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Aflatoxins

Nadeem A. Ramadan and Hadeel A. Al-Ameri

Abstract

The aflatoxin producing fungi *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*, although they are also produced by other species of *Aspergillus* as well as by *Emericella* spp. (Teleomorph). There are many types of aflatoxins, but the four main ones are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), while aflatoxin M1 (AFM1) and M2 (AFM2) are the hydroxylated metabolites of AFB1 and AFB2. Aflatoxin B1, which is a genotoxic hepatocarcinogen, which presumptively causes cancer by inducing DNA adducts leading to genetic changes in target liver cells. Cytochrome-P450 enzymes to the reactive intermediate AFB1-8, 9 epoxide (AFBO) which binds to liver cell DNA, resulting in DNA adducts, metabolize AFB1. Ingestion of contaminated food is the main source of exposure to aflatoxins, which adversely affect the health of both humans and animals. The compounds can cause acute or chronic toxic effects of a teratogenic, mutagenic, carcinogenic, immunotoxic or hepatotoxic character. You can reduce your aflatoxin exposure by buying only major commercial brands of food and by discarding that look moldy, discolored, or shriveled.

Keywords: aflatoxin, *Aspergillus flavus*

1. Introduction

Aflatoxins are a type of toxins produced by *Aspergillus* species, including *A. flavus* Link, *A. parasiticus* Speare, and *A. nomius* Kurtzman, Horn and Hesseltine. These toxins are responsible for harming 25 percent of the food crops in the world. The fungi produce both pre- and post-harvest contaminant toxins. Aflatoxin is responsible for major economic losses to agriculture in the United States and other developed countries, but aflatoxins also cause human and animal disease in developing countries where the use of contaminated grain cannot always be avoided. Aflatoxin exposure leads to the production of liver cancer in areas of the world where it is endemic, making it a major contributor to a serious public health epidemic. The presence in field samples of other mycotoxins, in particular fumonisins, along with aflatoxin, poses additional questions about the safety of food and feed supplies.

Until the 1980s, reviews and numerous studies on the effect of aflatoxin on livestock were available. To today from the 1990s, a number of clinical reports have been published on the toxicological issues caused by aflatoxins, concentrating mainly on the molecular biology of aflatoxin in both host and fungus, aflatoxin control by traditional breeding, and genetic engineering to develop resistant aflatoxin.

2. *Aspergillus* infection and aflatoxins development

A large family of fungi occupying very diverse ecological niches is the genus *Aspergillus*. Although there is a worldwide distribution of members, *Aspergillus* spp. Between latitudes north or south of the equator, 26° to 35°, the most abundant appear [1]. In subtropical and warm temperate climates, these fungi are thus more common. *Aspergillus* spp., generally known as saprophytes, In nutrient cycling, they grow on a large number of substrates and are very important. They are well suited to colonizing a number of grain and nut crops due to their ability to thrive in high temperatures and with relatively low. Some species have limited parasitic abilities under favorable conditions and can colonize crops in the field.

Some of the most significant fermentation fungi, e.g. *A. niger*, *A. sojae*, *A. oryzae* are grown for their ability to generate industrial enzymes and metabolites and to provide food with flavor. However, other members are infamous for the mycotoxins created by them. *Aspergillus* species-associated mycotoxins include aflatoxins, ochratoxins, versicolorins, sterigmatocystin, gliotoxin,

3. *Aspergillus flavus*

The major class of mycotoxins formed by *Aspergillus* spp. are aflatoxins. Aflatoxins are produced by only four species of fungi and each belongs to *Aspergillus* section Flavi [2, 3]. These species are *A. parasiticus*, *A. flavus*, *A. pseudotamarii* and *A. nomius*. But only *A. flavus* and *A. parasiticus* are economically important. These two fungi have overlapping niches in the production of maize, peanut, cotton, almond, and pistachio seeds and may produce aflatoxin. Other tree nuts are also affected, such as walnuts and Brazilian nuts. It is also possible to infect figs, but the occurrence is poor. These fungi may also develop aflatoxin on much of the substrate that is poorly preserved. The predominant species for all commodities is *Aspergillus flavus*, while *A. parasiticus* is prevalent in peanuts [4].

As early as 1920, *Aspergillus flavus* cause an ear mold of maize, but until the 1960s, when it was shown to produce the factor (later recognized as aflatoxin) associated with Turkey X disease, the fungus was of little concern. The meaning of *Aspergillus flavus* preharvest corn infection. Prior to 1971, was largely discounted as aflatoxin contamination was thought to be just a storage problem. In the Southern and Midwestern United States in the 1970s, the study of aflatoxin contamination awakened the scientific community to the importance of preharvest contamination [5, 6].

The occurrence of aflatoxin contamination is sporadic and highly dependent on environmental conditions. Contamination with aflatoxin is intermittent and highly dependent on environmental factors. Each year in the southern United States, large populations of *A. flavus* and aflatoxin infection occur, but significant outbreaks are related to above-average temperatures and below-average rainfall. In the United States' corn belt between 1983 and 1988, these two environmental factors were related to a high incidence of aflatoxin pollution. Aflatoxin has been found in high concentrations in southern China, Southeast Asia, and Africa [7].

In corn, the infection process of *A. flavus* is better represented [5]. It is reproduces through asexual conidia, inhabiting the soil. Shortly after pollination, conidia carried to the corn silks by wind or insects may expand into the ear and colonize kernel surfaces. The fungus can directly invade seeds and cobs if environmental conditions are favorable, or it may enter through wounds caused by insects. Major infection and aflatoxin contamination do not happen in either case until the moisture of the kernel is below 32 percent. In kernels, Aflatoxin will continue to be

produced until the moisture reaches 15%. While insects are not necessary to contaminate with aflatoxin, their presence raises the level of contamination and high levels of aflatoxin are almost always associated with injury to insects, in particular *Ostrinia nubilalis*, the European corn borer [6].

There is proof that peanut flowers can be contaminated with *A. flavus* when compared to infection of the pods, this route of infection seems minor. It is not known the exact route of infection in pods, but insects tend to play a major role. Established vectors of the fungus are both mites and lesser stalk borer larvae (*Elasmopalpus lingosellus*). And microscopic damage to the pods increases fungus infection [8].

Even though there is evidence for direct infection of cotton by *A. flavus* [9, 10], A high level of aflatoxin in the environment is often associated with insects. The entry point for the fungus tends to be the exit holes created by the Pink Boll Worm Larvae (*Pectinophora gossypiella* Saunders). Pistachios infection by *A. flavus* Early splits are associated with a disorder in which the hull splits before maturation of the nut. High aflatoxin contamination is associated with the damage of navel orange worm larvae in both pistachios and almonds [11, 12].

Temperature and moisture are the two major factors causing aflatoxin contamination [5, 6]. High temperatures and drought stress lead to high levels of aflatoxin contamination in maize and peanuts. Under field conditions where soil humidity and temperature have been regulated, [13] showed that neither by itself is sufficient. The researchers discovered that there was no aflatoxin in peanuts grown with sufficient moisture. Similarly, peanuts grown under prolonged drought were aflatoxin free at temperatures lower than 25°C or higher than 32°C. Colonization by *A. flavus* and aflatoxin contamination at 30.5°C was maximized. The airborne inoculum of the fungus is increased by high temperatures and drought conditions [14]. Increased growth and reproduction at higher temperatures of the fungus is probably linked to its relatively high optimum temperature of growth. Over a wide range of temperatures (12 to 48°C), the fungus can grow, but its optimum for growth is 37°C. Higher temperatures and conditions of drought can also favor *A. flavus* over other fungi due to its capacity to thrive on low water activity substrates. The fungus can grow at an aW as low as −35 Megapascals (MPa). Interestingly, the optimal temperature is 25 to 30°C for the processing of aflatoxin. The plant is also likely to be predisposed to increased infection by temperature and drought stress, but little is known about the mechanisms [1].

The effects of temperature on cottonseed aflatoxin contamination appear more nuanced and poorly understood [5]. While cottonseed aflatoxin contamination is rarely a problem in the southern United States, western-grown cotton can be a serious problem. High night temperatures are important, they have argued. Higher aflatoxin levels in almonds have also been related to high day and night temperatures [15].

Source of Inoculum for *A. flavus* is the soil, but there is no known prevailing survival structure. In the southern United States in society and in cornfields, the fungus produces sclerotia; in the Midwest, however, sclerotia has not been identified. The fungus is likely to survive as mycelium and, to some degree, as sclerotia and conidia [5]. The temperature and humidity of the soil significantly affect the amount of conidia in the soil and air [14].

4. Types of aflatoxins

While aflatoxins are of global concern, in developing countries located in the tropical and sub-tropical regions, their negative effect on health, the economy and

social life is greater. Agricultural products from SSA countries, e.g. Uganda, Gambia Tanzania, Kenya, and SEA countries, e.g. Thailand, China, Indonesia, Vietnam, have historically been associated with the highest incidence of aflatoxin, which has been associated with the highest incidence of hepatocellular carcinoma and the frequency of episodes of acute aflatoxicosis in the region [16]. In fact, these regions were the primary destination for scientists to perform epidemiological studies on the relationship between dietary exposure to aflatoxins and liver cancer, which was a major contributor to the production of aflatoxins as an etiological factor in human disease. Among more than 18 different forms and metabolites currently recognized, four major aflatoxin types [aflatoxin B1, aflatoxin B2 (AFB1), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2)] are the best known and the most studied [17].

The production of aflatoxins has been reported in members of three sections of *Aspergillus* genus; section Flavi (B- and G-type aflatoxins), section Ochraceorosei (aflatoxins B1 and B2), and section Nidulantes (formerly *Emerella* genus; aflatoxin B1) [18, 19]. However, the most widespread and potent aflatoxin-producing moulds are species of Section Flavi, with *A. flavus* and *A. parasiticus*. Due to their widespread distribution in the agricultural environment and their versatility to grow and produce aflatoxins under different ecological conditions, *A. parasiticus* is the most commonly found in agricultural products [17]. A recent polyphase-based classification revealed that 18 of the 33 species in the Flavi segment are aflatoxigenic and each of the 16 species is capable of producing four major aflatoxins (AFB1, AFB2, AFG1 and AFG2), while the other two species produce either AFB1 alone (*A. togoensis*) or AFB1 and AFB2 respectively (*A. pseudotamarii*) [20] (Table 1). The latter writers observed that *A. flavus* strains of Korean origin generate G aflatoxins exclusively, while strains of African origin generate B aflatoxins [21]. Contrary to the prevalent view that this species exclusively generates B aflatoxins, strains of Korean origin generate G aflatoxins [21]. Currently, the production of G aflatoxin by *A. flavus* was mentioned when these aflatoxins were first identified [20, 22, 23].

But when G-aflatoxin-producing strains NRRL 2999, 3000, and 3145 were originally classified as *A. flavus* a dispute was raised, re-classified as *A. parasiticus* [19]. Subsequently, Wicklow and Shotwell confirmed the production of aflatoxins G and B by other *A. flavus* strains; NRRL strains 3357, 6412, 6554, 6555, and 13003. However, *A. flavus* inability to produce G aflatoxins was later confirmed and validated by genetic research connecting indel genes to aflatoxins. (short insertions or deletions) mutations in the cyp A/nor B region in *A. flavus* to the impairment of the expression of genes coding for P450 monooxygenase enzyme required for the biosynthesis of G aflatoxins [24, 25]. It has been argued, however, that this mutation does not occur in all strains, and some *A. flavus*. Depending on the morphotype (S or L) and phylogenetic group (I or II) to which they belong, the strains may still produce B or G aflatoxins. The morphotypes are described by the strain-formed sclerotia size; 'S' for small sclerotia (diameter < 400 µ) and 'L' for large sclerotia (diameter > 400 µ). In this regard, it was revealed that phylogenetic group I contains both S- and L-morphotype strains that only produce B aflatoxins, while group II only contains S-morphotype strains that produce aflatoxins G and B [26]. However, it was later shown that, irrespective of the morphotype, the phylogenetic group I strains develop both B and G aflatoxins, and that the phylogenetic group II is not limited to the S-morphotype strains, but also includes the 'L' morphotype strains [27, 28]. In addition, some S-strains (SBG) have been shown to produce both B and G aflatoxins, while others (SB) only produce B aflatoxins [29]. Latest studies in taxonomy using a blend of advanced analytical methods have verified that *A. flavus* can indeed produce B and G aflatoxins regardless of the morphotype [20, 28]. However, it is well known that S-morphotype strains are more aflatoxigenic than their counterparts of the L-morphotype and accumulate greater quantities of aflatoxins regardless of the type of aflatoxin [28, 29]. This has been clarified by the fact

Aflatoxin	Source	Frequently Contaminated Products
Difurocoumarocyclopentenone		
Aflatoxin B1	Section Flavi: <i>A. flavus</i> , <i>A. togoensis</i> , <i>A. pseudotamarii</i> , <i>A. austwickii</i> , <i>A. aflatoxiformans</i> , <i>A. arachidicola</i> , <i>A. cerealis</i> , <i>A. mottae</i> , <i>A. minisclerotigenes</i> , <i>A. luteovirescens</i> (formerly <i>A. bombycis</i>), <i>A. novoparasiticus</i> , <i>A. parasiticus</i> , <i>A. nomius</i> , <i>A. pipericola</i> , <i>A. pseudonomius</i> , <i>A. pseudocaelatus</i> , <i>A. transmontanensis</i> , <i>A. sergii</i> , Section <i>Ochraceorosei</i> : <i>A. ochraceoroseus</i> , <i>A. rambellii</i> Section <i>Nidulantes</i> : <i>A. miraensis</i> , <i>A. astellatus</i> <i>A. venezuelensis</i> , <i>A. olivicola</i>	Cereals (like, rice, sorghum, wheat, barely, maize), oil seeds (like., cotton seeds, rape seeds, seeds of sunflower), seeds of nuts (like, pistachio, groundnut, peanuts), spices (like, black and red pepper, turmeric, allspices, ginger), dairy products, meats, dried fruits, fruit juices, eggs, foods derived from these products
Aflatoxin B2	Section Flavi: <i>A. flavus</i> , <i>A. aflatoxiformans</i> , <i>A. pseudotamarii</i> , <i>A. cerealis</i> , <i>A. austwickii</i> , <i>A. minisclerotigenes</i> , <i>A. arachidicola</i> , <i>A. luteovirescens</i> , <i>A. mottae</i> , <i>A. novoparasiticus</i> , <i>A. nomius</i> , <i>A. pipericola</i> , <i>A. parasiticus</i> , <i>A. pseudonomius</i> , <i>A. pseudocaelatus</i> , <i>A. transmontanensis</i> , <i>A. sergii</i> Section <i>Ochraceorosei</i> : <i>A. ochraceoroseus</i> and <i>A. rambellii</i>	Cereals (like, rice, sorghum, barely, wheat, corn,), seeds oil (like, sunflower seed, oilseed rape cotton seed,), nuts (like, groundnut, pistachio, peanuts), Spices (like, black and red pepper, ginger, turmeric), milk products, meats, dried fruit, eggs, fruit juices, and foodstuffs derived from such products.
Aflatoxin B2a	Aflatoxin B1 hydroxylated metabolite obtained by water addition to the terminal furan double bond under acidic conditions in the liver, stomach or soil (no evidence of the involvement of particular enzymes) produced naturally by <i>A. Parasiticus A. flavus</i> .	NA
Aflatoxin M1	Hepatic microsomal mixed-function oxidase (MFO) system (mainly cytochrome) hydroxylated aflatoxin B1 metabolite in mammalian liver Formed in vitro by liver homogenates from aflatoxin B1 produced naturally by <i>A. Parasiticus A. flavus</i> .	Milk (human milk included) and dairy products Meat products (liver, kidney) Groundnut and corn moulds
Aflatoxin M2	Hydroxylated B2 metabolite by mammalian hepatic microsomal MFO produced naturally by <i>A. parasiticus</i>	Idem as aflatoxin M1
Aflatoxin M2a	Hydration of the dilute acid terminal furan ring of aflatoxin M1 to yield the hemiacetal derivative Homogenates in the liver in vitro	dairy products and Milk
Aflatoxin P1	Demethylated aflatoxin B1 metabolite by O-demethylase-catalyzed liver microsomal oxidase	Dairy products, Mainly excreted in the humans urine and urine animals.
Aflatoxin Q1	Hydroxylated metabolite of aflatoxin B1 by microsomal enzymes in higher vertebrate and poultry liver (main monkey metabolite of aflatoxin B1)	Assumed to be in edible parts of bovine fed on aflatoxin B1-contaminated feed
Aflatoxin Q2a	Acid hydration of aflatoxin Q1	NA
Aflatoxicol (R0)	In vitro biotransformation of aflatoxin B1 by a soluble cytoplasm reductase system in fish, rats and human liver preparations In vitro biotransformation of aflatoxin B1 in fish, rodents, and human liver preparations by a soluble cytoplasm reductase system A naturally occurring hybrid of <i>A. parasiticus</i> and <i>A. flavus</i> .	Predominantly avian goods (primary metabolite in B1-contaminated feed fed to avian species). Dairy products Does not accumulate in edible parts of aflatoxin B1-infected bovine and pig feed.

Aflatoxin	Source	Frequently Contaminated Products
Aflatoxicol M1	Reduced metabolites of liver-catalyzed aflatoxin B1, aflatoxin R0 or aflatoxin M1 by soluble NADPH-dependent reductases	Dairy products and milk
Aflatoxicol H1	Reduced metabolites of soluble NADPH-dependent reductases catalyzed by aflatoxin B1 and aflatoxin Q1 in the liver	Dairy products and milk
Difurocoumarolactone		
Aflatoxin G1	<i>A. aflatoxiformans</i> , <i>A. flavus</i> , <i>A. cerealis</i> , <i>A. austwickii</i> , <i>A. minisclerotigenes</i> , <i>A. arachidicola</i> , <i>A. luteovirescens</i> , <i>A. mottae</i> , <i>A. novoparasiticus</i> , <i>A. nomius</i> , <i>A. pipericola</i> , <i>A. parasiticus</i> , <i>A. pseudonomius</i> , <i>A. pseudocaelatus</i> , <i>A. transmontanensis</i> , <i>A. sergii</i> .	Cereals (like, rice, sorghum, wheat, barely, maize), oily seeds (like, cotton seeds, rape seeds, sunflower seeds), nuts (like, peanuts, groundnuts, pistachio nuts), spices (like, ginger, black and red pepper, turmeric), milk products, meats, dried fruits, fruit juices, poultry, and feed and foods extracted from such products.
Aflatoxin G2	<i>A. flavus</i> ¹ , <i>A. austwickii</i> , <i>A. aflatoxiformans</i> , <i>A. arachidicola</i> , <i>A. cerealis</i> , <i>A. mottae</i> , <i>A. minisclerotigenes</i> , <i>A. nomius</i> , <i>A. luteovirescens</i> , <i>A. transmontanensis</i> , <i>A. parasiticus</i> , <i>A. novoparasiticus</i> , <i>A. pseudocaelatus</i> , <i>A. pipericola</i> , <i>A. sergii</i> , <i>A. pseudonomius</i>	Same as aflatoxin G1
Aflatoxin G2a	A hydroxylated aflatoxin G1 metabolite obtained by catalytic addition of water to the terminal furan double bond in the presence of acidic conditions in the liver, intestine, or soil (no evidence of unique enzyme involvement). Manufactured naturally by <i>A. flavus</i>	NA
Aflatoxin GM1	MFO produces a hydroxylated metabolite of aflatoxin G1 in the liver of mammals. <i>A. parasiticus</i> fed aspertoxin as a precursor produced it in vitro. <i>A. flavus</i> creates it naturally.	Dairy products and milk
Aflatoxin GM2	Hydroxylated mammalian liver derivative of aflatoxin G2 by MFO In vitro, developed by <i>A. Dihydro-O-methylsterigmatocystin-parasiticus</i> (DHOMST)	Dairy products and milk
Aflatoxin GM2a	Metabolite of aflatoxin GM1 in the mammalian liver Hydration of the dilute acid terminal furan ring of aflatoxin M1 to generate hemicetal in vitro in liver homogenates	Dairy products and milk
Parasiticol (aflatoxin B3)	An aflatoxin G1 metabolite from biodegradation (hydrolysis and decarboxylation reactions) in <i>Rhizopus stolonifer</i> , <i>A. flavus</i> , <i>Rhizopus oryzae</i> , <i>Rhizopus arrhizus</i> ,	such as aflatoxins G1 and B1
Others		
Parasiticol (aflatoxin B3)	An aflatoxin G1 metabolite from biodegradation (hydrolysis and decarboxylation reactions)in <i>Rhizopus stolonifer</i> , <i>A. flavus</i> , <i>Rhizopus oryzae</i> <i>Rhizopus arrhizus</i> , produced naturally by <i>A. flavus</i> , <i>A. parasiticus</i> , <i>A. nomius</i> , <i>A. novoparasiticus</i> , <i>A. mottae</i> .	Aflatoxin B1 and G1 are the same

Aflatoxin	Source	Frequently Contaminated Products
Aspertoxin b	<i>A. parasiticus</i> and <i>A. flavus</i>	Mainly plant products that are vulnerable to contamination with <i>A. parasiticus</i> and <i>A. flavus</i> ; Food products of animal origin are not considered to be important.
^a It is not a standard G-type producer of aflatoxins, but some strains have been reported to produce aflatoxins in addition to B1 and B2. [20].		
^b Typically regarded as an <i>A. flavus</i> produced sperate mycotoxin. Because of structural variations between aflatoxins and the difurocoumarin structure that characterizes them. Abbreviations: NA: Not available.		

Table 1.
Origins of aflatoxins and the products most exposed to contamination.

that aflatoxin production increases as the size of sclerotia decreases during its development [20, 30]. Indeed, in the low-elevation regions in Kenya where the S-morphotype is predominating (>90%), the concentration of aflatoxin B1 in maize was reported to exceed 1000 µg/kg [26, 31]. This was practically illustrated by the higher incidence of deadly acute aflatoxicosis in these regions compared with those where the S-morphotype strains are less common [32].

5. Physical, chemical, and toxicological properties of aflatoxins

More than 18 different forms of aflatoxin are currently known to occur naturally or as a result of feed and food carryover phenomena (Table 1). There are about 13 forms of aflatoxins that are naturally produced by toxic fungi, some of which can be metabolized to produce toxicity-retaining derivatives by humans, animals, or other microorganisms, Compared to the parent molecules, but typically with a lower potency. AFB1, AFB2, AFG1, and AFG2, especially AFB1. has high incidence and toxicities, they are of the greatest concern to the economy and public health, Aflatoxin M1 (AFM1), on the other hand, is of particular concern for the safety of dairy products because it is commonly found in the milk of lactating animals feeding on aflatoxin B1-contaminated feed, in addition to its high toxicity and possible carcinogenicity in humans [33, 34]. Other aflatoxins, however, should not be underestimated because of their inherent toxicity, which may not be negligible, or because the most active AFB1 can readily be inverted. They may also be intermediates for the more toxic mycotoxin biosynthesis [35, 36]. The physicochemical and toxicological properties of major aflatoxins are summarized in Table 2.

6. Structural diversity of aflatoxins

Structurally, aflatoxins are difuranocournarins/difurocoumarins synthesized through the polyketide pathway and consist of a coumarin nucleus (Figure 1A, B, center green) to which one side of the difuran moiety (Figure 1A, left blue) and one side of the pentene ring (Figure 1A, left red) or the other side of the six-sided lactone ring (Figure 1A, left blue) are linked (Figure 1B, red on the right). On this basis, aflatoxins fall into two main groups: (i) difurocoumarocyclopentenones comprised typically of aflatoxin B series and derivatives (Table 1 and Figure 1A), and (ii) difurocoumarolactones with the aflatoxin G series as the main representatives, typically including AFG1, AFG2, AFGM1, AFGM2, and AFG2a

Aflatoxin	MW (g/mol)	Formula	Melting Point (°C) ^a	Toxicity			Adverse Health Effects ^b
				LD50 (mg/kg bw)	Test Organism	Route	
Aflatoxin B1	312.063	C17H12O6	268.5	0.24–60 3.0	Various human chick embryo and species of animals and	In vitro experiments of injection in chick embryo, Oral intraperitoneal	Genotoxicity, carcinogenicity, hepatotoxicity, teratogenicity, immuno- toxicity
Aflatoxin B2	314.079	C17H14O6	286–289	1.7	Duck	Orally	Carcinogenicity, hepatotoxicity, Week mutagenicity
Aflatoxin B2a	330.074	C17H14O7	240	>400 µg showed a weak toxicity	Ducklings	Orally	Low toxicity (200-fold less than B1)
Aflatoxin M1	328.058	C17H12O7	297–299	0.32 1.5	Duck Rat	Unreported Oral	Carcinogenicity, nephrotoxicity, Hepatotoxicity
Aflatoxin G2	330.074	C17H14O7	237–240 226–229	2.5 Weekly mutagenic	Duckling S. typhimurium	test of oral Ames'	Low toxicity, no evidence in animals for carcinogenicity
Aflatoxin G2a c	346.069	C17H14O8	243.13 (Predicted)	NA	NA	NA	Low toxicity to inactive (a detoxified form of G1)

Data compiled from PubChem of the National Center for Biotechnology Information (Pubchem. Explore Chemistry. 2020 <https://pubchem.ncbi.nlm.nih.gov>) and ChemSpider of the Royal Society of Chemistry (Chemspider. Search and Share Chemistry. 2020. <http://www.chemspider.com>) databases, unless references are indicated beside the data.

^aDetails collected on the ChemSpider website (<http://www.chemspider.com>) The IARC claimed in the most recent classification of mycotoxins that 'sufficient evidence' exists for the carcinogenicity of aflatoxins B1, G1 and M1 in laboratory animals, unless indicated by an imbedded citation.

^bBut there is "limited evidence" or "insufficient evidence" for the carcinogenicity of aflatoxin B2 and G2 respectively, respectively, in laboratory animals.; However, in the light of mechanistic studies showing the ability of major aflatoxins (B1, G1, B2, G2, M1) as a first step in genotoxicity to form DNA adducts, They were listed as carcinogens in group 1 [IARC (International Organization for Cancer Research.; c Salmonella typhimurium-induced mutagenicity is <1 percent of aflatoxin B1 taken as a guide [37]. Abbreviations: NA: Not available.

Table 2.
Key properties of aflatoxins and their metabolites.

(Table 1 and Figure 1B). Parasiticol (also designated as aflatoxin B3) despite the absence of the characteristic six-membered lactone ring, it is generally categorized as a member of the latter group (Figure 1C, right). There is also a doubt as to whether or not aspertoxin is an aflatoxin that is not linked to members of any of the difurocoumarin groups due to its bifuroxanthone structure (Figure 1C, left). This mycotoxin, which is associated with sterigmatocystin structurally (an intermediate metabolite of aflatoxins B1 and G1) [38] A precursor of aflatoxin GM1 may also be [39], Which may explain why it is regarded by some writers as a member of the category of difurocoumarolactones. Aspertoxin has earned the

(C) Structure of aflatoxins B1, B2, G1, G2 and M1
AflatoxinB₁ Aflatoxin B₂

(D) Other aflatoxins

Figure 1.
Diversity of aflatoxin chemical structures in difurocoumarocyclopentenone (A) and in difurocoumarolactone (B) Structure of aflatoxins B1, B2, G1, G2 and M1 (C). groups. a difuranoxanthane, Aspertoxin,, parasiticol, lacking the lactone ring of its parent aflatoxin G1, are occasionally considered as standalone mycotoxins (D).

least publicity, unlike other aflatoxins, considering its demonstrated toxicity in chicken embryos where it causes malformations, generalized oedema, muscle tone loss, and umbilical vessel haemorrhage leading to death. It should be noted that saturated (AFG2, AFGM2, and AFM2) or hydrated (AFB2a, AFG2a, AFM2a, AFQ2a, AFG2a, AFGM2a) terminal furan ring aflatoxins are the least toxic, suggesting that the C8 = C9 double bond of this furan moiety plays a crucial role in aflatoxin toxicity [40].

7. Preharvest control strategies

To optimize plant output and minimize plant tension, any management activity can reduce aflatoxin contamination [5, 6, 13]. This involves planting adapted varieties, proper fertilization, control of weeds, and irrigation required. In years favorable for disease growth, even the best management methods can not eradicate aflatoxin contamination. It has been shown that previous cropping history affects soil fungus populations [4]. However, the meaning of the initial inoculum has not been established. In addition, *Aspergillus* molds grow from pistachio trees on litter, but it is not known whether infection and aflatoxin contamination can be minimized by practices to minimize this litter. Decreasing damage to the navel orange worm could decrease the contamination of aflatoxin. Cropping history and plant debris potentially play a minor role in aflatoxin contamination relative to plant stress [11, 12].

Breeding projects for all major crops affected by aflatoxin contamination are underway, but no genotypes with sufficient resistance to aflatoxin accumulation are commercially available. Inbred corn lines [6] And peanut genotypes with some resistance to the accumulation of aflatoxin have been reported. There is evidence of tolerance in maize and peanuts to the production of perse aflatoxin. In almonds, breeding for resistance to aflatoxin contamination is also ongoing.

Because chemical control procedures for contaminating mycotoxins are not economically feasible for most grain crops, there is an interest in developing effective biocontrol agents to reduce the contamination of mycotoxins. Recent research shows the potential for a biocontrol agent to minimize cotton, peanuts, aflatoxin contamination [41, 42], and corn. These crops were treated by researchers with nonaflatoxigenic isolates Either *A. flavus* or *A. parasiticus*. The reason for using nonaflatoxigenic isolates of the two fungi is that they are possibly the better biocompetitors, since they occupy the same or similar ecological niche as the aflatoxigenic strains, So far, there is no evidence that the ability to produce aflatoxin confers a competitive advantage to *A. flavus* or *A. parasiticus*.

Bock and Cotty carried out the most rigorous trials of a biocontrol agent for the prevention of aflatoxin contamination (1999). They received a U.S. permit. Environmental Protection Agency (EPA) for the treatment of wheat seed colonized by a naturally occurring nonaflatoxigenic strain (AF36) of *A. flavus* in Arizona cotton fields. This procedure has increased the AF36 population and decreased the cottonseed toxin strain and aflatoxin concentrations.

7.1 Aflatoxins: human and animal health; economic impact

The biologically active secondary metabolites produced by certain strains of *Aspergillus parasiticus* are aflatoxins (*Aspergillus flavus* toxins). These ubiquitous fungi are capable of infecting a large variety of crops that can be infected with this powerful mycotoxin under certain conditions. Acute toxicity, including hepatotoxicity, teratogenicity, immunotoxicity and even death, can result from ingestion of

food or feed that is highly contaminated with aflatoxin. The most abundant and toxic chemical form of Aflatoxin B1 (AFB1) is highly mutagenic and is one of the most active carcinogens ever tested in rats [43], suggesting that chronic exposure to very low levels of aflatoxin is cause for concern.

In humans, hepatotoxicity is correlated with ingestion of aflatoxin. Epidemiological studies have also shown that areas with elevated aflatoxin levels in the world are associated with a high incidence of liver cancer. The prevalence of the hepatitis B virus in these areas makes it difficult to create a clear cause-effect relationship. However, the International Agency for Research on Cancer has, on the basis of the available evidence, designated AFB1 as a possible human carcinogen. The US Food and Drug Administration developed action levels of 20 p.p.b. for food for human consumption (except milk, where the level is 0–5 p.p.b.) and 20–300 p.p.b. for most animal feeds because of this high level of concern regarding aflatoxin [44]. In the world, other countries have set even lower standards of intervention.

From an economic point of view, mycotoxins impact approximately 25 of the world's crops annually [44, 45]. This is equivalent to a direct expense of billions of dollars due to the loss of crops and livestock, plus the secret indirect costs of tracking crop aflatoxin levels and the reduced output of farm animals that eat aflatoxin and other mycotoxins. In the US and in many other regions of the world, the removal of aflatoxin is a critical economic and health issue.

In recent years, aflatoxins have been the subject of multiple reviews covering ecology as a testimony of their significance [46], Incidence [47], identification [48, 49], human health consequences (toxicity, carcinogenicity) [43, 50–52], genetics [53], biosynthesis [54, 55] and substances that interfere with biosynthesis [56], as well as the avoidance of aflatoxin contamination [57–59]; (for general reviews see [44, 60, 61]). Recent efforts in several laboratories have centered on developing an in-depth understanding of the molecular biology of the aflatoxin biosynthetic pathway due to the difficulties of effectively and economically regulating aflatoxin contamination of food and feed by conventional agricultural methods (see below). The purpose of this analysis is to provide current knowledge on the molecular biology of aflatoxin biosynthesis and how this information is used to: (1) extract toxin from the food chain; (2) understand aflatoxin pathway regulation and evolution; (3) to comprehend aflatoxin's biological significance to the fungus that creates it. This analysis is timely because it provides a summary of several major breakthroughs that have resulted from intensive research activity over the past 2 years - knowledge not available in previously published articles on the molecular biology of aflatoxin biosynthesis [62–64]. Since *Aspergilli* contains opportunistic mammalian, insect and plant pathogens, promoting our understanding of gene expression regulation, production and secondary metabolism in this diverse genus may provide important clues to their ecology and biology, leading not only to efficient aflatoxin management, but also to more general means of controlling this whole community of pathogens.

7.2 Biological significance of aflatoxins: a role in fungal development

The size of the cluster of aflatoxins and the striking serving of genes and organization of the cluster clearly imply that aflatoxins play a key role in the fungi' life cycle or survival. Are there any hints as to what that feature might be? The two key origins of inoculum for the survival or dissemination of these filamentous fungi are conidia (asexual spores of *Aspergilli*) and sclerotia (resting/survival structures).

There is no direct correlation between sclerotia development and aflatoxin (if any). Bennett and Horowitz [65] found no association between sclerotia production and aflatoxin production in toxigenic and atoxigenic strains of *A. flavus*. Other

study, on the other hand, has found that the regulation of aflatoxin synthesis in toxigenic strains has an effect on sclerotia growth. (reviewed in [46]). Mutations have been shown to cause cancer in preliminary experiments using molecular biology methods (UV or gene disruption) that result in the accumulation of certain intermediate aflatoxin pathways also result in the inhibition of sclerotic growth. Genetic blocks that remove AFB1 and intermediate synthesis result in improved development of sclerotia. Restoration of function by complementation often restores normal growth of sclerotia. These findings indicate that the synthesis of aflatoxin and fungal growth may be related. The existence and value of such a relation can be revealed by the continuation of these studies [66, 67].

7.3 Aflatoxicosis

The association of aflatoxins with animal diseases has been extensively studied [68]. The relationship of aflatoxins to hepatocellular carcinoma and other human diseases is still being studied, while acute aflatoxicosis is well known in humans. In Africa, the Philippines, and China, multiple epidemiological studies have implicated aflatoxins in the increased occurrence of human gastrointestinal (GI) and hepatic neoplasms. In human liver cell carcinoma, aflatoxin B1 was also involved [69].

7.4 Acute aflatoxicosis

In humans, acute disease due to ingestion of aflatoxin has been manifested as acute hepatitis, typically associated with highly contaminated foodstuffs, in particular corn. Exposure to aflatoxins in selected tissues was acceptable in some cases, and histopathological evidence was convincingly adequate to allow for the diagnosis of aflatoxicosis. Jaundice, low-grade fever, depression, anorexia, and diarrhea are common but nonspecific changes in patients with acute aflatoxicosis, with fatty degenerative changes in the liver apparent upon histopathological examination, such as Centro lobular necrosis and fatty infiltration. In patients with acute, aflatoxin-caused hepatitis in Kenya, tenderness was evident near the liver; ascites can develop. In outbreaks in India, mortality reached 25%. The liver samples collected from patients who died contained detectable aflatoxin B1 levels [70].

The ingestion of aflatoxin-contaminated foods was associated with two human diseases of undefined etiology: kwashiorkor and Reye's syndrome. The seasonal occurrence and distribution of aflatoxin in food has been geographically correlated with Kwashiorkor. Some of the same attributes of kwashiorkor, namely, hypoalbuminemia, fatty liver, and immunosuppression, were present in animals given dietary aflatoxin. Aflatoxins have been found in liver tissue by autopsy in 36 children with kwashiorkor, contributing to the reputation of aflatoxin as the cause of this human disease without any other known etiology. In some patients with kwashiorkor, malnutrition may change the metabolism of dietary aflatoxin, resulting in its detection [71].

However, the etiology of Reye's syndrome is more troublesome. Aflatoxin has been associated with this condition, which includes acute encephalopathy with viscera fat degeneration, since this mycotoxin was detected in patients with Reye's syndrome in Thailand, New Zealand, the former Czechoslovakia and the United States. In addition, aflatoxin B1 in macaque monkeys developed a disease similar to Reye's syndrome in [71].

Nelson, *et al.* [72], however, found no substantial variations in serum and urine between matched controls and patients with Reye's syndrome compared to aflatoxins. Similar differences were noticed concerning the incidence in patients of aflatoxins in tissues and Reye's syndrome. The U.S. cases often seem to lack any

geographical relationship to the exposure to aflatoxin. Again, in some patients, Reye's syndrome, which involves the liver, can alter dietary aflatoxin metabolism [71].

7.5 Chronic aflatoxicosis

The association of this mycotoxin with hepatocellular carcinoma typically suggests chronic aflatoxicosis in humans. Several epidemiological studies have investigated the importance of dietary aflatoxin and other factors associated with this disease in countries or localities with a high incidence of liver cancer. Most of the research, which occurred predominantly prior to 1980, attempted to determine and compare dietary levels of aflatoxin B1 with the existence of hepatocellular carcinoma.

Some earlier studies have been criticized for not understanding the hepatitis B virus exposure of the studied populations (HBV). In relation to the incidence of hepatocellular carcinoma, most post-1980 studies investigated hepatitis B surface antigen (HBsAg) as well as aflatoxin exposure. Most of them considered an aflatoxin effect independent of the prevalence of HBsAg [73]. No aflatoxin effect on liver cancer was observed when all racial, social, and cultural groups were included, but a positive association was found by an independent assessment of the Bantu people.

The consequence is that aflatoxin has been related to unique p53 mutations where codon 249 has a G → T trans-version in the third position. These particular tumor mutations may provide substantial evidence as to their origin. Epidemiological research on the relationship between aflatoxins and human hepatocellular carcinoma greatly benefit from the armament of biomarkers. Epidemiological research on the relationship between aflatoxins and human hepatocellular carcinoma greatly benefit from the armament of biomarkers. The findings showed that human liver cancer is associated with a particular biomarker for aflatoxin and that HBV and aflatoxin B1 interact as risk factors for liver cancer [74].

8. General effects of aflatoxins on health and productivity

Aflatoxins are powerful toxins in the liver. In animals, their effects differ with dosage, period of exposure, species, race, and diet or nutritional status (**Figure 2**). When ingested in large doses, these toxins can be lethal. Sublethal doses cause chronic toxicity, and low levels of chronic exposure in some species can lead to cancer, mainly liver cancer [75]. In general, young animals are more sensitive to the toxic effects of aflatoxin than older animals. Due to their widespread occurrence in many dietary staples, such as peanuts, tree nuts, milk, maize, dried fruits, and their potential as human carcinogens, aflatoxins have created the greatest public health concern.

In the early tests of aflatoxicosis, one of the experimental species used was trout. The LD50 was estimated to be equal to 0.5 to 1.0 mg/kg of crystalline aflatoxins B1 and G1 in the same amounts, and they seem to be very sensitive to the effects of the aflatoxins. Eighty components per billion total dietary aflatoxins produced a very high incidence of hepatomas in the trout. Rainbow trout are very susceptible to hepatogenicity in the early stages of development. Nine months later, immersion of fry or embryo in 0.5 ppm aflatoxin B1 for 0.5 h resulted in a 30 to 40% incidence of hepatocellular carcinoma. Aflatoxicosis epizootics also occur in fish and were possibly the cause of epizootic trout liver cancer that occurred in hatcheries in California from 1939 to 1942. In this outbreak, aflatoxin-contaminated cottonseed meal was implicated as the causative agent.

Aflatoxin fed trout grow hepatic cancer. In suckling piglets, rising and finishing swine, and breeder stock, aflatoxin toxicity has been reported. Decreased weight gain rates, decreased feed conversion performance, toxic hepatitis, nephrosis, and

Figure 2.

Guinea pig livers are given increasing doses of aflatoxin for the same period of time. From left to right, starting with the liver of a guinea pig given no aflatoxin in the upper left corner, and the liver of a guinea pig given the maximum dose of aflatoxin in the lower right corner. With increasing doses of aflatoxin, remember the increasingly pale livers. With increasing doses of aflatoxin, remember the increasingly pale livers. Picture courtesy of John L. Richard, USDA, ARS, Ames, Iowa, National Animal Disease Center; now at Romer Labs, Inc., Missouri, Union.

systemic hemorrhages are clinical and pathological symptoms. Depending on age, diet, concentration, and duration of exposure, the effects of aflatoxin in pigs differ. Swines tend to be immune to dietary aflatoxin levels of up to 300 ppb from weaning to marketing [76].

Acute aflatoxicosis has been comprehensively identified in cattle. Decreased feed intake, dramatic declines in milk production, weight loss, and liver damage are clinical symptoms. However, due to reduced feed quality, immunosuppression, and lower reproductivity, chronic exposure of dairy and beef cattle to naturally occurring aflatoxin levels may have an even greater economic effect. Aflatoxins affect the function of the rumen in vitro and in vivo by reducing the digestion of cellulose, volatile fatty acids and proteolysis [77, 78] showed reduced motility of the rumen in steers given a single aflatoxin dose.

Significant health concerns resulting from prolonged exposure of a dairy herd to aflatoxin-contaminated corn (120 ppb). In addition, breeding performance decreased by 2 percent for a five-month period after exposure, while milk production increased by 28 percent after the diet was removed from aflatoxin-contaminated corn. The birth of smaller and unhealthy calves, diarrhea, acute mastitis, respiratory disorders, prolapsed rectum, hair loss, and reduced feeding intake are other concerns.

The conversion of aflatoxin B1 to the hydroxylated metabolite, aflatoxin M1, which is excreted in milk, is another feature of aflatoxin exposure in dairy cattle. Aflatoxin M1 is present in milk from Holstein cows given aflatoxin B1 for seven days, while aflatoxin M1 is not detected in milk for four days after the end of aflatoxin B1 administration [68]. As a percentage of aflatoxin B1, the excreted quantities of aflatoxin M1 average 1 to 2 percent, but values as high as 6 percent have been recorded at kg of aflatoxin B1 daily intake levels. In the poultry industry, Aflatoxicoses have caused significant economic losses affecting ducklings, broilers, layers, turkeys, and quail. Anorexia, reduced weight gain, decreased egg development, bleeding, embryotoxicity, and increased vulnerability to environmental and microbial stressors are clinical symptoms of intoxication [79].

In chickens given a high level (1.5 ppm) of dietary aflatoxins, histopathologic changes, including fatty liver, necrosis, and bile duct hyperplasia, are observed. Clinical responses include hypoproteinemia; decreased hemoglobin; and decreased

serum triglycerides, phospholipids, and cholesterol in chickens provided half of this dose. Aflatoxins can reduce the activity of several enzymes in broiler chickens that are essential for the digestion of starches, proteins, lipids and nucleic acids. The decreased activity of the enzymes pancreatic amylase, trypsin, lipase, ribonucleic acid (RNA) and DNase could lead to the malabsorption of aflatoxicosis-associated nutrients [80].

Hamilton [81] reported a decrease in egg production to 5% of normal in laying hens given near- LD50 aflatoxin levels in naturally contaminated maize. Egg production and size are decreased by aflatoxin-contaminated feed (up to 10 ppm) ingested by layers for 4 weeks. As a percentage of total egg weight, total yolk and yolk weight decreased, followed by higher yolk and plasma carotenoid concentrations [82].

9. Immunologic effects

While they are mainly referred to as hepatotoxins and hepatocarcinogens, aflatoxins tend to have been implicated in domesticated animal outbreaks of infectious diseases. Salmonellosis, a bacterial infection, and candidiasis, a yeast infection, were related to outbreaks of aflatoxin-induced Turkey X disease in 1960. Following the discovery of high levels of aflatoxins in the regional corn crop in 1977, outbreaks of salmonellosis in swine occurred in the southeastern United States [83]. Several animal species' resistance to bacterial, fungal, and parasitic infections has been shown to be lowered by aflatoxins, according to comprehensive experimental evidence [84].

Table 3 summarizes the general characteristics of aflatoxin immunosuppression. Special care is required to interpret the findings of the aflatoxin and immunity studies, since some used aflatoxin mixtures, while others used purified aflatoxin B1. In this regard, differences were seen between aflatoxin B1 and its metabolites [85].

Data from experimental models generally supports the argument that aflatoxin B1 suppresses the cell-mediated immune response in particular. Several reviews have addressed Aflatoxin-induced immune modulation [86, 87].

Since aflatoxin poses an economic threat to the poultry industry, there has been comprehensive analysis of its effects on avian immunity. As reflected by decreased thymus weight and lower peripheral T lymphocyte numbers in chickens fed aflatoxin B1, cell-mediated responses are especially responsive [88, 89]. Graft versus host response in chickens given 300 ppm of aflatoxin B1 is suppressed [90]. In broiler

Cellular responses	Effects
Macrophage phagocytosis	was reduced.
Cutaneous hypersensitivity	Reduced with a delay
Reduced Lymphoblastogenesis	(response to mitogens)
Graft versus host response	reduced
Humorous factors' impact	
Concentrations of Immunoglobulins (IgA and IgG) in serum	may be reduced
Complementary activity	has declined.
Reduced of Bactericidal activity	of serum

Table 3.
Aflatoxin's effect on immunity.

chicks given 1 ppm of aflatoxin B1 feed, the delayed hypersensitivity response to dinitro-fluorobenzene is decreased [88]. Oral administration of aflatoxin B1 to chicks at 0.1 and 0.5 mg/kg body weight decreases the proliferation of peripheral blood lymphocyte responses to mitogenic T cell concanavalin A (Con A) [91].

Aflatoxin B1 is reliably inhibited by the phagocytic functions of macrophages and of the reticuloendothelial system. In chickens, aflatoxin B1 (0.3 to 1.0 ppm) depresses the percentage of nitroblue tetrazolium positive cells in spleen tissue, suggesting depressed macrophage activity [88]. In rats, oral administration of aflatoxin B1 (0.35 to 0.7 mg/kg bw) depresses both the amount and function of macrophages [92]. In chicks given aflatoxin B1 (0.3 mg/kg feed), the clearance of circulating colloidal carbon is reduced, indicating a decreased phagocytic status of the reticuloendothelial system. In vitro results suggest in vivo phagocytic activity suppression in chickens and rats as well. In rat peritoneal macrophages exposed to aflatoxins in vitro, phagocytosis, intracellular killing of *Candida albicans* and spontaneous superoxide anion (O₂⁻) development are suppressed [93]. For macrophage toxicity, activation of aflatoxin B1 by mixed-function oxidases is apparently needed [94, 95].

Aflatoxins inhibit the activity of the mononuclear phagocyte system of more than one cell type. This decrease in activity appears to be linked to effects on phagocyte cells (**Figure 3**) but, perhaps more importantly, to the serum heat-stable substance needed for phagocyte activity [91].

In many immune reactions, complement, a serum constituent formed by the liver, plays an important role. The deficiency of this operation indicates a reduction in the immunological capacity of an essential part of the host. In pigs given feed containing 500 ppm aflatoxin B1, serum complement activity is decreased; in pigs given 300 ppm aflatoxin B1 in feed and in rabbits given 95 ppm aflatoxin B1 in feed, complement activity is not affected [96]. Aflatoxins decrease the activity of a hemolytic complement in guinea pigs [97] and other species [98].

Aflatoxin B1 modulatory effects on humoral immunity are less clear than those on cell-mediated immunity, particularly in cross-species comparisons. There is no substantial difference in antibody titers in swine fed up to 500 ppm aflatoxin B1 and inoculated with *Erysipelothrix rhusiopathiae* bacteria compared to inoculated swine fed uncontaminated feed [99]. Aflatoxin (0.045 mg/kg bw) administered orally is not impaired by the ability of guinea pigs to develop *Brucella abortus* antibodies [100]. The antibody-forming response to sheep red blood cells (SR-BCs), a T cell-dependent

Figure 3.
Decreased macrophage phagocytosis with increased doses of rabbit aflatoxin [84].

antigen, is unchanged in rabbits given aflatoxin (approx. 24 ppm feed) relative to animals given aflatoxin B1-free diets [96].

To sum up, aflatoxin B1 suppresses immunity that is mediated by cells to a greater degree than humoral immunity. Aflatoxin B1 also inhibits some aspects of innate immunity, especially phagocytic responses. It is clear that aflatoxins are immunomodulatory in the low ppm range.

10. Hematopoietic effects of aflatoxins

The presence of aflatoxin-producing fungi has been associated with hemorrhagic anemia syndrome, caused by the ingestion of poultry moldy feed. Large hemorrhagic lesions in the main organs and musculature are typical of the condition. In broiler chicks that ingested aflatoxin, a suspected hemolytic anemia with bone marrow hyperplasia and a rise in bone marrow nucleic acid occurred. Hemoglobin, packed cell length, and erythrocytes circulating decreased significantly [101]. In broiler chicks fed aflatoxins for 3 weeks, Aflatoxins have triggered substantial period increases in whole blood clotting, recalcification, and pro-thrombin [102]. However, aflatoxins in the feed (20 ppm) of mature broilers induced only mild anemia for 4 weeks without raising erythrocyte fragility [103]. Lanza, *et al.* [104] provided evidence indicating that a secondary result of extreme hypoproteinemia was the production of anemia in aflatoxin-treated animals. As mentioned in the following section, these effects can be secondary to primary liver damage. Exposure to aflatoxins may also affect haemostasis in embryo development. Hatched chicks had substantially reduced cell counts, hematocrite, and hemoglobin concentrations following embryonic exposure to aflatoxin B1 [105]. However, no variations were observed between erythrocytes in the treated and control groups.

10.1 Aflatoxin biosynthetic pathway

A. flavus, *A. nomius* and *A. parasiticus* are the only fungal species known to produce aflatoxins [46]. Nevertheless, as many as 20 various Aspergilli, including *A. Sterigmatocystin* (ST) [106], a highly toxic intermediate in the biosynthetic pathway of AFBI, is produced by *nidulans* and species of *Bipolaris*, *Chaetomium*, *Farrowia* and *Monocillium*. Even though the AFBI biosynthetic pathway in *A. flavus* and *A. parasiticus* and the ST biosynthetic pathway in *A. nidulans* are believed to be similar, In order to recognize any key differences that may occur in biosynthesis or regulation and to shed light on the evolution and acquisition of the Aspergilli pathway and other genera, cooperative studies using all three species are being pursued.

Primary contributions in elucidating the biochemistry and molecular biology of the aflatoxin pathway have been the isolation and characterization of many mutants blocked in aflatoxin biosynthesis. Our current understanding of the order and mechanism of reactions in this complex biosynthetic pathway, which includes approximately 17 different enzymes, was developed by bioconversion experiments using these aflatoxin-blocked mutants, metabolic inhibitors and stable radio-isotope or isotopelabelled precursors or pathway intermediates [55].

Polymerization of acetate and nine malonate units (with CO loss) by polyketide synthetase (PKS) in a manner analogous to fatty acid biosynthesis is proposed as the initial step in the generation of the polyketide backbone of AFBI [54, 55]. The synthesis of a 6-carbon hexanoate starter unit by a fatty acid synthase (FAS), which is then expanded by a PKS (without further ketoreduction) to produce a 20-carbon decaketide, noranthrone, is an alternative and maybe more probable hypothesis

[107]. Noranthrone is then oxidized by a hypothesized oxidase into anthraquinone norsolorinic acid (NA) in either scheme. The rest of the proposed pathway is summarized in **Figure 4** [54, 55, 57, 108]. Versicolorin A (VA) is important because it is the first molecule containing a double bond in the difuran moiety at the 2.3 position in the AFB₁ pathway. This double bond is the target of microsomal cytochrome P450 enzymes that produce a highly reactive epoxide resulting in DNA and protein activation and adduct formation. (reviewed in [52]). In contrast, aflatoxin B₂ (AFB₂), which lacks this double bond, is hundreds of times less carcinogenic [52].

It is stated that many enzymes involved in the aflatoxin pathway have been purified for homogeneity. Two distinct O-methyltransferases include them [109, 110].

NA reductase (or probably two different enzymes) is involved in the conversion of ST to O-methylsterigmatocystin [111, 112]. NA to averantin (AVN), which is involved in the conversion of versicon to versicolorin B, is transformed by cyclase reversible conversion [107, 113].

NA reductase, which is involved in the conversion of ST to O-methylsterigmatocystin (or possibly two separate enzymes) [111, 112]. Involved in the cyclase reversible conversion of NA to averantin (AVN), which is involved in the conversion of versicon to versicolorin B [107, 113]. The reaction between versiconal hemiacetal acetate and versiconol acetate is catalyzed by two versiconal hemiacetal acetate reductases (VHA reductase I and II; possibly isozymes). (VHA reductase I and II; probably isozymes) [114]. Such purified enzymes have provided essential tools for gene cloning.

10.2 Gene cloning strategied/structure and function of cloned genes

The secret to understanding the molecular biology of the pathway is the cloning of genes involved in aflatoxin biosynthesis. Cloned genes are useful probes to elucidate the molecular mechanisms that govern these genes' timing and level of expression. In the cloning of aflatoxin biosynthetic genes, two separate methods have been successfully used.

The isolation of genes encoding three enzymes in the pathway was achieved using a genetic complementation method, *nor-1*, *ver-1* and *uvm8*, and aflR, a

Figure 4.
Aflatoxin B₁ and B₂ biosynthetic pathway.

regulatory gene. Transformation systems for *A. parasiticus* were established to incorporate DNA into the fungus [115, 116] and *A. flavus* [117].

The *nor-1* (*nar-1* was developed for NA-related problems [118] and *ver-1* genes [66] By complementing aflatoxin-resistant mutants B62, they were cloned (an *niaD* mutant derived from *A. parasiticus* ATCC 24690, *nor-7*, *bm-7*, [119] and CS10 (an *niaD* mutant derived from *A. parasiticus* ATCC 36537, *ver-7*, *wh-7*, NA (brick-red) and VA (yellow) are two brightly colored pathway intermediates. The addition of a cosmid DNA library derived from genomic DNA from a wild type aflatoxin-producing *A. parasiticus* strain produced complementation (SU-1). By hybridizing *ver-1* to an *A. nidulans* genomic DNA library, the functionally homologous *ver-1* gene of *A. nidulans* was isolated. *Ver-1* and *ver-1* gene products have almost identical predicted amino acid sequences [120], demonstrating the high degree of similarity among these *Aspergillus* species' aflatoxin biosynthetic genes The predicted amino acid sequences of *nor-1*, *ver-1*, and *verA* show significant identity and contain a NAD(P)H binding motif near the amino terminus (*ver-1/verA* 33%; *nor-1* 23%) Several NADPH and NADH-dependent reductase/dehydrogenase enzymes are involved. A short-chain alcohol dehydrogenase motif occurs in each sequence [121].

Recombination inactivation (gene disruption) was performed in the toxigenic strains of *A. parasiticus* to confirm the function of these genes in aflatoxin biosynthesis (*nor-1*, [121]; *ver-1*, [122]) and *A. nidulans* *verA*, [120]. *A. nidulans* lost measurable ST and accumulated VA after the *verA* gene was disrupted, confirming its role in the conversion of VA to ST. Likewise, As a result of the disruption of *ver-1*, the aflatoxin pathway was blocked, resulting in VA accumulation. The disruption of *nor-1* resulted in a significant accumulation of NA. The ability of disrupted strains to generate low levels of aflatoxin was maintained, suggesting that the aflatoxin pathway has one or more alternative routes (or enzymic activities) for synthesizing averufin from NA [108]. In *E. coli*, a *nor-1*/maltose-binding protein (MBP) fusion protein was recently expressed [123]. Crude *E. coli* cell extracts containing the fusion protein transformed NA to AVN only when NADPH was present, supporting the prediction that *nor-1* encodes a NA-to-AVN reductase.

Complementation of an aflatoxin-blocking mutant, *avm8*, derived from UV mutagenesis of *A. parasiticus* mutant strain B62, was used to clone the gene *avm8* (*niaD*, *brn-1*, *nor-1*) [124]. Metabolite conversion studies showed that *avm8* has two AFB1 pathway blocks, one at *nor-1* and the other prior to *nor-1*. The P-subunit of FASs (FAS1) from *Saccharomyces cerevisiae* and *Yarrowia lipobtica* had a high degree of similarity (67%) and identity (48%) to the predicted peptide sequence of extensive regions of the *avm8* gene product [125]. As a result, it was proposed that *avm8* encodes a FAS activity required for the synthesis of the proposed hexanoate starter. Due to the complete reduction of two keto groups in hexanoate to hydrocarbon, a FAS involved in its synthesis would be expected to contain three main enzyme activities, ketoreductase, dehydratase, and enoyl reductase, in addition to a P-ketoacyl- synthase. Limited nucleotide sequencing revealed an enoyl reductase domain in *zvm8* (based on similarity to *S. cerevisiae* FAS1), which is not needed for aflatoxin PKS in theory.

In *A. parasiticus* the *zvm8* gene has been disrupted. No detectable AFB1 or pathway intermediates consistent with a functional role in polyketide backbone synthesis were accumulated by the disrupted strains.

A second approach to gene isolation, reverse genetics, was based on the enzymes of the purified pathway described above. Where purification was necessary, the generation of enzyme antibodies and the isolation of the gene from the library of cDNA expression in *E. coli*, it can be done.

The *omt-1* gene from *A. flavus*, which encodes the O-methyltransferase activity responsible for the conversion of ST to O-methylsterigmatocystin, was cloned

using this process [126]. Antibodies raised against the native methyltransferase were used to screen an *A. parasitica* scDNA library made from RNA from an aflatoxin-induced culture. A motif found in other S-adenosylmethionine-dependent methyl-transferases was found in the predicted amino acid sequence derived from the cloned cDNA. Both the purified native protein and a fusion protein produced in *E. coli* from the cDNA showed substrate-specific methyltransferase activity. Omt-I is the only pathway gene that has been cloned using a reverse genetics approach to date. However, cloning several other genes encoding purified pathway enzymes should be possible using this process.

Feng, *et al.* [127] have used another molecular genetic method for gene cloning, subtractive hybridization, to isolate many genes whose expression pattern coincides with the development of aflatoxin in *A. parasiticus*. As in the two previous approaches, this approach is not dependent on precise knowledge of the role of the gene product and can therefore be beneficial when the timing of gene expression induction is understood, but pure enzymes or blocked pathway mutants are not usable. To date, no clear identification has been recorded of the activities of genes isolated by this process.

10.3 Regulation of aflatoxin gene expression

Polyketides are a wide and diverse family of secondary metabolites that are mainly formed by actino-mycetides, fungi, and higher plants, but are also synthesized in animals, including other species. The regulation of synthesis of these secondary metabolites is different from the regulation of primary metabolism, since secondary metabolism relies on energy, enzyme cofactors and building blocks of primary metabolism (i.e. acetate). Luchese and Harrigan [128] have reviewed the impact of primary metabolism on aflatoxin biosynthesis.

during idiophase, *A. parasiticus* and *A. flavus* produce aflatoxins. In culture, When it has slowed or stopped exponential growth and secondary metabolites are produced. Buchanan, *et al.* [129] demonstrated, using transcription and translation inhibitors, that de novo protein synthesis is necessary for the development of aflatoxin. Other studies have shown that the activity of at least four of the enzymes involved in the pathway is not detected before idiophase is formed [130–132]. During fermentation of batches of *A. parasiticus* During the transition between active growth and stationary phase, the *ver-1* and *nor-1* RNA transcripts accumulated most quickly [133]. A similar pattern was shown to follow the accumulation of RNA transcripts from the *aflR* gene, proposed to encode a key regulatory protein (see later) [134]. Coordinate transcription of these genes indicated that they are partly regulated at the transcription level, possibly by a common regulatory factor. A gene suggested to encode one significant regulatory factor, the *afE-2* gene, was cloned using a wildtype genomic DNA library from *A. flavus* by complementing an aflatoxin-nonproducing mutant [134]. *Afl-2* is involved in aflatoxin biosynthesis before NA, according to genetic evidence and metabolite feeding studies. *A. flavus* mutant strain, missing *afE-2* was unable to convert a number of exogenously supplied pathway intermediates to aflatoxin, suggesting the absence of main pathway enzymes. Complementation of mutant strains with the wild-type *afl-2* gene restored expression of several aflatoxin pathway enzyme activities in crude cell extracts, which is a requirement for a trans-acting regulatory factor encoding gene.

In *A. parasiticus* After transformation, *apa-2* was cloned with a single cosmid clone (NorA) containing both the aflatoxin genes *nor-1* and *ver-1* on the basis of overproduction of aflatoxin pathway intermediates [118]. The *apa-2* was replaced by an A. The *Favas afE-2* mutant strain shows that *apa-2* and *4-2* are functional homologues for the development of aflatoxin. The genetic data was confirmed by

nucleotide sequence analysis, which revealed that these genes share more than 95% nucleotide sequence identity [118]. In the predicted amino acid sequences of *apa-2* and *aj-2*, a cysteine-rich zinc cluster motif, Cys-Xaa2-Cys-Xaab-Cys-XaaG-Cys-Xaa2-Cys-Xaa6-Cys, was discovered [118, 135]. This zinc cluster motif is found in a family of fungal transcriptional activators, the most well-studied of which is *S. cerevisiae* GAL4. GAL4 controls the expression of genes involved in galactose utilization in yeast. The homologues *apa-2* and *a/-2* have been renamed *aflR* because the overwhelming evidence indicates that they are positive regulators of aflatoxin synthesis [134].

10.4 The gene cluster for aflatoxin

Since there is no known perfect (sexual) stage for *A. parasiticus* and *A. flavus*, Classical genetic experiments using the parasexual cycle have been performed. Parasexual study of eight blocked mutants with aflatoxin in *A. flavus* reported that they were all genetically related to linkage group VIII markers [136]. However, attempts to demonstrate parasexual studies to connect *nor-1* and *ver-1* were confounding due to problems inherent in analyzing segregant ploid levels and the non-random segregation of certain genes during haploidization (reviewed by [53]).

Many of the genes involved in aflatoxin biosynthesis in *A. parasiticus* and *A. flavus* are physically clustered on one chromosome, according to molecular genetic studies. One cosmid, Nor A, was discovered during the cloning and characterization of the *nor-1* and *ver-1* genes from *A. parasiticus*, and it hybridized to probes of both genes. Physical mapping of the corresponding area in the fungal genome in *A. parasiticus* later supported this preliminary evidence for linkage [67]. Later, the genes *aftR*, *uvm8*, and *omt-1* were found to belong to this cluster, as well as a cluster of aflatoxin genes in *A. flavus* [67, 137]. The physical order of genes in the cluster tends to be identical to the order of enzyme reactions catalyzed by their gene products, which is an interesting finding. It's unclear if this feature has any practical meaning.

Since 17 enzyme activities are thought to be needed for complete aflatoxin synthesis, it was hypothesized that several other pathway enzymes were encoded by the cosmid Nor A (and the corresponding region in *A. flavus*). In order to determine the size, Several more pathway enzymes were encoded. A transcriptional map of the genomic DNA insertion in cosmid Nor A was completed to determine the size, location and pattern of expression of other genes in the cluster. This cluster was localized to twelve separate RNA transcripts. They were tentatively known as aflatoxin genes because the timing of their expression was close to that observed for *nor-1* and *ver-1*. In a VA-accumulating mutation, CS10, and OMST development in an OMST-accumulating strain, genetic disruption of a gene (encoding a 7–0 kb transcript) located adjacent to *nor-1* in the gene cluster blocked VA production. Predicted amino acid sequence data from an extensive region of this gene showed a high degree of identity to the β - ketoacyl-synthase (Identity 67%.) and the acyltransferase (Identity 32%) functional domains [67]. In *A. nidulans*, the V A gene product encodes a PKS involved in conidial pigment development. P.-K. Chang and others (personal communication) found high homology between the acyl carrier protein domain of the w A gene product and the acyl carrier protein domain of the w A gene product. It's likely that this putative aflatoxin PKS is involved in extending the *tlvm8*-produced hexanoate starter cell [138].

A nucleotide sequence approach combined with biochemical studies of genetically disrupted strains can similarly classify the unique function of other genes in the cluster located by transcript mapping. Feeding interrupted strains with intermediates of the pathway of aflatoxin and analyzing their ability to transform. These

substrates to subsequent intermediates can aid in determining the stage of gene disruption. This approach to function of gene recognition is extended to another interesting gene adjacent to *nor-1* (encodes a 6.5 kb transcript).

A limited portion of this gene in nucleotide sequence analysis showed that The predicted protein has a high degree of similarity to the real thing (51% over 150 amino acid residues) with the enoyl-reductase domain in the same yeast FASl products as observed in the uvm88 analysis [67]. It's probable that the hexanoate starter requires two FAS subunits (and; encoded by separate genes) that are similar to those found in yeast. This hypothesis will be tested by combining gene disruption with feeding experiments. A common phenomenon is the clustering of genes involved in secondary metabolism. For example, a number of polyketide-derived antibiotics, including erythromycin, tetracenomycin, actinorhodin, griseusin and granaticin, are produced by different species of *Streptomyces* (reviewed in [139, 140]). Several genes contained in their biosynthetic pathways display a high degree of identity in comparable pathways with genes and are clustered on the chromosome in identical patterns.

There have also been studies of the clustering of fungal genes involved in the synthesis of secondary metabolites. The genes that encode penicillin and cephalosporin enzymes (members of the antibiotic β -lactam class) in the *Penicillium chrysogenum* and *Cephalosporium acremonium* pathways (reviewed in [141]), *A. nidulans* [142], *Fusarium sporotrichioides* genes involved in the trichothecene process (toxic sesquiterpenes) [143], Gene clusters can be found. However, recent studies indicate that the clustering of fungal genes is not limited to secondary metabolite synthesis. Some of the genes involved in melanin biosynthesis (a dark-brown polyketide-derived pigment) are clustered within a 30 kb stretch of genomic DNA in the filamentous fungus *Alternaria alternata* [144].

The role of gene clustering in the aflatoxin biosynthetic pathway's significance (if any), regulation or evolution has not yet been elucidated. However, with growing evidence that the structure of chromatin is involved in gene regulation [145–147], A role in cluster expression can be played by the chromosome structure. This is an unexplored area that can benefit from molecular biology techniques.

10.5 Aflatoxin gene duplication

The presence of at least two copies of the *ver-1* gene, *Per-1A* and *ver-1B*, in different regions of the *A. parasiticus* genome was discovered during physical mapping studies of the cosmid NorA. [122]. It was established that the gene originally cloned was *ver-1A* by comparing the restriction enzyme polymorphisms present in these two chromosomal copies with the cloned *ver-1* gene. Subsequently, *ver-1B* was cloned and its nucleotide sequence was calculated. These genes were found to share 93% of the identity of the nucleotide series. Near the center of the predicted *ver-1B* gene transcript, a stop codon was found, indicating that it may encode a truncated polypeptide that has little to no role. A duplicate chromosomal region extending from *ver-1A* and *ver-1B* approximately 12 kb upstream was found, which also includes an additional copy of *aflR* [122]. The higher stability of toxin production in *A. parasiticus* compared to *A. flavus*, which does not have such a duplication, may be explained by duplication of the *ver-1* and *aflR* genes. More than 90% of *A. parasiticus* isolates contain aflatoxin, while only 50% of *A. flavus* isolates are toxigenic (50 YO or less) [53].

10.6 Aflatoxin genes chromosomal organization

Keller et al. [148] successfully used pulsed field gel electrophoresis as a tool for genetic analysis of aflatoxigenic fungi. Genetic karyotyping and Southern blot

analysis using many different gene probes revealed similarities and differences between the genomes of *A. parasiticus* and *A. flavus*, as well as those of *A. niger* and *A. nidulans*.

Under identical electrophoretic conditions, *A. flavus* (5 to 8 chromosomal visible bands), *A. parasiticus* (5 to 6 bands of chromosomes) and *A. versicolor*, a related species reported to produce precursors (six chromosomal bands) in pathway of aflatoxin, showed chromosome numbers that were identical but varied.

The total size of the genome of these fungi was close to that of the size recorded for *A. nidulans*, as well as *A. niger* (31–38.5 Mb). These studies have yielded an additional, potentially significant finding. The karyotype patterns in 19 different *A. flavus* isolates were all different, suggesting that genetic diversity in this species is widespread [53].

An indicator of chromosomal rearrangement via gross translocation leading to specific karyotype patterns may be the size variation. Imperfect fungi can tolerate such rearrangements because, unlike sexual reproduction, Asexual reproduction (via mitosis) necessitates only the separation of similar chromatids, which necessitates the pairing of identical chromosomes and is strictly regulated genetically. The heterogeneity in the genomes of different isolates of *A. flavus* can be linked to the apparent instability in the ability to generate aflatoxins, which is of practical importance. Keller et al. [120] recently demonstrated that the *verA* gene of *A. nidulans* hybridizes strongly to chromosome IV in a similar sample (2.9 Mb in size). It should now be possible to identify the positions of duplicated aflatoxin gene clusters on the same or different chromosomes in *A. parasiticus* using identical procedures.

10.7 Ongoing studies

The molecular biology of aflatoxin synthesis is currently being studied in two areas: (1) The genes structure, role, organization, and comparative mapping and gene clusters of aflatoxin (or ST) in *A. parasiticus*, *A. flavus* and *A. nidulans*; and (2) the discovery of pathway genes controlling molecular pathways (Aflatoxin promoter structure and function; regulatory genes).

10.8 Structure and function of gene cluster

Analysis of the nucleotide transcript mapping and sequence of most of genes in *A. parasiticus* and *A. nidulans* should be finished in the immediate future while studies of disruption continue as candidate genes are identified. The function and localization of enzymes pathway is being pursued in related work. For instance, maltose-binding protein fusion products *nor-1* and *ver-1* were expressed in *E. coli*. It has developed and polyclonal antibodies (pAb) that seem to recognize the native fungal proteins [122, 123]. These antibodies will be used to locate these proteins in the cell, along with the available antibodies to the *nor-1* protein, and to decide if proteins function individually or in enzyme complexes. Preliminary results using the polyclonal antibody *ver-1* indicate that *ver-1* proteins are primarily localized in the fungal cell membrane fraction [122]. For studies on plant resistance mechanisms against the development of aflatoxin, immunolabelling will also be helpful in exploring the kinetics and level of expression of aflatoxin enzymes in host plant tissues.

10.9 Pathway genes regulated molecular mechanisms

Afl-1, a second putative regulatory locus (besides aflR), was discovered by Leaich and Papa [149] in *A. flavus* by use UV mutagenesis and determination to be

linked to *nor-1* by parasexual analysis (reviewed by [53]). In diploids, afl I mutants are functionally dominant, resulting in aflatoxin development loss. The afl- I mutation suppresses transcription of the three structural genes studied, according to recent research [150] (*nor-1*, *ver-1* and *omt-I*). In these strains, aflR transcription was natural. Cloning afl-1 and determining its position in regulation will be the subject of future research.

Following the cloning of aflR and the discovery of a δ -I, the next logical step is to investigate the mechanisms of influence exerted by the genes' products. Identifying the cis-acting sites and trans-acting proteins that control aflatoxin gene function is the latest strategy for conducting these studies. The *nor-1*, *ver-1*, and aflR promoters have been fused to the *E. coli* gene encoding β -glucuronidase (*zcidA*), also known as the GUS gene, whose gene product can be easily detected using colorimetric or fluorometric assays [151, 152]. This reporter construct is now being used in fungal strains: (1) to monitor fungi in plants under various conditions and to detect the induction of aflatoxin genes in fungi grown under various crop conditions; (2) classify, by deletion or site-directed mutation analysis, the cis regulatory regions related to the control of these promoters. By mobility shift assays, promoter regions are also being studied. It is then possible to identify and purify proteins which bind specifically. Preliminary data indicate that in the *nor-1* promoter, there are at least two distinct DNA/protein interactions [151]. To be demonstrated, the practical importance of these relationships remains. The study of aflatoxin biosynthesis in culture offers a model framework for understanding the biosynthesis of aflatoxin on natural substrates. Applications of molecular biology to the reduction, evolution and biological significance of the aflatoxin pathway. Factors that are essential for the regulation of aflatoxin biosynthesis in the host plant, however, can differ from those that work in the culture. More studies of the fungus in the host plant must require future work. In turn, these studies can lead to new toxin control techniques and increased understanding of the aflatoxin pathway's evolution and biological function.

10.10 Elimination of aflatoxins from food and feed

Figure 5 shows how the molecular biology contributions mentioned above can be extended to the removal of aflatoxin from food and feed. They will quickly summarize each of these applications.

Several methods (grouped into pre-harvest and post-harvest strategies) are currently being used or have been suggested for use in the food chain reduction or elimination of aflatoxin. Preharvest strategies are designed to block the host plant (crop) from fungal infection or to block the fungal pathogen's ability to grow or synthesize aflatoxins on the plant and are likely to have the greatest effect on human and animal health in the future. In reversing aflatoxin screening/detection, removal/adsorption, decontamination or altered aflatoxin metabolism/DNA adduct formation, expensive and/or inadequate post-harvest elimination strategies would not need to be relied on as vital treatment measures, but can provide a safety net to eliminate low levels of aflatoxins that can escape pre-harvest monitoring. Existing approaches to pre-harvesting, including irrigation, the use of fungicides or insecticides and the use of resistant or regionally adapted crop varieties, lack successful control. It is also psychologically inappropriate or too expensive to use pesticides or irrigation, though genetically stable, highly resistant crops have not been successfully obtained using traditional breeding methods. However, for potential use, several promising preharvest techniques have been suggested for aflatoxin regulation. These strategies concentrate on two main areas: (1) genetically modified crops to minimize the growth of fungi or inhibit biosynthesis of aflatoxin (long-term

Figure 5. Elimination of aflatoxin by applications of molecular biology. (a) Targets for inhibiting aflatoxin gene expression. Natural plant products or other agents that can be identified using GUS reporter constructs or polyclonal antibodies for each gene mentioned in the text can theoretically inhibit each step in gene expression, transcription, RNA transport and processing, translation, protein processing, and localization. TAF stands for trans-acting component. (regulatory protein). (b) The potential application of tools derived from cloned aflatoxin genes to increase host plant resistance to fungal growth, infection, or toxin biosynthesis. Biocontrol strains, fungal strains with GUS reporter constructs (i.e. tester strains), and polyclonal antibodies raised to pure native proteins or proteins expressed in *E. coli* are the main tools in development.

approach); and (2) the use of biological control species to exclude the toxigenic fungus from infecting the crop competitively (short-term approach). These other possible applications of molecular biology for the removal of aflatoxin have recently been studied in detail [57]. However, they are briefly outlined here to provide a basis for the discussion of aflatoxin synthesis molecular biology crop genetic engineering. This method employs molecular genetics to increase the expression of genes that control natural (endogenous) resistance and/or to introduce resistance genes from other sources into susceptible plants. *A. parasiticus* and *A. flavus* are weak pathogens of the plant's reproductive organs that are particularly aggressive in mature seeds with high oil concentrations [46]. Successful genetic manipulation of crops should be aided by the identification of the signals exchanged between host and pathogen that stimulate aflatoxin production in susceptible plants under host stress or that inhibit toxin formation in 'naturally resistant' crops. There is still a lot of work to be done in this field; however, the approach is promising because it may be reasonably easy to increase natural or endogenous resistance by modulating gene expression that is a common part of the plant genome.

In principle, resistance genes from other sources can be obtained by finding naturally occurring plant compounds that inhibit *A. flavus* and *A. parasiticus*. Growth and/or aflatoxin production. Crude botanical extracts that display these characteristics have been recognized (reviewed by [56]). By genetic engineering, genes that encode the synthesis of these novel compounds can be inserted into crops. Clearly, if one or two genes enable the biosynthesis of the compound, success will be more easily obtained and will only be obtained if the compound is non-toxic to humans, animals and engineered plants. In addition, at the correct time, the additional genes must be expressed in the engineered plant in the right organ. For the identification of plant compounds (or other agents) that promote or inhibit

fungal invasion, growth or toxin biosynthesis, the aflatoxin gene/GUS reporter constructs are extremely useful tools.

10.11 Biological control

Strains of *A. flavus* and *A. parasiticus* showed potential to reduce the level of the resident fungal population and showed a substantial reduction in aflatoxin contamination (80–90%) in greenhouse and field studies. ([42]; reviewed in [46]). Since this strategy depends on the survival and successful occupation by the biocontrol strain of an ecological niche, identification of environmental factors that benefit certain isolates of *A. flavus* and *A. parasiticus* must be recognized by others. The strains of *A. flavus* are an interesting aspect of this strategy, which must be considered for its successful execution. *A. flavus* appears to be a replacement for other strains of more successfully than *A. parasiticus*. Therefore, combinations of strains of both species are likely to be needed [153]. Recent studies have suggested that nontoxigenic isolates of *A. flavus* occur naturally. In as yet undetermined environmental conditions, weaknesses may have the genetic capacity to synthesize AFB1 [154]. Under as yet undetermined conditions of the environment. Using a molecular genetics approach, genetically stable *Aspergillus* toxigenic biocontrol strains that are known to compete well can be produced once these genes have been identified by specific deletions of key genes in the biosynthetic pathway. Using a molecular genetics approach, genetically stable *Aspergillus* toxigenic biocontrol strains that are known to compete well can be produced once these genes have been identified by specific deletions of key genes in the biosynthetic pathway. A minimum of one genetically engineered fungal biocontrol strain (tlvm8 disruption strain - Dis3) has been made available for field research using this gene disruption technology [124].

10.12 Evolution

A high degree of sequence identity between the genes of aflatoxin (*ver-1*, aflR, omt-1) in *A. parasiticus*, *A. nidulans* and *A. flavus*. The organization of the gene cluster also is well conserved. Interestingly, the *nor-1* and *ver-1* genes are present in *A. sajae*, *A. oryzae* and nontoxigenic *A. flavus* strains [154]. These data indicate that the AFB1 or ST pathway was also included in the progenitor *Aspergillus* strain that gave rise to the present species under analysis (it will be interesting to determine if *A. nidulans* has the genetic capacity to produce AFB1; i.e. genes for the O-methyltransferase and oxido reductase required to convert ST to AFB1). Physical clustering can also mean that the progenitor strain has acquired the intact pathway from some other organism by horizontal transfer (i.e. *Streptomyces* spp. produce anthraquinone polyketide antibiotics, structurally related to intermediates in AFB1 synthesis). Alternatively, cluster organization preservation can indicate that an intact structural organization relies on the role or control of aflatoxin synthesis. The aflatoxin pathway formed from a pre-existing pathway for the synthesis of a fungal polyketide, perhaps a mycelial or spore pigment, is another possibility that should receive further research. The putative aflatoxin PKS demonstrates a high degree of sequence identity to the PKS involved in conidial pigment synthesis in *A. nidulans* in support of this notion. Interestingly, the chemical intermediate structure in the synthesis of conidial pigment in *A. Parasiticus* (a polyketide called naphthapyranone) also has a good resemblance to NA [155]. A similar study determined that an ascospore pigment (ascoquinone A) in *A. nidulans* is a dimer of an anthraquinone and is likely to be polyketide in origin [156]. Recent studies on melanin biosynthesis in *Magnaporthe oryzae* have provided additional data that may support a correlation between pigment synthesis and aflatoxin synthesis [157].

A. Parasiticus 56% identity of the *ver-1* gene product in A was reported to share the predicted amino acid sequence of the gene encoding a polyhydroxynaphthalene reductase involved in melanin biosynthesis. This may indicate that a common ancestral polyketide pathway is used to derive these biosynthetic pathways (or parts of the pathways).

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Author details

Nadeem A. Ramadan and Hadeel A. Al-Ameri*
Department of Biology, College of Sciences, University of Mosul, Republic of Iraq

*Address all correspondence to: hadeelahmed.um@gmail.com

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