et al.* 2010), etc.

3.2.1 Vegetable Oils

Coconut oil

Coconut oil is the most widely used vegetable oil in Sri Lanka (Boateng *et al.* 2016). It is directly added to and/or used in frying food during cooking or in food processing industries, and it is preferred over animal oils due to health concerns. The growing population and the discovery of new uses for oils have increased the demand for vegetable oils. Around 15-20% of the annual fresh coconut produce is used for the production of coconut oil (primarily copra oil), which mainly serves the domestic market (Aiome *et al.* 2022).

The contamination of coconut-based products, including coconut oil, with aflatoxin in Sri Lanka, has been studied previously during the 1970s and 1980s (Arsecularatne and de Silva 1971; Samarajeewa 1975; Samarajeewa *et al.* 1983). According to the given standards by Sri Lanka Standard Institute (2017) on Specification for Coconut oil, the product is expected to contain an AFB1 aflatoxin level not exceeding 5.0 µg/kg and 10.0 µg/kg for total aflatoxin.

As per the reported results of the study by Arsecularatne and de Silva (1971), aflatoxin contamination was found in six out of ten coconut oil samples taken for testing, ranging from 50 g/kg to over 1000 g/kg. Two subsequent studies reported a mean AFB1 level of 50 g/kg in 116 coconut oil samples (Samarajeewa 1975), and a mean AFB1 level of 186 g/kg in 115 coconut oil samples, ten of which had AFB1 contamination in the range of 500-5000 g/kg (Samarajeewa *et al.* 1983). Another study by (Samarajeewa and Arseculeratne 1983) found AFB1 contamination ranging from 50 to 1000 g/kg in 45% of 95 coconut oil samples.

In recent decades, it has been discovered that nearly 20-30% of locally produced as well as imported coconut oil samples were contaminated with aflatoxins. Aflatoxin contamination levels in coconut oil ranged from 2.25 to 72.70 g/kg. The contamination level of aflatoxin B1 in coconut oil ranged from 1.76 to 60.92 g/kg (Karunarathne 2019). Aflatoxin contaminants detected in recent studies are comparatively lower than in

previous studies (Karunarathne 2019). The highest AFB1 level in a recent study 60.92 g/ kg vs. 5000 g/kg in Samarajeewa *et al.* (1983) is an obvious example of this. It further likely provides indirect evidence for improvements in the coconut oil industry over the years (in terms of controlling aflatoxin contamination), which may have resulted from increased consumer and industry awareness of the potential health impact of aflatoxins, as well as global and local food safety initiatives such as the implementation of maximum permissible levels. While some of the determined values exceed the current European Union regulatory limits, aflatoxin contamination in the coconut oil industry remains a significant concern in Sri Lanka, and consumers are at risk for health problems. Aflatoxin contamination in coconut oil is suspected to be caused by poor agricultural practices that promote toxigenic fungal growth on copra. The only way to solve the problem is to have market coconut oil tested at accredited laboratories for the presence of aflatoxins. Furthermore, introduce good manufacturing practices to the industry.

As to Guarte *et al.* (1996), aflatoxins in coconut oils are primarily attributable to the contamination of copra/ dried coconut kernels with aflatoxins. Coconut kernels are typically dried in the sun before being extracted to reduce their water content in the production of regular coconut oil. Inadequate or insufficient drying of the kernels, poor handling practices, insect and rodent damage during improper storage, rainy weather drying, inefficient storage in damp rooms, and poorly ventilated rooms frequently promote fungal colonization in copra, increasing the likelihood of aflatoxin contamination. Moreover, Einolghozati *et al.* (2021) have highlighted that during the oil extraction process, aflatoxins can transfer to the final edible coconut oil product. Virgin coconut oil, in contrast to regular coconut oil, is extracted directly from fresh, matured coconut kernel meat rather than from copra, which limits the potential for fungus growth. According to the annual report of the Coconut Development Authority (2015), various adulterations were found in 76% of the 150 coconut oil samples tested in Sri Lanka. Because the samples were obtained from retail stores rather than directly from oil mills, unbranded regular coconut oil samples could be adulterated with cheaper substitutes. This may be a reason for the low levels of aflatoxins detected in them (Aiome *et al.* 2022).

Other vegetable oils

The other more commonly used types of vegetable oils include olive oil, soybean oil, sunflower oil, palm oil and sesame oil. Aflatoxin contaminations in these edible oils have been investigated by various scientific works to assure their level of safety with regard to consumption in the cooking and food industry.

A research study conducted by Karunarathna *et al.* (2019) on contamination of aflatoxin in various edible oils in Sri Lanka, palm olein, sunflower oil, sesame oil, olive oil, soya bean oil, and corn oil had been reported with negligible levels of total aflatoxin contamination, making them compliant with the European Union's regulations on the maximum permissible limit for aflatoxins. The lack of higher aflatoxin levels in these oils could be attributed to proper harvesting and handling practices, as well as storage conditions of oilseed crops, which limit aflatoxigenic fungal growth in oil seeds/fruits. Aflatoxin levels in final products are effectively reduced by refining procedures used in vegetable oil processing as well as detoxification steps. Furthermore, studies indicate that soybean and olive are poor substrates for aflatoxin production (Gupta and Venkitasubramanian 1975; Nesheim and Wood 1995; Eltem 1996).

3.2.2 Spices

Sri Lanka is a tropical nation in Asia known as "Spice Island" that produces several highly valued spices. In Sri Lankan cuisines, chilies/ hot peppers are mainly used, along with cumin, turmeric, black pepper, cinnamon, cardamom, and mustard (Kunnumakkara *et al.* 2009). The island's prevailing climatic conditions are ideal for spice cultivation, but they are also ideal for mold infestations and thus, contamination with mycotoxins. The

ability of molds to produce mycotoxins is heavily influenced by environmental factors, the most important of which are temperature, relative humidity, insect damage, drought, and insufficient storage conditions (Miraglia *et al.* 2009; Prandini *et al.* 2009).

The AFs and/or OTA contamination of spices has been reported in several countries, including Turkey, Hungary, Malaysia, Spain, India, and Pakistan. (Reddy *et al.* 2001; Fazekas *et al.* 2005; Aydin *et al.* 2007; Paterson 2007; Santos *et al.* 2010; Jalili *et al.* 2010). Recent studies report the presence of multiple mycotoxins in Sri Lankan pepper and chili, the two most commonly used spices in Sri Lankan cuisine (Yogendrarajah *et al.* 2014a; Yogendrarajah *et al.* 2014b).

Hot peppers (*Capsicum annuum* L.), also known as chili, are becoming increasingly popular as spices, vegetables, and for other purposes. Powdered chili is a popular spice in South Asia, and it is commonly used to flavor, season, and add aroma or color to foods. Chili is the main ingredient in curry and chili powder, and it can also be used to make pepper sauce, red pepper, and paprika. Chili, on the other hand, are highly susceptible to aflatoxin contamination, which is influenced by atmospheric temperature, humidity (a temperature between 27 ºC and 38 ºC with a relative humidity of 85% is ideal for the growth of *Aspergillus flavus*), insects, and drying and processing conditions. Furthermore, as a developing country, Sri Lanka faces challenges in producing high-quality spices for domestic consumption as well as adhering to trade regulations imposed by importing countries (Rajasinghe *et al.* 2010). At the lower bound scenario, the mean exposure to AFB1 in the Northern (3.49 ng/ kg BW/day) and Southern (2.13 ng/kg BW/ day) regions of Sri Lanka exceeded the tolerable daily intake due to chili consumption, indicating the foreseeable need for Sri Lankan chili producers to pay close attention to their processing lines in order to achieve safer production for consumption (Yogendrarajah *et al.* 2014).

When conditions are favorable, aflatoxin contamination can occur in the field during crop production and during storage. According to Rajasinghe *et al.* (2009), long-term storage of spices causes aflatoxin production regardless of storage temperature; therefore, it is best to consume spices such as chili powder, turmeric, and so on within a relatively short period of time to avoid the accumulation of aflatoxins. In some nations, spreading chili on soil in a single layer and letting it dry in the sun is a common post-harvest practice known as sun drying. Some chili is contaminated with fungus as a result of the drying processes on the soil.

Black pepper, also known as the "King of Spices," can be used for a variety of purposes, including preservatives, biocontrol agents, and immense pharmacological benefits (Butt *et al.* 2013). The dried mature peppercorns of the vine *Piper nigrum* L. are used to make black pepper. The buff-colored seeds of white pepper are obtained by removing the outer pericarp of ripened red pepper berries (Raghavan 2000). After Vietnam, Brazil, Indonesia, and India, Sri Lanka is the fifth largest exporter of black pepper (Institute of Policy Studies 2017). Because of the high pungent principle piperine (15%), Lankan pepper commands a premium price in the global market (Jansz *et al.* 1983). A study on the presence of aflatoxins in Sri Lankan chili and pepper by Yogendrarajah *et al.* (2014), found that chili samples (87%) were more frequently contaminated with mycotoxins than pepper samples (65%) (Lisbeth *et al.* 2016). Among the mycotoxins discovered were AFG2, AFG1, AFB2, AFB1, FB1, OTA and sterigmatocystin. In terms of AFs contamination, 20% of the pepper samples were contaminated with at least one of the AFs (Limit of Quantification to 17.3 mg/kg), with AFB1 contaminating 73% of them.

Spices are primarily grown in developing countries with tropical and/or semi-tropical climates, such as Sri Lanka, and are exported worldwide (Ribeiro-Santos *et al.* 2017). In these growing areas, high temperatures, high rainfall, and relative humidity are ideal for fungal proliferation and mycotoxin production. Moreover, in developing countries

where peppers are grown, a lack of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) is the main consideration. Fungi from the genera *Aspergillus, Fusarium*, and *Penicillium* pose serious phytopathological and mycotoxicological risks in a variety of food products because they can produce several mycotoxins that cause serious problems for animal and human health around the world (Tsitsigiannis *et al.* 2012).

Spices are subjected considerably to microbial contamination from farm to fork due to poor collection conditions, an unpretentious production process, and long drying times. The traditional method of drying spices in the open air under the sun is still widely used in Sri Lanka, potentially exposing them to contamination and thus, need a proactive approach for combating the aflatoxin contamination throughout the process.

3.2.3 Cereals

Corn

There are a number of cereals grown in Sri Lanka that have been tested for aflatoxin contamination. Corn is one such agricultural produce for which aflatoxin levels have been reported, though there have been fewer scientific studies conducted in the Sri Lankan context. (Jayaratne *et al.* 2020). Corn is high in carbohydrates, making it an ideal substrate for *Aspergillus flavus* conidial and sclerotial development. *A. flavus* is visible on corn in the field as a greenish-yellow to yellowish-brown powdery mold growth on or between the corn kernels. According to the US FDA (2000), the toxic or deleterious level of aflatoxin from corn consumption is 20 ppb (Erdogan 2004).

Recently in 2021, Sri Lanka's maize prices suddenly soared and fears of aflatoxin in poultry feed became a burning issue due to the aflatoxin contamination in corn. It was reported by Economynext (2021), that the inappropriate storage conditions to maintain the moisture content of maize stores, especially during the rainy season, as the major cause of aflatoxin contamination.

A Sri Lankan study conducted by Jayarathne *et al.* (2020) on the detection and quantification of aflatoxin levels in one of Sri Lanka's major agricultural areas revealed the presence of aflatoxin B1 contamination in corn and corn growing soils, indicating a potential risk for consumers, but with a lower risk than the other major aflatoxin detected agricultural produce in Sri Lanka. The study has shown that aflatoxin B1 is extensively distributed throughout the study sample. Corn kernel samples obtained from areas including Rambewa,Punewa,Kebithigollewa, Mihintale, Galenbindunuwewa,Horowpathana,Kahatagas digiliya, Palugaswewa, Thirappane and Mahavilachchiya which exceeded the acceptable level of aflatoxin B1, showed a higher level of aflatoxin B1 in corn grown soil as well according to the Wilcoxon rank sum test. It demonstrates that the majority of the people in Anuradhapura are subsistence farmers with small-scale corn-growing fields. They cultivated corn using Pacific 999, Pacific 989, Jambo, and Jet corn breeder seeds and a fertilizer mixture of Triple Super Phosphate (TSP), Urea, and Muriate of Potash (MoP). Wilson *et al.* (1989) discovered that high levels of nitrogen-based fertilization increase aflatoxin levels in corn, whereas Diener *et al.* (1987) discovered that low levels of fertilizer also increased aflatoxin B1.

Furthermore, Jayarathne *et al.* (2020) identified seven different types of soil in Anuradhapura, all of which could have contributed to the presence of aflatoxin B1. Soil type, landform, irrigation, fertilization, and cultivation seemed to have a significant impact on spore numbers in soil, which are responsible for aflatoxin B1 contamination (Zhang *et al.* 2017). Although there is a link between aflatoxin B1 contamination in corn kernels and corn-grown soil, the influence of other environmental factors on aflatoxin B1 contamination cannot be ruled out. Weather conditions, as well as other physical factors such as pH and temperature, play an important role in growth. Furthermore, soil type, crop rotation, previous soil usage for the same or different types of crops, and other soil chemistry factors would all have an impact.

Rice

Rice is the main staple in Sri Lanka, and it is mostly processed into parboiled rice. Rice and corn are the cereals most contaminated by aflatoxins in natural conditions as a result of changes in agricultural practices. Because high relative humidity and temperature during storage promote microorganism growth, improperly stored processed rice could provide a suitable medium for the growth of toxigenic strains of *Aspergillus flavus* (Reddy *et al.* 2009).Aflatoxin contamination of rice, even at low levels, could be disastrous because the average Sri Lankan rice consumption per day is at a higher level (Bandara *et al.* 1991). Moisture levels above l0% in rice occur as a result of under-drying of paddy (rough rice), particularly during the processing of parboiled rice, as well as absorption of moisture from the atmosphere at high relative humidities during storage. Fungi growth in market rice is a common occurrence, and the presence of aflatoxins in such rice has been documented (Breckenridge *et al.* 1986).

Aflatoxin B1 and aflatoxin G1 levels in parboiled and raw milled rice collected from major rice-producing areas and rice-consuming townships in Sri Lanka have been studied by Bandara *et al.* (1991). Aflatoxin B1 and G1 levels were significantly higher in almost all of the parboiled rice samples tested than in raw milled rice. The highest AFB1 content was 185 g/kg, and the highest AFG1 content was 963 g/kg. These samples were collected from a major rice-producing district in Sri Lanka, where the mean relative humidity is 78% and the mean annual temperature is 27 °C, the highest among rice-growing areas. Raw rice was either free of aflatoxins or, if they were found, they were found in less than 10% of the samples. The presence of surface fungal flora (*Aspergillus/ Penicillium*) and aflatoxin content in market samples were found to be closely related.

According to a study, most milling areas' interim outdoor storage after milling provides ideal humid and warm conditions for *Aspergillus* spp. growth (Hurburgh 2005). The parboiling process appears to make rice grains more susceptible to *Aspergillus* infection. Based on these findings, it is foreseeable that the most important way to ensure toxin-free rice is to implement more stringent quality control procedures and proper storage facilities. This would not only reduce the current post-harvest waste of this valuable staple but would also prevent a serious health risk.

3.2.4 Nuts

Peanut

As a food crop, peanuts are widely grown and consumed in Sri Lanka. Peanut oil is a good cooking substitute for coconut oil, and its pressed cake is used as a nutrient component in animal feeds (Ancuța and Sonia 2020). Furthermore, peanut products such as fried and roasted peanuts are popular food items among city dwellers, and peanut butter and other sweet preparations are important food items in daily meals.

Peanut has been shown to be an excellent substrate for the growth of *Aspergillus* species and the production of aflatoxins (Gourama and Bullerman 1995). Thus, it is commonly found to be contaminated with Aflatoxin B1, which is the worst toxic among the group and therefore, should be controlled within permissible levels strictly. The maximum allowable limit for aflatoxin in peanuts used for human consumption is 15 g/kg (Wu 2014). According to the findings of Dissanayake and Manage (2009) on aflatoxin contamination of commercially available peanuts in Sri Lanka, the tested peanut samples were only moderately contaminated (12.5 ppb) and the rest were uncontaminated according to FAO standards. The study's overall findings revealed that the samples were not significantly contaminated, eliminating the need for unnecessary concern about aflatoxin contamination.

Moreover, Samarajeewa (1984) has further studied aflatoxin contamination in peanuts in Sri Lanka. According to the findings, the levels of aflatoxin AFB1 detected in peanuts from Colombo city using the densitometric concentration method and the calculation method were lower than previously reported

data. In his most recent study, the mean concentration of AFB1 was recorded as 1.98 ± 4.28 ppb. In this study, two of the eight samples were contaminated with AFB1 at levels greater than 1 ppb, with only one sample containing 12 ppb. As a result, according to the FAO and WHO standards, 6.6% of the tested samples were classified as having moderate hazards (15 ppb) of AFB1 for human consumption.

3.2.5 Dairy

Cow's Milk

Aflatoxin M1 (AFM1) is the hydroxylated metabolite of AFB1 and is found in high concentrations in milk or milk products obtained from livestock fed with contaminated feed. (Ardic *et al.* 2009).Milk and milk products are high in nutrients such as protein and calcium, and are primarily consumed by children (Omar 2016). Milk is not only consumed as liquid milk, but it is also used to make infant formulas, yogurt, cheese, and milk-based confectioneries such as chocolate and pastry, posing a higher risk of toxin contamination at the consumption level (Gürbay *et al.* 2006).

The European Union has set regulatory limits for AFM1 in milk and infant formula at 0.05 g/kg and 0.025 g/kg, respectively. The Codex Alimentarius Commission, on the other hand, has set a maximum limit of 0.5 g/kg for AFM1 in milk (Yoshinari *et al.* 2016). Although the consumption of liquid milk and powdered milk is high in Sri Lanka, published information on AFs in milk is limited. The study by Keskin *et al.* 2009 has shown that the AFM1 levels in the tested powdered milk samples in their study did not exceed the Codex limit of 0.5 g/kg. However, 19 (37.3%) of the 51 UHT milk samples tested positive for AFM1 (range = 0.539- 5.132 g/kg). AFB1 (7 samples; range = 0.03-0.31 g/kg) and AFB2 (3 samples; range = 0.03-0.067 g/kg) were also found in some of the above 19 samples, making them even more dangerous, whereas AFG1 and AFG2 were not found in any of the samples. This study demonstrates the evidence for the presence of AFM1 in milk as a result of feeding AF-contaminated feed to dairy cattle, which is then consumed by Sri Lankans, including young children. As a result, immediate attention should be paid to including a maximum limit for AFM1 in milk in national food regulations, as well as monitoring milk and milk products in the country to protect consumers.

4.0 Aflatoxin detection techniques

Aflatoxin toxicity and potency make them the primary health hazard, and the cause of losses associated with contamination of processed foods and feeds. As a result, determining the concentration of aflatoxins in food and feed is critical. However, due to the low concentration of aflatoxins in foods and feedstuffs, analytical methods for detecting and quantifying aflatoxins must be specific, sensitive, and simple to implement (Wacoo *et al.* 2014). There have been numerous analytical methods developed, which can be broadly classified into three categories: chromatographic, immunochemical, and spectroscopic (Mahfuz *et al.* 2018). They are, however, typically expensive, low-throughput, labor-intensive, and immobile, making them unsuitable for use in the field.

The basic scientific methods for detecting aflatoxins are based on chromatography, more specifically on High-performance liquid chromatography (HPLC)/ Ultra-performance liquid chromatography (UPLC) (Papp 2002).

4.1 Chromatographic Methods.

Chromatographic techniques are among the most widely used analytical methods for detecting aflatoxins in a wide range of samples (Wacoo *et al.* 2014). These methods rely on the physical interaction of a mobile phase and a stationary phase. The components to be separated are divided into two phases; stationary phase and mobile phase. Typically, the mobile phase is a fluid that percolates through or along the fixed stationary phase. Liquid, gas, and supercritical fluids are currently used as mobile phases, and chromatographic techniques are named after the nature of the mobile phase: liquid, gas, and supercritical fluid chromatography, respectively. In practice, the sample to be analyzed is dissolved in the mobile phase and applied to the stationary phase as a spot. The

mobile phase transports the analyte or sample, and the sorbent is the partition between the solid and liquid stationary phases. Because the constituents in the analytes travel at different speeds, they are partitioned differently between the mobile and stationary phases (Zhang and Banerjee 2020). Thin-layer chromatography (TLC), HPLC, and Gas Chromatography (GC) are the most commonly used chromatography techniques for aflatoxin analysis (Lin *et al*. 1998). Although many chromatographic techniques are extremely sensitive, they necessitate trained skilled technicians, time-consuming sample pretreatment, and expensive apparatus/equipment.

4.1.1. Thin-Layer Chromatography (TLC)

TLC is a popular separation technique in aflatoxins analysis (Liang *et al*. 1996). It is made up of a stationary phase made of silica, alumina, or cellulose that is immobilized on an inert material called the matrix, which is made of glass or plastic. The mobile phase is a mixture of methanol, acetonitrile, and water that transports the sample through the solid stationary phase. The distribution of aflatoxins between the mobile and stationary phases in TLC is primarily determined by differences in analyte solubility in the two phases. Depending on their molecular structures and interactions with the stationary and mobile phases, different analytes either adhere to the stationary phase more or remain in the mobile phase, allowing for rapid and effective separation (Kotinagu *et al.* 2015).

Thin-layer chromatography has been widely used in the detection of aflatoxins in various foods, with levels as low as 1-20 ppb of aflatoxins reported (Shekhar *et al.* 2017). The TLC method has the advantage of being able to detect multiple types of mycotoxins in a single test sample. While TLC has excellent sensitivities, it also necessitates skilled technicians, sample pretreatment, and expensive equipment. Furthermore, TLC lacks precision due to errors that accumulate during sample application, plate development, and plate interpretation.

4.1.2. High-Performance Liquid Chromatography (HPLC)

HPLC is the most widely used chromatographic technique for the separation and determination of organic compounds. HPLC is used to determine approximately 80% of organic compounds in the world (Reemtsma 2003). The HPLC technique employs a stationary phase contained within a glass or plastic tube, as well as a mobile phase composed of aqueous/organic solvents that flow through the solid adsorbent. When a sample is layered on top of a column, it flows through and distributes between the mobile and stationary phases. The liquid (mobile) phase that emerges from the column separates the sample into fractions containing individual components. Typically, the sample to be analyzed is injected into the stationary phase, and the analytes are carried through the stationary phase by the mobile phase under high pressure delivered by a pump. Chemical and physical interactions with the stationary and mobile phases cause analytes to be distributed differently within the stationary phase. Normal-phase and reversed-phase high -pressure liquid chromatography techniques are used for the detection of aflatoxins in foods. For the separation and determination of aflatoxins, the reversed-phase HPLC method is the most widely used. Chemical derivatization of aflatoxins B1 and G1 may be required occasionally to improve HPLC sensitivity during analysis because the natural fluorescence of aflatoxins B1 and G1 may not be high enough to reach the required detection limit (McCullum *et al.* 2014).

Aflatoxin detection results are fast and accurate when using HPLC. The disadvantage of using HPLC for aflatoxins analysis is the need for stringent sample purification with immunoaffinity columns. Furthermore, HPLC necessitates time-consuming pre- and post-column derivatization processes to improve the detection limits of aflatoxins B1 and G1 and the capacity of the machine restricts its use to the laboratory environment rather than field conditions (Wacoo *et al.* 2014).

4.1.3. Gas Chromatography (GC)

The mobile phase in GC is a carrier gas, and

the stationary phase is a liquid coated onto inert solid particles. Sample analysis by GC, like other chromatographic methods, is primarily based on the differential partitioning of analytes between the two phases. The stationary phase is composed of inert particles coated with a layer of liquid. A carrier gas transports the sample through the stationary phase after it has been vaporized into the gaseous phase (Zhang 2020).

The chemical constituents of the sample will be distributed between the mobile phase and the stationary phase. The components of the sample mixture with a higher affinity for the stationary phase move more slowly through the column, while those with a lower affinity move more quickly. In fact, each component of the analyte should have its own partition coefficient, which will govern its rate of passage through the column. Once separation has been achieved, the detection of the volatile products is carried out using either a flame ionization detector (FID) or an electron capture detector (ECD) and mass spectrometer (MS). Owing to their non-volatility in nature, aflatoxins may need derivatization in order to be detected. However, due to the availability of less expensive chromatographic methods, gas chromatography is becoming less common in commercial analyses of aflatoxins. Furthermore, because gas chromatography requires a preliminary cleanup step before analysis, it is limited to the analysis of a few mycotoxins, such as A-trichothecenes and B-trichothecenes (Rasooli and Abyaneh 2004; Rasooli and Owlia 2005; Zhang and Banerjee 2020).

4.2 Immunological methods

Highly specific antibody-based tests for measuring aflatoxins are now commercially available. Aflatoxins are low-molecular-weight small molecules. They have no effect on the immune system's ability to produce antibodies (Pitt 2000). Aflatoxin must be conjugated to a larger carrier molecule, such as a protein, because of this property. Radioimmunoassay (RIA), Enzyme Linked Immunosorbent Assay (ELISA), Immunoaffinity Column Assays (ICA), immunosensors, and immunodipsticks for aflatoxin analysis are all immunochemical methods (Mahfuz *et al.* 2018). ELISA is the most commonly used method for the estimation of aflatoxins (Leszczyńska *et al.* 2001). Several researchers have used the ELISA technique to detect aflatoxin in Sri Lankan foods such as edible oil (Karunarathna *et al.* 2009), corn (Jayaratne *et al.* 2020) chili (Yogendrarajah *et al.* 2014) and cow milk (Pathirana *et al.* 2010)

4.3 Spectroscopic Methods:

The maximum absorption wavelength for all aflatoxins is around 360 nm. "B" and "G" in aflatoxins stand for blue (425 nm) and green-blue (450 nm) fluorescence, respectively (Akbas and Ozdemir 2006). The fluorescence emission of the G toxin is over ten times that of the B toxin (Alcaide-Molina *et al.* 2009). As a result, scientists use fluorescence and spectroscopic methods to determine the aflatoxin content of various foods and feeds. The black light test, Laser-Induced Fluorescence (LIF) screening method, Photomultipliers (PTM), Ion mobility spectrometry (IMS) and Fourier-transform near-infrared (FT-NIR) spectrometry are all techniques for detecting AFs related to fluorescence, and spectrophotometry detection techniques include Ion mobility spectrometry (IMS), and FT-NIR spectrometry (Mahfuz *et al.* 2018).

The HPLC technique is the most widely used technique for the detection of aflatoxins in Sri Lanka. Accredited food quality analyzing institutions, such as Industrial Technology Institute (ITI), use HPLC to analyze four major aflatoxins naturally found in food: aflatoxins B1, B2, G1, and G2. For many years, the ITI has used TLC and HPLC to test for aflatoxin in various matrices such as spices, cereals, tea, milk, coconut oil, and so on. Currently, aflatoxins are tested using LC-MS-MS, which produces more accurate and consistent results and allows for much lower aflatoxin detection limits. The ITI issues numerous test reports on aflatoxins in chillies, curry powder, masala powder, cereals, ayurvedic preparations, food, and oils on a monthly basis in response to requests from the

respective industries (Industrial Technology Institute 2014).

5.0 Actions in regulating Aflatoxin contaminations

5.1 Regulations and Limitations

Since the discovery of aflatoxins in the 1960s, many countries have implemented regulations to protect consumers from the harmful effects of these toxins, which can contaminate both foodstuffs and feedstuffs. Permissible mycotoxin levels are determined by a number of factors. These include evidence-based data that supports risk assessment, such as toxicological data, food consumption data, data on the level and distribution of mycotoxins in goods intended for human and animal consumption, and data on analytical methodology (Pleadin *et al.* 2013). Economic factors such as commercial and trade interests, as well as food safety concerns, play a role (Wagacha and Muthomi 2008). The European Union (EU) has the most extensive and detailed regulations governing AFB1 presence in various types of food and feed when compared to other regions of the world. Furthermore, many of the EU candidate member states have mycotoxin presence-governing regulations that are as detailed as the regulations currently in force across the EU (Rodríguez-Blanco *et al.* 2020).

The Commission Regulation establishes maximum permitted levels (MPLs) of aflatoxins in food, including AFB1 and total aflatoxins. AFB1 is the only individual mycotoxin whose MPLs are set because it is a genotoxic carcinogen and a strong acute toxin that affects a variety of animal species (Pleadin *et al.* 2014). Considering human health, the most stringent criteria apply to feedstuffs intended for dairy cattle due to AFB1 conversion into AFM1 in milk and dairy products (MPL= 5 g/kg across the EU) (Faulkner 2014). To safeguard the health of consumers, many countries have set MPLs for aflatoxin and other mycotoxins, in human food and animal feed. For example, within the European Union (EU), the permitted levels range from 0.1 to 12 ppb for AFB1, from 4 to 15 ppb for total aflatoxins and 0.025–0.05 ppb for AFM1 in particular foodstuffs (Commission Regulation EU No 165/2010) (Joo *et al.* 2017). Whereas, in the United States of America (USA), the Food and Drug Administration (FDA) have set a maximum concentration of 20 ppb for AFB1 in foods (Marshall *et al.* 2020). Likewise, different guidelines exist between the EU and USA for aflatoxin levels in food and feed.

5.2 Aflatoxin Control

Aflatoxins are highly stable chemical compounds with decomposition temperatures ranging from 237 to 306 ºC (Rustom 1997) and are thus not destroyed by standard thermal processing or cooking. Unfortunately, no single step can be taken to prevent or eliminate mycotoxin contamination, but there are a variety of control strategies that can be implemented to help address the problem.

Control of aflatoxin can be targeted at two points along the supply chain: pre-harvest and post-harvest. Pre-harvest management primarily focuses on preventing toxin formation by preventing or eliminating fungal growth. Pre-harvest mitigation focuses on GAPs such as pest control and fungicide application (Pandey *et al.* 2019; Torres *et al.* 2014).

Biocontrol measures underpin a novel pre-harvest approach. Nontoxigenic *Aspergillus* fungi strains that are unable to produce aflatoxin are applied to crop fields, where they establish themselves, compete, and displace toxigenic strains, resulting in a reduction in aflatoxins (Senghor *et al*. 2020). Reducing the risk of aflatoxin contamination after harvest is largely dependent on proper crop storage, which must ensure that the crop remains dry, as *Aspergillus*, like many fungi, thrives in humid environments. Although fungal contamination prevention may be the key to reducing the impact of mycotoxins on both human and animal health, current practices do not adequately address this issue, particularly in less economically developed countries (Wielogorska *et al.* 2019). To reduce the potential for mycotoxin contamination, GAPs and the use of properly controlled storage conditions are used; however, these strategies have been shown to be ineffective in ensuring

the elimination of mycotoxin-producing organisms. Decontamination techniques are also required to reduce the risk of aflatoxin contamination. (Yilmaz *et al.* 2018). Decontaminating aflatoxins from food ingredients to a safe level is a top priority for avoiding consumer risks. There are three methods of aflatoxin decontamination as physical processes consist of three steps: separation of contaminated items, removal of mycotoxins, and reduction of mycotoxins content via exposure to sunlight, radiation, or microwave heating; chemical processes use ozone, ammonia, and citric acid, among other things, whereas biological processes use chlorophyllins, sulforaphane, isoimperatorin isolated from *Poncirus trifoliata* Raf. among other things (Samarajeewa *et al.* 1990; Ozer *et al.* 2018).

5.2.1 physical methods

Cleaning and segregation of aflatoxins

The first option for reducing aflatoxin is to physically separate the mold-contaminated grains or feed (kernel, seeds, and nuts) from the seemingly uncontaminated intact product. Cleaning, sorting, and handpicking are all examples of physical procedures that do not significantly alter the products. For people in developing countries having little or no access to mycotoxin testing of their foods, hand sorting is still practiced as the most common way to remove AFB1. Hand sorting of maize grains contains 6% AFB1 and 5% fumonisin B1 when compared to other methods such as flotation and dehulling. As a result, hand sorting maize grains is recommended as a last line of defense against mycotoxin exposure among subsistence consumers (Pankaj *et al.* 2018; Javanmardi *et al.* 2022).

Heating treatment

Aflatoxins are well known for their high temperature stability. Heating treatment is always used in modern food manufacturing technology to degrade mycotoxins to some extent during processing (Proctor *et al.* 2004). According to recent research, AFB1 can be significantly removed at high humidity. However, several possible facts, such as initial mycotoxin concentration, the extent of binding between mycotoxin and food or feed products, heat penetration, moisture content, and processing conditions, are associated with the prediction of the extent of mycotoxin reduction (Kabak 2009). Nonetheless, heat treatment to partially reduce mycotoxin concentrations in foodstuffs is still a viable physiological method because heating techniques are simple and inexpensive. Extrusion cooking is widely used in the food industry and is an efficient process in the food/feed process. In the industry, high-temperature, short-time extrusion is common (Castells *et al.* 2005).

Microwave heat treatment

Perez-Flores *et al.* (2011) discovered that microwave thermal-alkaline treatment significantly reduced aflatoxin content in traditional Mexican food tortillas. The results showed that the aflatoxin reduction was almost permanent when using extract acidification methods to mimic human stomach digestion procedure to quantify AFB1 concentration. However, this thermal-alkaline treatment for tortilla-making could only remove the majority of AFB, leaving some AFB in the food (Farag *et al.* 1996; Pérez-Flores *et al.* 2011).

Irradiation treatment

Radiation is another commonly reported physical decontamination technology (Pankaj *et al.* 2018). Radiation has been used on a variety of food substrates, including groundnuts, grains, palm juice, soybeans, and animal feed. Irradiating food with a ɣ-ray source is moderately effective, with a 65% average reduction at high irradiation dose. Gamma irradiation is a promising method for improving the safety and cost-effectiveness of moderately fungi-damaged feedstuffs (Ismail *et al.* 2018).

Electrolyzed water (EOW) treatment

Electrolyzed water treatment is a relatively new technique for treating AFB1-contaminated foods or feeds (Yang 2019). When treated with EOW, particularly neutral electrolyzed oxidizing water (NEW), the AFB1 was significantly reduced. The presence of OH in EOW may be an important factor in the significant fungicidal efficiencies

against *A. flavus.* AFB1 was mostly degraded after 15 minutes of EOW treatment. Fan *et al.* (2013) discovered that alkaline electrolyzed water (AIEW) could remove AFB1, with the best working conditions at pH 12.2.

Pulsed light technology to remove AFB1

Pulsed light has been shown to be an effective decontamination technique capable of destroying bacteria, viruses, fungi, and spores on food and material surfaces (Montie *et al.* 2000). Moreau *et al.* (2013) are the first to demonstrate a nonthermal technology for mycotoxin destruction and inactivation of their mutagenic activity. They assessed the efficiency of pulsed light technology in the degradation of mycotoxins.

5.2.2 chemical methods

The use of chemical additives on contaminated foods has been one popular method, especially the additives themselves would be used in the foods.

Ammonia decontamination treatment

Ammonization of maize, rice, barley, peanuts, and cotton seeds to alter the toxic and carcinogenic effects of aflatoxin contamination has been intensely researched by scientists from government agencies and universities around the world. Several studies have shown that aflatoxin B1 levels were reduced effectively and permanently by 1-hour ammonia treatment. Treatment with either NH_4OH at high temperatures or gaseous NH_3 can effectively reduce aflatoxin B1 content, sometimes reaching above 99% (Yang 2019).

Hydrochloric acid (HCl) treatment

The effect of hydrochloric acid on AFB1 degradation in contaminated corn gluten at various HCl concentrations is temperature, HCl concentration, and time dependent. During the wet milling process, 1 mol/L HCl at 100 °C resulted in AFB1 degradation of 27.6% after 4 hours and 42.5% after 8 hours. The degradation of AFB1 increases with increasing HCl concentration, and it will completely degrade AFB in the presence of 5 mol/L HCl after 4 hours at 110 °C (Yang 2019).

Lactic acid and citric acid treatment

Previous research has shown that some organic acids have the ability to detoxify aflatoxin-contaminated foods. Mendez-Albores *et al.* (2007) demonstrated that citric acid and lactic acid are effective at degrading aflatoxin. The amount of B-aflatoxins decreased as the acid concentration increased, and citric acid has a more noticeable effect on AFB degradation.

5.2.3 Biological treatments

One well-known strategy for reducing the level of aflatoxins in foods or feed products is to use microorganisms or enzymes for the biodegradation of aflatoxins. Biologically based interventions are being actively researched because they are more efficient, specific, and environmentally friendly than other non-biological degradation methods.

Soil bacteria

Many soil bacteria are capable of degrading aflatoxins. *Flavobacterium aurantiacum* NRRL B-184, a type of bacteria found in soils and water, demonstrated the ability to detoxify aflatoxins with high efficiency (Wu *et al.* 2009). Ciegler and Peterson (1968) discovered that *F. aurantiacum* NRRL B-184 removed aflatoxin from contaminated milk, oil, peanut butter, peanuts, and corn irreversibly and partially from soybeans. *F. aurantiacum* NRRL B-184 not only removes aflatoxins but also prevents the formation of new toxic products. It was also reported that the bacteria could remove AFM1 from milk (Pierides *et al.* 2000). While studying the effects of metal ions such as Cu^{2+}, Mn^{2+}, Zn^{2+}, and other chemical materials on AFB1 degradation by bacteria, they discovered that they could increase AFB1 degradation by 10-15%, implying that an enzymatic system was involved in aflatoxin B1 degradation by *F. aurantiacum*. Aside from *F. aurantiacum*, other microorganisms such as *Nocardia asteroides* and *Corynebacterium rubrum* can detoxify aflatoxin (Bhat and Hakeem 2021).

Fungi

Fungi can produce aflatoxins as well as degrade them. *Aspergillus niger*, *Eurotium herbariorum*, a *Rhizopus* sp., and a non-

aflatoxin-producing *A. flavus* were able to convert AFB1 to aflatoxicol-A (AFL-A), which was then converted to aflatoxicol-B (AFL-B) by medium components or organic acids produced by the fungi. The fungus *Penicillium raistrickii* NRRL 2038 has the potential to convert AFB1 into a new compound that is similar to AFB2 (Mishra and Das 2003).

Yeasts and lactic acid bacteria

The mechanism by which yeasts and lactic acid bacteria degrade AFB1 is due to their adhesion to cell wall components. Lillehoj *et al.* (1979) discovered that AFB1 concentrations drop dramatically during the brewing process, implying that *S. cerevisiae* yeasts absorb mycotoxin. During yogurt and dairy product fermentation, AFB1 was detoxified into a nontoxic new fluorescing compound corresponding to AFB2a. When fed diets contaminated with AFB1, drinking water containing *S. cerevisiae* strain showed a protective effect on the relative weight of the liver as well as histopathological and biochemical parameters. Lactic acid bacteria strains will bind specific dietary aflatoxin contaminants. Pierides *et al.* (2000) investigated the ability of 12 Lactobacillus, 5 Bifidobacterium, and 3 Lactococcus bacteria strains to bind AFB1. After a 72-hour incubation period, two *Lactobacillus amylovorus* strains and one *Lactobacillus rhamnosus* strain removed more than 50% AFB1. *Lactobacillus rhamnosus* strain GG (LBGG) and *L. rhamnosus* strain LC-705 (LC705) can remove approximately 80% AFB1 from culture media in a temperature- and bacteria-concentration-dependent manner.

5.2.4 Aflatoxin degradation by enzymes

Microbial systems have yielded some specific enzymes for the degradation of aflatoxins. Using enzymes to degrade aflatoxins has some advantages, such as not changing the flavor or reducing nutritional value. Motomura *et al.* (2003) studied the ability of 19 fungi to degrade AFB1 in cultured supernatants and purified 1 enzyme with aflatoxin degradation activity from *P. ostreatus* supernatants. The enzyme demonstrated that AFB could best degrade activity at 25 °C and pH 4.0-5.0. The novel enzyme has the ability to cleave the lactone ring of aflatoxin. Also it is found that an intracellular enzyme called aflatoxin-detoxifizyme had detoxification activity against aflatoxin B1, with the enzyme performing best at 35 °C and pH of 6.8.

5.2.5 Cold plasma technology to remove AFB1

Cold plasma has previously been used to sterilize sensitive materials. Cold plasma has recently received a lot of attention as a new microbial decontamination technology in the food industry (Kim *et al.* 2014). The study further elaborates that its benefits include high efficiency and short treatment time, no residue, and a low impact on the quality of treated food products. Cold plasma has recently been used to study the degradation of mycotoxins. AFB1 was successfully removed after 5 seconds of treatment with microwave-induced argon plasma. Within 15 minutes, nitrogen gas plasma could be efficiently degraded to 10% of its initial concentration. Within 10 minutes, a low-temperature radio-frequency plasma degraded 88% of AFB1.

The various preventive measures in place are clearly insufficient to eliminate all potential risks of aflatoxin contamination in feed and food commodities. The development and implementation of highly efficient novel decontamination strategies will become increasingly important for both human and animal health protection.

6.0 Conclusions and suggestions for Sri Lankan context

AFTs are secondary metabolites, difurano coumarins, produced by *A. flavus* and *A. parasiticus*. The presence of these toxins in unacceptable amounts is a global concern due to the harmful effects of aflatoxins on human health. Under the Sri Lankan context, the occurrence of four types of aflatoxins (AFB1, AFG1, AFB2, and AFG2), are reported in several food types including Vegetable oils, Nuts and Seeds, Cereals and Dairy products. Recent past, a higher incidence of aflatoxin in agricultural produce was reported in coconut oil. High levels of aflatoxin contamination in

coconut oil may have resulted from improper practices during the copra production that facilitates toxigenic fungal growth on copra. It is suggested that certain coconut oil products available in Sri Lanka may pose a health risk to consumers. Next to the edible oil spices such as chili and pepper are known to present aflatoxin contamination. However, the contamination of food products with aflatoxin shows a comparatively lower value which may be a result due to the higher level of awareness among people on actual causes of aflatoxin formation in food products during the food production line from food production up to harvesting and storage. Proper strategies should be formed for detection, decontamination, and further awareness.

The four pillars of food security availability, access, utilization, and stability as well as the agricultural sector's output in general, can all be impacted by the contamination of important staples with aflatoxin. Contamination in staples such as rice, maize, and groundnuts can reduce food availability directly. Producers of the affected crop may also earn less as a result of product rejection, a decline in market value, or a lack of access to the formal market and higher-value international trade.

AUTHOR CONTRIBUTION

PLNL devised the main conceptual ideas and proof outline. DMND and BEAUB wrote the manuscript in consultation with PLNL.

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