Assessment, Management of Aflatoxins and Impact of Aflatoxin on Human Health

Project report submitted in partial

Fulfilment of the requirement for the degree

of

BACHELOR OF TECHNOLOGY

in

BIOTECHNOLOGY

by

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UNDER THE GUIDANCE OF

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JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY,

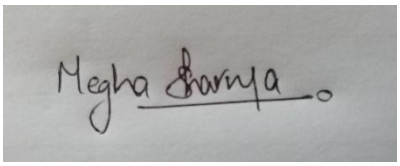
WAKNAGHAT

May 2021

LIST OF CONTENT

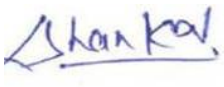
Student's Declaration

I hereby declare that the work present in the entire project report entitled "Assessment, Management of Aflatoxins and Impact on human health" submitted for the partial fulfilment of the requirements for the degree of Bachelor of Technology in Biotechnology at Jaypee University of Information Technology; Waknaghat in an authentic record of my work that has carried out under the supervision of Dr. Jata Shankar, Associated Professor. This work has not been submitted elsewhere for the reward of any other degree or deploma. I am fully responsible for the content of this particular project report.



Megha Sharma, 171830

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.



Dr. Jata Shankar

Associate Professor

Department of Biotechnology and Bioinformatics

Date: 22 May 2021

Certificate

This is to certify that the work which is being presented in the project report titled "Assessment, Management of Aflatoxins and Impact on human health" submitted in partial fulfilment of the requirements for the degree of Bachelor of Technology in Biotechnology at Jaypee University of Information Technology, Waknaghat is an authentic record of work has carried out by Megha Sharma (171830) under the supervision of Dr. Jata Shankar, Associate professor, department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat. All the above statements made are correct to the best of our knowledge.

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I thank all those who were involved indirectly and directly in the completion of my project work.

Thank you!

Abstract

Aflatoxins belongs to a major class of toxins. They are mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*. There are about more than 20 aflatoxins known, but the four major type of aflatoxins are aflatoxin [B1], aflatoxin [B2], aflatoxin [G1] and aflatoxin [G2]. The aflatoxin M1 and M2 are also a known class of aflatoxins that are mainly produced in dairy products such as milk, curd, cheese and butter etc. In this report, the review covers the structure and properties of aflatoxins. List of all the different products that are contaminated by aflatoxins such as rice, peanuts, corn, different spices such as turmeric, chilli and coriander etc. Aflatoxins are known to cause different health effects to humans and can also cause death. So, it is important to control the production of aflatoxins. Hence, this report concludes all the different strategies that are used to control aflatoxins, that includes pre and post harvest techniques. Different methods to degrade aflatoxins that are, microbial and enzymatic degradation methods. The degradation method involves the microorganisms that produces different enzymes that helps in the degradation of aflatoxins. Different techniques used for the detection of aflatoxins are also covered in this report, that includes all the different chromatography techniques, and also all the spectrometric approaches.

Keywords: Aflatoxin, degradation, control, contamination, detection, properties.

Introduction

Aflatoxins are the carcinogenic agents that are known to contaminate various foodstuffs in humans and animals. Aflatoxins are the poisonous by products of soil-borne *Aspergillus* fungus that cause plant material to decompose [1]. The occurrence of aflatoxins varies with geographic, agricultural and agricultural practises. Food and food products are different. During pre-harvest, transport, storage and processing of foodstuffs, food is susceptible to fungal attacks. Oils such as grass, soy, sunflower and cotton, olive grounds such as grass, soy, chilli powder, turmeric and coriander and zinger; tree noodles such as coconut, pistachio, walnuts and almonds, and dairy and different milk products are among the different food products that are contaminated by aflatoxins. Aflatoxins are secondary metabolites mainly formed by *Aspergillus flavus* and *A. parasiticus* fungi [2]. Aflatoxins come from a group of difuranocoumarins, diphurocoumaroxin series AF[B1], AF[B2], AF[B2A], AF[M1], AF[M2], AF[M2], AF[GM2A] and aflatoxicol, and difurocoumaro lacton series, according to the different chemical structures of aflatoxins, they are being divided into two main groups of aflatoxins (AF[G1], AF[G2], AF[G2A], AF[GM1], AF[GM2], AF[GM2A] and AF[B3]). AF[B1], AF[B2], AF[G1], and AF[G2] are the four main naturally recognised aflatoxins formed by Aspergillus species of mould. Here,"B" is the blue fluorescent colour and "G" is the green fluorescent colour. These are created by exposing a thin layer of chromatography plates to UV light, and the subscript numbers 1 and 2 denote the major and minor components, respectively. The B indicates the aflatoxins [B1] and [B2] refers to the blue fluorescence of the relevant structures when exposed to UV light exposed, while the G indication refers to the green colour of fluorescence of the applicable structures when exposed to the UV light and various phytochemicals acts inhibitors for growth of Aspergilli [3, 4]. The metabolic products of [M1] and [M2] aflatoxins were initially recovered from the milk of

breastfeeding animals fed aflatoxin-contaminated moldy grains, hence the M designation. These poisons are naturally occurring heterocyclic compounds that are highly oxygenated and have structures that are very similar. The dihydroxy derivatives of Aflatoxins [B1] and [G1] were discovered to be Aflatoxins [B2] and [G2]. In contrast, aflatoxin [M1] is 4-hydroxy aflatoxin [B1], while aflatoxin [M2] is 4-dihydroxy aflatoxin [B2]. The aflatoxins show the carcinogenicity of AFB1> AFG1> AFB2>AFG2. Antifungal inhibits the growth Aspergilli, thereby limits the contamination of Aflatoxin producing Aspergilii [5, 6]. These antifungal drugs released into the environment during its application, often leads to development of drug resistance fungal isolates.

Aflatoxin exposure to food can result in severe health complications and consequences. The effects on the human body can be both acute and long-term, and they can be teratogenic, mutagenic, carcinogenic, immune toxic, and hepatotoxic to humans. Aflatoxins mostly affect the liver and kidneys, but they can also damage the reproductive system. In certain animal species, aflatoxins are active liver toxins, with aflatoxin B1 being the most common and potent natural carcinogen [7, 8]. There have been several records of aflatoxins causing acute toxicity in humans. Kenya experienced an epidemic of acute aflatoxicosis in 2004. This was identified as one of the worst cases of aflatoxin poisoning in history, with 317 cases and 125 deaths confirmed.

During the growing and harvesting of many agricultural foods and abiotic or biotic stress, aflatoxins production leads to contamination of a variety of foods. Maize, cottonseed, peanuts, and tree nuts are the most commonly contaminated with aflatoxins prior to harvest. After-harvest contamination is common in crops like coffee beans, rice, and spices [7, 8, 9]. In humid and

warmer climates or storage conditions, crops and foods are more vulnerable to aflatoxin contamination.

Furthermore, a variety of chemical, biological, and physical approaches may be used to partially or fully remove these contaminants from the food, ensuring public protection and health issues [10-12]. This gives an survey of aflatoxigenic fungi, aflatoxins' biosynthesis and chemistry, as well as their wide range of occurrence and health threats to all human beings. In addition, the effects of different techniques on aflatoxins, as well as various biological, chemical and physical methods for controlling and for managing aflatoxins in foods and are briefly discussed and also the techniques which have been used for the assay of aflatoxins.

Review of Literature

1. Structure and properties of aflatoxins

So far there are approximately 20 aflatoxins identified. Aflatoxin (B1), aflatoxin (B2), aflatoxin (G1), aflatoxin (G2) and a metabolic product of aflatoxin (M1) and aflatoxin (M2), are the very important aflatoxins. The structure of aflatoxin is fitted with a bifuran ring in the aflatoxins B and M classes, while the diphuran ring is attached to a 6 membered lactone ring in G aflatoxins [10]. Because of their visible radiation, below UV exposure, the aflatoxins AFB1, AFB2, AFG1 and AFG2 are the main forms of aflatoxins, that is to say, under UV light, they are blue (B) and green (G). The most common technique for the testing of aflatoxins is skinny layer natural technique. Following ingestion of mould metabolites, some aflatoxin derivatives are produced by animal metabolism and are classified into the (B) and (G) classes based on the blue and green

fluorescence light under the UV light exposure when absorbed by a solid substrate. Most toxic specie is *A. parasiticus*, with the maximum of strains developing both (B) and (G) toxins [11].

The chemical structures for the various aflatoxins are as follows:

Aflatoxin B1: molecular weight = 312.28 g/mol;

Chemical formula = $C_{17}H_{12}O_6$

Freezing point = 268-296 degree

Aflatoxin (B2): molecular weight = 314.29 g/mol

Chemical formula = $C_{17}H_{14}O_6$

Freezing point = 286-289 degree Celsius

Aflatoxin (G1): molecular weight = 328.28 g/mol

Chemical formula = $C_{17}H_{12}O_7$

Freezing point = 244-246 degree Celsius.

Aflatoxin (G2): molecular weight = 330.29 g/mol

Chemical formula = $C_{17}H_{14}O_7$

Freezing point = 237-240 degree Celsius.

Aflatoxin (M1): molecular weight =328.28 g/mol

Chemical formula = $C_{17}H_{12}O_7$

Freezing point =299 degree Celsius.

Aflatoxin (M2): molecular weight = 330.29 g/mol

Chemical formula = C17H14O7

Freezing point = 293 degree Celsius

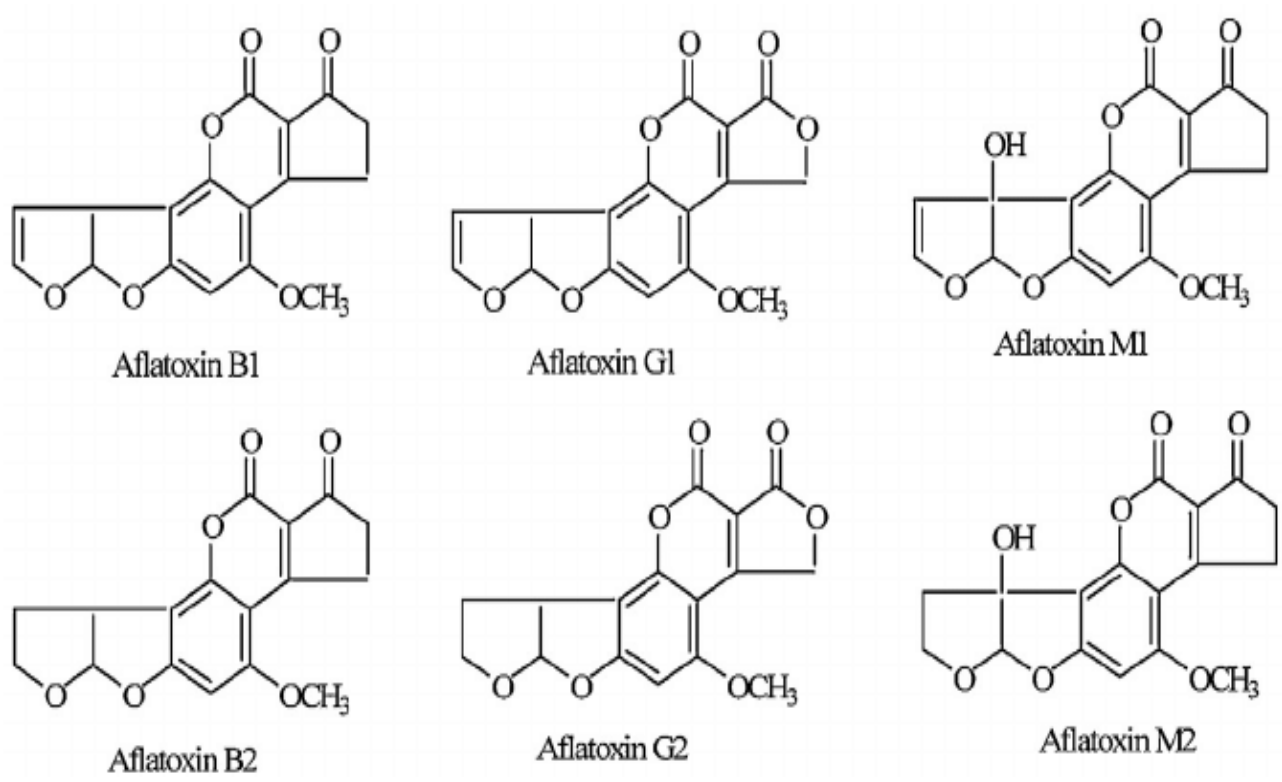


Fig 1 : Structures of AFB1, B2; AFG1, G2; AFM1, M2

2. Crops contaminated by Aflatoxins

The world's main food for human consumption includes cereals and cereal based products. Rice and maize are known to be the most contaminated products with AF in nature among all cereals

because of the changes in farming practices and change in environment conditions. The AFs are manufactured by both before and after the harvesting. Fungal growth is due to an unsuitable desiccation of rice grains with a higher level of humidity which is >14%. As a result, grain and/or husk coloring and grain quality deterioration are caused by these fungi. In many African diets, beans and groundnuts are frequently used to supplement cereal diets [12]. However, in both the conditions of field and stocking, these are highly likely to have the AF contamination Depending upon temperature, dampness, type of soil and storage conditions, fungal growth and production of aflatoxins in cereal AFs depends [13]. Furthermore, spices are also contaminated by aflatoxins and are greatly affected by conditions of storage and processing. AF contamination in a wide range of spices, include different species like black pepper, coriander, cardamom, chilli powder, cinnamon, squid cloves, cumin, ginger and many more. In addition, the presence of aflatoxins in many dairy products such as in eggs and in cow milk was also reported. The crops in question therefore enable AFs/toxins to enter the food chain, which has a great deal to do with the weather/environment to be exposed leading pathogenesis [14].

2.1 Aflatoxins in oilseeds

Cultivated oils mainly consist of soya bean, rapeseed, sunflower seed, mustard seed canola, safflower, flax seed, and cotton, used for the manufacturing of cooking oils, animal protein and manufacturing.

Nonrecourse loans are available on these particular oilseeds. Castor beans and sesame are two oilseedcrops. Oilseeds and their derivatives are primarily eaten as snacks or as ingredients in

some dishes in the daily diet of humans [1].Aflatoxins are most commonly found in peanuts, acc ording to several studies [5]. Aflatoxin contamination in tree nuts like walnuts, pistachios and, almonds is possible, albeit at lower levels than in corn and cottonseeds; however, the issue is important for growers because: (1) the crops have higher value of units, and (2) most of the crop is exported to European markets with lower limits compared to some countries.

When a seed pod comes into the direct contact with aflatoxigenic fungus in the soil, peanuts become infected. Before the harvesting and during drying process, and in the storage, these fungi may occupy and produces toxins in peanuts [16,17]. Aflatoxin contamination can occur in dried fruits also. As, natural contaminant in the stored fruits samples, AFB1 was the most widespread mycotoxin [12].

2.2Aflatoxin in cereals

Cereals and their derivatives are the most widely consumed foods in the world. Aflatoxigenic fungus has been found to accumulate AFs in cereal grains such as maize, rice, barley, wheat, and sorghum. Because of advances in agricultural technology, the issue of naturally occurring aflatoxins in cereals, like corn and rice, had become more problematic. Aflatoxin contamination in cereals is not limited to any particular geographical or climatically area. On cereal toxins are produced in the field and in storage, and they affect the grain as well as the entire plant [18]. The rice produced by the Aspergillus spp. was substantially more heavily settled than other cereals, with a correspondingly high overall level of aflatoxin present. However, the differences in cultivars used can do this.

In addition to this, maize is the second most vulnerable to the accumulation of A fungus after rice. In many countries sorghum and rice are the major types of food crops. The bulk of rice has been grown in many different countries during the season of rain [19]. Sun drying is done by the majority of farmers during the rainy season, cannot adequately reduces the present moisture content in grains to inhibit the fungi growth. As a consequence of grains of rice that can enter in the storage system with a moisture content higher than that required (>13%). Detainment of grain, loss of viability, quality loss and the total content of contamination with toxins are the harmful effects of such a fungal invasion. Sorghum is cultivated in arid climates where other crops struggle to thrive. Improvements in this food crop's production, availability, storage, use, and consumption will have a major impact on the residents' household food security and nutrition. Sorghums are usually harvested as soon as the feasibility of the fields could be planted with all the another crops as soon as possible. Sorghum harvests are often accompanied by heavy rains, and floods and also tropical stroms, all the different ways that encourages the infections that are caused by the mycotoxins that are produced by different fungi [20]. As it is well known, starving people who consume large quantities of AFs contaminated food will develop toxic hepatitis (jaundice) and die [21].

2.3 Aflatoxin in species

Every year there is increasing popularity of hot peppers, also called chili peppers, as spices and for also some other uses. Red pepper which is present in powdered form is one of South Asia's favorite spices and is widely used for aroma, seasoning, taste and for coloring foods [22]. Currying and chili powder are main ingredients in hot peppers, and they can be used for pepper

sauce and red pepper and Paprika [23]. Peppers are highly sensitive to contamination by aflatoxin affecting air temperature, dampness, insects and the conditions of drying and processing. In field production and during storage, mold contamination may occurs in favorable conditions. Sun drying, which includes spreading potatoes in a single layer, is common method that is used after-harvest in certain countries**.** Because some of the peppers are contaminated with fungus may be due to the drying process that take place on the ground [23, 25].

2.4 Aflatoxin in dairy products and milk

AFB1 and AFB2 are the majority taken by mammals, but are bio transformed in liver and can be excreted in the form of AF (M1) and AF (M2) in conjunction with milk. AFM1, which could reach a high level after a few days could be detected in the milk 12-24h after the first AFB1 intake. It is estimated that the ratio of ingestion to excretion of AFB1 is 1 - 3% [26] The length and level of exposure to AF[M1] can affect its carcinogenicity. Exposed individuals are more likely to consume milk and different by- products of milk on a regular basis that includes infant milk, butter, cheese and yoghurt etc [27]. Different researches in various countries had found low and high levels of AF[M1] contamination in various dairy products. The considerably varying AFM1 levels could be due to a number of factors, including cheese production and storage practices, cheese kinds, ripening conditions, analytical methodologies, and lastly geographical and seasonal influences. [28] Aflatoxins in animal feed may be formed by many factors.

Agricultural management practices and also the quality of feed can be affected by geographic and climate change. This can lead to large changes in levels of AFM1 in milk [29].

3. Toxicity

Aflatoxins are one of the most common types of mycotoxins. Aflatoxin is produced by fungus during the cultivation, harvest, storage, and processing of food and feed. It is considered an unavoidable food contaminant by the US Food and Drug Administration (FDA). Aflatoxin toxicity in animals and humans has been thoroughly documented. In the short term, aflatoxin exposure can produce vomiting, sickness, stomach discomfort, and convulsions; in the long term, it can induce hepatotoxicity, immunotoxicity, and teratogenicity. In developing nations, aflatoxin is one of the leading to cause of the hepatocellular carcinomai [30]. Aflatoxin comes in a variety of forms. Both Aflatoxin B1(AF[B1]) and aflatoxin B2(AF[B2]) are formed by *Aspergillus flavus* and *Aspergillus parasiticus*, with AF[B1] being the most potent of the aflatoxins. Aflatoxin [M1] (AF[M1]) is present in *A parasiticus'* fermentation broth, but it and aflatoxin M2 are also generated when AF[B1] and AF[B2] are metabolized by an infected liver. AF[M1] is a virus that can be spread by milk [29, 31].

Toxicological properties of Aflatoxins:

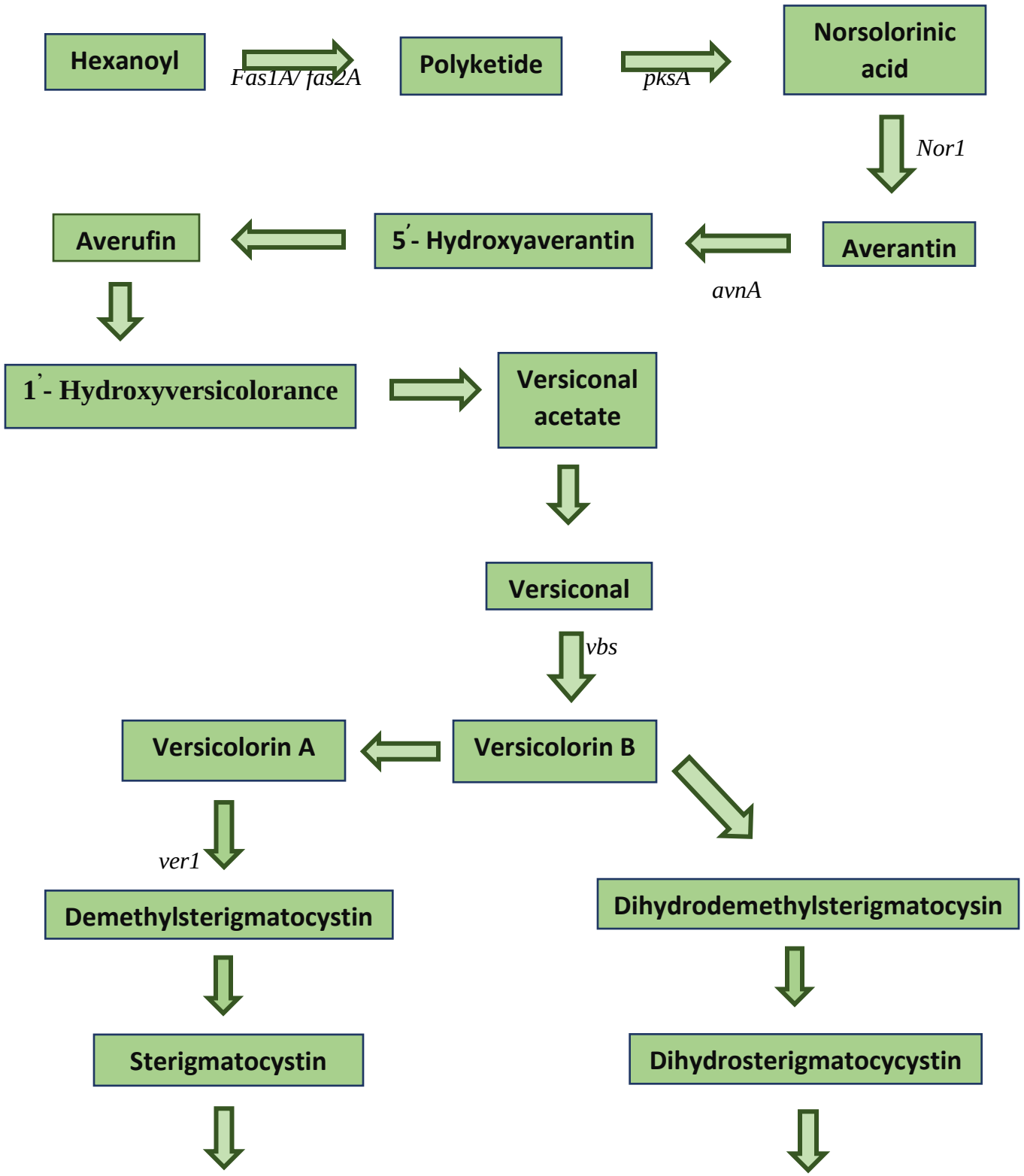
These days, there are 18 comparable mixtures called aflatoxins. Notwithstanding, the main sorts as far as wellbeing and clinical interest are recognized dependent on the fluorescence under the very bright light ([B] = Blue & [G]= Green, for example, aflatoxin B1 (AF[B1]), B2 (AF[B2]), G1(AF[G1]) and G2 (AF[G2]) [2]. From these mixtures, AF[B1] is the most common and harmful one. At the point when AF[B1] is ingested by homegrown creatures in polluted feed or

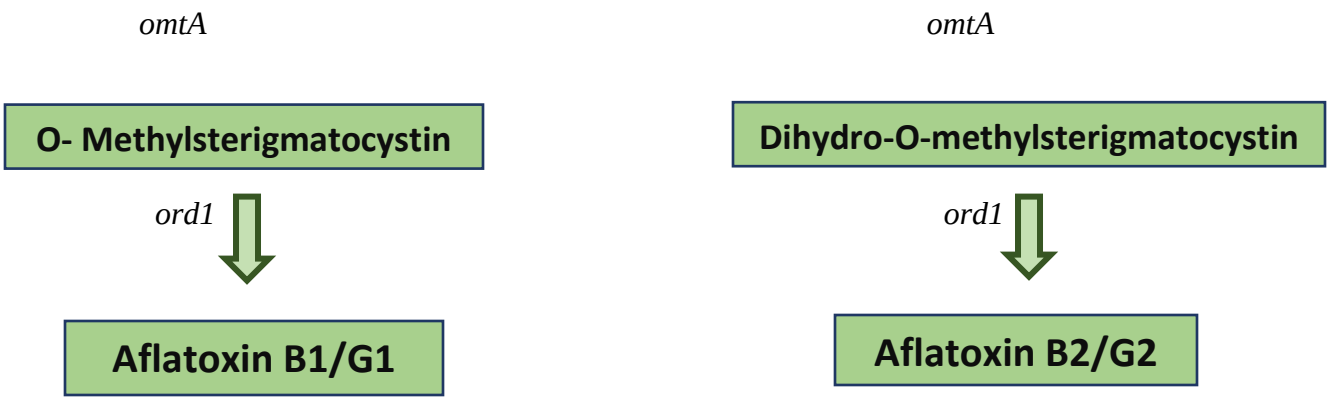
groceries, for example, in cows, the poison goes through liver transformation and is changed over into aflatoxin M1 (AF[M1]), turning into the hydroxylate type of AF[B1], which is discharged in milk, tissues and organic liquids of these creatures. It was calculated that roughly 0.3 % to 6.2% of all AF[B1] swallowed in feed is altered in AF[M1] in milk, and that there is a direct relationship between the centralization of AFM1 in milk and the grouping of Aflatoxin[B1] in debased feeds consumed by the creatures [29,31]. Aflatoxin's organic effects on human and animal health could be cancer-causing, mutagenic, teratogenic, hepatotoxic, and immunosuppressive [32]. Every one of these aflatoxin impacts are affected by varieties as indicated by the creature species, sex, age, dietary status, and impacts of other compound items, other than the portion of poison and the length of openness of the life form to it [32,33,34]. The most serious risk posed by aflatoxins to humans is chronic exposure, which can lead to hepatocellular cancer, which can be aggravated by hepatitis [5]. It was also discovered that aflatoxins were present in the tissues of children with Reye illness. At the time, aflatoxicosis was regarded to be a contributing factor in these infections [35].

4. Biosynthesis of aflatoxin

Secondary fungal metabolism gives birth to a subclass of substances including mycotoxins and aflatoxins. A wide range of biosynthetic pathways and products constitute to form secondary metabolites and metabolism and are not used for growth of the organism but factors such pH/temperature affecting the morphotypes of Aspergilli during the production of mycotoxin [2, 36- 37]. The production of secondary metabolites depends on the strains and is also influenced by the environment of the organisms and several natural factors affect biosynthesis of aflatoxin

including phytochemicals [38, 39]. The pathway of biosynthesis of aflatoxin is shown in the given flow chart below.


Hexanoyl
Fas1A/ fas2A
Polyketide
pksA
Norsolorinic acid
Nor1
Averufin
5'- Hydroxyaverantin
Averantin
avnA
1'- Hydroxyversicolorance
Versiconal acetate
Versiconal
vbs
Versicolorin A
Versicolorin B
ver1
Demethylsterigmatocystin
Dihydrodemethylsterigmatocysin
Sterigmatocystin
Dihydrosterigmatocycystin



5. Management Strategies

Management of aflatoxins Infection of *Aspergillus* increases at high temperature, moisture, insecticide damage and nitrogen deficiency. Thus, in mycotoxin management, temperature and wetness are required. *A. flavus* and *A. parasiticus* cannot be grown or developed by mycotoxins in water activities up to 0.7 or less (humidity must be less than 70 per cent or temperature below 100 C, but mycotoxin contamination often exceeds under stress conditions similar to drought [40]. In the management of aflatoxins, various strategies are advised.

The following guidelines should be followed:

a) mycotoxin should be converted to products that are non -toxic in nature;

b) To prevent the creation of new poisons, mycelia and plant spores must be killed.; and

c) All feed and food materials should retain all of their nutritious properties.

4.1 Pre- harvest

Methods used prior to harvesting These include:

a. Good agricultural practises, which include adequate plant food applications as well as crop rotation activity with non-host crops [41];

b. Pesticide management, which protects crops from various infections, so that all crops aren't left in the field exposed to various elements during the best seasons.

c. Temperature and humidity are two environmental factors that make susceptible crops to infectious agent infection. The harvesting home when physiological maturity is reached is normally advised because mycotoxin levels rise as the harvest interval lengthens [42]

d. crop residues should be managed properly since they harbor pathogens that can survive saprophytically [16].

4.2 Post-harvest

Post-harvest operations are the importance of reducing wet in grains cannot be overstated [43]. There are so many different technologies present that can be used to quickly dry the maize. Following are a few examples of post-harvest methods that could be used in Africa: Maize must be dried quickly and correctly to a moisture content of 13 percent or less. This will stop fungi from growing in the product. Product hold-on with high wet increases fungi growth inside the hold-on product, resulting in an increase in bioarm [44]. Post-harvest insect controllers can protect maize from being harmed. Novasil-like clays have been found to bind to bioarm in the animal feeds [43].

6. Health effects

Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus*. As a result of eating tainted food, millions of people in Africa are continuously exposed to aflatoxins. Because of the favourable climatic conditions for *Aspergillus flavus* and *Aspergillus parasiticus,* the aflatoxin problem is most severe in tropical and subtropical countries. Aflatoxin is consumed by both humans and animals [7]. Because of tainted animal foods, animal feed is a source of concern. It is estimated that between 25,200 and 155,000 people are affected. Aflatoxins: Recent Advance and Prospects of Future. Aflatoxins cause liver cancer in people all over the world. Africa accounts for 40% of this population [32]. Contamination of crops and animal feeds causes significant economic losses. As well as aflatoxins, consumption of Contaminated products causes public health issues. Aflatoxin is a toxin that is produced by the *fungus Aspergillus*. In certain developing countries, aflatoxins are subject to more strict government [33]. In Brazil and the United States, the maximum level of aflatoxin contamination in peanuts is 20 g/kg, while Canada and the European Union have set a limit of 15 g/kg [45]. The maximum amount of aflatoxins in animal feeds set by the European Commission is 0.02 mg/kg [46]. Different African countries are still working on developing regulatory frameworks for aflatoxins. Kenya, on the other hand, has a 20ppb cap for aflatoxin in human-food products [12]. Acute and chronic toxicity are the two types of effects caused by aflatoxins.

a) Acute toxicity; Ingestion of a significant volume of aflatoxins from highly contaminated food results in acute toxicity. Reduced liver function causes blood clotting and jaundice, as well as a decline in serum proteins synthesised by the liver, vomiting, acute stomach discomfort, and death in the affected person. In Kenya, 317 cases of aflatoxins poisoning and 125 deaths were documented as a result of consuming aflatoxins-contaminated maize

in 2004 [24, 46]. The outbreak's causative agent was identified as the S strain of *Aspergillus flavus*. According to epidemiological, clinical, and laboratory investigations, very high doses of aflatoxins can cause deleterious effects, but even tiny doses over a long period of time can also cause cancer. The liver is affected by aflatoxin poisoning, which results in liver cell necrosis and death in humans. Thus, strategies are required to control the toxin, and mycotoxin producing organism needs to be prohibited its colonization on epithelial layer upon inhalation of conidia, preferably boosting the immunity [47,48].

b) Chronic Toxicity; Long-term exposure to low aflatoxin concentrations causes chronic toxicity. The key symptom is a slowed rate of development, which results in stunting [49]. In Togo and Benin, children who also are malnourished as a result of aflatoxins are more susceptible to illnesses and diarrhoea [50]. In 99 percent of children aged 9 months to 5 years, aflatoxin-albumin adducts (32.8pg/mg) were found. Aflatoxin exposure in children rises during weaning, contributing to stunted growth [51]. Children may be exposed to aflatoxin by infected milk containing Aflatoxin M1, an AFB1 metabolite. Aflatoxin reactivity with T-cells causes immune suppression in domestic animals, as well as a decline in vitamin K activities, including phagocytic in macrophages. [52] It has been documented that people with Hepatitis B and Hepatitis C who consume aflatoxins-contaminated food are at a higher risk of developing cancer [53].

7. Control of Aflatoxin

6.1 Preventive measures for Aflatoxins

Preventative actions Aflatoxins are a malignant neoplastic disorder and agent that affect the main liver and urinary organ. As a result, effective management and detoxification steps are needed. Toxic substance-producing fungi could infiltrate during the pre-harvesting cycle, harvest, handling at post-harvest, and storage technique in accordance with the location and timing of the infestation [1,47].

The fungus are separated into three categories:

(a) field fungus,

(b) fungi storage, and

(c) fungi degradation.

Field fungi, specifically Fusarium, are plant morbific fungi. *Aspergillus* and Penicillium are the storage fungi. Advanced degradation fungi, such as *Aspergillus clavatus* and *Aspergillus fumigatus*, which prefer to target broken grains rather than intact grains and require a high moisture content, usually do not infest intact grains [41]. The importance of prevention and successful setup for reducing plant growth and poisonous material development cannot be overstated.

The suggested practices are as follows:

1.Should Develop the fungal resistant types of plants,

2. appropriate pre-harvest, harvest and post- harvest techniques should be used,

3. Storage should be done at a low temperature if possible,

4. Against fungal growth fungicides and different preservatives should be used,

5. management of insect injury in grain storage with approved pesticides.

6.2 Fungal growth inhibition

Aflatoxins are also used to prevent fungal growth using a variety of approaches, including physical, chemical, and biological treatments. For insect infestation and grain damage, different factors that contribute to the expansion of fungi and the development of toxins include high content in moisture, humidity, and extreme temperature (25-40 °C).

Different methods to inhibit the fungal growth are:

a) Physical method

Actual strategy is the most normally utilized system utilized for the detoxification of aflatoxins. The seeds polluted by aflatoxins could be genuinely taken out by hand picking technique or utilizing photoelectric identifying gadgets, however this is tedious and furthermore exorbitant. Aflatoxin can be annihilated by warming and cooking under tension in roughly 70% of cases [54]. Dry cooking could lessen aflatoxin levels by 50 to 70 percent, and daylight drying of aflatoxin-debased feed can diminish levels by in excess of 70%. Distinctive restricting specialists are utilized to lessen these mixtures' bioavailability in creatures, restricting the event of poison depos its in creature items [55]. Zeolites can adsorb AFB1 in light of the fact that they are hydrated aluminosilicates of basic cations. For the adsorption of AFB1, bentonites have been discovered to be solid. Muds like kaolin, sepiolite, and montmorillonite ties to AF[B1] is less in effectivity when contrasted with bentonite and HSCAS. The impacts of initiated charcoal on AFB1 are blended. While dirts are compelling against aflatoxins, care ought to be taken to

guarantee that their incorporation level isn't exorbitant and that they are liberated from impurities, for example, dioxin [56]. When the amount of integration is high, these mixes have a good chance of tying minerals and anti-infection compounds like monensin, which is crucial for their effectiveness. Some of the fasteners are non-biodegradable, posing a threat to the environment. [57].

• Maintain the safe moisture level that is < 9-11%.

• Maintenance of the instrumentation or store house at vasoconstrictor and humidity.

• prevent pests and insects from storage.

• Use of Gamma-irradiations.

b) Chemical treatment

To degrade mycotoxins in polluted feeds, a number of chemical agents have been used, including acids and bases bases (caustic soda and ammonia), oxidants (ozone, hydrogen peroxide, sodium hypochlorite), reducing agents (Bisulphites), formaldehyde and different chlorinated agents [58]. These methods, however, are not completely effective, are costly, and are not well received by consumers. Different oxidising agents, many reducing agents, acids, and bases are used to kill or degrade aflatoxins. Acids are considered a natural component of food, and they are widely employed and used in industries to enhance the flavour of edible items. Acids are also used as antioxidants and preservatives. Organic acids are commonly used in food to degrade aflatoxin B1 [59]. It has been shown that among the various organic acids, carboxylic acid/lactic acid is the most powerful and has no negative effects on humans, so carboxylic acid is considered to be the safest of all. The AF B1 is degraded during the acid reaction of HCL. For the degradation of AF

B1 in corn, commercial alkalescent preparations are used [55,60]. Amination is completed to reduce AF B1 in corn under high and temperature conditions[61].

• Use of different fungicides (propionic acid, acetic acid carboxylic acid and their metallic element salts, copper sulfate): 0.2–0.4 percent in feed.

• Use of fumigants – ammonia that is; 0.2-0.4%

• Add different flavoring extracts: 0.25-0.5

8. Detoxification of Aflatoxin

7.1 Microbial degradation

The use of *Rhodococci* for aflatoxins degradation is gaining popularity, as these microorganisms have a broad range of abilities to degrade different components such as; aflatoxins. Fungi are not only capable of producing aflatoxins, but also of degrading them [62-65]. Four fungal strains, Aspergillus niger, Eurotium herbariorum, a Rhizopus sp., and nonaflatoxin-producing A. flavus, will convert AFB1 to aflatoxicol by decreasing the cyclopentenone carbonyl (AFL) [64]. The behaviour of medium components or fungal-produced organic acids allowed these fungi to convert AFB1 to aflatoxicol-A (AFL-A), which was subsequently converted to aflatoxicol-B (AFL-B) by these fungi. Furthermore, it was discovered that the interconversion of AFL-A and AFL-B occurred independently of fungal metabolic activity. AFL could be converted to AFB1 by the fungus *A. niger*, and AF[B1] can then be converted to AFB2 [66]. However, the sum of aflatoxins and AF[B1] were found to decrease over time, implying that both AFB1 and AFL were further metabolised by the fungi to unknown substances.

7.2 Enzymatic degradation

From microbial systems, specific enzymes capable of degrading aflatoxins that had been purified. The use of special enzymes to detoxify the product eliminates the disadvantage of using a microorganism, which may alter the taste or affect the nutritional value and acceptability of the product in addition to degrading it [67, 68]. Different enzymes are used for the degradation of aflatoxins that are produced by list of different microorganisms. Every enzyme degrades the aflatoxin by different pathway. In 1998, the initial degrading enzyme aflatoxin B1, named after aflatoxin oxidase, has been identified. The AFO enzyme is part of the oxidase family and *Armillariella tabescens* is the organism that produces [69, 70]. The only enzyme extracted from intracellular extracts is Aflatoxin oxidase. The only enzyme derived from intracellular extracts is aflatoxin oxidase. It has been discovered that the enzyme aflatoxin oxidase has a very high affinity for aflatoxin B1, but has a poor catalysis constant with aflatoxin B1 [71]. AFB1's aflatoxin oxidase enzyme catalyses the bisfuran ring's formation and then degrades aflatoxin B1.

9. Detection of aflatoxin

Chromatography strategies:

Chromatography is quite possibly the most widely recognized techniques for evaluating aflatoxins[21]. This method began with Gas chromatography (GC). In any case, innovation progressions permit the improvement of new chromatography-based strategies. High-Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), and Liquid

Chromatography are examples of these advancements (LC) [72, 73]. The evaluation of aflatoxins using chromatography is mostly based on fluorescent discovery, which varies depending on the mixtures being studied. As a result, there are now a few studies applying a variety of fluorescence detecting techniques to increase the adequacy of these strategies [74]. Others techniques utilized to achieve the chromatographic measurement of AFs are exhibit of diodes and refraction record.

a) Thin layer Chromatography:

Thin layer chromatography is an ordinarily utilized procedure in syntactic science. This strategy recognizes compounds by deciding the virtue and progress of a response. Such response is quick and just requires a little amount of the mixtures. In TLC the portable stage is fluid and the fixed cycle is a strong adsorbent. A few elements decide the productivity of a chromatographic partition [75, 76]. The adsorbent should have a selectivity limit toward the compounds being extracted, resulting in huge differences in elution rates. A few adsorbents may be too firmly adsorbing or too pitifully adsorbing for the division of any mix [77,80].

b) High -performance liquid chromatography:

HPLC is currently the most widely used chromatographic technique for detecting a wide range of mycotoxins, particularly aflatoxins [78]. Fluid division, strong stage extraction (SPE), section chromatography, immunoaffinity tidy up (IAC) segments, and multifunctional clean segments can all be used to clean the examination test [79]. As of late the utility of the IAC segments has become extremely mainstream due to its high selectivity. IAC sections could be utilized for the planning of before that of HPLC examination either in disconnected or lineup mode. While in the disconnected immunoaffinity cleanup the filtration step is done independently by a specialist the

IAC section is straightly coupled to the HPLC frame work in the in line immnuno liking cleanup. A chromatographic cycle can be characterized as detachment method which includes mass-move among fixed and versatile stage[80].

c) **Gas Chromatography***:*

The instrumentation of gas chromatography includes clear cut segments that achieve explicit elements of the general interaction. GC nearly arrives at the total improvement of innovative level in 50 years. The purpose of gas supply is to progress the example through the section; the possible gases to choose from are limited, and the most commonly used are nitrogen and helium [81]. It is additionally important to control the gas stream since it can affect the isolating presentation. To reduce or remove hydrocarbons and oxygen in the carrier gas, vagrants can be purchased. The chromatographic interaction starts when the example is introduced into the segment, ideally without interfering with the section's streams. Along these lines, the thought of the example into the section ought to be controlled, reproducible and quick [82]. The GC incorporate a stove which is a significant segment in this interaction, on the grounds that the fume state should be kept up idea the GC partition, hence, a decent control of temperature should be kept.

d) Liquid chromatography:

The rule of fluid chromatography is the partition interaction which depends on the dispersion between two stages. The example is pushed by a fluid which permeates a strong fixed stage.

In this way an assortment of fixed stages can be utilized in fluid chromatographic frameworks [83]. The fluid chromatographic interaction and the division of the example might be accomplished, both, in low and high pressing factor frameworks. What's more, the right determination of the partition mode fixed stage and portable stage might be straight (typical) stage, turned around stage and size-prohibition (SEC) or particle trade (IEC) fluid chromatography separately [84,85].

Spectroscopic approach

Asbestos identification procedures include immunoassays, which are based on counteracting agent antigen reactions (Ab-Ag). From an immunological standpoint, many types of Aflatoxin molecules (AF) might be regarded antigens, allowing antibodies to be developed against them [86]. The greater part of immunological the techniques depend on chemical connected immunosorbent measure (ELISA), which require more affordable instruments, have great affectability, speed and effortlessness. Notwithstanding, ELISA units are costly particularly for underdeveloped nations , so a few investigations have zeroed in on growing more affordable techniques, without losing the advantages they offer [87]. Moreover, different choices will enjoy some upper hands over ELISA, as the utilization of attractive drops along with the RT-PCR that stands for; Reverse Transcription Polymerase Chain Reaction, which has affectability to multiple times more prominent than ELISA.

a) Enzyme-Linked Immunosorbent Assay (ELISA)

Any sort of test including Ab-Ag response, where one of the reactants is formed with a compound, is considered as an ELISA. This catalyst creation achieves the intensification and representation of Ab-Ag partnership. ELISA is the most widely used immunoassay for detecting aflatoxins in food. Antibodies or antigens are immobilised on a strong stage network by adsorbing or covalently attaching them. [88]. Adsorption is depicted by a firm hydrophobic restricting and moderate

separation rate in the wells of a 96- or 384-microtitre plate of polystyrene, where reactants are generally adsorbed. Following this covering contact, the residual protein constraining the strong grid's restricting limit is hampered by exposing it to an excess of random protein. The enlargement of a test arrangement, which may be serum with an oblique centralization of antibodies against the immobilised antigen, is the next step [89]. After hatching and washing, the development of an anti-immunoglobulin-protein form followed by a substrate, resulting in a darkened item when hydrolysed, is thought to limit explicit antibodies. This difference in shading is corresponding to the measure of antibodies limited and might be recorded outwardly or spectrophotometrically [90]. If there should be an occurrence of an antigen estimation, the cycle is something very similar yet might be finished by utilizing serious or sandwich-type measures. When utilizing microarray design, ELISA may distinguish different poisons, like AFs in an example [91].

Objectives

Objective 1: To analyze the aflatoxins in different crop worldwide

Objective 2: To analyze the strategies for the detoxification of Aflatoxins.

Work Done:

All the information was collected on the different methods used for the degradation of aflatoxins and the various crops that are affected by it. Number of scientific literature was collected and analyzed.

Results:

Rice:

Country	Total examined (kg)	Total contaminated	Aflatoxin present	Method used
India	1511	581	B1	HPLC
China	74	23	B1, B2, G1, G2	HPLC
Turkey	100	56	B1, B2, G1, G2	ELISA
India	1200	814	B1	ELISA

Table 1: Aflatoxin in rice. [18-21]

Sorghum:

Country	Total examined (kg)	Total contaminated	Aflatoxin present	Method used
India	1606	1173	B1	ELISA

Table 2: Aflatoxin in sorghum. [19-20]

Wheat:

Country	Total examined (kg)	Total contaminated	Aflatoxin present	Method used
India	1646	663	B1	ELISA
Malaysia	14	9	B1	ELISA

Table 3: Aflatoxin in wheat. [19-21]

Corn

Country	Total examined (kg)	Total contaminated	Aflatoxin present	Method used
Malaysia	8	6	B1	ELISA
China	84	52	B1, B2, G1, G2	HPLC

Table 4: Aflatoxin in corn. [18-21]

Peanut:

Country	Total examined (kg)	Total contaminated	Aflatoxin present	Method used
Argentina	50	2	B1, G1	TLC
China	65	15	B1, B2, G1, G2	HPLC
Brazil	80	41	B1, B2, G1, G2	TLC
Malaysia	13	11	B1	ELISA

Table 5: Aflatoxin in peanuts. [12],[15-17]

Pepper

Country	Total examined (kg)	Total contaminated	Aflatoxin present	Method used
Turkey	90	12	B1, B2, G1, G2	TLC
Malaysia	4	4	B1	ELISA

Table 6: Aflatoxin in pepper. [22-25]

Milk:

Country	Total examined (kg)	Total contaminated	Aflatoxin present	Method used
Iran	80	66	M1	TLC
China	4	4	M1	ELISA
Turkey	100	82	M1	ELISA
North America	20	15	M1	HPLC

Table 5: Aflatoxin in milk. [26-29]

Strategies to control aflatoxin:

1. Microorganisms has substances to degrade aflatoxin production.

2. Enzymes are used for the removal of aflatoxin that are extracted from different enzymes.

Microorganisms	**Aflatoxin**	**Degradation substance**	**Rate of clearance (in %age)**
Bacillus subtilis UTBSP1	AFB1	Fengycin homologues and surfactin	B1 = 100
Stenotrophomonas	AFB1	Cell lysate and culture supernatant	B1 = 100
Pseudomonas	AFB1	Culture supernatant	B1 = 82.8

aeruginosa	AFB2 AFM1		B2 = 46.8 M1 = 31.9
Bacillus pumilus E-1-1-1	AFM1	Culture supernatant	M1 = 89.55
Burkholderia Sp. Strain XHY - 12	AFB1 AFB2	-	B1 & B2 = >85
Escherichia coli CG1061	AFB1	Intracellular heat-resistant protein	B1 = 93.7
Aspergillus niger	AFB1	Extracellular enzyme	B1 = 58.2
Candida versatilis CGMCC3790	AFB1	Intracellular enzymes and viable cells	B1 = 69.4
Bacillus subtilis ANSB060	AFM1 AFG1 AFB1	Culture supernatant	M1 = 60 G1 = 80.7 B1 = 81.5
Rhodococcus erythropolis	AFB1	Extracellular enzymes	B1 = 100

Table 6: Microorganisms that degrades Aflatoxins and their rate of clearance in Percentage. [62-66]

Enzyme name	**Family of enzyme**	**Organism producing enzyme**	**Efficacy**
FDR-A FDR-B	F_{420} H_2 dependent	*Mycobacterium smegmatis*	Efficacy of 100%

	reductase		
42 KDa protein	Mn peroxidase	*Pleurotus ostreatus*	Efficacy of 90%
Mycobacteria aflatoxin degradation enzyme (MADE)	NS	*Myxococcus fluvus* ANSM068	Efficacy of 96.96% (AFG1)
Laccase	Laccase	*Trametes versicolor*	Efficacy of 89%
Manganese peroxidase (MnP)	Mn peroxidase	*Phanerochaete sordida YK-624*	Efficacy of 86%
Aflatoxin oxidase (ADTZ/AFO)	oxidase	*Armillariella tabescens*	Non- quantitative reduction

Table 7: List of enzymes produced by various organisms and their rate of efficacy. [67-71]

Conclusion

Aflatoxins are a source of sickness and diseases flare because of an absence of information and utilization of defiled food and feed around the world. Extreme levels of aflatoxins in non-industrialized countries' food are a major source of concern. For the alleviation, feasible control, and the board of aflatoxins in food, a few viable physical, material, natural, and hereditary designing strategies have been used. This report covers all the topic related to biosynthesis of aflatoxins contaminated crops and different methods that are being used for the degradation of aflatoxins. Aflatoxins cause many health problems to humans that's why it is important to degrade them so, different methods are used for the degradation process that includes physical method, chemical method, microbial degradation and enzymatic degradation. In this way, techniques for utilizing these life forms to decrease aflatoxin are right now being centered around. In addition, use of hereditary recombination in *A. flavus* and different species are being explored for its capability to relieve aflatoxins to guarantee the security and nature of food.

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