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Mitigating Aflatoxin Contamination in Grains: The Importance of Postharvest Management Practices

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Abstract

Grains are the most important food staples for humans and livestock animals. Grains are susceptible to fungal attacks while in the field and/or storage. The toxigenic fungi not only cause quality deterioration and grain loss but also produce toxic secondary metabolites. Known as mycotoxins, the most important ones regarding their occurrence and toxicity are aflatoxins (AFs), which can cause many bad effects on humans and animals ingested. AFs may cause acute or chronic diseases such as carcinogenic, mutagenic, teratogenic, and hepatotoxic. Numerous studies around the world indicate that the contamination of most grains with one or more types of AFs leads to large economic losses, such as food commodity rejection by many countries (EU authorities, for example). To protect human beings and animals from these health risks, many countries have established regulations to limit exposure to AFs, as well as several strategies and measures to mitigate both the presence and concentration of AFs. For that, the purpose of this state of art is to present the status of aflatoxin contamination at the local and global levels in the main grains (corn, wheat, and rice), along with the most important methods and strategies proposed to mitigate AFs, which can be divided into physical, chemical, and biological methods during post-harvest.

Keywords: Aflatoxins, Postharvest, Grains, Management, Mitigation, Mycotoxins

Abbreviations: AFs: Aflatoxins; TLC: Thin Layer Chromatography; JECFA: Joint Expert Committee on Food Additives and Contaminants; EU: European Union; RASFF: Rapid Alert System for Food and Feed; IGC: International Grains Council; UV: Ultraviolet; LAB: Lactic Acid Bacteria; AI: Artificial Intelligence

Introduction

Aflatoxins (AFs) are one of the most important mycotoxins produced by fungi, fundamentally by Aspergillus species, under specific conditions such as temperature, relative humidity during preharvest, post-harvest, transportation, and storage [1-3]. Four main types of AFs (B_1, B_2, G_1, and G_2) have been identified. AFB_1 has been reported to be carcinogenic, teratogenic, and mutagenic to a wide range of organisms and is known to cause hepatic carcinoma in humans [4,5]. The (IARC) has categorized AFB_1 as the most dangerous human carcinogens known, placing them in Group 1 [5]. AFs incidence has been documented in many global regions. However, a higher occurrence of AFs is associated with tropical and subtropical areas and some temperate regions, which are now more susceptible to the presence of AFs due to climate change and poor practices during preharvest, harvest, and postharvest activities [6]. Methods for controlling AFs are largely preventive and include good agronomic practices such as using sound, fungus-free seeds for planting, controlling insects and plant diseases, and proper irrigation practices. These methods are essential to avoiding contamination of raw materials and processed products; therefore, they are an option to guarantee product safety for consumers [7]. Once the contamination by aflatoxins has occurred, other strategies post-harvest have been proposed to reduce the risk of exposure to AFs, which include physical, chemical, and biological removal or combining more than one method to achieve the target. In this state of the art, I will present the global status of the occurrence of aflatoxins in the three main grains (corn, wheat, and rice) for the biggest producers. Also, discuss the different strategies and how they work to reduce or mitigate AFs post-harvest.

Definition and Properties of Aflatoxins

Aflatoxins (AFs) are a group of secondary metabolites produced by many Aspergillus species, such as *Aspergillus flavus, A. parasiticus, A. nomius, A. bombycis, A. pseudotamarii, and A. aflatoxiformans* [2,8] Aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2) are thse four main AFs that are produced naturally. B and G refer to the blue and green, fluorescent colors produced under UV light on Thin Layer Chromatography (TLC) plates (Figure 1). The AFs molecule contains a coumarin nucleus linked to a difuran and either a pentanone, as in AFB_1 and the dihydro derivative AFB_2, or a six-member lactone, as in AFG_1 and its corresponding derivative AFG_2. AFs are easily soluble in moderately polar organic solvents like methanol and chloroform, but only very weakly soluble in water and insoluble in non-polar solvents [9,10].

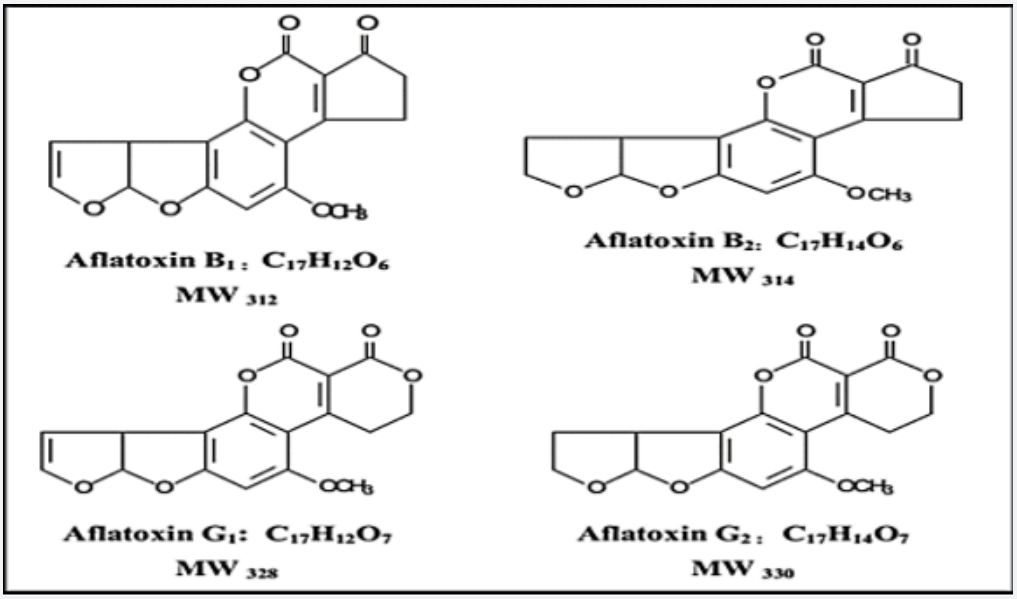

Figure 1: Chemical structures for the major four AFs.

Conditions of fungal growth and AFs production on grains during post-harvest

Fungal growth and the production of AFs in the stored-grain ecosystem are influenced by several factors, such as temperature, water activity, relative humidity, substrate, and fungal strain. In addition, there are conditions related to storage that encourage the formation of AFs, such as gas concentration, time, and interaction with insects, as well as grain defects like physical and chemical damage that affect the production of AFs [11,12].

Impact of temperature, water activity (aw), and relative humidity

Temperature and water activity play a key role in the association of fungal growth with grains. Aflatoxigenic fungi can grow in a wide range of temperatures (19–35°C), with 28°C optimum for growth and 28–30°C for AFs production (Lahourar et al., 2016; [13,14]. AFs produced with optimal relative humidity (85%), while 95% relative humidity increases AFs production to a considerable level [15,16]. The moisture content and aw of the grain increase during storage if the relative humidity of the surrounding air is higher than the grain's equilibrium relative humidity. A higher level of aw during storage makes grains more susceptible to fungus invasion, germination, growth, and AFs production [17].

Effect of fungal strains and pH on the production of AFs

Members of Aspergillus Section Flavi, which comprises 33 species, the majority of which are toxigenic (produce aflatoxin), are responsible for the production of AFs. Prominent toxigenic and economically important members of the section are A. flavus and *A. parasiticus*. According to [9], 18 of the 33 species in Flavi produce aflatoxins. In addition, 16 species could produce the four types (AFB_1, AFB_2, AFG_1, and AFG_2). The other two species, A. togoensis and *A. pseudotamarii*, produce either AFB_1 alone or AFB_1 and AFB2, respectively. Toxigenic fungi can grow in a wide range of pH (1.7-9.3), but the ideal range is (3-7). According to [18] the lower pH (3 > pH > 1) inhibition the growth and produce of AFs in contrast higher pH (6 > pH > 3) encourages to produce of AFs. In addition, light has an impact on the growth of fungi and formation of AFs. Whereas, darkness increases AFs production while sunlight inhibits it [19]. Short wave light and decreased water activity in the substrate work together to efficiently and persistently inhibit the growth of aspergilli that produce mycotoxin [20].

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Substrate and their effect on AFs production

The substrate and other nutritional components like carbon, nitrogen, lipids, amino acids, and a few trace elements also have a significant impact on aflatoxin formation. Substrate rich in carbohydrates supports more production of AFs, as carbohydrate easily provides carbon, which is needed for good fungal growth [21]. Among carbohydrates, glucose, ribose, sucrose, xylose, and glycerol act as excellent substrates, while peptone, lactose, and sorbose were unable to promote AFs production [22]. Lipids are also necessary for the biosynthesis of AFs, such as lipophilic epoxy fatty acids, which cause ergosterol oxidation-induced AFs generation and fungal proliferation. Additionally serve as a substrate for acyl-CoA starting synthesis [23,24]. Damaged grains: As compared to seeds with intact husks, damaged seeds are more susceptible to AFs infection, according to numerous studies. An infestation of insects, poor food processing and inappropriate harvesting techniques can all harm the seed husk [25].

Overview of the global occurrence of AFs in grains

Many reports about the occurrence of AFs in food and products are available, especially with the advancement of analytical instruments and techniques. Nevertheless, at this point in this review, I focus on the natural contamination of AFs in only three types of grains, including corn, wheat, and rice, in major grain-producing countries, as well as in Egypt. AFs contamination of grains and products based on grains affects trade and the economy in both developed and developing countries. In the United States, corn producers lose $160 million a year because of contamination by AFs [26]. According to [27], these numbers are higher in developing nations, particularly in sub-Saharan Africa, where losses total $450 million. Some grains often contain more than one type of AFs. According to [28], 18304 samples of corn, (15889) wheat (2210), and rice (205) were collected from 100 countries during January 2008–December 2017. They found that AFB_1 was detected in 24%, 10%, and 31% of the samples, respectively. In addition, 41.1%, 38.5%, and 20.9% of samples of corn from South Asia, Sub-Saharan Africa, and Southeast Asia, respectively, exceeded the maximum level for AFB_1 (20 µg/kg). [29] reported that of the 41 and 25 studies surveyed on the occurrence of AFs in corn and wheat from 2018 to 2020, they concluded that the results of this literature review showed AFB_1 was detected in 87.5 and 40% of corn and wheat samples, respectively.

A review study by [30] concluded that the data from around 17149 analyses by the European Food Safety Authority and data released from recent large surveys on aflatoxins occurrence across the world by Biomin suggest that aflatoxins prevalence is highest in Asia (25%), Europe (7%), the Middle East and North Africa (7%), and South and Central America (19%), as shown in (Table 1). Concerning AFB_1, it is one of the most widespread aflatoxins commonly found in cases of aflatoxicosis. Also, it has a specific clause within the permissible limits. Therefore, (Table 2) clarifies the occurrence of AFB_1 in the different regions of the world. Chandravarnan et al. (2024) in their systematic review of the prevalence of mycotoxins in rice from 2890 studies conducted from 2000 to 2023 showed that total aflatoxins ranked first (56%), while AFB_1 recorded the third rank (34%), for the highest prevalence of mycotoxins in rice. On the other hand, concentration AFB_1 (56.17 µg/kg).

Table 1: Total AFs and their levels in the grains produced in different regions of the world in 2020 according to [88].

Region	NO. of Samples Tested	Positive Rate (%)	Minimum (µg/kg)	Average (µg/kg)	Maximum (µg/kg)
Europe	3711	7	2	6	92
Asia	3350	25	9	47	2495
Middle East and North Africa	116	7	2	2	5
Africa (without North Africa)	1059	7	4	28	1032
North America	1655	4	4	26	482
South and Central America	7258	19	3	5	179

Table 2: Distribution of AFB_1 in the different regions in the world for 2019 according to [88].

Region	NO. of Samples Tested	Positive Rate (%)	Average (µg/kg)
Northern Europe	1958	5.9	3.1
Central Europe	21036	12.7	1.6
Southern Europe	3527	28.9	2.1
Eastern Europe	2382	17.0	3.4
South Asia	1136	82.2	20
Southeast Asia	4310	57.4	10
East Asia	13232	17.1	10

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Middle East and North Africa	1075	22.2	2.4
South African	1077	9	2.2
Sub-Saharan African	208	76	23.0
North America	5471	10.5	8.7
Central America	367	8.6	3.9
South America	17332	23.5	3.2

Monitoring the presence of AFs during food (including grain) trade between countries

In this section, I draw on a collection of sources that offer a worldwide overview of aflatoxins' distribution between 2018 and 2023. These sources include data from international organizations that monitor food safety globally, including the Joint Expert Committee on Food Additives and Contaminants (JECFA) and European Union (EU) Rapid Alert System for Food and Feed (RASFF). as well as certain recent systematic reviews. Statistics from [31] show that 400 cases of mycotoxin were reported as hazards; AFs only accounted for 367 (91.7%) alerts, which is approximately 10% of the total RASFF notifications this year [31].

[32] conducted a comprehensive analysis of risk assessments of aflatoxins published between 2016 and 2022. Based on the EU's Rapid Alert System for Food and Feed (RASFF) data, they came to the conclusion that grains and their products represent the fourth most significant food category contaminated by AFs (3%) from these notifications. Corn and rice have been found to be the main sources of dietary intake from grains for individuals with AF at 2.19% and 0.71%, respectively. It comes in seventh and tenth rank per food category. In this regard, [33] reported that AFs are present in 60–80% of the world's grain harvests. As well, for RASFF data, 2812 AFs notifications were found in different countries issued within the same period (January 2016 and March 2022), as shown in (Figure 2).

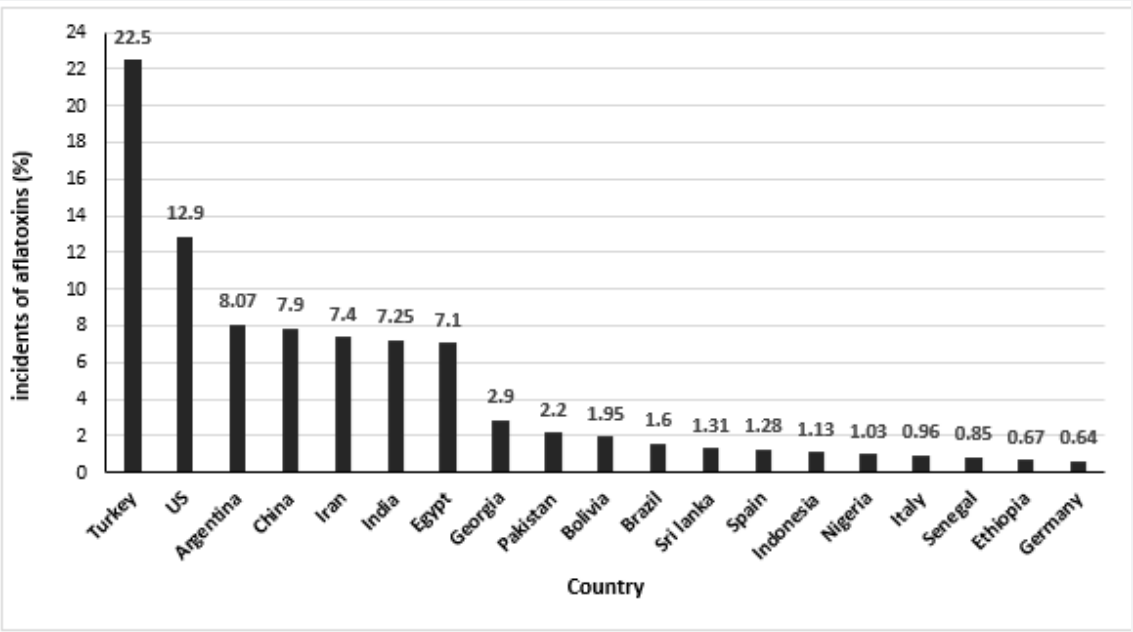

Figure 2: The percentages of incidents of AFs per country of origin of the food products, according to [116].

According to the data from [34] on the World Mycotoxin Survey, which was conducted on 21287samples from 86 countries, overall, the survey shows that the occurrence of fumonisins and deoxynivalenol remains high on every continent. Although the prevalence of mycotoxins is shifting, "due to climate change, mycotoxins, which were usually found in the southern part of the world, are now moving to the north. Mycotoxins are moving with the shifting climate," said Annelies Mueller, product manager at Biomin. (Figure 3) shown the rate of the prevalence of AFs in some geographical areas in world.

The global production of main grains

The production of grains increased by 64 million tonnes, or 2.1%, globally between 2020 and 2021, mostly because of a 4.1%

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increase in corn. Corn, wheat, and rice accounted for 90% of the total amount of grains produced in 2021. Corn, wheat, rice, barley, and sorghum are the five most produced species of grains (Figure 4). Corn showed the highest production (1.235 billion metric tons in 2023), followed by wheat (784.91 million metric tons) and rice (513.55 million metric tons). Concerning Egypt, according to CAPMAS (2022), the amount of production of corn, wheat, and rice was 7.2, 9.8, and 4.4 million metric tons, respectively. On the other hand, sorghum and barley recorded 750 and 90 metric tons, respectively [35]. The global production of corn produced by the United States and Brazil was 39%. Almost 23 percent of production was from China, making it the second-largest producer. Asia ranked first in the world for rice production, with the top three producers following China, India, and Bangladesh with percentages of 27, 25, and 7%, respectively. The total production of wheat in the world is centered in China (18% of the world total) and India (14%). The Russian Federation was the third-largest wheat producer, accounting for 10% of global production.

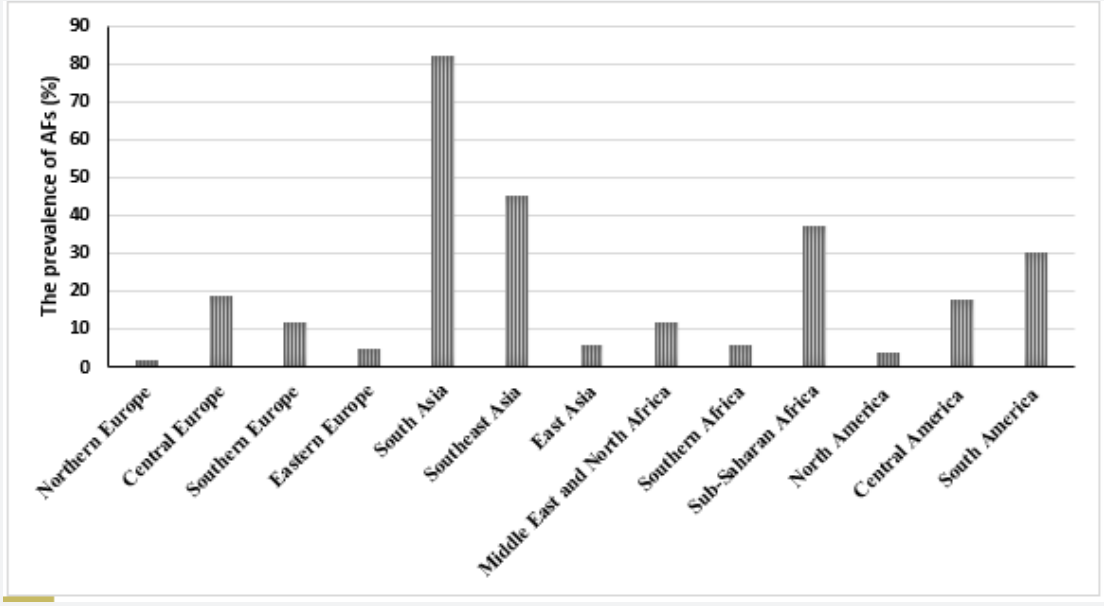

Figure 3: The percentages of prevalence of AFs around the world according to survey by [88].

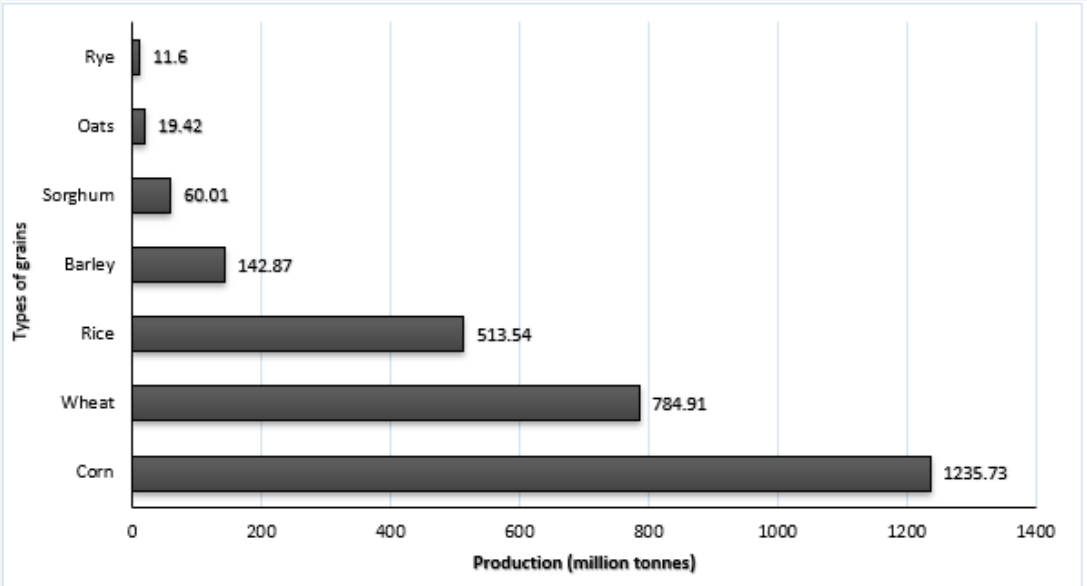

Figure 4: Worldwide production of grain in 2022/23 according to [40].

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Natural occurrences of AFs in Egypt and the biggest producers of corn grains

Corn is one of the most important crops in the world that are currently stored for animal feed and human consumption. According to the (IGC) International [36], the leading exporters of corn in the world are the US, Brazil, and Argentina by 369, 113, and 57 million tons, respectively, which are main producers of corn [36]. On the other hand, (Figure 5) displays the global corn production in 2023 as reported by [35]. The occurrence of AFs in corn can start before harvest, during harvest, and postharvest during storage when conditions are favorable for aflatoxigenic fungi for the growth and production of AFs [33]. AFs levels in corn grain vary from year to year but are typically highest in heat- and drought-stressed years [37]. Consequently, AFs production may also alter periodically and significantly at a given location. According to [38], hot and dry weather patterns with drought episodes proved favorable for higher AFB_1 contamination. (Figure 6) shows the percentage of AFs contamination of corn on the world's continents. Over the past five years, numerous investigations have detected AFs in corn and its derivatives. According to data published by [12], more than 76% of total AFs were detected, followed by 20% of AFB_1 and the remaining AFB_2. The concentrations for total AFs ranged between 0.01 and 3760 µg/kg and AFB_1 0.15–2072 µg/kg. Many studies have documented the contamination of corn by AFs. (Table 3) in this review displays the total AFs and AFB_1 natural occurrences in samples from Egypt, as well as the biggest country produced for corn.

Table (3): Natural occurrences of AFs in corn (maize) samples in Egypt and some main product countries.

Country	Type of AFs	Incidence Rate % (Sample Size)	Concentration (µg/kg)	Detection Technique	Ref.
Egypt	AFB_1 Total Afs	25 (61) 46.6 (15)	0.. 2.35-7.85	TLC HPLC	[89,90]
USA	Total AFs AFB_1	7.6 (711) 1.7 (711)	2.0-611 4.5-606	UPLC-MS/MS	[91]
China	Total AFs	13 (1649)	8.0-331	LC-MS/MS	[92]
Brazil	AFB_1	20.3 (3960)	1.8-8.9	NIR Spectroscopy	[93]
India	AFB_1	18.6 (150)	48-383	HPLC	[94]
Argentina	Total AFs	40 (270)	0.02-8.0	HPLC	[95]
Mexico	Total AFs	8 (3861)	Jul-30	HPLC	Odjo et al. (2022)
South Africa	Total AFs	27.6(123)	0.0 -65.0	LC-MS/MS	[96]
Nigeria	Total AFs	95 (140)	0.65–265	HPLC	[97]
Ethiopia	AFB_1 AFB_1	20 (30) 34 (90)	2.18-10.23 3.9–381.6	HPLC ELISA	[98,99]

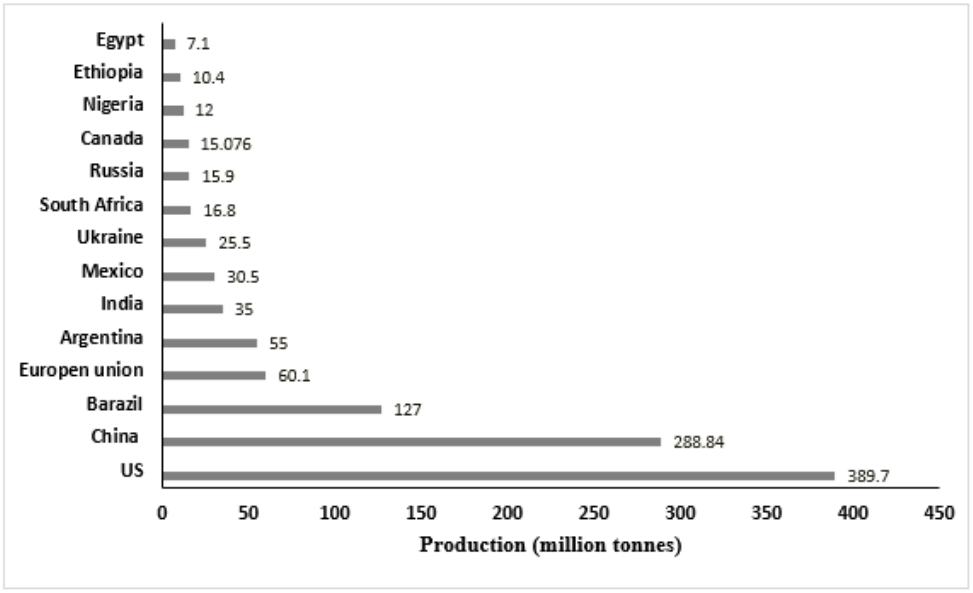

Figure 5: Corn production during 2023 in some different countries [40].

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Natural occurrences of AFs in Egypt and the biggest producers of wheat grains

Wheat is an economic and important grain that supplies a fifth of global food calories and protein. It is one of the main staple foods in developing countries. Egypt has one of the highest wheat per capita consumption levels in the world (156.1 kg/person/year). Egypt is eighth in the world in wheat consumption at a rate of 20.6 million tons annually, while the total production of wheat in Egypt was 9.1 million tons harvested from 3.2 million Feddan [39,40]. On the other hand, the top 10 wheat-producing countries in the world are display in (Figure 7). As shown in (Table 4), a number of studies have documented the presence of AFs in wheat. [28] reports that AFB_1 found in 23% of 74,821 samples of wheat collected from 100 different countries worldwide and that the average concentration of infected samples was rising in samples taken from East Asia.

Table (4): Natural occurrences of AFs in wheat grains in Egypt and some main product countries.

Country	Incidence Rate % (Sample Size)	Type of AFs	Concentration (µg/kg)	Detection technique	Ref.
Egypt	33.3 (36) 41.66 (36)	AFB_1, AFB_2 AFB_1	(0.13–49.8), (0.09–2.96) 0.89-3.79	HPLC HPLC	[100,101]
China	3.3 (338)	AFB_1	0.6-19.7	LC- MS/MS	[102]
USA	2 (141)	Total AFs	0.21-0.44	HPLC	[103]
France	14 (60) 19 (60) 6 (60)	AFB_1 AFB_2 AFG_1	1.03-9.5 0.34-0.67 0.53-1.05	LC- MS/MS	[104]
Ukraine	--------	AFB_1 Total Afs	0.13-0.46 0.767-1.6	HPLC	[105]
Pakistan	26 (48)	Total AFs	0.02–4.78	HPLC	[106]

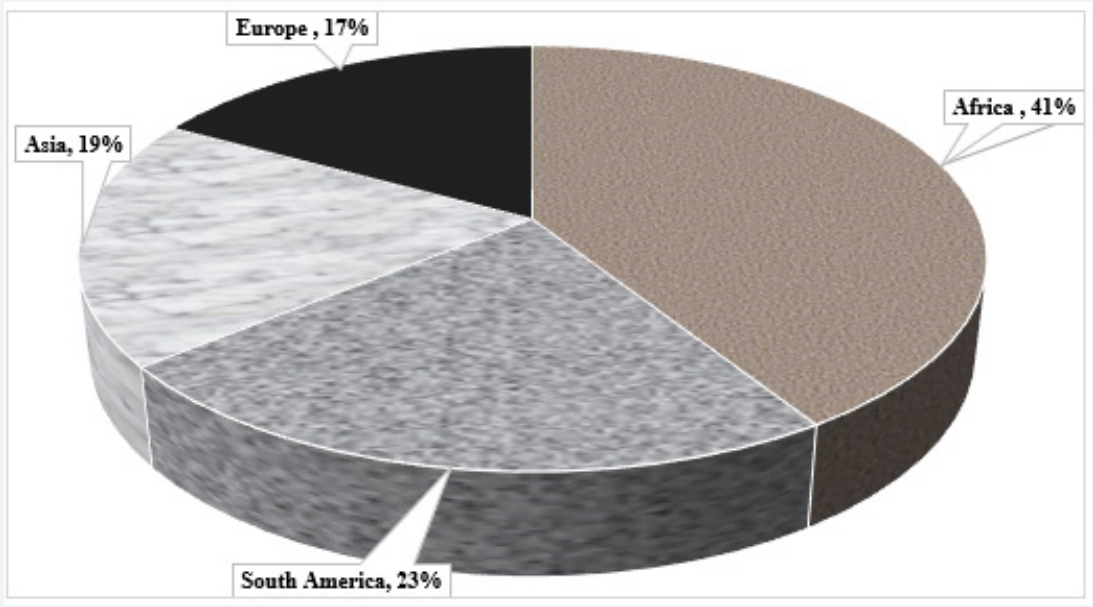

Figure 6: Global contamination of aflatoxins in corn, depending on some data reported by [12].

Natural occurrences of AFs in Egypt and the biggest producers of rice grains

Asia is the primary region for the cultivation and consumption of rice. Rice ranks third in the world after corn and wheat. As (Figure 8) illustrates, China and India are the world's top producers. In Africa, rice is mainly produced in Egypt and Nigeria. Fungi that produce AFs can contaminate rice during harvest, handling, and storage, as well as when the field's climate becomes conducive to their growth. Several studies have reported the presence of AFs in rice, which are highly prevalent in Asian nations. The significant frequency of AFs contamination in rice and rice-derived products highlights the significance of close observation of this staple

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food around the globe [41,42]. AFs prevalence in rice worldwide is displayed in (Table 5). According to [12], the number of AFs present in rice during the 2015–2020 period (with concentrations ranging from 0.014 to 921.93 µg/kg) accounts for 65% of the total aflatoxins. AFB_1 and AFB_2 make up 35%, with concentrations between 0.014 and 44.10 µg/kg.

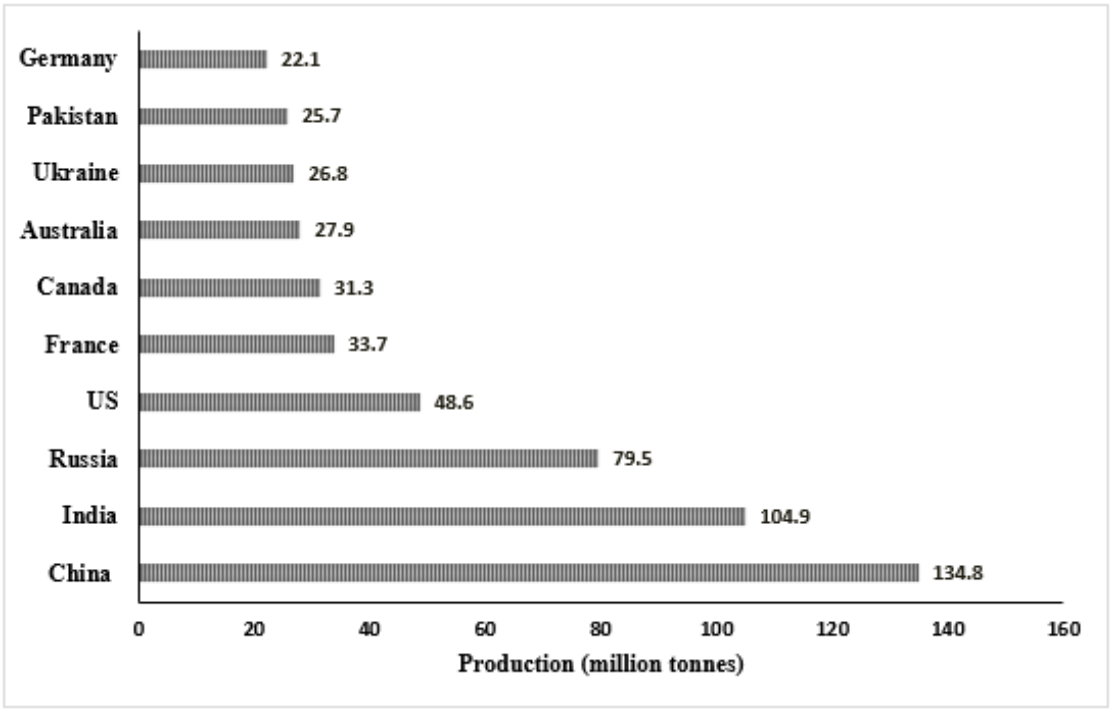

Figure 7: The biggest country producer of wheat in 2023, according to FAO.

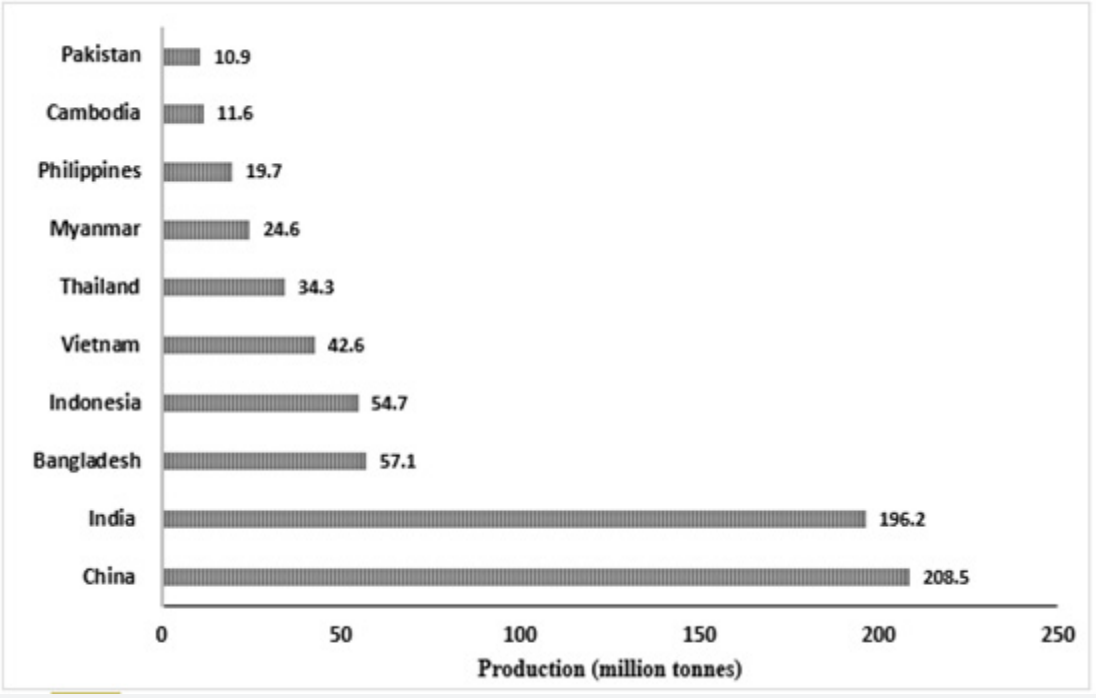

Figure 8: Paddy rice production in top ten countries in the world in 2023.

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Table 5: Natural occurrences of AFs in rice grains in Egypt and some main product countries.

Country	Incidence rate % (sample size)	Type of AFs	Concentration (µg/kg)	Detection technique	Ref.
Egypt	12.5(51) 46.6 (15)	AFB_1 Total Afs	100-200	TLC	[107]
China	247.1(792)	AFB_1	0.07-262.6	LC/MS/MS	[109]
India	2.3(87)	Total AFs	0.0-22.9	TLC	[110]
Bangladesh	7.1(14)	AFB_1	0.-1.46	HPLC	[111]
Vietnam	-50	Total AFs	1.4-4.0	ELISA	[112]
Philippines	95(78)	AFB_1	0.0-8.5	HPLC	[113]
Pakistan	66.0 (62)	AFB_1	504-11.9	UPLC-MS/MS	[114]
Nigeria	50.0(40)	Total AFs	1.75-22.8	ELISA	[115]

Regulatory limits and standards of aflatoxins in grains

Depending on the potential human health risks posed by the dietary intake of AFs that have been assessed by several scientific bodies, such as FAO/WHO, JECFA, and EFSA. Many countries and international organizations have developed regulatory limits for the presence of AFs in food, including grain (cereals). In Egypt, the regulatory limits as recommended by EOSQC are compliant with EC No. 1881/2006, as shown in (Table 6).

Table (6): Maximum Limits (MLs) set by different international organizations on AFs in cereals including corn, wheat, and rice.

	Wheat		Corn & Rice	
International regulatory organizations	**Total AFs**	**AFB_1**	**Total AFs**	**AFB_1**
EC (European Union)	4	2	10	5
FDA (USA)	20	Not stated	20	Not stated
FSSAI (India)	15	10		
NHFPC and CFDA (China)	Not stated	5	Not stated	10
FAMIC (Japan)	Not stated	10		10
EOSQC (Egypt)	4	2	10	5

EC: European Commission; FDA: Food and Drug Administration.

FSSAI: Food Safety and Standards Authority of India; FAMIC: Food and Agricultural Materials Inspection.

Centre; EOSQC: Egyptian Organization for standardization and Quality Control.

Post-harvest management to mitigate aflatoxins in grain

AFs can be found in all stages of grain production, from pre-harvest to processing, going through a harvest, and post-harvest stages; each stage has appropriate methods and strategies to mitigate and/or remove AFs. In the stage of pre-harvest, methods for controlling aflatoxins are largely preventive, like farming resistant varieties, reducing plant stress, proper fertilization, insect and weed control with the use of fungicides, and timely harvesting, as well as avoiding mechanical damage to the grain and rapid drying. However, this part will focus on the methods and strategies applied in the post-harvest stage, which includes the storage stage too. Strategies for mitigating or controlling aflatoxin in cereals are a viable means of ensuring that customers will receive safe and wholesome food because they prevent contamination of raw materials and processed goods [9]. (Figure 9) summarizes the various innovative strategies for the control of AFs in grain.

Any method or strategy used for the mitigation of AFs must meet a set of conditions, such as having no negative effect on either nutritional properties or food safety; not changing the physical-chemical properties of the treated foods significantly; and not leaving any toxic residues of the aflatoxins in the food products. In addition, its impact on AFs is an irreversible change; it is it is an environmentally friendly method. Lastly, these methods have easy-to-use, cost-effective, and safe post-harvest tools during storage and food processing [43-45]. Although the prevention of aflatoxins contamination in the field is the main goal of the agricultural and food industries, under certain environmental conditions, the contamination of several commodities with fungi produced for AFs may be unavoidable for producers. Once fungi or contamination with aflatoxins infects grains, different strategies

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and treatment options are available to improve the quality of the contaminated grains, which include chemical, biological, and physical treatments (Park, 2000). Irradiation, including gamma, ultraviolet (UV), and electron beams, for contaminated grain is considered an effective method for degradation of aflatoxins based on some factors like the UV dose and treatment time.

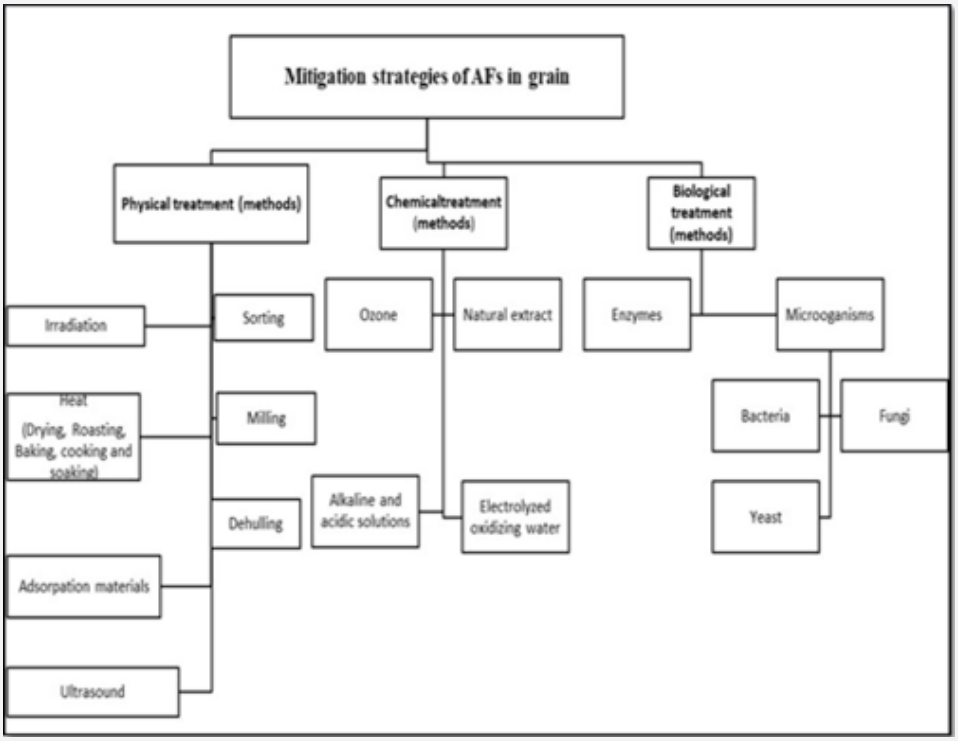

Figure 9: Some strategies (methods) for the mitigation of aflatoxins.

Post-harvest mitigation of AFs using physical methods or techniques

Numerous studies have revealed that physical strategies implemented after harvest are effective in reducing AF levels. For example, [46] reported that hand sorting by visible fungi infection is very efficient to decrease the AFB_1 concentration of corn. Nevertheless, this approach is only applicable on an industrial scale using optical sorting equipment. Hulling of corn removes more than 90% of the AFs content, and rice polishing is a recommendable process [47,48]. The degradation of AFs requires high temperatures ranging from 237 to 306°C. According to reports, to achieve partial elimination of the toxins, temperatures must be above 150°C. Most industrial processes do not detoxify AFs, so there must be detection of AFs in final products. However, rice contaminated with AFB_1 after cooking has shown a reduction of 34% that increased to 88% with pressure-cooking [49]. Sorting of grains leads to a decrease in AFs levels as clean grains are physically separated from contaminated ones. Nevertheless, this method is not very practical due to the incomplete removal of AFs-tainted grains Schaarschmidt and [50]. Milling of contaminated grain with AFs leads to the redistribution of toxin in certain mill fractions without destroying it [51]. Irradiation, including gamma, ultraviolet (UV), and electron beams, for contaminated grain is considered an effective method for the degradation of aflatoxins based on some factors like the dose and treatment time. According to [52], gamma (γ) is the most preferred radiation source for treating food with doses up to 10 kGy. On the other hand, UV irradiation is highly cost-effective and eco-friendly. Treatment of grains with moderate doses has no negative impact on their sensory and physicochemical properties. Many studies showed that UV treatment was effective. Most studies reported that the sensitivity of aflatoxins to UV was $AFB_1 > AFG_1 > AFB_2$ [53]. Treatment of AFs leads to the appearance of several degraded products, as shown in (Figure 10).

The application of ultrasound for the reduction or degradation of aflatoxins in grains is a promising technology because of its minimal impact on the physicochemical properties of foods. It also produces no secondary pollutants, so it is an eco-friendly and non-polluting technique. AFB_1 degraded by ultrasound by affecting the chemically stable furan moiety and by changing the lactone ring in the main structure of AFB_1 as shown (Figure 11 A &B) [54,55].

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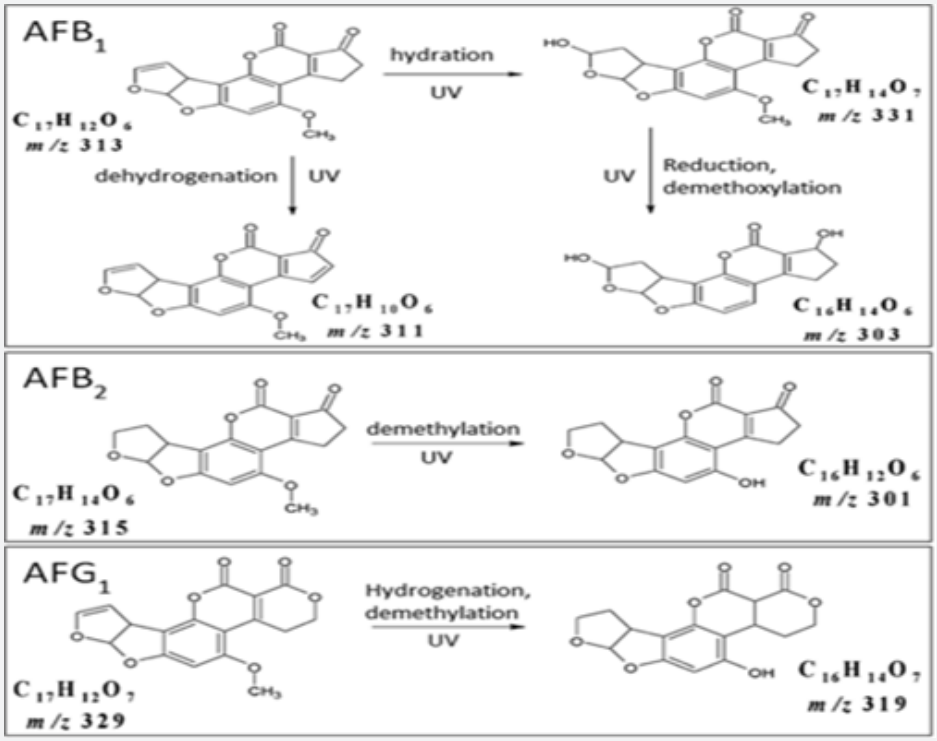

Figure 10: Compounds produced by AFB_1, AFB_2, and AFG_1 after degradation by UV, according to [117].

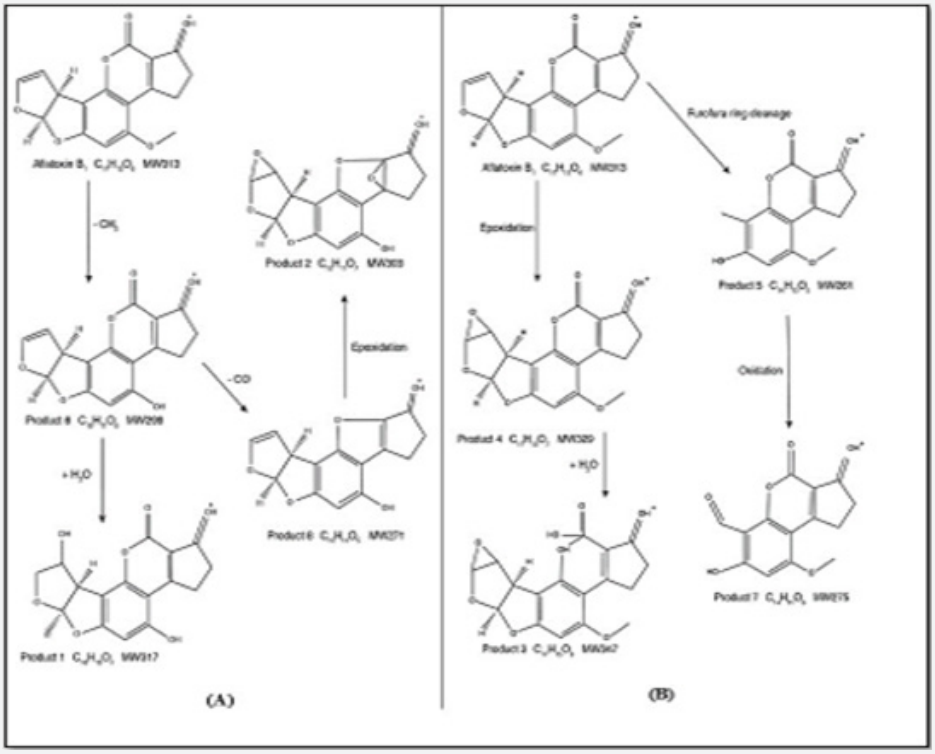

Figure 11: Showing first (A) and second (B) pathway of AFB_1 degradation mechanism by ultrasound according to [54].

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Post-harvest mitigation of AFs using chemical methods or techniques

Numerous chemicals have been tested for their ability to degrade or detoxify aflatoxins, including acids, ammonia (ammoniation), natural extracts from plants, and ozone gas (ozonation). Although many of the proposed treatments may successfully destroy aflatoxins, however, after many studies, it became clear that many of them are not suitable for application due to their many problems, such as sensory and nutritional properties [56]. In addition, the ammoniation process generates toxic products [57]. On the contrary, the ozonation process is one of the most important methods in this field and the most suitable for application with grains at all stages of production [58,59]. In this point, will shed light on some of these methods, their results, and their working mechanisms.

Ammoniation was one of the degradation methods for aflatoxins in the past, which used NH_4OH or NH_3. It can be utilized in two ways: as a high-pressure and high-temperature process or as an atmospheric pressure and ambient temperature process. (Figure 12) shows that this process begins with the opening of the lactone ring of AFB_1 (reversible) and followed by ammonium salt forming from the resulting hydroxy acid. According to [60], using higher ammonia concentrations resulted in an increase in the ammoniation process's efficiency. Organic acids have been used for AFs degradation through the soaking process of grains, for instance, soaking of grain in 1.0 N tartaric acid, lactic acid, and citric acid for 18 h at room temperature, which leads to a degradation of the AFB_1 level by 95.1, 92.7, and 94.1%, respectively [61]. To get the best results, it is preferable to combine them with other mitigation technologies (Rastegar et al., 2017). The use of organic acid for the treatment-contaminated grains is expensive [62].

Some authors have previously reported on the use of natural extracts and essential oils for the decrease or degradation of AFs, especially in vitro, in different ways, like inhibiting the growth of aflatoxigenic fungi, blocking AF biosynthesis, and removing or degrading AFs [63-65]. Because plant extracts contain a large group of active compounds, as shown in (Figure 13). The most important advantage of using plant extracts to control aflatoxin is that their effect on food quality is limited. However, most of them lack specific mechanisms of action. Therefore, it needs more research to benefit from it on a commercial scale [66]. In this regard, I have conducted a study using the aqueous extract of three leaves of wild edible plants (sow thistle, chicory, and Rajesh), which has proven effective in preventing the synthesis of AFs with percentages of inhibition of AFB_1 of 78.03, 68.8, and 81.7%, respectively [67]. Another study by [68] found that using an aqueous extract of carob pulp at 5 mg/mL reduced the production of AFs by 76.5 to 86.5%.

Ozonation has been considered an interesting method for the remediation of cereals contaminated by aflatoxins. The ozone reaction with AFB_1 in the site C8-C9 double bond at the terminal furan leads to the formation of aflatoxin molozonide, which is further changed to aflatoxin ozonide. This compound is unstable and changes to aldehydes, ketones, acids, and CO_2 (Figure 14). Ozone decomposes to form oxygen gas and therefore can be classified as a nonpersistent chemical; however, it must be generated at the location of its intended use (McKenzie et al., 1997). On the other hand, these features make ozone an important alternative for the food industry. Finally, many international organizations, including the WHO, FAO), and FDA, had regarded ozone as a safe and effective chemical applied in the food industry. A few factore, including temperature, moisture content, exposure time, and O_3 concentration, which effected on effectiveness of ozonation process on aflatoxins. A few factors, including temperature, moisture content, exposure time, and O_3 concentration, affect the degradation of aflatoxins by ozone [69,70]. In my opinion, it is one of the most effective ways to reduce aflatoxins. It also has ease of application in gaseous as well as liquid forms, no residue after contact, no hazardous disposal, and easy on-site generation of ozone [71].

Post-harvest mitigation of AFs using biological methods or techniques

Biological methods for degradation or mitigation of AFs involve microorganisms (fungi, bacteria, and yeast) or enzymes that lead to less toxic or non-toxic metabolites. This method has emerged as an efficient and eco-friendly strategy for the degradation of AFs. Due to its safety, sustainability, and economic viability, the biocontrol method offers an appealing and suitable substitute for the removal and degradation of AFB_1 from food [72]. However, it has some drawbacks, such as the difficulty of controlling microbial performance, the safety of the newly formed product to the body, and some of them are only active in certain environmental conditions [73].

There are many antagonistic microbes that can be used for this purpose, such as several bacterial species, nontoxigenic fungi, and trichoderma. The biological techniques are based on competitive exclusivity or biological interactions like antibiosis. Biodegradation for AFB_1 may work through catabolic pathways to detoxify the AFs into less toxic intermediates or final products. Such as, microbe is able to convert AFB_1 to aflatoxicol by reducing the cyclopentenone carbonyl of AFB_1. These fungi could convert AFB_1 to aflatoxicol-A, and then aflatoxicol-A was converted to aflatoxicol-B by the actions of medium components or organic acids produced from the fungi (Figure 15).

Lactic acid bacteria (LAB) such as Lactobacillus, Bifidobacterium, and Propionibacterium are the most studied of all bacteria used for the biodegradation of AFs. LAB has demonstrated a great potential for removing AFs and can be utilized as starter cultures in the fermentation of foods and as additives in food processing. The mechanism of reducing AFs by LAB is due to their adhesion to cell-wall components [74]. As well, LAB produces many active compounds that change the

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original structure of the AFS and convert them into substances that are low in toxicity or even completely non-toxic [8]. Lactic acid bacteria are a promising strategy to mitigate aflatoxins contamination of grains. Especially if it is used in liquid media like fermentation processes for some grains, such as barley and other grains [75]. In this field, I have conducted research with a group of researchers, such as Fouad and [76], they evaluated three strains to reduce aflatoxins in wheat grains and found that the highest inhibition rates of AFs ranged between 61.4 and 75.8% with Lactobacillus rhamnosus [76]. In another study of ours, we evaluated fourteen strains of LAB isolated from dairy products to reduce AFs. We found that all tested strains were able to reduce AFB_1 at different rates depending on the time of incubation. Increasing the incubation time to 36 hours led to removing more than 88 from AFB_1 [77]. As well as non-toxic fungi can be used. [78] used the fungus Trichoderma harzianum to biodegrade AFB_1. They found that the greatest degradation was 76.8%, which led to a 65% suppression of A. flavus growth.

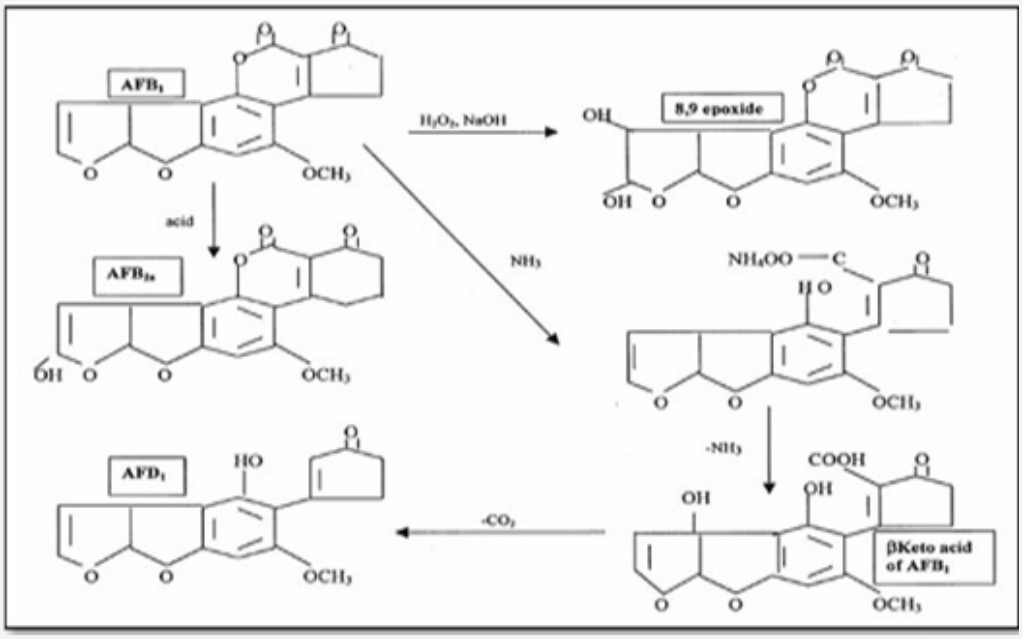

Figure 12: Proposed mechanism detoxification of AFB_1 by ammoniation according to [118].

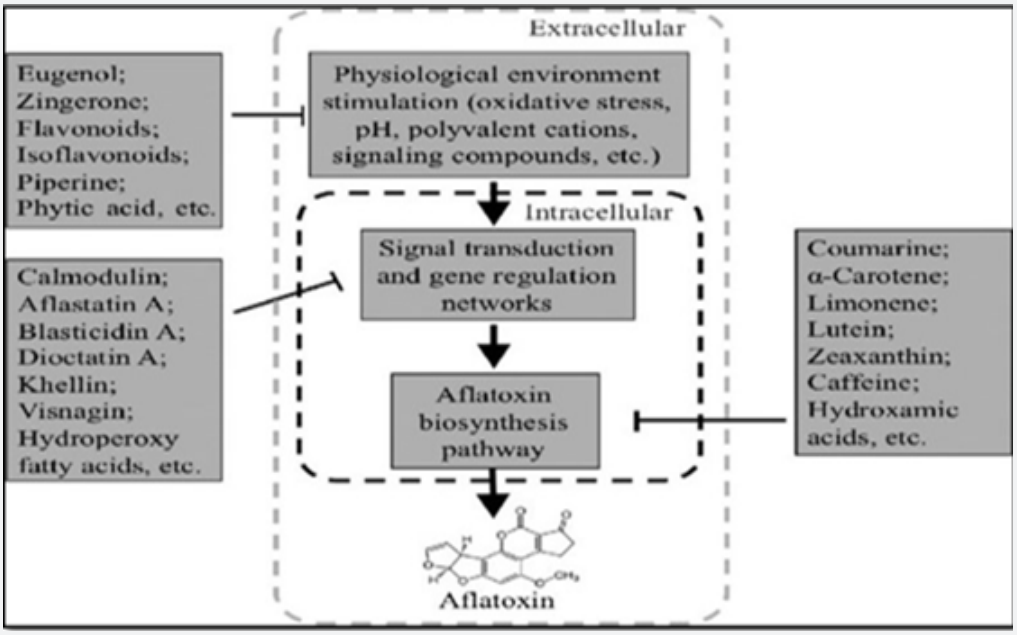

Figure 13: Shows some natural compounds for the production inhibition of AFs.

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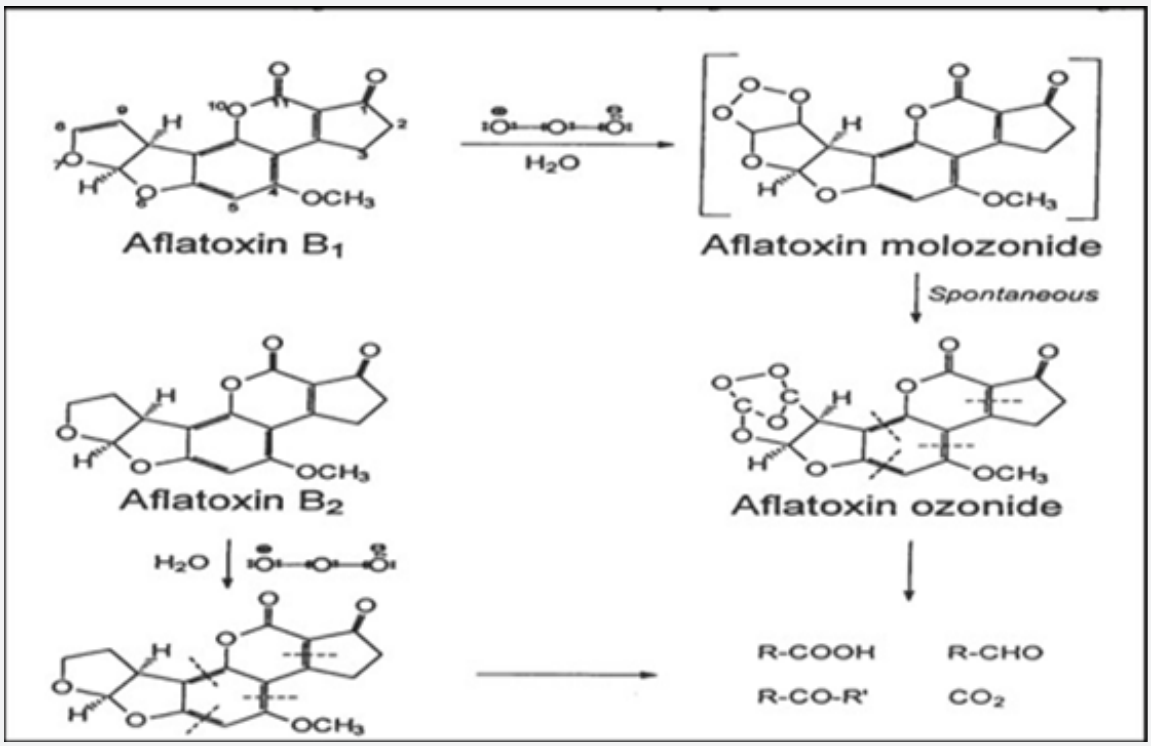

Figure 14: Mechanism of degradation of AFs by the ozonation process according to McKenzie et al., 1997.

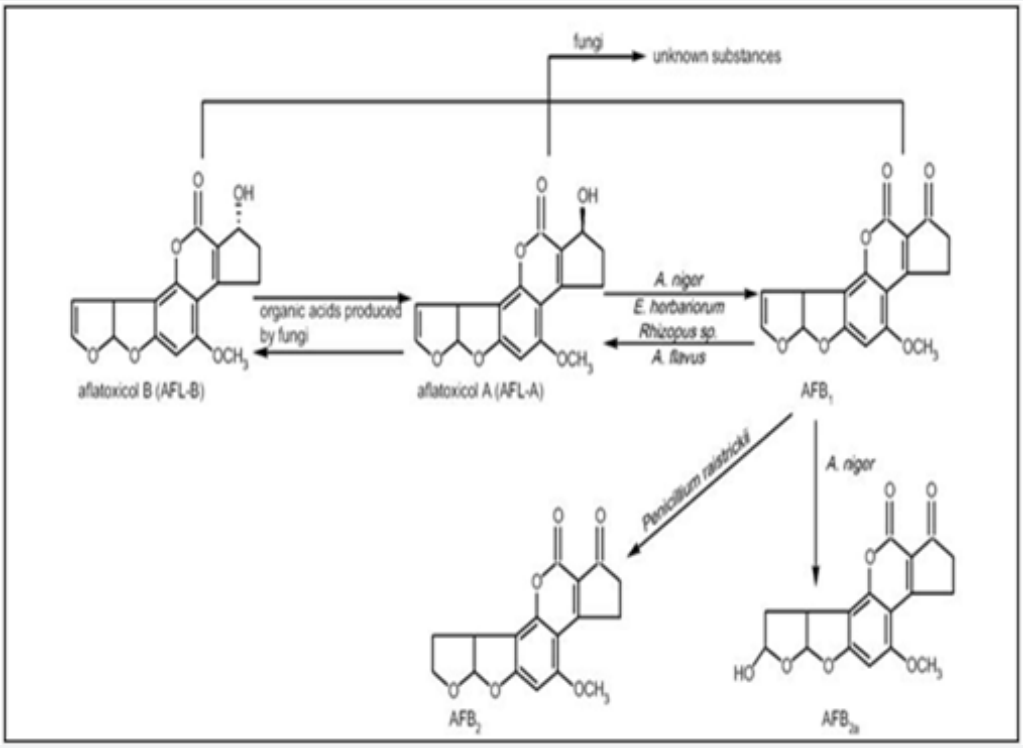

Figure 15: Biodegradation of AFB_1 by fungi according to [119].

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Recently, many reports on the isolation, identification, and purification of AFs-degrading enzymes from microorganisms have increased significantly. To avoid defects resulting from the use of whole organisms for biodegradation for AFs, the use of enzymes is far more convenient since they are substrate-specific, effective, and environmentally friendly; moreover, their application in the food and feed industries has been established [79]. The enzymatic degradation of AFs depends on a number of factors, including temperature, incubation time, enzyme concentration, and initial AFs concentration [80]. Alberts et al. (2009) first proposed the role of laccases that produced and purified from Pleurotus pulmonarius in degradation of AFB_1. They reported that enzyme laccase (Lac2) showed AFB_1 degradation up to 90%. According to [81] laccases act on AFB_1 in two ways as shown in (Figure 16).

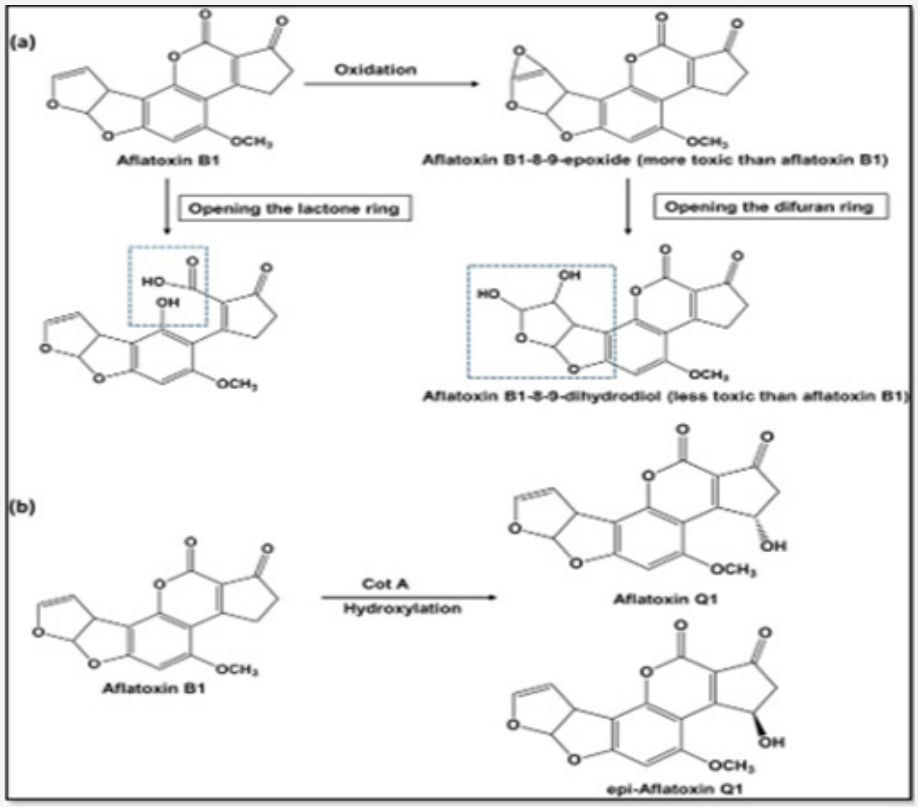

Figure 16: AFB_1 degradation pathway by laccase (a) attack on the lactone and furan ring; (b) C3-hydroxylation in AFB_1 to produce two isomeric compounds AFQ_1 and epi-aflatoxin Q_1.

Controlling and mitigation strategies for aflatoxins during storage grains

Sorting and cleaning

The most important thing about grain storage is that AFs levels will not increase if the grain is properly stored but may increase if it is not. Thus, the lower the level of AFs in the grain when it is stored, the lower the levels in the grain when it is taken out of storage. Although it would be better if the grains were free of AFs from the beginning, on the other hand, it is difficult for grains to be free of fungi and their spores, which develop under storage conditions and produce AFs. Therefore, it is extremely important to reduce the microbial load of stored grains before storage by any methods such as ozonation or washing. AFs contamination during storage can be greatly reduced by using a combination of cleaning techniques to effectively remove grain that is clearly moldy, sick, broken, and/or damaged. According to [82] and Schaarschmidt and [50], cleaning grains leads to removing 7–50% of the toxin contaminating the grain. AFs were shown to be reduced by 40–80% when damaged and contaminated corn grains were removed. Furthermore, sifting broken and damaged grains by hand eliminated 95% of AFs. The initial concentrations in grains and the percentage of pollutants removed throughout the cleaning process determine how much the cleaning process can reduce aflatoxin levels Park, 2000 [46].

Storage conditions and management techniques

The temperature, grain moisture content, and relative humidity during storage are the main factors that must be under control. As well, which must be managed efficiently and professionally.

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➢ **A moisture content**

10–14% is ideal for drying grain. Since grain is often dry when harvested, it is allowed to put it in storage if the a_w is less than 0.70 [83,84]. As well, proper monitoring of temperature and relative humidity.

➢ **Regulate the temperature**

At low or cold temperatures, fungal is not killed, but growth will be slow, and metabolism (production AFs) is more difficult to occur at lower temperatures. Keeping the grain piles at a consistent temperature and practicing proper hygiene are sufficient and essential storage precautions [48].

➢ **Control of insects**

It is necessary to manage the presence of insects since the majority of insects in storage systems have the ability to promote the growth of fungus by increasing the temperature of the grains and moisture, all of which promote the production of AFs [85,86].

➢ **Modification of atmosphere during storage**

Changes to the atmospheric gases, such as CO_2 and N_2, in storage silos could stop or at least lessen the generation of AFs. Certain aflatoxins have been shown to be inhibited and fungal growth on grain to be prevented by <1% from O_2 and/or increasing CO_2 or N_2 concentrations [87, 120-124].

Future vision for the development and sustainability of controlling methods of AFs

In this section, I highlight a few points that I think merit more investigation in the future.

➢ Designing a program to monitor and predict aflatoxins during grain storage

➢ Using artificial intelligence (AI) systems to combine more than one method of reducing aflatoxin toxins.

➢ Relying on sustainable methods to combat or reduce the presence of aflatoxins

➢ Use the equipped robots to monitor storage operations inside the silos.

➢ Developing packaging materials using nanotechnology to prevent the growth of fungi and the production of their toxins on grains

➢ Developing detection and analysis methods in order to reduce costs (sampling, analysis, and storage) during monitoring storage.

➢ The transformation pathways and transformation products of aflatoxins still require more research.

➢ We still need to work on developing some technologies to make it safer, more environmentally friendly, and faster.

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