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Original Article

Role of plant-derived saponins in reducing the growth of aflatoxins: A review

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ABSTRACT

Aflatoxins are toxic secondary metabolites produced principally by aflatoxigenic Aspergillus species and are major contaminants of cereals, oilseeds and other agricultural commodities. Their formation is influenced by fungal ecology, host stress, temperature, water activity, insect damage and post-harvest storage conditions. Aflatoxin B_1 (AFB_1) is of particular concern because hepatic cytochrome P450-mediated bioactivation generates the highly reactive AFB_1-8,9-epoxide, which forms DNA adducts and contributes to hepatocarcinogenesis. Current management relies on integrated pre-harvest, post-harvest, biological, chemical and technological measures, but practical constraints include variable efficacy, cost, environmental concerns, resistance and incomplete removal of preformed toxins. Plant-derived saponins are amphiphilic glycosides, broadly divided into triterpenoid and steroidal classes, whose hydrophobic aglycones can interact with membrane sterols while their sugar chains influence solubility and biological activity. Experimental literature supports antifungal activity for several saponins, with membrane–sterol interactions being a major mechanistic feature. However, direct evidence that saponins specifically inhibit aflatoxin polyketide synthase, cytochrome P450 pathway enzymes, or aflatoxin biosynthetic genes remains limited and should not be inferred solely from their general antifungal activity. This review therefore evaluates the biochemical basis of aflatoxin formation, established control measures, the chemistry and biological actions of plant-derived saponins, their plausible relevance to aflatoxin-producing fungi, safety and regulatory considerations, and the research gaps that must be addressed before saponins can be developed into validated food-preservation or biocontrol agents.

Keywords: Aflatoxin; *Aspergillus flavus; Aspergillus parasiticus*; plant-derived saponins; antifungal phytochemicals; membrane sterols; aflatoxin control; food safety.

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INTRODUCTION

Aflatoxin Contamination in Stored Grains

Aflatoxin contamination remains an important food-safety problem in tropical and subtropical agriculture, particularly in commodities such as maize, groundnuts, sorghum and other cereal-based products. Aflatoxigenic *Aspergillus* species can colonize crops before harvest and continue to grow during storage when temperature, moisture and handling conditions are favourable. Poor drying, insect damage and inadequate storage management increase the probability of fungal proliferation and toxin accumulation [1,2].

From a biochemical perspective, aflatoxins are secondary metabolites whose production is controlled by a coordinated biosynthetic gene cluster and by environmental, nutritional and developmental signals. The pathway depends on central carbon metabolism and reducing equivalents, while late-stage reactions involve oxygen-dependent enzymes. These features explain why changes in fungal physiology and the storage environment can alter toxin production even when visible fungal growth is limited [2-5].

Aflatoxins may be present without obvious changes in the sensory properties of a commodity [1]. They can persist through ordinary food-processing operations because substantial thermal stability limits the effectiveness of conventional heating alone [1]. Long-term dietary exposure is associated with important toxicological and public-health consequences (Figure 1), particularly hepatic injury and hepatocellular carcinoma. [6-8].

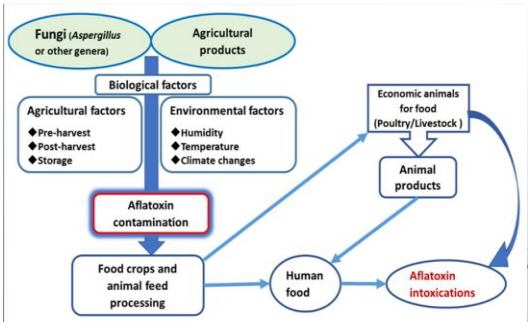

Figure 1. Aflatoxin intoxication via the feed and food supply chain [1].

Review Methodology

This paper is a narrative review of peer-reviewed literature on aflatoxin occurrence, fungal biology, aflatoxin biosynthesis and toxicity, current mitigation strategies, and the chemistry and antifungal actions of plant-derived saponins. Literature was identified from major biomedical and scientific databases, including PubMed, Scopus, ScienceDirect and Google Scholar, using combinations of the terms 'aflatoxin', *'Aspergillus flavus'*, *'Aspergillus parasiticus'*, 'plant-derived saponins', 'antifungal phytochemicals', 'mycotoxin control' and 'food safety'. Foundational papers were retained where they provide the primary biochemical framework, while recent reviews were used to synthesize contemporary evidence. The review is narrative rather than a formal systematic review and therefore does not claim PRISMA-level exhaustive searching [2-6,10-12]

1.0 Aflatoxins: Sources, Classification, and Occurrence

Aflatoxins are toxic secondary metabolites produced predominantly by members of *Aspergillus* section Flavi. The major naturally occurring aflatoxins are AFB_1, AFB_2, aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). AFB_1 is generally regarded as the most potent and clinically important

member of the group. Aflatoxin M_1 is principally a hydroxylated metabolite formed during the biotransformation of AFB_1 and may subsequently occur in milk and dairy products [1,2,6,8].

1.1 Biology and Ecology of *Aspergillus flavus* and *Aspergillus parasiticus*

Aspergillus flavus and *A. parasiticus* are filamentous fungi adapted to soil, plant debris, crops and stored agricultural commodities. Their success in food systems reflects efficient sporulation, broad metabolic capacity and the ability to tolerate environmental stresses. Although both species are aflatoxigenic, their toxin profiles differ *A. flavus* predominantly produces B-type aflatoxins, whereas *A. parasiticus* is capable of producing both B- and G-type aflatoxins [2,3].

Temperature, water activity, oxygen availability, substrate composition, insect damage and host stress all influence colonization and toxin formation. Consequently, aflatoxin control must address both fungal growth and the environmental conditions that activate secondary metabolism [1-4,10,11].

1.1.1 Fungal growth, colonization, and metabolic adaptation in stored grains

Fungal colonization begins with spore germination and hyphal development, followed by extracellular hydrolysis of host macromolecules. Enzymes such as amylases, proteases and lipases release smaller metabolites that enter glycolysis, the tricarboxylic acid cycle and the pentose phosphate pathway. The resulting metabolic intermediates provide carbon skeletons, ATP and reduce equivalents needed for growth and secondary metabolism [2,4,5].

When environmental or nutritional stress alters fungal physiology, carbon and redox metabolism can be redistributed toward secondary metabolite formation. The pentose phosphate pathway is particularly important as a cellular source of NADPH, although the relationship between NADPH supply and aflatoxin formation is regulated rather than simply proportional [4,5].

1.1.2 Secondary metabolism and aflatoxin biosynthesis pathway

Aflatoxin biosynthesis (Figure 2) is encoded by a clustered set of pathway genes within the Aspergillus genome. Current descriptions indicate a cluster of roughly 25-30 genes within an approximately 70-80 kb region, depending on the strain and genomic definition used [2-4].

At the biochemical level, acetate-derived carbon, including acetyl-CoA and malonyl-CoA, feeds the fatty acid synthase/polyketide synthase system that generates the early polyketide precursor. The pathway then proceeds through intermediates such as norsolorinic acid, averantin, versicolorin A, sterigmatocystin and O-methylsterigmatocystin before formation of the terminal aflatoxins. Multiple reduction, oxidation, cyclization and methylation reactions are involved, and several late steps require molecular oxygen [2-4].

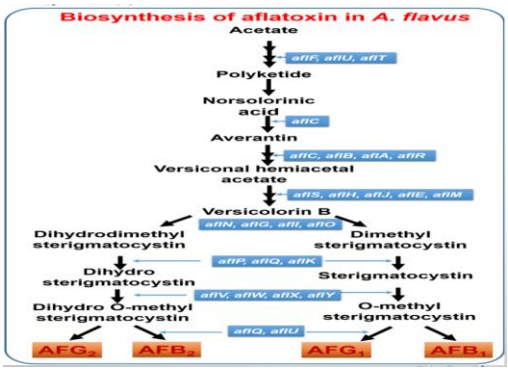

Figure 2. Biosynthetic pathway of aflatoxins in *Aspergillus flavus*. *[2].*

1.1.3 Structural biochemistry and toxicological activation of aflatoxins

Aflatoxins contain a coumarin-based core fused with bifuran and lactone-containing structural features. Their planar, conjugated architecture contributes to physicochemical properties relevant to biological activity. AFB_1 is particularly important because hepatic biotransformation produces a highly reactive epoxide intermediate capable of forming covalent adducts with nucleophilic cellular targets [1,6,8].

1.1.4 Genetic regulation of aflatoxin biosynthesis

The pathway-specific transcription factor AflR is a central regulator of many genes within the aflatoxin cluster. AflS acts as a co-regulatory partner, while pathway enzymes are encoded by structural genes such as aflD and aflQ. In addition, global regulatory systems involving LaeA and the Velvet complex integrate environmental and developmental signals with fungal secondary metabolism [2-5].

1.1.5 Role of oxidative stress, reactive oxygen species and signal transduction

Reactive oxygen species (ROS) participate in the regulation of fungal development and secondary metabolism. Experimental work in *A. flavus* has shown that disruption of ROS homeostasis can alter aflatoxin biosynthesis and expression of pathway-associated genes. However, ROS should be treated as part of a broader regulatory network rather than as a single on/off switch for aflR. [3,5,9]

Signal transduction pathways, including mitogen-activated protein kinase and cAMP-dependent systems, contribute to the coordination of fungal development, stress responses and secondary metabolism. The net effect of oxidative stress on aflatoxin formation depends on the intensity and duration of the stress and on the physiological state of the fungus [2,3,5,9].

1.2 Classification of Aflatoxins

The major aflatoxins as shown in Figure 3, are conventionally divided into B and G groups according to their fluorescence characteristics under ultraviolet light. AFB_1 and AFB_2 are classified as B-type aflatoxins, whereas AFG_1 and AFG_2 are G-type aflatoxins. AFB_1 is the principal toxicological concern because of its potent hepatocarcinogenicity and its efficient metabolic conversion to a DNA-reactive epoxide [1,2,6,8].

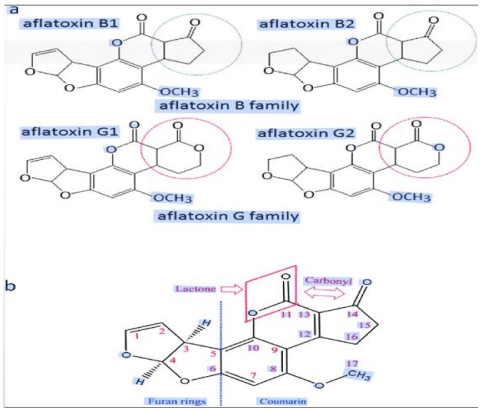

Figure 3. Structural classification of the major aflatoxins. [6].

1.3 Bioactivation of Aflatoxin B_1 in the Liver (Toxic Pathway)

Aflatoxin B1 AFB_1 is metabolized in the liver primarily by cytochrome P450 enzymes, particularly CYP1A2 and CYP3A4, with contributions from other P450 isoforms. Bioactivation can generate the highly electrophilic AFB_1-8,9-epoxide. This metabolite can react with DNA, initially forming AFB_1-N7-guanine adducts and downstream lesions that contribute to mutagenesis. Persistent genotoxic stress, especially in the context of chronic exposure and hepatitis B infection,

increases the risk of hepatocellular carcinoma. [6-8]

1.4 Environmental and Storage Factors Influencing Aflatoxin Contamination

Environmental conditions strongly influence both fungal growth and aflatoxin formation. Temperature, water activity, substrate moisture, oxygen availability, mechanical injury and insect infestation can change the probability that an aflatoxigenic population will establish and produce toxin. Warm, humid storage conditions are particularly conducive to contamination [1,2,8,11].

Post-harvest management therefore focuses on rapid drying, moisture control, protection against insects, hygienic handling, appropriate aeration and storage systems that limit environmental conditions favourable to *Aspergillus*. Hermetic storage can reduce oxygen availability and suppress fungal development when correctly implemented, but it is not a substitute for drying grain to a safe moisture status before storage [8,11].

1.5 Health Implications of Aflatoxin Exposure

High acute exposure can cause aflatoxicosis, characterized by severe hepatic injury and, in extreme cases, death. Chronic lower-level exposure is associated with hepatocellular carcinoma and has also been linked to impaired growth, immune dysfunction and other adverse outcomes. The magnitude of risk is influenced by dose, duration of exposure, nutritional status and co-exposure to other disease factors, particularly hepatitis B virus infection [6-8].

1.6 Biochemical Mechanisms of Toxicity

Aflatoxin B1 toxicity results from both direct macromolecular adduct formation and secondary cellular disturbances (Figure 4). The epoxide metabolite damages DNA, while metabolic activation and cellular responses can increase oxidative stress and disturb antioxidant defenses. These processes can promote lipid peroxidation, mitochondrial dysfunction, altered protein homeostasis, cell death and genomic instability [6-8].

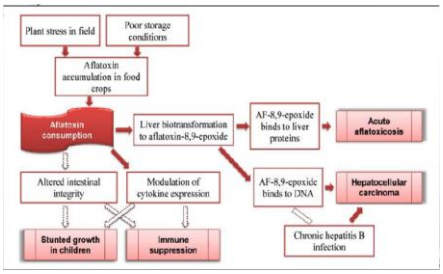

Figure 4. Aflatoxin disease pathways and associated health effects in humans [7].

1.7 Economic Impact and Food Security Implications

Aflatoxin contamination has consequences beyond toxicology. Rejection of contaminated lots, reduced market value, livestock productivity losses, increased monitoring costs and barriers to trade can all create economic burdens. In settings where staple foods constitute a large proportion of dietary intake, contamination also intersects with food security and public-health priorities [1,8,10,11].

1.7.1 Economic losses

Losses may arise from rejected produce, downgraded commodity quality, reduced export opportunities, increased testing costs and the need for disposal or detoxification. The magnitude varies by commodity, region, regulatory threshold and contamination prevalence [8,10,11].

1.7.2 Livestock and food-chain impact

Aflatoxin-contaminated feed can reduce animal performance and may lead to carry-over of metabolites such as aflatoxin M_1 into milk. This creates an additional food-chain exposure pathway and emphasizes the need for control measures that protect both feed and food [1,8].

1.7.3 Healthcare burden

Chronic aflatoxin exposure contributes to the burden of liver cancer and other health disorders, increasing the need for surveillance, diagnosis and long-term health care. The risk is particularly important in populations with continuous exposure to contaminated staple foods [6-8].

1.7.4 Food security challenges

Aflatoxin contamination reduces the usability and marketability of food commodities and can compound food insecurity in regions that depend heavily on susceptible crops. Effective control must therefore protect not only consumer health but also food availability, farmer income and market access [8,10,11].

1.7.5 Control strategies

Practical mitigation requires an integrated sequence of interventions, including good agricultural practice, resistant or less susceptible varieties, biological control where appropriate, rapid drying, insect management, improved storage, sorting and scientifically validated detoxification methods. No single intervention is sufficient under all contamination scenarios [2,8,10,11].

1.8 Current Strategies for Aflatoxin Control

Aflatoxin control can be viewed as a biochemical and systems problem involving fungal metabolism, host susceptibility and storage ecology. The most effective approaches reduce the opportunity for fungal colonization, interrupt conditions that support toxin biosynthesis, or remove/deactivate toxin after contamination [2,8,10,11]. These approaches as represented in Figure 5 are discussed in the following sections:

1.8.1 Pre-Harvest control measures

Pre-harvest interventions include resistant varieties, good agronomic practice, irrigation management, insect control, appropriate planting and harvesting schedules, soil and crop management, and biological control with selected atoxigenic *Aspergillus* strains. These approaches primarily reduce fungal infection, crop stress and the opportunity for aflatoxin formation before harvest [2,10,11].

1.8.1.1 Agronomic and cultural practices

Crop rotation and appropriate soil management can influence inoculum pressure and overall crop health. Irrigation is important because severe drought or heat stress can increase crop susceptibility to Aspergillus infection. Insect management is similarly relevant because kernel injury can create entry points for fungal invasion. The biochemical value of these measures is best understood as reduction of fungal establishment and host stress rather than as a simple direct block of a single aflatoxin enzyme [2,10,11].

Resistant cultivars can limit fungal penetration and aflatoxin accumulation through multiple physical and biochemical traits. Marker-assisted selection, genomics and proteomics have also been used to identify host factors associated with reduced susceptibility, although translating these traits into broadly

effective commercial varieties remains challenging [2,10].

1.8.1.2 Biological control

Biological control with non-aflatoxigenic *A. flavus* strains primarily operates through ecological competition with toxigenic strains for space and nutrients and can substantially reduce aflatoxin contamination when the biocontrol strain is well adapted to the local environment. Other microbial antagonists and natural products may also contribute through antibiosis or interference with fungal development. Direct claims that particular biocontrol metabolites universally inhibit the aflatoxin polyketide synthase should be avoided unless demonstrated experimentally for the strain and compound under study [2,10,11].

1.8.1.3 Genetic and biotechnological approaches

Genetic approaches include conventional breeding, marker-assisted breeding, omics-assisted selection, transgenic strategies and host-induced gene silencing. RNA interference can be used to reduce the expression of selected fungal genes involved in pathogenicity or aflatoxin production, but performance is dependent on target selection, RNA delivery and the stability of the silencing response. These technologies remain largely complementary to, rather than replacements for, agronomic and storage management [2,10].

1.8.2 Post-Harvest storage and handling techniques

Post-harvest management is critical because fungal growth and toxin production can continue after harvest when moisture and temperature remain favourable. Core measures include thorough drying, removal of damaged kernels, insect control, hygienic handling, appropriate packaging and storage conditions that restrict fungal growth [8,11].

1.8.2.1 Environmental control

Reducing water activity and controlling temperature slows fungal metabolism and lowers the likelihood of toxin formation. Low-oxygen or modified-atmosphere systems can further restrict fungal growth when properly designed, although their effectiveness depends on grain condition, storage duration and maintenance of the intended atmosphere. The correct biochemical interpretation is that environmental control alters the physiological state of the fungus; it should not be reduced to a single presumed effect on NADPH generation or aflR expression [8,11].

1.8.2.2 Physical and biological methods

Sorting, cleaning, density separation, milling and other physical approaches can remove a proportion of contaminated or visibly damaged material, although toxin distribution is heterogeneous and physical processing cannot guarantee complete removal. Biological detoxification approaches use microorganisms or enzymes to absorb, transform or degrade aflatoxins. Their suitability depends on demonstrated efficacy, safety of products formed during degradation, and compatibility with the food matrix [1,8,11].

1.8.2.3 Chemical and synthetic fungicides

Chemical fungicides can suppress fungal development through interference with specific cellular processes. Their use must be evaluated in relation to target specificity, residue limits, environmental effects and resistance development. The relationship between fungicide action and

aflatoxin reduction is not necessarily linear because toxin biosynthesis responds to fungal stress and developmental state. Consequently, fungicide efficacy should be assessed using both fungal growth and aflatoxin endpoints where relevant [8,10,11].

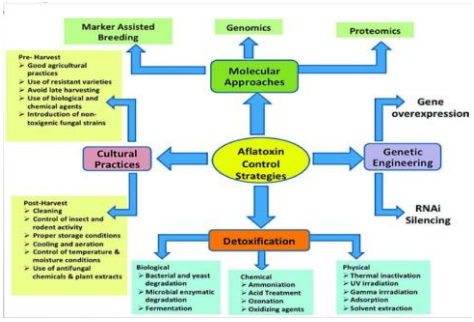

Figure 5. Integrated approaches to aflatoxin control and detoxification [2,8,10,11].

1.9 Limitations of Current Strategies

Current strategies are constrained by practical and biological limitations. Physical decontamination may be incomplete; chemical control can be limited by residues, cost and resistance; biological agents require ecological compatibility; and genetic approaches may involve long development timelines and regulatory or public-acceptance considerations. Moreover, toxin already formed in food may remain even after fungal growth has been halted [8,10,11].

These limitations support the development of complementary, food-compatible interventions that can reduce fungal growth or toxin formation without creating unacceptable toxicological, nutritional or environmental trade-offs. Plant-derived phytochemicals, including saponins, are therefore of interest, but their specific anti-aflatoxigenic actions require direct experimental validation [12,14,15].

2.0 Plant-Derived Phytochemicals in Fungal Control with Emphasis on Saponins

Plants synthesize diverse secondary metabolites that contribute to defense against microorganisms and herbivores. Important phytochemical classes include phenolics, terpenoids, alkaloids, glucosinolates and saponins. Their antimicrobial effects may arise from membrane disruption, enzyme inhibition, metal chelation, redox modulation or interference with signaling and nutrient acquisition as revealed in Table 1 [12-15].

Among these compounds, saponins are notable for their amphiphilic architecture, which combines a hydrophobic aglycone with one or more hydrophilic sugar chains. Their interaction with membrane sterols is a well-established basis for antifungal activity in a number of plant–fungus systems [12-15]

Tab

le 1: Classes of Antifungal Phytochemicals and Their Biochemical Mechanisms

Phytochemical class	Primary mechanism	Representative biochemical target/process
Phenolics	Oxidative-stress modulation and enzyme interaction	ROS-responsive proteins and fungal enzymes [12]
Terpenoids	Membrane and sterol perturbation	Membrane lipids/sterol-associated processes [12,15]
Alkaloids	Enzyme and nucleic-acid interference	Multiple cellular enzymes and macromolecular processes [12]
Glucosinolates	Generation of reactive breakdown products	Thiol-containing cellular enzymes [12]

Saponins	Sterol interaction and membrane disruption	Ergosterol-rich fungal membranes [14,15]

The available evidence supports direct antifungal membrane effects for some saponins, but it does not justify a universal model in which all saponins directly reduce fungal acetyl-CoA or NADPH pools and thereby inhibit the aflatoxin polyketide synthase. Such metabolic consequences remain mechanistic hypotheses that require compound-specific and organism-specific validation [12,14,15]

2.2 Structural Features of Saponins

Saponins are glycosides containing a hydrophobic sapogenin (aglycone) linked to one or more sugar residues. They are broadly categorized as triterpenoid or steroidal saponins (Figure 6). Triterpenoid saponins commonly contain C_{30} pentacyclic aglycones derived from oxidosqualene cyclization, whereas steroidal saponins generally contain C_{27} steroidal frameworks, including spirostanol and furostanol forms [12-14]

Sugar identity, linkage, chain number and aglycone substitution can markedly influence solubility, membrane affinity, haemolytic activity and antifungal potency. Structure–activity relationships are therefore compound-specific; it is not scientifically appropriate to state that all monodesmosidic saponins are invariably more antifungal than all bidesmosidic saponins [12-14]

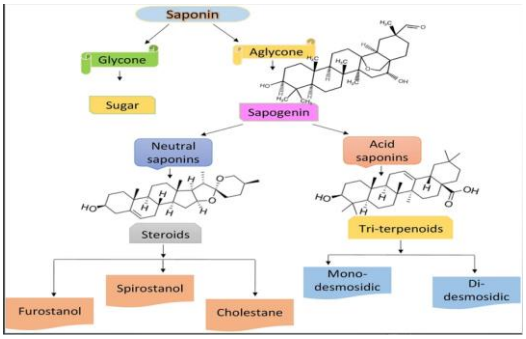

Figure 6. Classification and structural components of saponins. [13].

2.3 Biosynthesis of Saponins

Triterpenoid and steroidal saponins arise from isoprenoid metabolism. Sterol precursors originate from pathways supplying isopentenyl diphosphate and dimethylallyl diphosphate, followed by formation and cyclization of oxidosqualene and subsequent oxidation and glycosylation. The final structural diversity of plant saponins is generated by the combined activities of tailoring enzymes and glycosyltransferases [12,13].

Because saponin biosynthesis occurs in the plant, application of a saponin to fungi should not be described as causing direct competition with fungal polyketide synthase for shared cellular acetyl-CoA or NADPH pools. Antifungal activity occurs primarily through interactions between the applied saponin and fungal cellular structures or through downstream stress responses [12-15].

2.4 Extraction and Characterization Techniques

The extraction of saponins depends on the polarity and structural composition of the target compounds. Aqueous alcohols such as methanol or ethanol are commonly

used for crude extraction, while partitioning with n-butanol can enrich glycosylated saponins. Further purification may involve column chromatography or preparative high-performance liquid chromatography. Structural characterization commonly combines mass spectrometry, nuclear magnetic resonance spectroscopy and chromatographic profiling [12,13].

2.5 Saponins as Anti-Aflatoxigenic Agents

Saponins are biologically plausible candidates for suppressing aflatoxin-producing fungi because their membrane activity can inhibit fungal growth, and reduced fungal fitness can in turn reduce the opportunity for secondary metabolite formation. However, evidence should be distinguished between direct antifungal activity, inhibition of aflatoxin biosynthesis and detoxification of preformed aflatoxin. These are three different endpoints and require separate experimental demonstration [12,14,15].

3.0 Mechanisms of Saponin Action Against Fungal Growth and Aflatoxin Biosynthesis

3.1 Interaction with Fungal Cell Membranes (Ergosterol Binding)

Saponins can interact with membrane sterols and alter fungal membrane organization (Figure 7). Experimental work with the saponin aescin demonstrated that its antifungal activity can be reversed by ergosterol, supporting a role for sterol interaction. Depending on molecular structure and concentration, saponin–sterol interactions can increase membrane permeability and contribute to loss of cellular homeostasis [14,15].

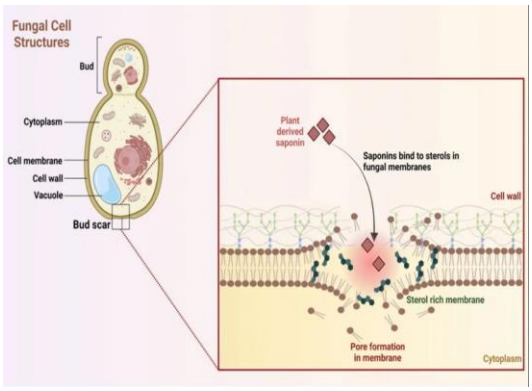

Figure 7. Schematic representation of saponin–sterol interaction and membrane pore formation in fungal cells. 14,15].

3.2 Disruption of Membrane Integrity and Cellular Homeostasis

Membrane disruption can lead to leakage of ions and metabolites, altered membrane potential and impaired cellular homeostasis. Secondary effects may include reduced nutrient uptake and changes in mitochondrial or energy metabolism. These consequences can inhibit fungal growth, but they should not automatically be interpreted as direct inhibition of an individual enzyme within the aflatoxin pathway. [12,14,15]

3.3 Effects on Fungal Growth, Sporulation and Metabolism

Antifungal saponins can inhibit biomass accumulation, hyphal development and spore-associated processes in susceptible fungi. The magnitude of the response depends on the chemical structure of the saponin, the fungal species or strain and the exposure conditions. Therefore, claims about universal suppression of cAMP–PKA or MAPK signalling by saponins require organism-specific evidence and should not be generalized from unrelated fungal models [12,14,15].

3.4 Modulation of Oxidative Stress and Reactive Oxygen Species

Saponin exposure can alter cellular redox homeostasis in fungi and in plant–pathogen systems. In some contexts, ROS contribute to antimicrobial activity or to the activation of plant defense responses. Experimental work with aescin has shown induction of oxidative responses in plants, while studies of aflatoxin-producing *A. flavus* demonstrate that disturbance of ROS homeostasis can affect aflatoxin biosynthesis. The two observations are related mechanistically but do not establish that saponins universally repress aflR or aflS through ROS [9,14,15].

3.5 Relationship between Saponin Activity and Aflatoxin Biosynthesis

Aflatoxin biosynthesis depends on fungal growth state, carbon metabolism, redox balance, oxygen availability and expression of the pathway gene cluster. Saponin-mediated membrane damage may therefore reduce the physiological capacity of a fungus to synthesize secondary metabolites. However, direct inhibition of the aflatoxin polyketide synthase or late pathway cytochrome P450 enzymes by saponins has not been established as a general mechanism. Claims that saponins mimic sterol substrates and competitively inhibit AflM or AflQ should therefore be treated as unverified unless supported by direct enzymology or validated molecular studies [2-5,12,14,15].

3.6 Effects on Aflatoxin Biosynthetic Gene Expression

Gene-expression changes may accompany chemical stress in *A. flavus*, and ROS-modulating compounds such as estragole can alter the transcriptional state associated with aflatoxin biosynthesis. Importantly, the currently identified literature in this review does not establish a reproducible, saponin-specific percentage reduction in aflR or aflD expression across *A. flavus* systems. Therefore, numerical claims such as '60% reduction in aflR' or '55% reduction in aflD' have been removed from the corrected review [9,12,16].

3.7 Synergistic Interactions with Other Phytochemicals

Combination strategies are attractive because phytochemicals can affect different cellular targets. In principle, membrane-active saponins may increase susceptibility to other antimicrobials, while complementary compounds can act on redox balance, cell-wall functions or other metabolic processes. Such synergy, however, must be demonstrated experimentally using defined concentrations and appropriate interaction models rather than assumed from chemical class membership [12,14,15].

3.8 Applications, Limitations and Challenges in the Application of Saponins

Saponins have potential as natural antifungal ingredients because they are plant-derived and possess established bioactivity against a range of organisms. Their translation into food-preservation systems nevertheless depends on the identity and purity of the saponin, effective concentration, food-matrix interactions, sensory consequences, stability and toxicological profile [12-15].

3.8.1 Application of Saponins in Grain Preservation

A rational application strategy would first establish the minimum inhibitory concentration or another validated antifungal endpoint for a defined saponin

fraction against the relevant *A. flavus* strain. Grain trials would then determine whether the compound remains active in the presence of proteins, starch, lipids and native microflora. This approach is preferable to assuming a universal grain-coating concentration because effective doses are expected to vary substantially with chemical composition and matrix [12,14,15].

Possible delivery approaches include surface coating, incorporation into food-compatible matrices or controlled-release systems. Any formulation intended for stored grain must demonstrate that the treatment does not compromise germination where seed viability matters, sensory quality or nutritional properties, while also achieving reproducible antifungal performance [11,12].

3.8.2 Integration into Storage Systems and Food Processing

Saponins are more plausibly viewed as one component of an integrated preservation system than as a stand-alone replacement for drying and storage management. Combining a validated saponin treatment with low water activity, insect control, hygienic storage and suitable packaging could reduce the ecological opportunity for *A. flavus* growth. However, laboratory inhibition does not automatically translate into effective control in real grain stores, so storage trials are essential [8,11-15].

Claims that saponins directly bind the lactone ring of AFB_1 and prevent its conversion to the reactive epoxide have not been established sufficiently to support their presentation as a general detoxification mechanism. Likewise, a pore-forming saponin should not be assumed to enhance delivery of detoxifying enzymes into contaminated matrices without direct evidence. These mechanisms have therefore been excluded from the definitive mechanistic model in this review [6-8,12].

3.9 Toxicological Profile and Safety Considerations

Saponin safety is strongly dependent on chemical structure, dose, route of exposure and matrix. Some saponins can interact with cholesterol-rich mammalian membranes and display haemolytic activity at sufficiently high concentrations. Interactions with bile acids and intestinal membranes may also affect nutrient handling. Consequently, an antifungal saponin intended for food use requires compound-specific toxicological evaluation rather than reliance on a generic safety label [12,14,16].

The use of the term 'generally recognized as safe' should also be approached carefully. Regulatory status is substance-specific and jurisdiction-specific; the existence of food uses for some saponins does not mean that any crude plant saponin extract is automatically approved for use as a grain preservative [12].

3.10 Regulatory Framework and Practical Application Challenges

Practical regulation requires a clearly characterized active substance or standardized fraction, evidence of efficacy, toxicological data, residue or exposure assessment, and a reproducible manufacturing process. The structural heterogeneity of plant saponins makes standardization particularly important because different plant sources, cultivars, extraction conditions and purification steps can produce fractions with different biological properties [12,13].

Analytical quality control may require chromatographic and spectrometric methods capable of identifying and

quantifying the major saponin constituents. Translation from laboratory studies to grain stores also requires evaluation of stability, storage temperature, water activity, microbial interactions and food-matrix effects. These requirements are particularly important where a proposed treatment is intended for routine application to staple foods [11-13].

4. Research Gaps and Future Directions

Several important knowledge gaps remain. First, most published evidence on saponin antifungal activity is not specifically focused on aflatoxin-producing Aspergillus strains or on reduction of aflatoxin concentrations in real stored grain. Direct studies that simultaneously measure fungal growth, aflatoxin production and saponin exposure are needed [12,14,15].

Second, the structural diversity of saponins makes cross-study comparison difficult. Future work should identify purified or chemically characterized saponins from relevant indigenous plants and relate specific structural features to antifungal potency, sterol binding, haemolysis and food-matrix behaviour [12-14].

Third, mechanistic studies should move beyond inference from general membrane damage and directly test whether selected saponins alter the expression of aflR, aflS or pathway structural genes, intracellular redox status, membrane sterol composition, aflatoxin intermediates or enzyme activities. Omics approaches coupled with targeted biochemical assays would be especially valuable [2,3,5,9,12].

Fourth, formulation research is required to improve stability and reproducibility. Encapsulation, controlled-release matrices and compatible grain coatings may be useful, but their performance must be assessed under realistic storage conditions and at scales relevant to farmers and food processors. Finally, long-term safety, residue behaviour and regulatory acceptability should be evaluated before any saponin preparation is proposed for routine food preservation [8,11-13].

5. Conclusion

Aflatoxin contamination is a complex biochemical and food-safety problem arising from the interaction of aflatoxigenic fungi, host susceptibility and environmental conditions. The strongest evidence supports integrated control based on good agricultural practice, biological control, drying and storage management, appropriate physical or chemical interventions, and validated detoxification technologies. Plant-derived saponins are attractive research candidates because their amphiphilic structures can interact with fungal membrane sterols and impair cellular homeostasis. Nevertheless, a rigorous biochemical interpretation requires a clear distinction between established membrane-level antifungal activity and mechanisms that remain hypothetical for aflatoxin biosynthesis. In particular, direct inhibition of the aflatoxin PKS system, specific cytochrome P450 enzymes or aflR/aflD transcription should not be claimed without direct evidence. Future studies should therefore employ chemically characterized saponins, relevant A. flavus strains, simultaneous fungal-growth and aflatoxin endpoints, mechanistic assays, food-matrix validation and compound-specific safety evaluation. This framework would provide a sound basis for determining whether selected

plant-derived saponins can be developed into credible bio-based tools for management of aflatoxin-producing fungi in stored foods.

Authors' Contribution

E.I.O. contributed to the conceptualization, extensive literature search, data curation, and preparation of the initial manuscript draft. N.M.O. provided supervision, critical review, and intellectual input to enhance the content, quality, and structure of the manuscript. H.K.M. contributed to supervision, technical guidance, and critical revision of the manuscript. A.O.O. provided supervision and final proofreading. E.C.E and H.O.A. contributed to the refinement and critical review of the manuscript. All authors reviewed and approved the final version for submission and agreed to be accountable for all aspects of the work.

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