

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,400

Open access books available

174,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter

The Menace of Aflatoxin: Understanding the Effects of Contamination by *Aspergillus* *Species* on Crops and Human Health and Advancements in Managing These Toxic Metabolites

*Amir Afzal, Sairah Syed, Rafiq Ahmad, Muhammad Zeeshan
and Ghulam Nabi*

Abstract

Food security and safety are essential global issues that require collaboration from governments, private industry, and individuals to ensure there is enough safe and nutritious food to meet the needs of a growing population. The three main elements impacting food security and safety are the availability of food, access to safe food, and the utilization of food for a healthy life. Aflatoxins, harmful mycotoxins produced by certain fungi, damage a significant proportion of the world's food supply, which is a factor in food insecurity. Effective strategies to prevent and manage aflatoxin contamination in crops include promoting sustainable and diversified agricultural practices, improving crop management, post-harvest handling and storage, and strict regulation and monitoring of food quality and safety. To date, there have been 20 different types of aflatoxins identified, with B₁, B₂, G₁, and G₂ being the most prevalent and dangerous. To mitigate the impact of aflatoxins, it is important to understand the mechanisms of contamination, the impact of aflatoxins, and the management strategies that can be employed to reduce contamination. An updated review on aflatoxin contamination, its impact and management strategies can provide valuable information for researchers, policymakers, and food safety professionals.

Keywords: food safety, aflatoxins, aflatoxin contamination, *aspergillus* spp.

1. Introduction

Food security and safety are indeed global issues that are becoming increasingly important as the world's population continues to grow. Food security refers to the ability of individuals and communities to access sufficient, safe, and nutritious

food to meet their dietary needs and preferences. With a growing population, it is essential to ensure that there is enough food available to meet the nutritional requirements of everyone. Food safety, on the other hand, refers to the measures taken to ensure that food is free from harmful contaminants and pathogens that could cause illness or disease. This includes everything from ensuring that food is properly stored and handled to conducting regular inspections of food processing facilities and enforcing strict food safety regulations. Ensuring both food security and food safety requires a coordinated effort from governments, private industry, and individuals. This includes investing in sustainable agriculture and food production practices, supporting local food systems, and promoting nutrition education and awareness. Ultimately, ensuring food security and safety is critical for the health and well-being of individuals and communities around the world, and it will require ongoing commitment and collaboration from all stakeholders to achieve this goal. Food security and safety are among the top priorities in today's world with a growing population. These issues are primarily influenced by three critical elements: (1) sufficient food availability, (2) accessibility to safe food, and (3) utilization of food with regard to its quality, nutritional value, and cultural significance for a healthy lifestyle. Having enough food is essential to ensure individuals have access to enough sustenance to meet their daily energy requirements and maintain good health. However, food security goes beyond mere availability and encompasses the quality and safety of the food as well. Access to safe food is also a concern, particularly for those who may not have access due to poverty, limited resources, or unavailability [1]. In some areas, food may become contaminated due to improper storage, handling, or transportation practices, making it necessary to implement measures to ensure proper food handling and storage. Finally, utilizing food in terms of quality, nutrition, and cultural significance is important for a healthy life. Food provides necessary nutrients for growth and body maintenance, and also has a significant cultural and social impact [2]. A balanced and varied diet is necessary to maintain good health and prevent chronic diseases. In conclusion, addressing these crucial elements of food security and safety is imperative for the overall well-being of the global population. If any of these components fail, it results in food insecurity and malnutrition that has a detrimental impact on human health and the social and economic well-being of society. Furthermore, contamination of food and feed by mycotoxins is a significant contributor to the problem of food insecurity [3]. Aflatoxins are a type of mycotoxin primarily produced by the fungi *Aspergillus flavus* and *A. parasiticus* and have been extensively studied [4]. In addition to the human and animal health risks associated with aflatoxin contamination, it can also have significant economic impacts. Aflatoxin-contaminated crops may be rejected by food processors and retailers, resulting in reduced market value and financial losses for farmers. In some cases, crops may need to be destroyed entirely, which can result in even greater economic losses. The contamination of a diverse range of foods and feeds with AF can lead to economic losses, and various factors such as season, post-harvest and management practices, food type, and geographic location can contribute to this contamination [5]. This review serves as a valuable resource for researchers as it provides crucial information that can be used to devise effective mitigating strategies.

According to the Centers for Disease Control and Prevention (CDC), mycotoxin exposure is a chronic issue affecting an estimated 4.5 billion people [6]. However, a recent study suggests that the global occurrence of mycotoxin contamination in crops is between 60 and 80% [7].

2. Aflatoxin-producing species of *Aspergillus* and their diversity

Aflatoxins, which are primarily produced by *Aspergillus flavus* and *A. parasiticus*, are the mycotoxins known to be produced by species of *Aspergillus*. However, other species such as *A. nomius*, *A. pseudotamarii*, *A. parvisclerotigenus*, and *A. bombycis* from section *Flavi*, *A. ochraceoroseus* and *A. rambellii* from section *Ochraceorosei*, as well as *Emericella astellata* and *Epipleoneura venezuelensis* from the *Nidulatan*s, have also been identified as producers of aflatoxins [8]. There are multiple types of aflatoxins that have been documented, and their presence in crops and food is a major global concern, particularly for crops of economic significance (**Figure 1**). Aflatoxins are toxic compounds produced by certain species of fungi, including *Aspergillus flavus* and *A. parasiticus* [10].

The genus *Aspergillus* is comprised of four subgenera and a total of 339 species [11]. The *Aspergillus* genus is a diverse group of fungi, which includes over 200 species [12]. The agriculturally important species of *Aspergillus* that produce aflatoxin belong to the *Flavi* section [13]. *A. flavus* and *A. parasiticus* are known to contaminate a variety of crops including maize, peanuts, cottonseed, tree nuts, and spices, among others [14]. There are two different types of *A. flavus*, a fungus that creates aflatoxins, based on the size of their sclerotia: L and S morphotypes. The L morphotype produces many spores and a range of aflatoxin levels, but only a few large sclerotia (>400 µm). The S morphotype produces fewer spores, but consistently high levels of aflatoxins, and many small sclerotia (<400 µm) [15].

3. Classification and types of aflatoxins

The names of these aflatoxins are derived from their property of absorbing and emitting light at distinct wavelengths. Aflatoxins B₁ and B₂ fluoresce blue when subjected to ultraviolet light with a wavelength of 425 nm, while aflatoxins G₁ and G₂ fluoresce green when exposed to ultraviolet light with a wavelength of 540 nm [5]. Aflatoxins are difuranocoumarins that can be divided into two categories based on their chemical structure: the difurocoumarocyclopentenone series, which includes AFB₁, AFB₂, AFB₂A, AFM₁, AFM₂, AFM₂A, and aflatoxicol; and the difurocoumarolactone series, which includes AFG₁, AFG₂, AFG₂A, AFGM₁, AFGM₂, AFGM₂A, and

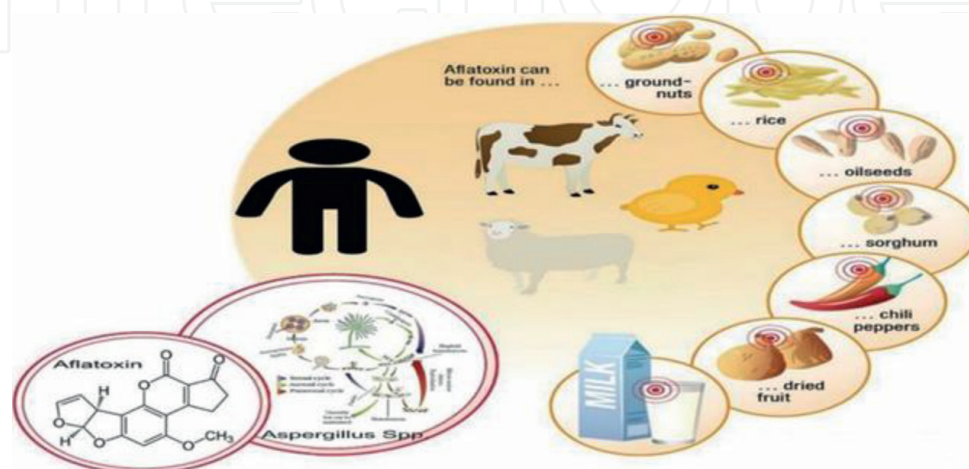


Figure 1. Contamination of aflatoxins in a wide range of regional crops and food commodities [9].

AFB₃ [16]. More than 20 distinct types of aflatoxins have been identified to date [17]. However, the most commonly and widely known aflatoxins are aflatoxins B₁, B₂, G₁, G₂, M₁, M₂, aflatoxicol, and aflatoxin Q₁ [18].

4. Environmental conditions favoring aflatoxin production on crops

The growth of these fungi is commonly observed in warm and humid conditions, which are prevalent in tropical and subtropical regions [19]. They are commonly found on crops such as corn, peanuts, cottonseed, and tree nuts, which are stored in warm and humid conditions for extended periods of time. This provides an ideal environment for the growth and spread of aflatoxin-producing fungi, leading to contamination of these crops and the potential health hazards and economic losses that come with it [20].

5. The carcinogenic properties of aflatoxins B₁, B₂, G₁, and G₂

Of all the types of aflatoxins, aflatoxins B₁, B₂, G₁, and G₂ have received the most extensive research attention and are the most commonly found, and they have been shown to have the most significant impact on human and animal health [21, 22]. These are considered to be more important due to their widespread prevalence in food [5]. Aflatoxins B₁, B₂, G₁, and G₂ are four major types of aflatoxins that are classified as Group 1 carcinogens. Among them, the most toxic and commonly found in crops is aflatoxin B₁ (AFB₁) [23]. Moreover, aflatoxin B₁ can bind to DNA and modify its structure, resulting in genotoxic effects [24]. Different species of *Aspergillus* fungi produce different types of aflatoxins. Aflatoxins B₁, B₂, G₁, and G₂ are produced by *Aspergillus bombycis*, *A. nomius*, *A. parasiticus*, *A. parvisclerotigenus*, *A. pseudocaelatus*, *A. minisclerotigenes*, and *A. arachidicola*. However, species such as *A. flavus*, *A. ochraceoroseus*, and *A. rambellii* only produce aflatoxin B₁ and B₂, while others such as *Aspergillus pseudonomius*, *A. pseudotamarii*, *Emericella astellata*, *E. olivicola*, and *Epipleoneura venezuelensis* produce only aflatoxin B₁ [25]. The occurrence of aflatoxins was observed in the following decreasing order: AFG₂ > AFG₁ > AFB₂ > AFB₁, where: AFG₁: Aflatoxin G₁, AFG₂: Aflatoxin G₂, AFB₁: Aflatoxin B₁, AFB₂: Aflatoxin B₂. Note that the ">" sign indicates "greater than" and is used to show the decreasing order of occurrence, with AFG₂ being the most prevalent and AFB₁ being the least prevalent with concentration ranges of 0.78 ± 0.04–234.73 ± 3.8 µg/kg, 0.47 ± 0.03–21.6 ± 0.33 µg/kg, 1.01 ± 0.05–13.75 ± 1.2 µg/kg, and 0.66 ± 0.06–5.51 ± 0.26 µg/kg, respectively. Of the 100 samples analyzed for total aflatoxins (total AFs), 68 (68%) exceeded the limits set by the EC, with concentration ranges of 4.98 ± 0.6–445.01 ± 8.9 µg/kg. Similarly, 58 (58%) of the samples exceeded the limits set by GSA, with concentration ranges of 12.12 ± 1.4–445.01 ± 8.9 µg/kg [26].

6. Understanding the health effects of aflatoxin exposure

Aflatoxin contamination is a significant public health concern, particularly in developing countries where food safety regulations may be less stringent and where poverty and malnutrition can exacerbate the health effects of exposure [27]. The European Commission and the U.S. Food and Drug Administration have set a

maximum limit of 20 ppb (parts per billion) of aflatoxins in food and feed products for human and animal consumption to help prevent these health hazards and economic losses. Aflatoxins are both carcinogenic and mutagenic in nature and can result in aflatoxicosis in humans and animals [5]. Recently, a form of pulmonary aspergillosis has been linked to the coronavirus disease (COVID-19). It has been established that patients with compromised immune systems are more likely to experience severe cases of COVID-19 when complicated by pulmonary aspergillosis. To date, there have been 20 reported cases of COVID-19 associated pulmonary aspergillosis (CAPA) worldwide [28]. Recently, a correlation between the coronavirus disease (COVID-19) and pulmonary aspergillosis has also been reported, with 20 cases of coronavirus disease-associated pulmonary aspergillosis (CAPA) recorded globally. It is noted that the severity of COVID-19 symptoms is increased in immunocompromised patients with pulmonary aspergillosis [29]. Given their widespread presence, around 4.5 billion people worldwide are estimated to be exposed to aflatoxin contamination [30]. The adverse effects of aflatoxins on living organisms have led to the setting of a maximum limit of 20 ppb (parts per billion) for aflatoxins in food and feed products intended for human and animal consumption by both the European Commission and the U.S. Food and Drug Administration [31]. The adverse health effects of aflatoxin contamination can include acute toxicity, immune suppression, liver damage, and an increased risk of developing liver cancer. Aflatoxin contamination can have a range of adverse health effects in both humans and animals, and these effects can vary depending on the level and duration of exposure. The most significant health effects of aflatoxin contamination include the following.

6.1 The adverse health effects of aflatoxin contamination and the importance of prevention and control measures

High levels of aflatoxin exposure can cause acute toxicity, which can result in a range of symptoms, such as vomiting, abdominal pain, convulsions, coma, and even death ([32] Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557781/>). These symptoms occur due to the liver's inability to detoxify aflatoxins effectively, leading to a buildup of toxic metabolites in the body. When consumed, aflatoxins are primarily metabolized in the liver, where they can cause damage to liver cells and impair liver function. The liver is responsible for breaking down and eliminating many toxins, including aflatoxins, from the body. However, in cases of high-level exposure, the liver may not be able to metabolize and eliminate the aflatoxins effectively, leading to a buildup of toxic metabolites in the body [33]. The severity of the symptoms of acute toxicity depends on several factors, including the level and duration of aflatoxin exposure, as well as the individual's age, nutritional status, and overall health. Young children and people with weakened immune systems or liver disease are particularly vulnerable to the adverse effects of aflatoxin exposure, as their bodies may not be able to detoxify the aflatoxins effectively. In addition, malnutrition can exacerbate the health effects of aflatoxin exposure, as it can weaken the body's ability to fight off infections and other health problems. This can lead to an increased risk of complications and death in cases of acute aflatoxin toxicity. Overall, the adverse health effects of aflatoxin contamination, including acute toxicity, can have significant impacts on public health and food safety, particularly in developing countries where food safety regulations may be less stringent, and where poverty and malnutrition can exacerbate the health effects of exposure. Implementing appropriate prevention and control measures, regular monitoring and testing, and appropriate

food safety regulations, as well as promoting public awareness and education about the risks of aflatoxin exposure, are critical to preventing the adverse health effects of aflatoxin contamination.

6.2 Aflatoxin exposure and the increased risk of infections and diseases

Aflatoxins are known to have immunosuppressive effects, which means that they can impair the body's immune system and its ability to fight off infections and other diseases. A weakened immune system can increase the risk of various health problems, including bacterial and viral infections, as well as chronic illnesses such as cancer. The immunosuppressive effects of aflatoxins can occur through various mechanisms, including the disruption of immune cell function and the impairment of cytokine production. Aflatoxins have been shown to reduce the production of cytokines, which are proteins that regulate the immune response and play a critical role in fighting infections and diseases. In addition, aflatoxins can damage the liver, which can impair its ability to produce proteins that are important for immune function. The immunosuppressive effects of aflatoxins are particularly concerning for individuals who are already immunocompromised, such as those with HIV/AIDS, cancer, or other chronic illnesses. For these individuals, exposure to aflatoxins can further weaken their immune system, making them more susceptible to infections and other health problems. Coulombe [34] described the various routes of exposure to mycotoxins, such as ingestion, inhalation, and dermal contact. He discussed the factors that can influence the toxic effects of mycotoxins, such as dose, duration of exposure, and the age and health status of the individual.

6.3 Aflatoxins as carcinogens: understanding the link to liver cancer

Aflatoxins are toxic compounds that are primarily metabolized by the liver. When ingested, they are absorbed from the gastrointestinal tract and transported to the liver, where they are metabolized into a range of byproducts, some of which are highly reactive and can cause damage to liver cells. The liver plays a critical role in the detoxification of aflatoxins and other harmful substances, but when exposed to high levels of aflatoxins, the liver can become overwhelmed, and its ability to metabolize and eliminate the toxins can be impaired. This can lead to the accumulation of aflatoxin metabolites in the liver and other tissues, which can cause liver damage and even liver failure. Liver damage caused by aflatoxin exposure can manifest in various ways, including acute and chronic forms. Acute liver damage caused by aflatoxin exposure is characterized by rapid onset and is associated with symptoms such as jaundice, abdominal pain, and liver failure. Chronic liver damage caused by aflatoxin exposure, on the other hand, is associated with long-term exposure to lower levels of the toxin and can result in the development of liver cirrhosis or liver cancer. Individuals who are at higher risk of liver damage caused by aflatoxin exposure include those who consume a diet high in aflatoxin-contaminated foods, as well as those who have pre-existing liver disease or other risk factors that compromise liver function [35].

HCC is a primary liver cancer that arises from the hepatocytes, which are the main functional cells of the liver. HCC is a major global health problem, and its incidence has been increasing in many countries worldwide. The risk factors for HCC, which include chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), alcohol consumption, obesity, and exposure to aflatoxin. The prevalence of these risk factors varies in different regions of the world and among different populations [36].

Chronic exposure to aflatoxins has been shown to increase the risk of developing liver cancer, a type of cancer that affects the liver [30]. Aflatoxins are naturally occurring toxins produced by certain molds, primarily *Aspergillus flavus* and *Aspergillus parasiticus*, which can contaminate food and feed crops, particularly those that are stored in warm and humid conditions. When contaminated crops are consumed by humans or animals, aflatoxins can enter the bloodstream and be transported to the liver, where they can cause DNA mutations and other cellular damage that can lead to the development of cancerous cells.

Aflatoxins are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC), which means that there is sufficient evidence to suggest that they are carcinogenic to humans. Aflatoxin exposure is a significant risk factor for liver cancer in areas where food and feed contamination are common, such as sub-Saharan Africa, Southeast Asia, and parts of South America. The liver is the primary organ responsible for detoxifying aflatoxins, but chronic exposure to high levels of aflatoxins can overwhelm the liver's capacity to metabolize and eliminate the toxins, leading to the accumulation of DNA-damaging metabolites and increased risk of liver cancer [37]. The risk of developing liver cancer from aflatoxin exposure is further increased in individuals with pre-existing liver disease, such as viral hepatitis B or C, as well as those who consume alcohol or have a weakened immune system. Aflatoxins are highly toxic and carcinogenic to both humans and animals, and exposure to these toxins can lead to liver damage, immune system suppression, and even death in severe cases. As a result, there are ongoing global efforts to better understand the genetics, biochemistry, and regulation of aflatoxin biosynthesis, as well as the taxonomy, biology, toxicology, and evolution of aflatoxigenic fungi.

Some of the key areas of research in this field include identifying the genes and pathways involved in aflatoxin biosynthesis [38], developing new methods for detecting and quantifying these toxins, and exploring potential strategies for preventing or reducing aflatoxin contamination in crops. By gaining a better understanding of these factors, researchers hope to develop new tools and approaches for mitigating the risk of aflatoxin exposure and protecting public health.

7. Understanding the genetics and biochemistry of aflatoxin biosynthesis: implications for food safety and public health

Identifying the genes and pathways involved in aflatoxin biosynthesis is an important area of research for several reasons. First, it can help us to better understand the mechanisms by which aflatoxins are produced by fungi, which can in turn inform strategies for preventing or reducing their production. Second, it can help us to develop new tools for detecting and quantifying aflatoxins in crops and food products, which is essential for ensuring food safety. Over the years, researchers have made significant progress in identifying the genes and pathways involved in aflatoxin biosynthesis. This work has been facilitated by advances in genomics and bioinformatics, which have enabled researchers to sequence and analyze the genomes of aflatoxin-producing fungi. Some of the key genes and pathways involved in aflatoxin biosynthesis include the following:

1. The aflatoxin biosynthetic gene cluster (AF cluster): This cluster contains around 25 genes that are involved in the biosynthesis of different intermediates in the aflatoxin pathway [39]. The cluster is regulated by a complex network of transcrip-

tion factors that respond to environmental signals, such as temperature [40], pH [41], and nutrient availability [42, 43].

2. Polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) enzymes: These enzymes are responsible for assembling the complex carbon skeletons of the aflatoxin molecules. There are several PKS and NRPS enzymes involved in the biosynthesis of aflatoxins, each of which produces a different intermediate in the pathway [44]. The genes encoding these enzymes are often clustered in fungal genomes and are regulated by complex networks of transcription factors, which can respond to environmental cues such as temperature, pH, nutrient availability, and stress. Additionally, the production of mycotoxins can be influenced by the interactions between fungi and their hosts, as well as by the presence of other microorganisms in the same environment [45].
3. Cytochrome P450 monooxygenases: Cytochrome P450 enzymes are a family of heme-containing enzymes that play a crucial role in the metabolism of xenobiotics, including drugs and environmental pollutants. Cytochrome P450 enzymes are highly variable and have evolved to meet the demands of different organisms and environments. These enzymes are responsible for oxidizing the intermediates produced by the PKS and NRPS enzymes, which creates the different types of aflatoxins [46].

By studying the function of these genes and their products, researchers can gain a deeper understanding of how aflatoxins are produced and regulated in fungi, and develop new approaches for preventing or reducing their production. Payne & Brown [47] highlights the importance of understanding the genetic and physiological mechanisms of aflatoxin biosynthesis in developing effective strategies for controlling its production and minimizing its impact on human and animal health.

8. Advances in methods for detecting and quantifying aflatoxins in crops and food products: implications for food safety

Monitoring and controlling aflatoxin contamination in food products is crucial to ensure food safety and protect public health. By establishing reliable testing methods and implementing prevention strategies, we can minimize the risk of exposure to aflatoxins and mitigate their adverse health effects [48]. Developing new methods for detecting and quantifying aflatoxins is essential for ensuring food safety and protecting public health. Traditional methods for detecting aflatoxins include thin-layer chromatography (TLC) [49] and high-performance liquid chromatography (HPLC), which are effective but time-consuming and labor-intensive [50]. In recent years, several new methods have been developed for detecting and quantifying aflatoxins in crops and food products. Some of these methods include the following:

8.1 Immunoassays

Immunoassays are based on the use of antibodies that specifically recognize and bind to aflatoxins. Immunoassays can be performed using a range of formats [51], including ELISA (enzyme-linked immunosorbent assay) [52, 53], lateral flow tests [54], and fluorescent assays [55].

8.2 Mass spectrometry

Mass spectrometry is a powerful analytical technique that can detect and quantify the presence of aflatoxins with high sensitivity and specificity. Mass spectrometry can be coupled with different separation techniques, such as liquid chromatography (LC) or gas chromatography (GC), to achieve high levels of separation and detection [56].

8.3 Biosensors

Biosensors are devices that use biological components, such as enzymes or antibodies, to detect and quantify the presence of aflatoxins in food samples [57]. Biosensors can be based on different transduction principles, such as electrochemical, optical, or piezoelectric transduction [58].

8.4 DNA-based methods

DNA-based methods use DNA probes or PCR (polymerase chain reaction) to detect the presence of aflatoxin-producing fungi in crops or food products. DNA-based methods can be faster and more sensitive than traditional methods, but they require specialized equipment and expertise [59].

By developing new methods for detecting and quantifying aflatoxins, researchers can improve our ability to monitor and control the presence of these toxins in food products, and ensure that they meet regulatory standards for food safety.

9. Aflatoxin prevention for food safety

Exploring potential strategies for preventing or reducing aflatoxin contamination in crops is critical for ensuring food safety and protecting public health. Some of the key strategies that researchers are investigating in this area include the following:

9.1 Breeding for resistance against aflatoxin contamination

Breeding groundnut varieties with stable resistance to aflatoxin contamination is a sustainable and effective approach to reducing the problem. However, this poses challenges to breeders due to the limited availability of improved germplasm and the significant genotype-by-environment (GxE) interaction for aflatoxin contamination. The limited germplasm restricts the range of genetic variability that breeders can work with, making it difficult to identify suitable parental lines for breeding. Additionally, the significant GxE interaction means that the performance of a genotype in one environment cannot be used to predict its performance in another environment, making it hard to select for resistance across diverse environments. Overcoming these challenges will require breeders to utilize innovative breeding techniques, such as marker-assisted selection, genomic selection, and multi-environment testing, to identify and incorporate favorable alleles for resistance across diverse environments [60, 61]. To combat this problem, researchers have been working on developing crops that are resistant to aflatoxin contamination. Aflatoxin biosynthesis in *Aspergillus* spp. is regulated by oxidative stress responses, which are induced by environmental stresses such as drought and heat stress. Host-derived reactive oxygen species (ROS) may play a role in cross-kingdom communication between host plants and *A. flavus*.

9.1.1 Application of RNA interference (RNAi) technology

Recent advances in plant breeding technology have enabled the study and application of metabolomic, proteomic, and transcriptomic knowledge in productive breeding populations [62]. Researchers are exploring ways to engineer crops that are resistant to aflatoxin-producing fungi or that produce antifungal compounds that can inhibit the growth of these fungi. However, there have been some promising advancements in engineering aflatoxin-resistant crops. One approach is to use RNA interference (RNAi) technology to silence genes in the fungi responsible for aflatoxin production [63, 64]. Another approach is to introduce genes from other organisms, such as bacteria or plants, that can break down aflatoxins [65]. Breeders now have more options for their improvement programs as there are a greater number of maize breeding lines that demonstrate resistance to both *A. flavus* infection and aflatoxin accumulation. The majority of these resistant lines have a tropical background, but newer lines have been created through crosses and backcrosses between tropical and temperate germplasm [66]. The GEM project has been instrumental in developing many of these lines through breeding crosses and hybrids [67]. Genomic regions that offer a consistent increase in resistance to aflatoxin or *A. flavus* induced ear rot in resistant maize lines have been identified through various studies, including several QTL and one meta-QTL analysis. Each of these QTL typically accounts for 5–20% of the observed variation in resistance. However, it is possible that the effects of some of these QTL may have been overestimated due to the Beavis effect [68].

9.1.2 The introduction of genes from other organisms

Another approach is to use genetic engineering to introduce genes that can enhance plant defense mechanisms against fungi, such as the expression of antifungal proteins or enzymes [69]. For example, researchers have successfully introduced genes from a wild peanut species into commercial peanut cultivars to increase their resistance to aflatoxin-producing fungi [70]. Overall, while there are still many challenges to overcome in developing aflatoxin-resistant crops, there is promising progress being made. The development of such crops could have significant benefits in improving food safety, reducing health risks, and increasing food security, especially in developing countries where aflatoxin contamination is a major problem.

10. Crop management practices

Aflatoxin contamination is strongly influenced by environmental factors, such as temperature, humidity, and rainfall, as well as cultural practices, such as planting density, irrigation, and fertilization. Researchers are exploring ways to optimize these factors to reduce the risk of aflatoxin contamination in crops. To prevent the adverse health effects of aflatoxin contamination, it is important to implement appropriate prevention and control measures.

10.1 Preharvest amendments

Preharvest amendments are treatments or additives applied to crops or soil before harvest in order to improve crop quality, yield, or post-harvest performance. The use of preharvest amendments is common in agricultural practices, as they can help to address

a range of issues affecting crop production and quality. For example, preharvest amendments may be used to control pests and diseases, improve soil fertility and nutrient availability, enhance plant growth and development, or reduce post-harvest losses and spoilage. However, it is important to carefully consider the potential risks and benefits associated with the use of preharvest amendments, as some may have negative impacts on the environment or human health. In addition, regulations governing the use of preharvest amendments may vary depending on the crop, location, and other factors.

10.1.1 The role of high-quality seeds in preventing aflatoxin contamination in agriculture

Using high-quality seeds is an important part of preventing aflatoxin contamination in food production. High-quality seeds are typically produced using good agricultural practices, such as careful selection and handling of parent plants, testing for disease and pests, and appropriate storage and transport conditions. By using high-quality seeds, farmers can ensure that their crops are healthy and better able to resist fungal infections that can lead to aflatoxin contamination. This is because healthy plants are better able to defend themselves against pathogens and are less likely to develop the kinds of stress conditions that can make them more susceptible to fungal infections.

10.1.2 Managing aflatoxin contamination in groundnuts through soil amendments

Ijaz et al. [71] proposed the use of soil amendments has been as a potential strategy for managing aflatoxin contamination in groundnuts. Several studies have investigated the effects of different soil amendments on aflatoxin levels in groundnuts, including organic amendments such as poultry manure, vermicompost, and green manure, as well as Inorganic amendments such as lime and sulfur. Overall, the results of these studies have been mixed, with some studies reporting significant reductions in aflatoxin levels following the application of soil amendments, while others have found little to no effect. The effectiveness of soil amendments in reducing aflatoxin levels may depend on a variety of factors, including the type and amount of amendment used, the timing and frequency of application, and the local environmental conditions. Despite the inconsistent results, the use of soil amendments may still be a promising approach for managing aflatoxin contamination in groundnuts, particularly in areas where other strategies, such as chemical fungicides, are not available or feasible. However, more research is needed to better understand the mechanisms underlying the effects of soil amendments on aflatoxin contamination, as well as to optimize their use in different agricultural settings.

10.1.3 The importance of crop rotation in preventing aflatoxin contamination

Proper crop rotation is an important practice that can help reduce the risk of aflatoxin contamination in agriculture. This is because crop rotation helps to interrupt the life cycle of the fungi that produce aflatoxins, which can help prevent the buildup of fungal spores and reduce the risk of contamination in subsequent crops. Crop rotation involves planting different crops in a field over successive growing seasons. This helps to break the cycle of plant-specific pests and diseases, which can build up in the soil over time and infect subsequent crops. By alternating crops, farmers can help to disrupt the life cycle of these pests and diseases, which can help to reduce their populations and limit their impact on subsequent crops. In the case of aflatoxin contamination, crop

rotation can help to reduce the buildup of fungal spores in the soil. This is because the fungi that produce aflatoxins typically infect specific crops, such as corn and peanuts. By rotating these crops with other types of crops, such as legumes, cereals, or grasses, farmers can help to reduce the buildup of fungal spores in the soil, which can in turn reduce the risk of contamination in subsequent crops [72–74].

10.1.4 Preventing aflatoxin contamination through effective Pest control measures

Aflatoxigenic fungi can grow on crops both in the field and during storage, and their growth is often facilitated by the presence of pests and insects. Pests such as insects and rodents can damage crops, creating entry points for fungi that produce aflatoxins. Proper pest control measures can help prevent crop damage and reduce the risk of fungal infections. Effective pest control measures can include the use of chemical or biological pesticides, crop sanitation practices, and appropriate storage and transport practices. Integrated pest management (IPM) is a holistic approach to pest control that uses a combination of techniques to minimize the use of pesticides while still effectively managing pests. IPM can be an effective approach to preventing aflatoxin contamination while also minimizing the use of potentially harmful chemicals in food production [75].

10.1.5 Biological control of aflatoxins

10.1.5.1 Using non-toxigenic strains of *aspergillus* spp.

Biological control of aflatoxin using non-toxigenic strains of *Aspergillus* spp. has shown promising results in both laboratory and field studies. These non-toxigenic strains can outcompete and displace the toxigenic strains, reducing the overall levels of aflatoxin contamination in crops [3]. The use of non-toxigenic strains for biological control of aflatoxin is considered to be a safe and environmentally friendly approach. These strains are naturally occurring and do not produce harmful toxins, making them ideal for use in agricultural settings [76]. Several non-toxigenic strains of *Aspergillus* spp. have been identified and tested for their ability to control aflatoxin contamination in crops, including *Aspergillus flavus* AF36 and *Aspergillus parasiticus* NRRL 2999. Studies have shown that these strains can significantly reduce the levels of aflatoxin contamination in crops, including maize, peanuts, and tree nuts. PCR assays can be used to distinguish between genetically similar toxigenic and atoxigenic isolates of *A. flavus* [77]. Specifically, atoxigenic isolates with deletions within the aflatoxin gene cluster can be readily identified using this method. While the use of non-toxigenic strains for biological control of aflatoxin is still in the early stages of development, it shows great potential as a strategy for reducing aflatoxin contamination in crops and improving food safety. Further research is needed to better understand the effectiveness of this approach in different agricultural settings and to develop practical methods for implementing it on a large scale [78].

10.1.5.2 Effectiveness of *Trichoderma* species and cattle dung as soil amendment in reducing aflatoxin contamination in groundnut

A study conducted recently aimed to investigate the effect of using *Trichoderma* species in combination with cattle dung as a soil amendment on the yield and preharvest aflatoxin contamination of groundnut. The researchers found that the application of *Trichoderma* species, in combination with cattle dung, significantly improved

the yield of groundnut and reduced preharvest aflatoxin contamination. Moreover, the study also showed that the application of *Trichoderma* species and cattle dung improved soil fertility and increased the availability of nutrients in the soil. The study suggests that the use of *Trichoderma* species in combination with cattle dung can be an effective strategy to improve crop productivity and reduce aflatoxin contamination in groundnut cultivation [79].

10.2 Post-harvest management

Post-harvest management plays a crucial role in reducing aflatoxin contamination in crops. Aflatoxins are a group of mycotoxins produced by the fungus *Aspergillus flavus* and *Aspergillus parasiticus* that can contaminate crops during preharvest, harvest, and post-harvest stages. Here are some post-harvest management practices that can help reduce aflatoxin contamination:

10.2.1 Mitigating aflatoxin contamination through appropriate storage and drying conditions

Proper storage and drying conditions are critical to mitigating the risk of aflatoxin contamination in food production. After harvest, crops should be stored in dry, cool, and well-ventilated facilities to prevent the growth of fungi that can produce aflatoxins. Crops dried to the appropriate moisture content prevent fungal growth and minimize the risk of contamination. Proper drying conditions may vary depending on the type of crop and the local climate, but generally involve careful monitoring of temperature, humidity, and airflow. Drying can be done using a variety of methods, such as natural drying in the sun or with fans and heaters, or using specialized equipment such as dryers and dehumidifiers. Proper storage and drying practices can significantly reduce the risk of aflatoxin contamination in food production and help ensure the safety and quality of food products [80]. Mousavi Khaneghah et al., [81] emphasizes the importance of understanding and addressing the risks associated with aflatoxin contamination in cereals. While the production of these toxins is a natural occurrence, there are several factors that can contribute to increased contamination, such as improper storage and handling practices.

11. Conclusion

Food security is crucial for human health and socioeconomic stability, but mycotoxins produced by fungi can contaminate crops, leading to health problems and economic losses. Aflatoxin is a mycotoxin that commonly contaminates crops such as corn, peanuts, and cottonseed, and can cause serious health consequences. Preventing and managing aflatoxin contamination requires effective strategies such as improved crop management practices, post-harvest handling and storage, and strict regulation and monitoring of food quality and safety. There are several strategies that researchers are exploring to prevent or reduce aflatoxin contamination in crops. Some of these strategies include the following:

Good agricultural practices: One of the primary ways to prevent aflatoxin contamination is to implement good agricultural practices, such as crop rotation, proper irrigation, and the use of high-quality seeds. These practices can help minimize the growth of *Aspergillus* fungi, which are responsible for producing aflatoxins.

Biological control: Researchers are also investigating the use of biological control agents, such as non-aflatoxigenic strains of *Aspergillus* fungi or other microorganisms, to reduce the growth of aflatoxin-producing fungi. This strategy involves introducing these microorganisms into the soil or onto crops to compete with and displace the aflatoxin-producing strains.

Chemical control: Chemical control methods, such as the use of fungicides or insecticides, can also be effective in preventing aflatoxin contamination. However, these methods can be costly and may have negative environmental impacts.

Post-harvest interventions: Another strategy for reducing aflatoxin contamination is to implement post-harvest interventions, such as proper drying and storage techniques, to prevent the growth of *Aspergillus* fungi and minimize aflatoxin production.

Genetic modification: Researchers are also investigating the use of genetic modification to develop crops that are more resistant to aflatoxin contamination. This strategy involves modifying the genetic makeup of crops to enhance their ability to resist *Aspergillus* fungi and reduce the production of aflatoxins. Breeding groundnut varieties with stable resistance to aflatoxin contamination is a sustainable approach but poses challenges due to limited germplasm and significant genotype-by-environment interaction. Overcoming these challenges will require breeders to utilize innovative breeding techniques and strategies, through collaborative efforts among breeders, geneticists, and agronomists.

In summary, preventing the adverse health effects of aflatoxin contamination requires a multi-pronged approach that involves implementing appropriate prevention and control measures, regular monitoring and testing, and appropriate food safety regulations, as well as promoting public awareness and education about the risks of aflatoxin exposure. In addition to safeguarding the health of consumers, such practices can enhance the quality and market value of their produce. Overall, a combination of these strategies may be necessary to effectively prevent or reduce aflatoxin contamination in crops and ensure food safety.

Conflict of interest

None.

IntechOpen

Author details

Amir Afzal^{1*}, Sairah Syed¹, Rafiq Ahmad¹, Muhammad Zeeshan²
and Ghulam Nabi³


1 Barani Agricultural Research Institute, Chakwal, Pakistan

2 Barani Agricultural Research Station, Fateh Jang, Pakistan

3 Groundnut Research Station, Attock, Pakistan

*Address all correspondence to: rajaamirafzal@gmail.com

IntechOpen

© 2023 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Vågsholm I, Arzoomand NS, Boqvist S. Food security, safety, and sustainability—Getting the trade-offs right. *Front. Sustain. Food Syst.* 2020;**4**:16. DOI: 10.3389/fsufs.2020.00016
- [2] Kumar A, Pathak H, Bhadauria S, Goyal AK. Aflatoxin contamination in food crops: Causes, detection, and management: A review. *Food Prod. Process. Nutr.* 2021;**3**(1):17. DOI: 10.1186/s43014-021-00064-y
- [3] Udomkun P, Wiredu AN, Nagle M, Müller J, Vanlauwe B, Bandyopadhyay R. Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application—a review. *Food Control.* 2017;**76**:127-138. DOI: 10.1016/j.foodcont.2017.01.008
- [4] Nazhand A, Durazzo A, Lucarini M, Souto EB, Santini AJF. Characteristics, occurrence, detection and detoxification of aflatoxins in foods and feeds. *Food.* 2020;**9**(5):644
- [5] Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: A global concern for food safety, human health and their management. *Frontiers in Microbiology.* 2017;**7**:2170. DOI: 10.3389/fmicb.2016.02170
- [6] Emmott A. Aflatoxins: Finding Solutions for Improved Food Safety. Market-Led Aflatoxin Interventions: Smallholder Groundnut Value Chains in Malawi. IFPRI. (International Food Policy Research Institute) A member of the CGIAR Consortium | A world free of hunger and malnutrition 2033 K Street, NW, Washington, DC 20006-1002 USA; 2013:1-2
- [7] Eskola M, Kos G, Elliott CT, Hajšlová J, Mayar S, Krska R. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. *Critical Reviews in Food Science and Nutrition.* 2020;**60**(15):2773-2789
- [8] Ahmad MM, Ahmad M, Ali A, Hamid R, Javed S, Abdin MZ. Detection of *aspergillus flavus* and *aspergillus parasiticus* from aflatoxin-contaminated peanuts and their differentiation using PCR-RFLP. *Annales de Microbiologie.* 2014;**64**:1597-1605
- [9] Gholami-Shabani M, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. Aflatoxins and aflatoxigenic fungi in Iran: A systematic review of the past, present, and future. *Mycol. Iran.* 2018;**1**:65-84. DOI: 10.22043/MI.2017.116760
- [10] Gourama H, Bullerman LB. *Aspergillus flavus* and *aspergillus parasiticus*: Aflatoxigenic fungi of concern in foods and feeds - a review. *Journal of Food Protection.* 1995;**58**(12):1395-1404
- [11] Campione E, Gaziano R, Doldo E, Marino D, Falconi M, Iacovelli F, et al. Antifungal effect of all-trans retinoic acid against *aspergillus fumigatus* In vitro and in a pulmonary Aspergillosis In vivo model. *Antimicrobial Agents and Chemotherapy.* 2021;**65**(3):e01874-e01820. DOI: 10.1128/AAC.01874-20
- [12] Samson RA. Current taxonomic schemes of genus *aspergillus* and its teleomorphs. *Biotechnol.* 1992;**23**:355
- [13] Frisvad JC, Hubka V, Ezekiel CN, Hong SB, Novakova A, Chen AJ, et al. Taxonomy of *aspergillus* section Flavi and their production of aflatoxins,

- ochratoxins and other mycotoxins. *Studies in Mycology*. 2019;**93**:1-63. DOI: 10.1016/j.simyco.2018.06.001
- [14] Probst C, Bandyopadhyay R, Cotty PJ. Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. *International Journal of Food Microbiology*. 2014;**174**:113-122. DOI: 10.1016/j.ijfoodmicro.2013.12.010
- [15] Cotty PJ. Virulence and cultural characteristics of two *aspergillus flavus* strains pathogenic on cotton. *Phytopathology*. 1989;**79**:808-814
- [16] Cortés G, Carvajal M, Méndez-Ramírez I, Avila-González E, Chilpa-Galván N, Castillo-Urueta P, et al. Identification and quantification of aflatoxins and aflatoxicol from poultry feed and their recovery in poultry litter. *Poultry Science*. 2010;**89**(5):993-1001. DOI: 10.3382/ps.2009-00019
- [17] Bbosa GS, Kitya D, Odda J, Ogwal-Okeng J. Aflatoxins metabolism. *Health*. 2013;**5**:14-34. DOI: 10.4236/health.2013.57A3003
- [18] Vijaya Kumar V. Aflatoxins: Properties, toxicity and detoxification. *Nutr. Food Sci. Int. J.* 2018;**6**:555696
- [19] Magan N, Aldred D. Post-harvest control strategies: Minimizing mycotoxins in the food chain. *International Journal of Food Microbiology*. 2007;**119**:131-139
- [20] Luo S, Du H, Kebede H, Liu Y, Xing F. Contamination status of major mycotoxins in agricultural product and food stuff in Europe. *Food Control*. 2021;**127**:108120
- [21] Abdin M, Ahmad MM, Javed S. Advances in molecular detection of *aspergillus*: An update. *Archives of Microbiology*. 2010;**192**:409-425
- [22] Benkerroum N. Aflatoxins: Producing-molds, structure, health issues and incidence in southeast Asian and sub-Saharan African countries. *IJERPH*. 2020;**17**:1215. DOI: 10.3390/ijerph17041215
- [23] Alshannaq A, Yu JH. Occurrence, toxicity, and analysis of major mycotoxins in food. *IJERPH*. 2017;**14**(6):632
- [24] Santini A, Ritieni A. Aflatoxins: Risk, exposure and remediation. In: *Aflatoxins: Recent Advances and Future Prospects*. London, UK, London, UK: Intech Open; 2013. pp. 343-376
- [25] Coppock RW, Christian RG, Jacobsen BJ. Aflatoxins. In: Gupta RC, editor. *Veterinary Toxicology*. 3rd ed. London, UK; Academic Press; 2018. pp. 983-994. DOI: 10.1016/B978-0-12-811410-0.00069-6
- [26] Kortei NK, Annan T, Dzikunoo J, Mensah DL, Ababio PF. Exposure assessment and risk characterization of aflatoxins intake through consumption of maize (*Zea mays*) in different age populations in the Volta region of Ghana. *Food Contam.* 2022;**9**(1):13
- [27] Wild CP, Gong YY. Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis*. 2010;**31**(1):71-82
- [28] Marr KA, Platt A, Tornheim JA, Zhang SX, Datta K, Cardozo C, et al. Aspergillosis complicating severe coronavirus disease. *Emerging Infectious Diseases*. 2021;**27**(1):18-25
- [29] Salmanton-García J, Sprute R, Stemler J, Bartoletti M, Dupont D, Valerio M, et al. COVID-19-associated pulmonary aspergillosis, march-august 2020. *Emerging Infectious Diseases*. 2021;**27**(4):1077-1086

- [30] Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *The American Journal of Clinical Nutrition*. 2004;**80**(5):1106-1122. DOI: 10.1093/ajcn/80.5.1106
- [31] Kaale L, Kimanya M, Macha I, Mlalila N. Aflatoxin contamination and recommendations to improve its control: A review. *World Mycotoxin Journal*. 2021;**14**(1):27-40
- [32] Dhakal A, Hashmi MF, Sbar E. Aflatoxin Toxicity. *Treasure Island (FL): StatPearls*; 2022
- [33] Abreham S, Manoj VR, Hailu M, Chen YY, Lin YC, Chen YP. Aflatoxins: Source, detection, Clinical Features and Prevention. *Processes*. 2023;**11**(1):204. <https://doi.org/10.3390/pr11010204>
- [34] Coulombe RA. Biological action of mycotoxins. *Journal of Dairy Science*. 1993;**76**(3):880-891
- [35] Sherif SO, Salama EE, Abdel-Wahhab MA. Mycotoxins and child health: The need for health risk assessment. *International Journal of Hygiene and Environmental Health*. 2009;**212**:347-368
- [36] McGlynn KA, London WT. Epidemiology and natural history of hepatocellular carcinoma. *Best Practice & Research. Clinical Gastroenterology*. 2005;**19**:3-23. DOI: 10.1016/j.bpg.2004.10.004
- [37] Marchese S, Polo A, Ariano A, Velotto S, Costantini S, Severino L. Aflatoxin B1 and M1: Biological properties and their involvement in cancer development. *Toxins*. 2018;**10**:214
- [38] Yu J. Current understanding on aflatoxin biosynthesis and future perspective in reducing aflatoxin contamination. *Toxins*. 2012;**4**(11):1024-1057. DOI: 10.3390/toxins4111024
- [39] Flaherty JE, Payne GA. Overexpression of aflR leads to upregulation of pathway gene transcription and increased aflatoxin production in *aspergillus flavus*. *Applied and Environmental Microbiology*. 1997;**63**(10):3995-4000
- [40] Gallo A, Solfrizzo M, Epifani F, Panzarini G, Perrone G. Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in *aspergillus flavus* on almond medium. *International Journal of Food Microbiology*. 2016;**217**:162-169
- [41] Luo Z, Ren H, Mousa JJ, Rangel DE, Zhang Y, Bruner SD, et al. The PacC transcription factor regulates secondary metabolite production, stress response, but has only minor effects on virulence in the insect pathogenic fungus *Beauveria bassiana*. *Environmental Microbiology*. 2017;**19**:788-802
- [42] Calvo AM, Wilson RA, Bok JW, Keller NP. Relationship between secondary metabolism and fungal development. *Microbiology and Molecular Biology Reviews*. 2002;**66**:447-459
- [43] Maggio-Hall LA, Wilson RA, Keller NP. Fundamental contribution of beta-oxidation to polyketide mycotoxin production in planta. *Molecular Plant-Microbe Interactions*. 2005;**18**:783-793. DOI: 10.1094/MPMI-18-0783
- [44] Stachelhaus T, Mootz HD, Marahiel MA. The specificity-conferring code of adenylation domains in non-ribosomal peptide synthetases. *Chemistry & Biology*. 1999;**6**:493-505
- [45] Huffman J, Gerber R, Du L. Recent advancements in the biosynthetic

- mechanisms for polyketide-derived mycotoxins. *Biopolymers*. 2010;**93**(9):764-776. DOI: 10.1002/bip.21483
- [46] Gonzalez FJ, Gelboin HV. Human cytochromes P450: Evolution and cDNA-directed expression. *Environmental Health Perspectives*. 1992;**98**:81-85. DOI: 10.1289/ehp.929881
- [47] Payne GA, Brown MP. Genetics and physiology of aflatoxin biosynthesis. *Annual Review of Phytopathology*. 1998;**36**:329-362
- [48] Herzallah SM. Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Food Chemistry*. 2009;**114**(3):1141-1146
- [49] Braithwaite A, Smith FJ, Stock R. Plane Chromatography. In: *Chromatographic Methods*. London: Chapman and Hall; 1985:24-84. DOI: 10.1007/978-94-009-4093-2_3
- [50] Namjoo M, Salamat F, Rajabli N, Hajihoseini R, Niknejad F, Kohsar F, et al. Quantitative determination of aflatoxin by high performance liquid chromatography in wheat silos in Golestan Province, north of Iran. *Iranian Journal of Public Health*. 2016;**45**(7):905-910
- [51] Nardo FD, Cavallera S, Baggiani C, Chiarello M, Pazzi M, Anfossi L. Enzyme immunoassay for measuring Aflatoxin B1 in legal cannabis. *Toxins*. 2020;**12**(4):265. DOI: 10.3390/toxins12040265
- [52] Chu FS, Fan TS, Zhang GS, Xu YC, Faust S, McMahon PL. Improved enzyme-linked immunosorbent assay for aflatoxin B₁ in agricultural commodities. *Journal of AOAC International*. 1987;**70**(5):854-857
- [53] Sun DD, Gu X, Li JG, Yao T, Dong YC. Quality evaluation of five commercial enzyme linked immunosorbent assay kits for detecting aflatoxin B₁ in feedstuffs. *Asian-Australas. J. Anim. Sci*. 2015;**28**(5):691
- [54] Cvak B, Warth B, Atehnkeng J, Parich A, Moritz A, Sulyok M, et al. Evaluating the performance of lateral flow devices for Total aflatoxins with special emphasis on their robustness under sub-Saharan conditions. *Toxins (Basel)*. 2021;**13**(11):742. DOI: 10.3390/toxins13110742
- [55] Zheng MZ, Richard JL, Binder J. A review of rapid methods for the analysis of mycotoxins. *Mycopathologia*. 2006;**161**:261-273
- [56] Iram W, Anjum T, Iqbal M, Ghaffar A, Abbas M. Mass spectrometric identification and toxicity assessment of degraded products of aflatoxin B₁ and B₂ by *Corymbia citriodora* aqueous extracts. *Scientific Reports*. 2015;**5**:14672. DOI: 10.1038/srep14672
- [57] Oliveira IS, da Silva Junior AG, de Andrade CAS, Lima Oliveira MD. Biosensors for early detection of fungi spoilage and toxigenic and mycotoxins in food. *Current Opinion in Food Science*. 2019;**29**:64-79
- [58] Polat EO, Cetin MM, Tabak AF, Bilget Güven E, Uysal BÖ, Arsan T, et al. Transducer Technologies for Biosensors and Their Wearable Applications. *Biosensors*. 2022;**12**(6):385. DOI: 10.3390/bios12060385
- [59] Ortega SF, Siciliano I, Prencipe S, Gullino ML, Spadaro D. Development of PCR, LAMP and qPCR assays for the detection of aflatoxigenic strains of *aspergillus flavus* and *A. parasiticus* in hazelnut. *Toxins*. 2020;**12**(12):757. DOI: 10.3390/toxins12120757
- [60] Nigam SM, Waliyar F, Aruna R, Reddy SV. Breeding peanut for resistance

to aflatoxin contamination at ICRISAT. Peanut Sci. 2009;**36**:42-49

[61] Yu B, Huai D, Huang L, Kang Y, Ren X, Chen Y, et al. Identification of genomic regions and diagnostic markers for resistance to aflatoxin contamination in peanut (*Arachis hypogaea* L.). BMC Genetics. 2019;**20**:32. DOI: 10.1186/s12863-019-0734-z

[62] Fountaina JC, Kheraa P, Yanga L, Nayakb SN, Scullye BT, Lee RD, et al. Resistance to *aspergillus flavus* in maize and peanut: Molecular biology, breeding, environmental stress, and future perspectives. Crop J. 2015;**3**:229-237. DOI: 10.1016/j.cj.2015.02.003

[63] Arias RS, Dang PM, Sobolev VS. RNAi-mediated control of aflatoxins in peanut: Method to analyze mycotoxin production and transgene expression in the peanut/*aspergillus* pathosystem. Journal of Visualized Experiments. 2015;**106**:e53398. DOI: 10.3791/53398

[64] Cheng W, Song X-S, Li H-P, Cao L-H, Sun K, Qiu X-L, et al. Host-induced gene silencing of an essential chitin synthase gene confers durable resistance to Fusarium head blight and seedling blight in wheat. Plant Biotechnology Journal. 2015;**13**:1335-1345

[65] Guan Y, Chen J, Nepovimova E, Long M, Wu W, Kuca K. Aflatoxin detoxification using microorganisms and enzymes. Toxins. 2021;**13**(1):46

[66] Smith JS, Williams WP. Aflatoxin accumulation in a maize Diallel cross containing inbred lines with expired plant variety protection. Agronomy. 2021;**11**:2285. DOI: 10.3390/agronomy11112285

[67] Williams WP, Windham GL. Registration of Mp718 and Mp719 germplasm lines of maize. J. Plant Regist. 2012;**6**(2):200-202

[68] Beavis WD. QTL analyses: Power, precision, and accuracy. In: Patterson AH, editor. Molecular Dissection of Complex Traits. New York, NY, USA: CRC Press; 1998. pp. 145-162

[69] Sher Khan R, Iqbal A, Malak R, Shehryar K, Attia S, Ahmed T, et al. Plant defensins: Types, mechanism of action and prospects of genetic engineering for enhanced disease resistance in plants. 3 Biotech. 2019;**9**(5):192. DOI: 10.1007/s13205-019-1725-5

[70] Arias RS, Sobolev VS, Massa AN, et al. New tools to screen wild peanut species for aflatoxin accumulation and genetic fingerprinting. BMC Plant Biology. 2018;**18**:170. DOI: 10.1186/s12870-018-1355-9

[71] Ijaz M, Afzal A, Ali SR, Nawaz HH, Altaf R, Jannat FT, et al. Aflatoxin: Can be managed by soil amendments in groundnut? Pak. J. Phytopath. 2022;**34**(01):65-70. DOI: 10.33866/phytopathol.034.01.0749

[72] Abraham A, Saka V, Mhango W, Njoroge SMC, Brandenburg R. Effect of crop rotation on aflatoxin contamination in groundnuts. In: Proc. 5th RUFORUM Biennial Reg. Conf. Cape Town, South Africa: RUFORUM Working Document Series No. 14(3); 2016. pp. 173-178

[73] Fandohan P, Hell K, Marasas WF. Food processing to reduce mycotoxins in Africa. In: Leslie JF, Bandyopadhyay R, Visconti A, editors. Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. Wallingford, UK: CABI (Centre for Agriculture and Bioscience International); 2008. pp. 47-59

[74] Mutegi CK, Ngugi HK, Hendriks SL, Jones RB. Prevalence and factors associated with aflatoxin contamination of peanuts from western

Kenya. International Journal of Food Microbiology. 2009;**130**:27-34

[75] Nesci A, Baara P, Etcheverry M. Integrated management of insect vectors of *aspergillus flavus* in stored maize, using synthetic antioxidants and natural phytochemicals. Journal of Stored Products Research. 2011;**47**(3):231-237

[76] Zanon MSA, Barros GG, Chulze SN. Non-aflatoxigenic *aspergillus flavus* as potential biocontrol agents to reduce aflatoxin 594 contamination in peanuts harvested in northern Argentina. International Journal of Food Microbiology. 2016;**231**:63-68

[77] Mylroie JE, Ozkan S, Shivaji R, Windham GL, Alpe MN, Williams WP. Identification and quantification of a toxigenic and non-toxigenic *aspergillus flavus* strain in contaminated maize using quantitative real-time PCR. Toxins (Basel). 2016;**8**(1):15

[78] Weaver MA, Abbas HK, Falconer LL, Allen TW, Pringle HC III, Sciumbato GL. Biological control of aflatoxin is effective and economical in Mississippi field trials. Crop Protection. 2015;**69**:52-55

[79] Dania VO, Eze SE. Using Trichoderma species in combination with cattle dung as soil amendment improves yield and reduces pre-harvest aflatoxin contamination in groundnut. Agrivita J. Agric. Sci. 2020;**42**:449-461

[80] Pitt JI, Taniwaki MH, Cole MB. Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of food safety objectives. Food Control. 2013;**32**(1):205-215

[81] Mousavi Khaneghah A, Eş I, Raeisi S, Fakhri Y. Aflatoxins in cereals: State of the art. Journal of Food Safety. 2018;**38**:e12532. DOI: 10.1111/jfs.12532