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Chapter

# Aflatoxins: A Postharvest Associated Challenge and Mitigation Opportunities

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## Abstract

In agriculture, Aflatoxins are of major concern as they affect the nutrient quality of crops like Groundnut, Maize, and Coffee which are global economic commodities. Aflatoxin-contaminated products cause substantial financial losses and significant health problems in living beings. *Aspergillus* produces aflatoxins during environmental stress conditions. The International Agency for Research in Cancer (IARC) conducted studies on aflatoxins and found that Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) can cause cancer in both humans and animals and are classified into the Group 1 category of chemical hazards for potentiation mutagens. In India, the Food Corporation of India (FCI) monitors Aflatoxin levels in food and feeds. Aflatoxin contamination reduces the quality of groundnuts, maize, and coffee, affecting their exports. Consumption of aflatoxins contaminated feed induces liver cancer, immune suppressions, shunted growth, and in higher amounts, causes death. The current review provides information based on previous studies and newly adapted guidelines and methods showing the impact of aflatoxins on crops such as groundnut, coffee, and maize. The use of artificial intelligence to detect aflatoxin and mitigation opportunities using technologies such as Aflasafe, Aflaguard, hermetic bags, and Purdue Improved Crop Storage (PICS).

**Keywords:** *aspergillus*, aflatoxin, groundnuts, maize, coffee, biological control, artificial intelligence

## 1. Introduction

A new disease was identified with unknown characteristics in England during the 1950s and 1960s, which increased turkey mortality. Later, aflatoxin was recognized in 1960 in England as a causative agent of the mysterious Turkey 'X' disease that causes excessive mortality in the poults of Turkey (**Table 1**) [1, 5]. The term aflatoxin (*Aspergillus flavus* toxin) was coined for this toxic agent [2]. Aflatoxins are low molecular weight and extremely toxic compounds. These are classified as the largest group of mycotoxins. The mold that includes the species of *Aspergillus* and *Penicillium* accounts for the spoilage of stored grains. Fungi under extreme

<b>Year</b>	<b>Findings</b>
1960	Outbreak of Turkey 'X' disease in England Aflatoxin discovery
1961	Identified <i>Aspergillus flavus</i> associated with toxicity of groundnuts
1962	Studies conducted on physicochemical properties of aflatoxins Aflatoxin B and G identified in TLC analysis Isolation and synthesis of crystalline aflatoxins
1963	Aflatoxin B2, G1, and G2 were identified and chemically characterized as Difurocoumarin derivatives
1965	FDA approved the first regulation on aflatoxins 30 µg/kg
1966	Milk toxins were designated as AFM1 and AFM2 AFM1 was detected in Milk, Urine, Kidney, and liver
1967	Chemical synthesis of Aflatoxin B1
1969	FDA approved new limits for aflatoxins 20 µg/kg
1972	First IARC evaluation to detect the possible relationship between aflatoxins and liver Cancer in Humans
1974	The first outbreak of Aflatoxins affecting humans (106 deaths)
1975	IARC confirmed the carcinogenicity of Aflatoxins
1977	FDA approved new limits for aflatoxins AFM1 0.5 µg/kg DNA adducts of Aflatoxin B1 were identified
1981	DNA adducts of Aflatoxin B1 were identified in Urine
1987	IARC classified Aflatoxin in Group 1 as carcinogens
1991	Point mutation was identified on Codon 249 of the p53 gene
1992–1993	IARC confirmed Aflatoxin B1 as Group 1 carcinogen
1992	AFB1 and Liver cancer are linked was identified
1996	Aflatoxins producing fungi were detected in Grains by PCR analysis
2001	Establishment of Aflatoxin legal limit commission regulation (EC) no. 466/2001
2003	New terms of Aflatoxin legal limit commission regulation (EC) no. 2174/2003 for specific types of species
2004	An outbreak of aflatoxicosis in Kenya by consuming contaminated
2006	New regulations of Aflatoxin legal limit commission regulation (EC) no. 401/2006 for the limits of aflatoxins in foodstuffs
2012	Due to climatic conditions, cereals were affected, and a potential increase in aflatoxin B1 in the EU was observed IARC identified AFB1, AFB2, AFG1, and AFG2 are highly carcinogenic and are classified as Group 1 carcinogens
2014–2019	Neonatal exposure and Aflatoxin traces were found in the Umbilical cord (Nepal and Bangladesh)
2019	Around 25 Species of Aflatoxin-producing fungi were identified in sections Flavi, Nidulantes and Ochraceorosei

Year	Findings
2020	From section Flavi- <i>A. texensis</i> , <i>A. agricola</i> and <i>A. toxicus</i> are able for production of aflatoxins
2021	Aflatoxins Poisoning in Dogs (US) by consuming contaminated feed

*Adopted and modified from Refs. [1–4].*

**Table 1.**  
*Timeline for Aflatoxin (1960–2021).*

stress and inadequate maintenance, such as low nitrogen content, temperature, and drought, enhance aflatoxin accumulation in *Aspergillus spp.* *A. flavus* contaminates oilseed, stored grains, and coffee in pre and postharvest conditions. Globally, FAO (The Food and Agricultural Organization of United Nations) has provided regulations on mycotoxin content in both food and feeds, and FDA (Food and Drug Administration) has assigned specific limits for aflatoxins for human consumption, i.e., 20 ppb (parts per billion) and 0.5 ppb in food and dairy products respectively. A person infected with the hepatitis B virus who ingests aflatoxin-contaminated food has a higher risk of developing hepatic cancer [6]. European Union (EU) has specified stringent standards of value 4 ppb for aflatoxins. Several approaches have been explored to examine Aflatoxins present in foods such as TLC, HPLC, ELISA, LC-MS/MS, and others. HPLC, combined with fluorescence detection, is an analytical method widely used for detecting aflatoxins in different food samples (Table 2).

## 1.1 Aflatoxins and stored grains

### 1.1.1 Groundnut

Groundnut is a leading oil production crop and a highly traded commodity. China ranks first for groundnut production globally with 17.57 million metric tons (MMT) of quantity. India produces 6.93 MMT, Nigeria, 4.45MMT, Sudan 2.83 MMT, and US 2.49MT. Furthermore, groundnut production will be 81.56 MMT in 2020–2022. The estimated groundnut production in 2021–2022 will be 82.54 MMT. The fungi, namely *Aspergillus flavus* and *Aspergillus parasiticus* that produce aflatoxins, contaminate the field, resulting in significant loss of groundnut production. Due to this, the yield is reduced by 13–59%, particularly during warm and humid conditions. Pests account for an estimated 39% of all losses, leading to increased economic loss. Drought is yet another contributor to the decline in groundnut production. These conditions favor the growth of opportunistic molds, which enhances field contamination higher and loss of yield. An estimated 20–30% of groundnut loss occurs due to damaged pods during the postharvest. This merely relies on the techniques used for post-harvesting and the soil's moisture content. Insect activity in storage conditions increases the chances of contamination up to 6–10%. In Gujrat, groundnut is harvested during June and October twice a year. In both cases, there is a high risk of experiencing rain during drying harvested crops which causes heavy damage to a pod. Around 50% turned black due to mold infections. In such conditions, molds produce aflatoxins, which reduces seeds' quality and market value (Figure 1).

S. No.	Mycotoxins	Major producing fungi	Major substrates in nature	US FDA µg/Kg	EU µg/Kg	FSSAI µg/Kg	IARC No.*
1	Alternaria (AM) Mycotoxins	<i>Alternaria alternata</i>	Cereal grains, tomato, animal feeds	—	—	15	—
2	Aflatoxin (AF) B1	<i>Aspergillus flavus</i>	Peanuts, corn, cottonseed, cereals	20	2–12 for AFB1 4–15 Total AF	15	1*
3	Other aflatoxins	<i>Aspergillus parasiticus</i>	Walnuts	20 for total	4–15 total	15	1*
4	Citrinin (CT)	<i>Penicillium citrinum</i>	Barley, corn, rice, walnuts	—	—	—	2B*
5	Cyclopiazonic acid (CPA)	<i>Aspergillus flavus</i> , <i>P. cyclospium</i>	Peanuts, corn, cheese	—	—	—	—
6	Deoxynivalenol (DON)	<i>Fusarium graminearum</i>	Wheat, corn	1000	200–50	1000	3*
7	Cyclochloronitine (CC)	<i>P. islandicum</i>	Rice	—	—	—	—
8	Fumonisin (FM)	<i>F. moniliforme</i>	Corn, sorghum	2000–4000	200–4000	—	2B*
9	Luteoskyrin (LT)	<i>P. islandicum</i> , <i>Prugulosum</i>	Rice, sorghum	—	—	—	—
10	Moniliformin (MN)	<i>F. moniliforme</i> , <i>A. ochraceus</i>	Corn, Barley, cereals, feeds, maize, oats, rice	—	—	—	—
11	Ochratoxin A (OTA)	<i>A. verrucosum</i>	Rice, wheat	not set	02–10	20	2B*
12	Patulin (PT)	<i>Ppatulum</i> , <i>Purticae</i> , <i>A. clavatus</i>	Wheat, apple, beans	50	10–50	50	3*
12	Penicillic acid (PA)	<i>Aspergillus ochraseus</i>	Foodstuff, corn	—	—	—	—
14	Penitrem A (PNT)	<i>Ppalitance</i>	Barley, corn	—	—	—	—
15	Roquefortine (RQF)	<i>Proqueforti</i>	Cheese	—	—	—	—
16	Rubratoxin B (RB)	<i>Prubrum</i> , <i>Ppurpurogenum</i>	Corn, Soyabean	—	—	—	—
17	Sterigmatocystin (ST)	<i>Aspergillus versicolor</i> , <i>A. nidulance</i>	Corn, grains, cheese	—	—	—	—

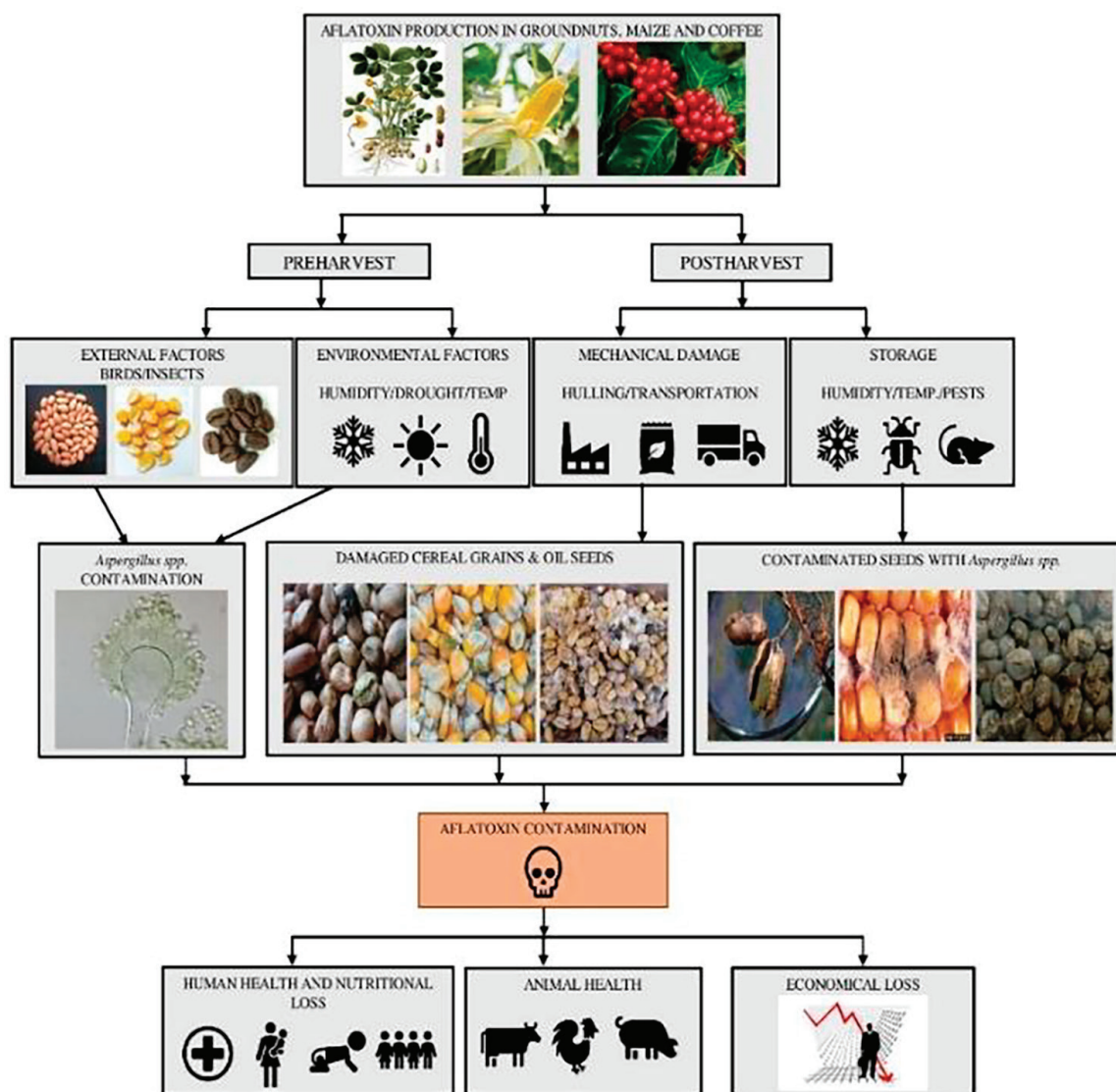
18	T-2 Toxin	<i>F.sporotrichioides</i>	Corn, feeds, hay	15	25-1000	—	3*
19	12-13, Epoxy trichothecenes other than T-2 and DON	<i>F.nivale</i>	Corn, feeds, hay, peanuts, rice	15	25-1000	—	3*
20	Zearalenone (ZE)	<i>F. gramineum</i>	Cereals, corn, feeds, rice	—	20-100	—	3*

Adopted and modified from Refs. [7-9].

NOT SET = —.

**Table 2.**

*Mycotoxins and their respective substrates. (IARC defines 1 for carcinogenicity to humans; 2B as Possible carcinogenic; 3 as not classified as a carcinogen to humans).*



**Figure 1.**  
Effects of Aflatoxin contamination in Groundnut, Maize, and Coffee.

### 1.1.2 Maize

The US produces a high quantity of maize, around 360.252 MMT of maize/year; after that, China produces 260.67 MMT, Brazil 109 MMT, EU 63.6 MMT, Argentina 47.5 MMT, Ukraine 29.5 MMT, India 28.5 MMT, Mexico 28 MMT. In 2021, the United States Department of Agriculture (USDA) estimated a world corn production in 2020–2021 to be 1133.89 MMT compared to 2019–2020. The 1133.89 MMT shows a surge of 1.57% in corn production around the globe. In India, 50%–60% of the harvested crops are stored by traditional methods in households and fields for consumption during scarcity. However, poor management causes 40%–45% damage to stored maize. In maize, contamination appears because of unexpected weather conditions, and insect infestation causes 0.2–11.8% weight loss [10]. During corn filling, birds eat kernels and damage the plant, which causes fungal spores deposition and eventually infects the plant and imparts increased agricultural losses. *A. flavus* is a saprotrophic and opportunistic parasitic fungus that causes severe loss of crops in the field. The average loss by mycotoxins and pests per year is around 20%–30% in maize due to inappropriate practices in agriculture and improper storage management, accounting for 5%–7% loss (Figure 1) [11].

### 1.1.3 Coffee

Coffee is a global commodity consumed daily by millions of people. It is a highly traded commodity worldwide. Asia will be the third-largest coffee producer during 2019–2020. Indian coffee accounts for 3.14% of global coffee production, i.e., approximately 299,300 MT of coffee production. In contrast, the total export was US \$490.59 Million between April–November 2019 and 298000MT of coffee production, approximately US \$459.87 million in April–November 2020. There is an increased risk of infection by molds during harvest and storage by the fungi, including *Aspergillus flavus*, *Aspergillus paraciticus*, *Penicillium*, etc. [12]. These molds grow in humid and moist environments. The temperature also influences their growth. Before storage, coffee beans are dried until the moisture level reaches 11%–12%. The semi-dry method is a combination of both dry and wet methods. Recent studies found that at 10% humidity, molds can produce 4.387 µg/g of aflatoxin. In 25% humidity, they produce 10.436 µg/g of aflatoxin. In 45% humidity of storage, the coffee beans are highly susceptible to Aflatoxins and produce 4.604 µg/g of aflatoxin resulting in quality loss of beans (Figure 1).

## 1.2 Aflatoxin outbreaks

In India, the disease was initially recognized in 1966 in chicks, leading to thousands of chicks' mortality. The cause of mortality was recognized by the Government poultry breeding unit in Bangalore. The first aflatoxin poisoning outbreak in India in 1974 caused 106 deaths by ingesting contaminated maize [13]. Besides this, in 1982, the Chittoor, a district in Andhra Pradesh, reported a loss of heavy chick mortality as a cause of aflatoxicosis. Due to this, poultry farms remained shut during this period. To combat this, in 1985, egg production was discontinued to reduce loss during outbreaks of aflatoxicosis in the Warangal district of Andhra Pradesh. In the Mysore district of Karnataka, the first reports of aflatoxicosis were in 1966. This outbreak resulted in the mortality of 2219 chick's poultry farms. Similarly, South Canara and Ranga-Reddi districts of Andhra Pradesh recorded 200,000 chickens dying due to aflatoxin poisoning [14].

In the study conducted in 1993 by the International Agency for Research in Cancer (IARC), it was concluded that sufficient data is available for trials in animals for the AFM1 carcinogenicity. However, the information on AFM1 in humans is inadequate [14]. Researchers suggest that seasonal variations in aflatoxin exposure could be correlated with food availability [15]. Humans can experience various symptoms due to Aflatoxins depending on their health conditions, age factors, duration of infection, and level of contamination in their body. Prolonged aflatoxin consumption in humans can result in hepatic cancer. They are much more susceptible to acquiring hepatitis B and cause initial stages of jaundice [6]. India has 40 million active hepatitis B virus (HBV) carriers, approximately 10.15% of the global population. Around 15–40% of infected patients develop symptoms like cirrhosis, liver failure, and hepatic carcinoma [16]. The same symptoms are observed in aflatoxicosis, so it may be concluded that both Aflatoxin and HBV are closely associated. Coffee is also consumed by billions of people worldwide. This crop is vulnerable to aflatoxin and may result in a pandemic if the toxin levels are not controlled. It leads to adverse human health effects. In India, 86 million people live in extreme poverty, accounting for approximately 6% of the country's total population (May 2021), whose access to low-quality foods and unhygienic environments increases the vulnerability of rural communities as a whole



perfect hotspot for such toxicants. In such instances, food protection and policies for aflatoxin control must be established and regularly monitored in these regions. The World economic forum conducted a study that states that India may suffer from aflatoxicosis in the future since they cannot afford a portion of good quality food and are still coping with scarcity and consuming contaminated food.

### **1.3 Food safety governing bodies and the impact of aflatoxins on the food chain**

Food Corporation of India (FCI) was set up under the Food corporation act 1964 to manage food policies for farmers. They also maintain satisfactory operational Safety stock of food grains for the nation's safety. Food Safety and Standards Authority of India (FSSAI) was established under the Food safety and standard act 2006, a law regulating food products and enforcing food safety standards in India. FSSAI promotes and regulates public health through regulations and regular food safety management in India. On 19 August 2020, FSSAI notified a publication that a first amendment regulation limited the various contaminants, including metals, aflatoxins, and mycotoxins. In a new regulation by governing bodies, aflatoxin's permissible value for grains and food products is 10 µg/kg.

After ingesting aflatoxin-contaminated food products, experience abdominal pain, vomiting, pulmonary edema, liver necrosis, extensive proliferation of bile duct, and fat infiltrations; few studies on aflatoxins stated that it causes growth suppression. It is also reported that Aflatoxins cause carcinogenic activity in animal models, confirming their carcinogenic potency. According to the studies conducted by Wogan et al. [17], rats are prone to toxins. It was observed that the clinical administration of rats with doses of 1, 5, 15, 50, 100 ppb could induce tumors in the liver, implying that even a microgram of aflatoxin can induce cancer. Among the 14 naturally occurring aflatoxins, AFB1, AFB2, AFG1 and AFG2 are potentially carcinogenic and can damage the coffee beans in storage. AFB1 is categorized as extremely carcinogenic and is added to the group 1 category by IARC. The remaining aflatoxins are categorized as a possible carcinogen that belongs to group 2B because of their toxicity and carcinogenicity [18]. In a study on coffee beans, 20–30% of *Aspergillus flavus* are widely isolated that can produce B type of aflatoxin. *Aspergillus parasiticus* produces both AFB and AFG in coffee beans. The toxic nature of the AFG family is lower than the AFB family [19].

Aflatoxins are usually identified based on the fluorescence they emit in UV light. Fluorescence blue (B) series toxins are represented as the fusion of cyclopentane ring with lactone ring of coumarins (**Table 3**), and green fluorescence (G) series toxin contains fused lactone rings. AFB2 and AFG2 remain non-toxic unless the metabolic oxidization internally by humans and animals forms AFB1 and AFG1. These forms of aflatoxins (AFB1 and AFG1) possess unsaturated bonds at the 8–9 position of the terminal furan ring. The epoxidation at this position, catalyzed by P450 monooxygenase, results in the generation of epoxide, which can further react with DNA adduct, i.e., Aflatoxin N7 guanine, which is critical for its carcinogenic potency. Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2) are hydroxylated forms of AFB1 and AFB2 in cattle milk by consuming contaminated feeds. Several studies suggest the correlation between aflatoxin and point mutation. This mutation occurs at a specific location, i.e., at the third base of codon 249 in the p<sup>53</sup> gene, resulting in the transversion of guanine to thymidine, which was observed in liver cancer in African patients [20].

In contrast, few studies reveal that aflatoxins carcinogenicity is independent of p<sup>53</sup> gene mutations [21]. Structure elucidation of AFB1 was identified in early 1963,

S. No	Aflatoxins	Aflatoxin producing fungi
Difuranocoumarins series		
1	AFB1	<i>A. flavus</i> , <i>A. arachidicola</i> , <i>A. bombycis</i> , <i>A. nomius</i> , <i>A. rambelli</i> , <i>A. ochraceoroseus</i> .
2	AFB2	<i>A. nomius</i> , <i>A. parasiticus</i> , <i>A. flavus</i> , <i>A. arachidicola</i> , <i>A. minisclerotigenes</i>
3	AFB2A	<i>A. flavus</i>
4	AFM1	<i>A. flavus</i> , <i>A. parasiticus</i>
5	AFM2	Metabolite of AFB2
6	AFM2A	Metabolite of AFM1
7	AFLATOXICOL	<i>A. flavus</i> , Metabolite of AFB1
8	AFLATOXICOL M1	Metabolites of AFM1
Difuranocoumarolactone series		
9	AFG1	<i>A. flavus</i> , <i>A. arachidicola</i> , <i>A. minisclerotigenes</i> , <i>A. nomius</i> , <i>A. parasiticus</i>
10	AFG2	<i>A. flavus</i> , <i>A. arachidicola</i> , <i>A. minisclerotigenes</i> , <i>A. nomius</i> , <i>A. parasiticus</i>
11	AFG2A	Metabolite of AFG2
12	AFGM1	<i>A. flavus</i>
13	AFGM2	Metabolite of AFG2
14	AFGM2A	Metabolite of AFGM2
15	PARASITICOL (P)	<i>A. flavus</i>
16	AFLATREM	<i>A. flavus</i> , <i>A. minisclerotigenes</i>
17	ASPERTOXIN	<i>A. flavus</i>
18	AFQ1	The major metabolite of AFB1

**Table 3.**  
*Aflatoxin-producing fungi and aflatoxin derivatives depending on the Difurocoumarin and Difurocoumarolactone series.*

further validated by its total biosynthesis [22]. Foods with carbohydrate and lipid content are more susceptible to aflatoxin contamination. Once produced, they are highly stable and heat resistant. Therefore, removing aflatoxin is tedious and not eliminated by heat treatment. AFB1 is a highly heat-stable form of aflatoxin [23].

#### 1.4 Aflatoxins in groundnut

Groundnuts (*Arachis hypogaea* L.) are extensively grown in semi-arid regions worldwide. It is produced and supplied in over a 100 nations to aid the global demand. Among the Asian continents, China is a massive cultivar. India contributes 65% of the groundnuts produced; the US and the African countries (26%) also cultivate groundnuts. Groundnuts are also termed peanuts, earthnuts, goobers, and monkey nuts. Globally, groundnuts are the 4th most traded among the oil seeds. Groundnuts are rich in proteins (20–50%), fats (40–50%), carbohydrates (10–20%) and soil content of around 33.6–54.95%. Groundnuts are safe to be consumed directly as raw or can be roasted and boiled.

Groundnuts are estimated to be approximately 95% of production in developing countries. Nevertheless, they are contaminated with aflatoxins and are impossible to

be traded globally [24]. Aflatoxin contamination is a severe problem for groundnuts. These contaminations can arise during pre and postharvest periods, extreme weather, increased drought, or excessive precipitation. These conditions lead to increased humidity surrounding the kernels, resulting in groundnut pod infection, eventually affecting stored kernels. The ideal conditions that accelerate aflatoxins in groundnuts are high temperature (up to 45°C) and humidity (65–90%). Infestations by rodents, insect activity and inadequate storage management are the secondary causes that enhance the severity of contamination. Groundnut is an economically important crop. Poor management while cultivating and harvesting can cause considerable loss of cultivation and increase the economic burden. Aflatoxins are colorless, odorless, and flavorless, making these compounds untraceable. Both humans and animals can unintentionally consume the contaminated foods.

Aflatoxins are accountable for the contamination of many economically important cash crops. Many genetically modified species of groundnuts have been developed to address this issue. Planting the crop varieties resistant to aflatoxin-producing fungal species can be a cost-effective way to decrease the infections and eventually reduce the level of aflatoxin in groundnut. Aflatoxins are the group of mycotoxins [7] closely related to groundnuts as it has all the essential components for fungal growth. Due to contamination, most groundnuts are not exported to the international market due to strict guidelines about aflatoxin. Above the permissive limit, the food commodity is neither restricted for trade in the market nor used for other purposes. Since the groundnuts are not being used causes an economic loss. In temperate countries such as India and Africa, it is most likely to get contaminated with soil-borne fungal infections, as groundnuts are in direct exposure to the soil, which elevates the risk of getting infected in changed environmental conditions, eventually causing infestation on pods and plants. *Aspergillus flavus* and *Aspergillus parasiticus* invade groundnuts while harvesting, storage, and processing. These fungi infect the plant, reducing its yield, and produce aflatoxins in seeds. At the same time, if harvested groundnuts are kept in the field for a longer duration without proper management, there is a high risk of pods getting infected with many opportunistic aflatoxin-producing fungi. The insect activity also damages a kernel and results in gaps in the pods. From these gaps, fungi can enter the seeds, which remain inactive until the conditions are favorable for the growth and production of aflatoxins [25, 26].

Preharvest management of groundnuts includes several measures to avoid fungal infection, including cultivating resistant varieties, irrigation, managing insect activity, and crop rotation. These practices can decrease the level of fungal infestation in groundnut. Resistant varieties are not entirely immune to *Aspergillus spp.* However, it can be moderately resistant, which helps reduce aflatoxin-causing fungal growth. Irrigation practices aid in decreasing the temperature of the field as the fungus produces aflatoxins in higher temperatures. It is recommended that regular field irrigation can be beneficial for contamination and the proper management of plant pathogens. Irrigation can relieve drought stress and maintain the temperature of the soil [27]. During the initial stages of crop development, insect activity is a serious concern as it could damage plant parts. Insects also act as carriers of fungal spores from an infected plant to a non-infected plant and transfer the spores through minor incisions. Hence, insects are considered the primary cause of infections in plants. Its proper management in reducing the infections on crops is substantial. Insects including mites, beetles, grasshoppers, and thrips damage peanut kernels and make them more prone to infection by the *Aspergillus spp.* that results in the production of Aflatoxins in seeds.

Similarly, crop rotation is favorable for reducing fungal infections in groundnuts as aflatoxin-producing fungi are soil-borne. Using the non-host plant is an effective strategy for reducing the contamination level for subsequent harvest. Selection of the appropriate crop is crucial because if both plants are susceptible to the same fungi, it can be infectious to both the plants, contaminating the entire field in much more significant quantities and causing inconvenience for market export after the harvest [28].

Postharvest management includes steps after the crops have been harvested from the field. Groundnuts are adequately cleaned and dried until the seed's moisture content reaches 7%. It is crucial for storage since high moisture content in kernels can promote fungal activity leading to aflatoxin production in seeds. Storage spaces must be cleaned thoroughly before storage to avoid fungal and insect infestations. After the drying process, the kernels were transferred to storage rooms and kept for nearly 1 year at 25–27°C [29]. Insects can infect groundnuts during storage but can be managed with chemical pesticides. There is a higher risk of seed damage during transportation which the proper management of transport services can prevent. Recently, newer packing materials have been developed to protect groundnut against fungal infections. Infections can also be decreased by segregating infected and non-infected seeds. Although this is a laborious process, it effectively reduces the aflatoxin level. After extraction of healthy pods, the damaged pods must be immediately incinerated as many cultivars adulterate them with healthy pods for for-profit and endangering the lives of both humans and animals. Altered seeds are restricted from the international market due to the aflatoxin regulations. However, these are sold and utilized at the local markets at much lower prices. Government bodies should regularly monitor these practices to avoid aflatoxin contamination (**Figure 1**).

#### *1.4.1 Phytochemical composition of groundnuts*

Groundnuts are rich in nutritional value with high phytochemical components to prepare oils and animal feeds. The phytochemical analysis of groundnut seeds reveals the following compounds; tannins ( $822 \pm 3.78$  mg/100gm), saponins ( $438 \pm 2.12$  mg/100gm), nitrogen ( $1.33 \pm 0.03$  mg/100gm), phenolic acids ( $218.2.11$  mg/100gm), phytic acid ( $572 \pm 4.37$  mg/100gm), flavonoids like catechins, epicatechins, apigenin, luteolin and phosphorus ( $700 \pm 3.62$  mg/100gm) [30]. Flavonoids have been shown to protect against heart diseases. It inhibits the oxidation of low-density lipoproteins and cholesterol, reducing the formation and circulation of free radicals in the body [31]. Recent researchers have identified a compound phytosterol  $\beta$ -sitosterol (SIT) in groundnut seeds, oils, and flour. This compound has been protective against different types of cancer such as Colon, Prostate and Breast cancers by blocking cholesterol absorption [32].

### **1.5 Aflatoxins in maize**

Maize is an essential and staple agricultural crop that is consumed worldwide. In most regions of the world, maize is infected with aflatoxins, especially in tropical and subtropical areas. In terms of production, consumption, and revenue, maize is an essential commodity worldwide. Several countries do not impose safety standards on maize as they lack proper infrastructures, sampling protocols, and qualified personnel to monitor the standards. Due to high aflatoxin poisoning, more than a 100 countries have set specific limits for aflatoxin tolerance levels. Maize is the most traded

food among cereal grains and significantly supports the world economy. However, the contaminations are the concerns that affect the quality of maize, lowering the market price that primarily impacts the underdeveloped countries, affecting the country's economic progress. The FDA and EU have issued guidelines for aflatoxins, which most countries accept [33, 34]. Many farmers continue using local varieties of maize. They have poor agricultural practices while farming, leading to plant infections and maize being contaminated with fungal spores [24]. When there are unfavorable weather conditions, the fungus produces aflatoxins and contaminates the maize.

Regions with high humidity increase the possibility of maize infection with aflatoxin-producing fungi such as *A. flavus* and *A. parasiticus*. Apart from these fungi, a section of Flavi, consisting of more than 18 fungi, can produce aflatoxins. To avoid infections from the aflatoxin-producing fungi, infected maize should be predisposed during its development. During maturation, the maize is more vulnerable to diseases as many bugs, insects, mites, beetles, and grasshoppers carry fungal spores on their body. When it attacks the plant, it gets damaged and injured. Fungal spores enter the plant through these incisions and inhabit the plant until favorable conditions for growth. During adverse drought and elevated temperatures, fungi produce aflatoxins in response to environmental stimuli. Genetically modified species of maize can be a solution for decreasing contamination, but this technique is moderately effective. Another approach is that maize inhabits various atoxigenic fungi that may act as biocontrol agents to cope with aflatoxin contamination [35, 36].

Most farmers cultivate maize for self-consumption and store it in houses with less or no management, increasing the risk of contamination to a greater extent. Consumption of contaminated maize can cause stunted growth, immune suppression, cirrhosis and liver cancer. Safety regulations are followed in the global market to protect and safeguard human and animal health. However, in most developing countries, even with many regulations on aflatoxins, individuals tend to consume contaminated maize that has not been subjected to regulatory checks, which is a significant concern. Consumers should be well informed of the consequences of aflatoxin contamination, and specific awareness meetings should be organized to limit the aflatoxin level in food [24].

Aflatoxins that contaminate maize and its products can be of significant health concern to commercial and substantial farming. According to the FAO, globally, most of the cereal grains in the United States are affected by mycotoxin-producing fungi, increasing the economy's loss [34]. In the US, contamination of aflatoxin is less. However, animal feeds were often recalled due to higher levels of aflatoxin content [37]. Contaminated maize has been least recommended for food and feed consumption. To avoid losses, the contaminated maize, which contains high aflatoxin levels, is transferred to industries to be utilized as feed products that increase animal health risks. Low levels of aflatoxin-contaminated food that are regularly fed can induce liver cancer [38]. Animals express symptoms such as vomiting, feed refusal, weight loss, infertility, and impaired organ functions. Good agricultural management comprises all the steps from plantation to harvest and postharvest handling. The effective practices that help reduce the infections of opportunistic fungi use of Aflatoxin resistant plant varieties, irrigation, use of fungicides and insecticides, sorting, the safe disposal of the infected plant, moisture control measures, for instance, solar drying, trap drying, and improved storage conditions are effective practices that reduce the chances of the crop getting contaminated with fungi producing aflatoxins. The allowed limit of aflatoxins in maize for humans is 20 ppb, and for animals, it is

300 ppb. According to Nyandieka et al. [39], maize crops placed in a sealed container for 1–2 weeks, applied with ammoniation gas can decrease the aflatoxin level by 90%.

Similarly, Whitlow's studies show that to prevent the hazardous effects of aflatoxins on animals. Binding agents can be used in animal feeds to neutralize up to 90% of the aflatoxins from maize while processing [40]. Sorting good and contaminated maize, winnowing, washing, and crushing, combined with dehulling, effectively removes mycotoxins in maize grains. Maize can also be contaminated with other classes of mycotoxins, such as Fumonisin [7, 41].

### 1.5.1 Phytochemical composition of maize

Maize kernels are rich in polyphenolic components, phenolic acids, vitamins, carotenoids, polysaccharides, flavonoids, and sugars. Studies on maize silk flavonoids have shown antifatigue activity and reduced oxidative stress in mice. Even corn tassels are an excellent source of phytochemicals. In a study by Duangpapeng et al. [42], they found that 92.4% of antioxidants in a variety P4546 for DPPH (2,2-diphenyl-1-picrylhydrazyl) [42]. Cornhusk consists of anthocyanins, and corn pollen contains phenolic compounds. Phytochemical analysis helps genetically modify the species to produce chemicals with better insecticidal activity and increased nutritional quality [27].

## 1.6 Aflatoxins in coffee

Coffee is consumed globally due to specific tastes and its various medicinal properties. Globally, coffee is a beverage widely consumed due to its health benefits, taste, aroma, pharmacological properties, and stimulant effects [43]. Various species are cultivated worldwide, but coffee arabica (60%) and robusta (40%) provide the global supply. It has been the second most exported commodity after petroleum and is crucial in supporting the economy. Wide coffee varieties are cultivated worldwide. Coffee Arabica and Coffee Robusta are major cultivars and dominate the coffee market. Coffee Arabica is mild and has more aroma than robusta.

In contrast, Robusta beans are dark with high caffeine content than coffee arabica. More than 80 species of coffee are grown in different regions of the world. In every society, coffee has historical, social, cultural, and economic value. Brazil has been the largest producer and exporter of raw coffee beans globally [44]. In 2018 2.13MT of coffee was exported, earning the US \$ 5.14 Billion.

In India, coffee cultivation started in the 1600s. Beans were brought by Baba Budan from yamen and first grown in Chikmagalur district, Karnataka. Globally, India is the sixth-largest cultivar and trader of coffee. In the post-monsoon season (2020–2021), 342000MT of coffee has been harvested in India. The country's prominent coffee-producing regions are Karnataka, Kerala, Tamilnadu, Tripura, Nagaland, Assam Meghalaya, Manipur, Orissa, Andhra Pradesh, Arunachal Pradesh, And Chikhaldera [45]. In Chikmagalur, Karnataka, the average coffee production is 80300MT, 35800MT Coffee arabica, and 44500MT Coffee robusta. Around 250,000 cultivars are currently present in India, whose source of revenue is coffee cultivation. Indian coffee beans with production in different regions have different properties and qualities that can be attributed to several factors such as climatic conditions, soil topography, agricultural practices and harvest conditions that include both preharvest and post-harvest techniques (drying, wet process, storage, roasting, and grinding) [46]. Karnataka state contributes almost 71% of total coffee cultivation in

India, Kerala contributes 21%, Tamil Nadu 5%, and the remaining are small growers. The total coffee harvest consumed domestically in the country is 25–30%, and the remaining 70–75% is exported globally. Numerous factors directly or indirectly affect the cultivation in these regions where coffee arabica and Coffee robusta are produced.

Coffee has various metabolites, terpenes, phenols, and antioxidants. Caffeine is the principal constituent in coffee beans, a psychoactive compound. Regular coffee consumers are less likely to develop psychological disorders like Alzheimer's and Parkinson's. Due to environmental factors and infections caused by molds that produce aflatoxins, coffee cultivars face many issues. AFB1 has many secondary derivatives. The FDA sets specific limits safe intake of coffee and food products. AFB1 is highly resistant to heat, making it challenging to eliminate from the coffee beans. Aflatoxin endemics have resulted in the death of several people in India. Coffee is a major source of caffeine [47].

The caffeine content in coffee beans is a significant concern in the global market, as high caffeine tastes bitter. Caffeine, a psychoactive drug, can be beneficial if its intake is limited and harmful to health in higher doses. Besides health concerns, high caffeine is associated with coffee quality, eventually affecting the cost and consumer preference for coffee beans [48]. There are certain limits to caffeine content. For adults, 3–4 cups of coffee/day, i.e., 300 mg/day of caffeine, is considered moderate intake, and above 400 mg/day is the highest consumption limit [43, 49]. In 2006 Canadian health agencies set caffeine's upper limit as 450 mg/day, which is considered safe. In Australia, the recommended limit is 160 mg/day [49]. Caffeine can cause deleterious effects on health that include cardiovascular disturbance, miscarriage, restlessness, headache, excitement, muscular tension, increased blood sugar levels, sleep disturbance, increased pepsin secretion, and gastric acid secretions [49]. Caffeine can pass through the placenta. It is a stimulant; it increases the foetal heart rate and metabolic processes. Higher doses of caffeine elevate the risk of spontaneous abortion and impair fetus growth [50]. In adults, the moderate caffeine content in the blood can cause cardiovascular stimulatory effects and behavioral changes [51].

Coffee is rich in antioxidants. Several commercial companies market energy drinks with high caffeine content, and unknowingly, many consumers having a history of cardiovascular diseases ingest it, placing their lives at risk. In the Tromso heart study [52], it was observed that consumption of coffee could reduce the gamma-glutamyl transferase (GGT) level. [53], and yet another study documented shows inverse relationships between coffee consumption and levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [54]. Besides caffeine, coffee has many aromatic compounds that give coffee its particular smell and taste. Its perception is dependent on the volatility of the compound present. Grosch studied many aromatic compounds in coffee. The study identified around 900 volatile compounds, but lesser than 20 compounds have been associated with coffee aroma. Consumption of coffee has beneficial effects on health and was found to have hepatoprotective effects. In 2015, Lui F. conducted a study on the consumption of coffee and found out that those who consume coffee have reduced incidences of cirrhosis compared to non-consumers of coffee (**Figure 1**) [55].

### *1.6.1 Phytochemical composition of coffee*

Caffeine (1, 3, 7 trimethyl xanthine) is found in coffee beans, and apart from beans, it occurs in other plant parts [52, 56]. Caffeine is a psychoactive drug utilized

by pharma companies to treat Alzheimer's and Parkinson's disease [57]. It has non-selective adverse effects on adenosine receptors, specifically at A1 and A2A positions. These receptors (A1 and A2A) are in a consistent reaction and only work in the presence of stimulatory G protein. A1 receptors affect the portion of the brain that controls the sleep and wake cycle, and the A2A receptor is present in the dopamine-rich areas of the brain. By forming heterodimers, A2A receptors coexist with dopamine D1 and D2. Chlorogenic acid (polyphenol) [52, 58] is primarily a phenolic ester of trans-cinnamic acid and quinic acid, also known as 5-o-caffeoylquinic acid [59]. It regulates glucose and lipid metabolism, cardiovascular diseases, cancer, and hepatic steatosis [60]. Also, few studies reported anti-diabetic, anti-carcinogenic [46], and anti-inflammatory activities.

Coffee beans have fat-soluble lipids such as kahweol and cafestol [52, 61]. These diterpenes originated from the isoprenoid metabolic pathway. An isoprenoid is a varied group of plant metabolites [62]. These diterpenes increase the blood cholesterol level in humans if consumed unfiltered. Post et al. [63] found that coffee brew consumption increases the cholesterol level [63]. In an experiment, bile acid mass production was measured on 24 hours basis with 8 hours of preincubated rats fed with cafestol, kahweol, and isokahweol mixture in a proportion of 48:47:5w/w, which resulted in a decline of bile production showing  $91 \pm 5\%$  and  $68 \pm 3\%$  inhibition [63]. Kahweol is highly unstable when purified. Thus, its properties are studied in combination. They are diterpenes with anti-cancerous properties. These diterpenes have been reported to act against AFB1 in humans. Also, these compounds can produce various biochemical processes that decrease the genotoxicity of cancer-causing agents such as DMBA, AFB1, BaP, and PhIP. The studies by IARC categorize coffee as non-carcinogenic for humans.

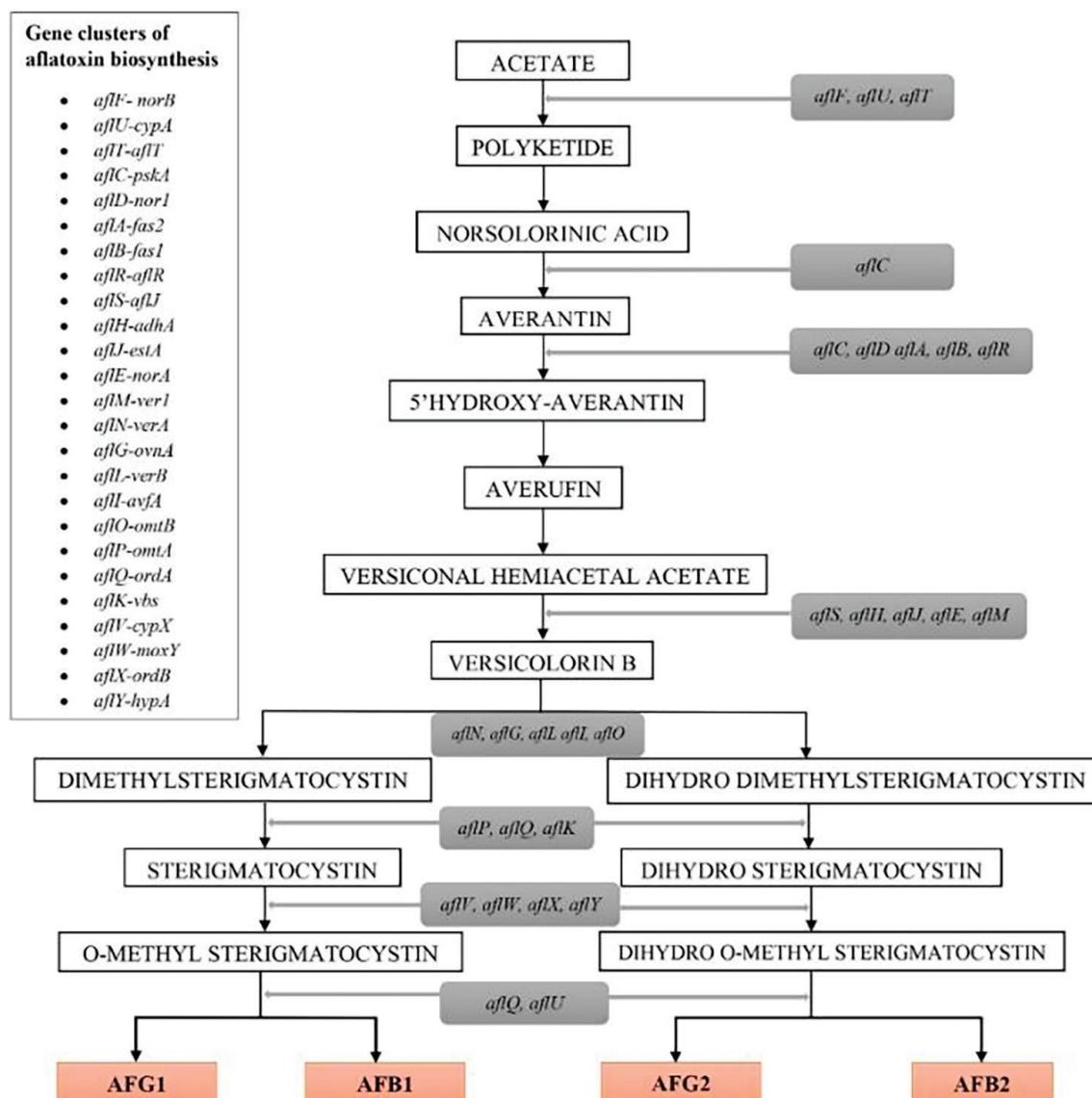
Several bioactive were isolated and identified from coffee leaves, including alkaloids, flavonoids, terpenoids, xanthonoids, phenolic acids and catechins, tannins, and sucrose [64]. Emura reported that the floral parts have aromatic nitrogenous compounds and phenylethane derivatives. In fruits, caffeine and tannins are present, which have antioxidant properties. Green beans of coffee consist of 7–17% of lipids, phenolic, and chlorogenic acids. These compounds can inhibit oxidative damage by free radical scavenging in biosynthesis and improve CCl4-induced liver damage by inhibiting the CYP2E1 liver-inducing enzyme and forming free radicals. Coffee oil consists of various bioactive compounds, and it was found that these metabolites can cure diabetes type 2 conditions [43, 65], cancer [65], and inflammation [52]. According to Velazquez Pereda Mdel et al. [66], green coffee oil affects elastin, collagen, and glycosaminoglycan synthesis [67].

## 2. Biosynthesis of aflatoxins

In 1967, Birch first proposed that a polyketide pathway is required for aflatoxin production [68]. Aflatoxins have similar structures, which are dihydrofuran coumarin derivatives (**Table 3**) [69]. Biosynthesis of Aflatoxins is a long series of processes requiring a minimum of 30 genes grouped inside 75 bp gene clusters and regulated by specified transcription factors. In the early 1990s, molecular biologists studied aflatoxin biosynthesis. In 1992 first gene was identified, isolated and transcribed (nor 1 and ver 1); later, a complete gene clustered for aflatoxin biosynthesis was identified. Concerning sequencing, *A. flavus* and *A. parasiticus* have similar gene clusters. However, they vary in deletion, ranging from 0.8 kb (L strain) and 1.5 kb



(S strain) [70]. In the case of *A. flavus*, deletion extends from 5'end of *aflF*, *aflU* to whole 279 bp intrinsic loci, which prevents it from producing the Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2). The DNA analysis of *A. flavus* and *A. parasiticus* shows a 96% affinity for the gene clusters. For the aflatoxin biosynthesis in *A. flavus*, there are 30 pathway genes involved, including the antisense gene (*aflR*), three sugar utilization genes, and ORF genes, all of which are clustered within the 80 kb DNA region. *A. parasiticus* has 25 pathway genes consisting of sugar utilization genes and ORF genes clustered within the 80 kb DNA region [69]. For Norsolorinic acid (NOR) synthesis, three transcription factors are involved, the *aflA* gene (Fatty acid synthase A), *aflB* gene (Fatty acid synthase B), and *aflC* gene (Polyketide synthase), which helps in Norsolorinic acid synthase (NorS) production [71]. NorS utilized in synthesizing hexanoyl primer by integrating with the molecules of malonyl CoA. Hexanoyl primer shifts towards  $\beta$ -ketoacyl synthase and attaches to malonyl CoA, forming Norsolorinic acid anthrone (NAA). In the presence of NAA oxidase, it turns into Norsolorinic acid. This step is a crucial metabolite for aflatoxin biosynthesis [72]. Next, the *aflD* gene (Reductase), a NOR 1'keto group, is reduced by ketoreductase to the AVN 1'hydroxyl group. Even if it has defined work, the mutant strain of the *aflD* gene does not always lead to the formation of AVN. The gene *aflG* encodes cytochrome P450 mono-oxygenase which catalyzes the breakdown of AVN on the 5'keto group, which then converts into a 5' hydroxyl group of 5'Hydroxyaverantin (HAVN) in *A. parasiticus* [73]. The *aflH* gene (dehydrogenase) 5'hydroxyaverantin dehydrogenase help in the dehydrogenation of the 5'hydroxyl group of HAVN to the 5'oxide group of oxoaverantin (OAVN) [74]. *AflK* gene (OAVN Cyclase) catalyzes the hydration of 5'oxide of OAVN to form the 2'-5' Averufin (AVF). *aflV* gene encodes P450 oxidoreductase, which reduces the hydride groups from AVF, and the *aflI* gene encodes the function of an oxidoreductase. *aflW* gene encodes monooxygenase, which incorporates O<sub>2</sub> atoms within 4'-5' ketone groups of HAVN, producing Versiconal hemiacetal acetate (VHA). VHA acetate is stimulated by the *aflJ* gene (Esterase) enzyme, eradicating and converting it into Versiconal (VAL) [75]. Afterwards, the *aflK* gene encodes the cyclase enzyme, which helps catalyze the cyclodehydration of VAL and converts it into Versicolorin B (VERB). In this step, bisfuran ring closure has occurred. It acts as a final precursor for the aflatoxin biosynthetic pathway and is an important step. *AflL* gene (desaturase) converts the tetrahydrofuran ring to dihydrobisfuran in the presence of the enzyme cytochrome P450 monooxygenase. VERA is utilized for the formation of Demethylsterigmatocystin (DMST), *aflM*(*ver1*), *aflN*(*ver1*), *aflY*(*hypA*) and *aflX*(*ordA*) enzymes are needed to produce AFB1-AFG1 [76]. Similarly, for the AFB2-AFG2 biosynthetic pathway, VERB is utilized as a substrate, leading to Dihydro-Demethylsterigmatocystin (DHDMST). *aflO* gene encodes O-methyltransferase and helps convert the S-adenosylmethionine methyl group, DMST hydroxyl group, and synthesis of sterigmatocystin from DHDMST and DHSD based on the Aflatoxin biosynthetic pathway. *aflP* is another gene which encodes O-methyltransferase, suitable for substrates like sterigmatocystin but can catalyze DHST and DHOMST. *aflQ* gene encodes cytochrome P450 monooxygenase, which helps transform OMST into the Aflatoxin B1 (AFB1)/Aflatoxin G1 (AFG1). Yu suggested *aflQ* gene replicated in C-11 hydroxylation. In contrast, the keto tautomer 11 hydroxy of the OMST *aflL* gene might serve as an Oxygen (O<sub>2</sub>) source [77]. *aflM* demethylates a ring that works with cytochrome P450 as a final aflatoxin biosynthetic pathway. The *aflV* gene oxidizes the metabolites produced in the process, which are then utilized as a substrate for the formation of the final intermediate, which is then



**Figure 2.** Modified and adapted Flow diagram for Biosynthetic pathway of Aflatoxins [25, 77].

catalyzed by the *nadA* gene and the *aflF* gene. The product is obtained as an AFG1 [69, 78]. *aflU* encodes a reductase and cytochrome p450 monooxygenase, and the *nadA* gene helps catalyze the DHD MST to form an AFG2. So the *aflE(norA)* and *aflF(norB)* genes are suitable substrates for enhancing the activity for the production of AFG1 and AFG2 [69, 70] (Figure 2).

### 3. Prevention and management of Aflatoxin contamination

To detoxify and reduce aflatoxin levels in crops, it is crucial to intervene in both preharvest and postharvest processes. During pre-harvest, good agricultural practices, environmental factors, soil conditions, and certified fertilizers are the vital factors that help in plant development. The use of biological agents aids in reducing aflatoxin-producing fungi by eradicating them from the field. It is an effective preharvest strategy.

Postharvest techniques involve chemical methods, ammonification, the use of hydrated oxides, and biological agents. Oxidation, reduction, hydrolysis, and absorption have been performed in the chemical process [7]. Treatment with ammonia reduces aflatoxin to an untraceable level, but ammonia can be harmful to human health. Thus, European Union (EU) forbids treatment with bases for food intended for consumption by humans. Calcium hydroxide is used to degenerate the structure of aflatoxin AFB1 and reduce the toxicity. These chemicals can have harmful effects on food products and human health. Sorbent additions like silica, alumina, and aluminosilicates can bind to aflatoxins, minimizing the toxic effects. Sodium bisulfate can deprive Aflatoxins (AFB1) molecules in humans and animals on DNA reaction sites, thus decreasing the mutagenic potential of aflatoxin [7]. Another method for reducing aflatoxin includes epoxide hydrolase and glutathione-S-transferase [61]. It detoxifies the activated AFB1, glutathione conjugates with AFB1 8, 9 epoxides converted into glutathione aflatoxin conjugate and finally removed from the cell. CYP1A2 is effective in the hydroxylation of AFB1 to less potent AFM1, a poor substrate of epoxidation by the catalysis of cytoplasmic reductase enzyme, which converts AFB1 into AFQ1 that is excreted from the body by urination [61].

### **3.1 Aflasafe®**

Aflasafe is a biological control method for managing the fungal strain of *Aspergillus flavus*, which contaminates food and feeds with aflatoxin. Aflatoxins are harmful metabolites produced in farms and storage rooms during stressful conditions such as high humidity and extreme heat. Afla safe is a developed strain of *Aspergillus flavus* that does not produce aflatoxin. When the Aflasafe is introduced in farming fields, it removes toxic strains with non-toxic strains. This phenomenon is known as competitive exclusion, as it increases competition between two strains of the same fungi [79]. Bandopadhyay and their research group have reported around 80% aflatoxin reduction in fields treated with Aflasafe strains compared to non-treated fields [27]. Aflasafe shows a promising result, particularly in crop fields and storage rooms. Even after the harvest, it does not allow the toxic strain of *Aspergillus flavus* to produce aflatoxins. This has long-term benefits, lowering the need for fungicides in storage rooms. For commercialization, farmers should utilize Aflasafe as it helps reduce contaminated crops and helps produce safe staple food [80]. Adopting Aflasafe can be beneficial for the reduction of contaminations in a staple food (maize), resulting in higher prices and a rise in the economy. Food safety levels can be maintained and stored for longer durations with proper management [27, 79].

The International Institute of Tropical Agriculture (IITA) has developed Aflasafe SN1 in a Nigerian laboratory according to the methodology adopted by Atehnkeng et al. 2014. Since this region is at a higher risk for aflatoxin contamination, trials were conducted to determine the efficacy of the Aflasafe SN1 in reducing aflatoxin contamination in oilseeds (Groundnut) from 2010 to 2013 results suggested reduced aflatoxin contamination. AG RESULTS Projects have promoted Aflasafe adoption [79].

### **3.2 Aflaguard®**

Aflaguard has a solid brown appearance with a barley-like odor. Aflaguard is a biologically designed aflatoxin controlling agent which reduces *Aspergillus flavus* growth [81]. It consists of the non-toxicogenic *Aspergillus flavus* strain (NRRL21882), which does

not produce aflatoxin but can be competitive. Aflaguard is used in both corn and groundnut fields. The company recommends a quantity of 20 lb./acre which can be applied with the help of a tractor-mounted Grandy box or broadcast applications. Aflaguard should be applied to plants only after 40–80 days of a plantation. Aflaguard can be applied through the air with the same quantity, i.e., 20 lb./acre for groundnuts. In maize, Aflaguard GR can be applied during the V7 and R1 growth stages (the V7 stage refers to the growth period where seven visible leaf colors are present, and the R1 stage refers to the onset of silking) with the broadest application equipment. Precautions should be taken that all the cultivation and mechanical activities have been completed before the application of Aflaguard GR. The recommended limit of Aflaguard® GR is per season. Precautionary measures, such as PPE kits, must be used using Aflaguard® [82].

### **3.3 Hermetic bags**

Grain pro® is a global company in concord, Massachusetts, established in 1992 and specialized in Ultrahermetic technologies for storage, transportation, and drying agricultural solutions. Grain pro company has many collaborations with various organizations worldwide, which help them develop a sustainable system for postharvest management. Grain pro products are Grain pro cocoon, Grain pro bags zipper, and Grain pro transafeliner. These developed products support chemical-free and organic. Hermetic bags has been utilized to dry, store, and transport agricultural commodities. It supports moisture-free, insects free and mold-free conditions. Grain pro bags helps in protecting the commodities against mold interactions, insecticidal activities, oxidation, and rancidity. These products contribute to keeping the food safe, preventing significant costs associated with pests, and manage mold during storage. Grain pro products used by farmers can help avoid food spoilage losses and maintain the quality of seeds in these bags [83].

### **3.4 Purdue improved crop storage (PICS)**

PICS bags were developed by Purdue University in collaboration with private entrepreneurs and vendors. PICS bags are of 50 kg and 100 kg capacity and cost around \$2USD-\$4USD. PICS bags are high-density polyethene bags that are 80 µm in thickness. PICS comprises three bags; the inner bag is filled with grains and covered with another bag, the middle. Then these two bags are covered by a bag made from woven polypropylene. Woven bags are thick. Inner liners have less permeability for oxygen. The middle bag is tightly packed above the innermost bag and should surround without gaps. The outer woven bag is tied over those two bags. The advantage of PICS bags is that this method is devoid of fumigants and insecticide use. Thousands of smallholder farmers adopt PICS bags to store grains, showing promising results by reducing contamination levels and insecticidal activity. Farmers can utilize the same bags multiple times for a longer time. These bags can help achieve food security goals and preserve nutritional value. PICS bags helps in minimizing mold growth, management of insect pests, controlling mycotoxin accumulation, and postharvest loss [84].

### **3.5 Artificial intelligence (AI) and aflatoxin**

Artificial intelligence is a program designed to develop intelligent machines that can work with higher accuracy. Artificial intelligence aids in understanding the processes that can predict and develop 3D structures of materials in scientific fields.

Pure scan AI is a newly developed technology that is a portable and easy-to-use device that can detect aflatoxins in groundnuts and maize via spectroscopic (UV) accessible scan technology integrated with Artificial Intelligence (<https://purescanai.com>) [85]. It is cost-effective and can identify contamination in less than 30 seconds, with an accuracy of 10 ppb. Results can be monitored on any device. The mechanism of this device is that cameras with filters capture an aflatoxin fluorescence. The captured images are processed through a program to detect the degree of fluorescence patterns, which helps predict the level of aflatoxin contamination in a sample. Pure scan AI is a startup company. The International Crops Research Institute for the SemiArid Tropics (ICRISAT) Hyderabad scientists Dr. Hari Sudini and Dr. Shrikanth Rupavatharam have collaboratively developed an Aflascan AI device to detect aflatoxin-contaminated groundnuts and maize with a 1 ppb accuracy. It is an integrated system of UV lights, and the results can be observed on any android device. This device costs around INR50000 (Indian Rupees) and per test INR8.

#### **4. Discussion**

Aflatoxin contamination is a significant cause of contamination on oilseeds (groundnuts), staple food (maize), and commodities (coffee). These food products are widely traded worldwide and are supplied for making food products for human and animal consumption. Due to improper management, fungi damage and contaminate the groundnut, maize, and coffee during their initial harvesting and storage conditions. Excessive drought and humidity increase the chances of infection. This is likely to increase if the crops are prone to insects, rodents, and birds attacking during the pre-harvest. The damages that occur in the crop pave the way for the opportunistic fungi *Aspergillus flavus* to penetrate the plant and stay dormant until favorable environmental conditions occur. Fungal spores can stay inactive for more extended periods. That is why, after postharvest, fungi are still attached to the harvested product. In storage rooms, deposited fungi grow and infest the whole batch of bags under optimum temperature and produce aflatoxins, which are harmful toxins for humans and animals.

Groundnut is the most traded oilseed which is cultivated worldwide. Just after removing the oils from seeds, the remaining residues of seeds are utilized to make animal feeds. All the parts of the seeds are ultimately used, making it a perfect food. Groundnuts are rich in proteins and are a valuable crop. It is susceptible to fungal infections, and *Aspergillus* is a significant contaminant that produces aflatoxins in groundnuts. It is challenging the elimination of Aflatoxin contamination from the seeds; the entire batch needs to be discarded, resulting in a loss for farmers, which are not being compensated. Aflatoxins contamination is one of the leading causes of economic loss in groundnuts [1]. Maize is a staple food crop widely cultivated in all regions around the globe. Aflatoxin contamination leads to the significant loss of maize kernels. Maize kernels are filled with starch and proteins, a good source for fungal growth, and the plant suffers contamination. Insect activities induce infections.

Contamination can be prevented in the initial stages of infection but must be monitored regularly. Due to aflatoxin-contaminated maize consumption, Kenya had high mortality. A high aflatoxin concentration in food can be lethal and must be impeded by traditional and newly developed technologies [7]. Several technologies have been developed to manage aflatoxins, such as Aflasafe®, Aflaguard®, Hermetic bags, and

PICS bags, which show promising results in reducing contamination [27, 83]. Newer technology, such as Aflascan, was developed by Proscan and ICRISAT researchers. This device can visually detect aflatoxins in groundnuts with a high accuracy level. Coffee consumption is widely spread globally and plays an essential role in the economy with additional health benefits.

Contaminated coffee beans containing aflatoxins can cause aflatoxicosis in humans and animals. There is a need for specific limits on aflatoxin content in food products with accurate monitoring. Many aflatoxin-producing fungi grow in temperate and humid regions during harvest and storage [86]. Aflatoxin contamination causes a massive loss of coffee beans worldwide and affects the economy. Many strategies are used to reduce aflatoxin contents in coffee. However, there are certain limitations to the chemical method, and there is a risk of other diseases due to chemical impurities in coffee. In the traditional method, some aflatoxins are heat-resistant AFB1. Novel techniques need to be developed for the best results. Aflatoxin has a series of global health issues, and it causes hepatic cancer and cirrhosis in humans and animals [6]. Aflatoxin control strategies will provide a better quality of coffee beans and reduce several harmful diseases.

It is a fundamental need to ensure food safety against deadly mycotoxins and aflatoxins. The first limits for aflatoxins were set in the late 1960s. By 2003, many countries had developed their limits for aflatoxins according to their needs. There is a need for research on aflatoxins to explore the short and long-term effects on human health [87].

## 5. Conclusion

In this review, comprehensive data has been presented from previous studies by researchers. Aflatoxins are a significant issue worldwide; they cause a substantial economic burden on developing countries. It is responsible for contaminating the crops like groundnut, maize, and coffee. These crops are economically important as their never-ending demand in the local and international market makes them perfect crops for trading. *Aspergillus* is soil-borne fungi that produce Aflatoxins in stress conditions caused due to environmental factors. In research of IARC, it was found that aflatoxins have the potential to induce cancer, and they categorize it as a group 1 carcinogen. Groundnut, maize, and coffee are considered commodity crops. They are on the list of 10 highly traded crops, increasing their importance in the market. Due to fungal infections, farmers face huge losses as when aflatoxin is produced in seeds, it cannot be eradicated, and it is very stable, having heat resistance capability. The level of aflatoxin in seeds can be reduced to a certain level by chemical treatments, which are costly and hazardous. Aflatoxins are untraceable and which makes them more dangerous to humans and animals. There are very few solutions available to reduce aflatoxins in crops. Recently, some modified technologies were developed and used in preharvest conditions as a suitable time to stop the infection from fungi like *Aspergillus*. Aflasafe and Aflaguard are genetically developed products that help reduce the aflatoxin-producing *Aspergillus* on farms. Hermetic bags and PICS are technologies utilized in postharvest conditions. These bags are integrated with specific compounds that inhibit fungi infections. Still, even after these technologies are available. Aflatoxins are a concern because this technology can not altogether remove the contamination in seeds; it only prevents them to some extent. Still, further research is needed to find the eco-friendly and less costly methods to eradicate

aflatoxins from seeds completely. This study will help develop techniques and technologies to control and manage aflatoxins in preharvest and postharvest conditions.

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## **Author contributions**

AK and VBCS conceptualized the topic; AK wrote the manuscript; VBCS & AR corrected the manuscript. The authors read and approved the final manuscript.

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