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REVIEW ARTICLE

A review on aflatoxins in oilseeds: Sustainable strategies for detoxification through physical and microbial approaches

K Nanthini[1], K Nelson Navamaniraj[2]*, V Manonmani[1], S Marimuthu[3], P Masilamani[4] & Vinoth Kumar Muniyappan[1]

[1]Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[2]Office of Controllerate of Examinations, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[3]Centre for Agricultural Nanotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[4]Agricultural Engineering College and Research Institute, Kumulur, Trichy 621 712, Tamil Nadu, India

*Correspondence email - nelsonnavamaniraj@tnau.ac.in

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Abstract

Aflatoxins (AFs) are highly toxic and carcinogenic secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, posing significant risks to food safety and public health. These mycotoxins commonly contaminate oilseeds such as peanuts, sunflower, cotton and maize seeds, particularly under warm and humid conditions. Controlling and reducing aflatoxin levels in these products is critical to ensuring food safety and complying with international regulatory standards. This review provides a comprehensive overview of current strategies for aflatoxin degradation in oilseeds, with a particular emphasis on physical and microbial approaches. Physical methods including thermal treatment, ammoniation, irradiation and advanced radiation techniques have been explored for their effectiveness in reducing aflatoxin levels. Microbial strategies involving specific bacteria, fungi and their enzymatic systems offer environmentally friendly and biologically based solutions for detoxification. The review critically examines the efficacy, mechanisms of action and limitations of each approach, highlighting the key factors that influence their success in practical applications. Moreover, it discusses the advantages and challenges associated with integrating these methods into existing food processing systems, considering factors such as cost-effectiveness, preservation of nutritional quality, regulatory acceptance and scalability. Finally, the review identifies key areas for future research, emphasizing the need to develop more efficient, sustainable and industrially viable technologies for large-scale aflatoxin decontamination in oilseeds. These advancements are essential for enhancing global food safety, protecting public health and supporting international trade.

Keywords: aflatoxins; biological degradation; microbial degradation; oilseeds; physical degradation

Introduction

Oilseeds play a crucial role in global agriculture as a source of edible oils, protein-rich meal and bio-based industrial products. Their economic and nutritional significance makes them a vital component of food and energy security, particularly in developing countries. Among the leading oilseed crops worldwide, soybean, groundnut, rapeseed-mustard, sunflower and sesame are widely cultivated due to their adaptability and diverse uses (1).

In the Indian context, oilseeds are a cornerstone of the agricultural economy, contributing significantly to rural livelihoods and the edible oil industry. During 2017-2018, oilseeds occupied 15.7 % of the total arable land, reflecting their agricultural importance. Among the nine major annual oilseed crops, groundnut, soybean, rapeseed-mustard and sesame together accounted for 89.2 % to 94.4 % of the total cultivated area and 90.4 % to 95.0 % of the total production between 2012-13 and 2018-19 (2). Over the long term, from 1950-51 to 2018-19, India witnessed a substantial transformation in oilseed production. The area under oilseeds expanded by 131.1 %, production increased by 510.9 % and productivity improved by 164.2 % (3). These gains have been driven by the adoption of high-yielding varieties, improved agronomic practices and policy support under initiatives such as the Technology Mission on Oilseeds (TMO).

Despite being one of the largest producers, India still faces challenges in meeting its domestic edible oil demand and ensuring quality standards for export. Contaminants such as AFs in oilseeds like groundnut and maize pose a serious threat to food safety and trade. Therefore, enhancing productivity while ensuring safe and contamination-free oilseed production is critical for the sector's sustainable growth and global competitiveness.

Crop productivity can be significantly enhanced through the use of healthy, high-quality seeds. The physiological and pathological quality of seeds plays a vital role in determining their performance, as seeds are known to act as carriers of plant pathogens. According to studies, infected seeds can not only reduce germination and vigour but also serve as a primary source of disease transmission in the field. Pathogens may be present either on the seed surface or internally and can affect plants before or after germination. Ensuring seed health through proper testing and treatment is therefore essential for sustainable crop production and effective disease management (4-6).

Among seed-borne pathogens, aflatoxin-producing fungi are of major concern due to their toxic and carcinogenic effects. Agents that can be detected inside or outside of seeds are known as seed-borne pathogens, which can cause serious plant diseases and also contribute to post-harvest contamination. AFs, primarily produced by *A. flavus* and *A. parasiticus*, pose a significant threat to food safety. Many crops, including oilseeds, spices, cereals and tree nuts are susceptible to AFs contamination. Therefore, the development of efficient methods for AFs degradation is essential. A variety of physical, chemical and biological methods have been investigated to reduce AF levels. Physical methods, though often time-consuming, include heating, radiation and the use of absorbents to bind toxins (7-9). While the primary focus of this review is on physical and microbial detoxification, chemical methods are included to provide a comprehensive perspective, given their established efficacy and widespread industrial use. Chemical detoxification has shown great promise in breaking down or changing AFs into less harmful substances, despite its somewhat contentious nature. To detoxify aflatoxin B_1 (AFB_1) in oilseeds like groundnut and maize, for e.g. ammoniation that has been approved in number of countries including USA and parts of Africa can effectively transform it into less toxic or nontoxic products such as aflatoxin D_1 (AFD_1) (10). Chemical treatments such as formaldehyde, ammoniation and calcium hydroxide have shown promise in detoxifying contaminated products (11). These interventions, when applied strategically and safely, can reduce the AF burden and help preserve both seed health and food safety.

The two most dangerous AFs that contaminate crops like peanuts, cereals, spices and tree nuts are AFB_1 and AFB_2 (aflatoxin B_2). The most favourable conditions for AF-producing fungi to infect peanut oilseeds typically occur in hot and humid regions, which are common in major peanut-growing areas (12). As secondary metabolites, *A. flavus* and *A. parasiticus* produce AFs that are highly toxic, carcinogenic, mutagenic, immunosuppressive and teratogenic in nature (13). In groundnut, AF contamination can result from infection by *A. flavus* and *A. parasiticus* and may occur in the pods and seeds at any stage as before harvest, during harvest and post-harvest (14). This contamination renders the groundnut unfit for both human consumption and commercial sale due to health risks and failure to meet food safety standards (15).

Aflatoxin especially Aflatoxin B_1 represent highly toxic and cancer-causing secondary metabolites that primarily develop from two *Aspergillus* species such as *A. flavus* and *A. parasiticus* (16). The mycotoxins affect oilseeds including peanuts, sunflower seeds, cottonseed and sesame seeds for endangering food security, global trade activities and human health (17, 18). AF detection in oilseeds is a major international concern, particularly because these crops are predominantly cultivated in tropical regions where storage conditions are often inadequate, especially in developing countries. A useful post-harvest method for reducing AF contamination is the use of hermetic storage technologies such as Purdue Improved Crop Storage bags (PICS), which restrict moisture and oxygen to prevent fungal development and creation of AF (19). Complete prevention of AF contamination is challenging, which is why increasing attention is being given to post-harvest degradation strategies. These strategies are often employed as critical control points within the food supply chain to minimize AF levels and reduce associated health risks (20, 21).

Researchers have developed various physical and microbial degradation methods to detoxify AFs in oilseeds, many of which are considered safe and environmentally friendly (22). The breakdown of AFs by whole microbial cells (fungi, yeasts, bacteria) is referred to as microbial degradation. This occurs frequently as result of metabolic activity of cells during development or fermentation (23). Any biological activity that results in breakdown of AF, including microbial and enzymatic processes referred to as biodegradation (24). The breakdown of AFs by extracellular or isolated enzymes, either purified or produced by microorganisms is specifically known as enzymatic detoxification (25). Among the physical approaches, thermal processing, irradiation and adsorption are widely used. These methods reduce AF concentrations by applying heat, light radiation or binding agents that inactivate or remove the toxins from the contaminated matrix (26). The effectiveness, simplicity and scalability of these physical methods can vary depending on several factors, including the composition of the food matrix and environmental conditions during processing (27).

The microbial degradation process employs specific bacteria, fungi and yeasts that utilize enzymatic pathways to convert AFs into non-toxic degradation products including aflatoxicol (AFL), aflatoxin B_{2a}(AFB_{2a}), AFD_1 and AFQ_1. Laboratory studies have demonstrated that oxidative enzymes produced by microorganisms such as *Bacillus subtilis*, *Trichoderma* spp. and *Saccharomyces cerevisiae* are capable of breaking down AFs through the activities of laccase, peroxidase, AF oxidase, cytochorome P450 monooxygenases and esterase enzymes (27-30). These biotransformation methods, when applied to oilseeds, offer environmentally friendly detoxification while preserving the nutritional quality of the seeds, without causing undesirable changes (31). Despite their potential, the industrial adoption of physical and microbial detoxification methods faces several challenges. These include issues related to process standardization, cost-effectiveness, regulatory approval and scalability, which continue to hinder widespread implementation in commercial food systems (32).

Physical treatments often pose challenges for complete AF detoxification and may negatively impact the quality and nutritional value of oilseeds, especially when high temperatures are applied for extended periods (33). Additionally, some adsorbents may bind essential nutrients during processing or fail to effectively degrade AFs, potentially resulting in the formation of toxic residues. Microbial-based solutions offer targeted and eco-friendly detoxification, but their industrial application faces several limitations. These include the specific growth requirements of microorganisms, the need for prolonged processing times and concerns regarding the safety, consistency and reliability of microbial byproducts (34). Furthermore, regulatory approval and public acceptance of food products derived from microbial interventions require more extensive research, particularly in the areas of standardization, risk assessment and compliance with food safety norms. Given these constraints, there is a clear need for the development of more efficient, scalable and safe degradation technologies for managing AF contamination in oilseeds (32-34). The categorization of AF degradation strategies such as physical, chemical and biological methods is illustrated in Fig. 1.

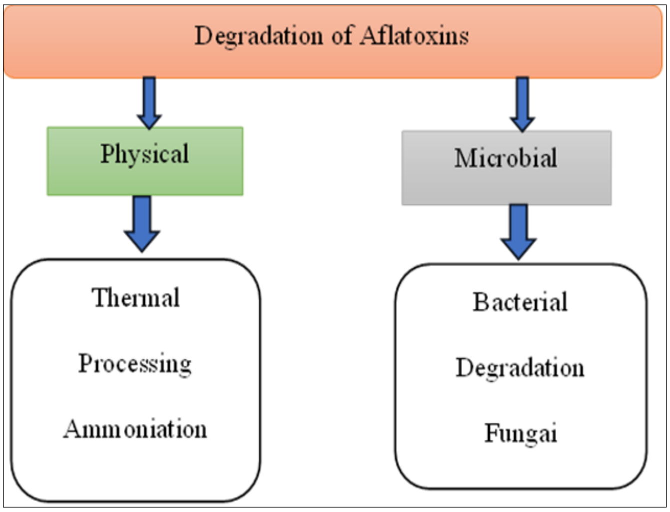

Fig. 1. Categorization of AF degradation strategies.

Physical degradation of AFs

In this study, physical degradation of AFs in oilseeds is explored through methods such as thermal processing, ammoniation, irradiation and radiation. These techniques aim to reduce toxin levels by breaking down AFs or removing them using heat, radiation or binding agents (Table 1 & 2).

Thermal processing of AF-contaminated seeds

Solar heat treatment has shown promise as a sustainable thermal processing method for AF degradation in oil-bearing crops. For instance, treating coconut oil using a parabolic solar concentrator for 10 -30 min resulted in 100 % removal of total AFs (16). This method is environmentally friendly, energy-efficient and economically advantageous. However, its effectiveness is highly dependent on favourable weather conditions and further technological refinement is necessary for industrial-scale applications. In another approach, heat-treated microorganisms have been evaluated for their efficacy in AF removal. The effect of thermal processing on *S. cerevisiae* and *Lactobacillus rhamnosus* GG in removing AFB_1. The heat-treated *L. rhamnosus* GG exhibited a high toxin adsorption capacity of 97.74 % (17). Heat treatment enhanced the AF-binding ability and thermal stability of microbial cells. However, one limitation noted was that excessive heating might increase AF formation under certain conditions.

Roasting is another widely applied thermal detoxification technique. For example, roasting peanuts at 150 °C for 40 min led to reductions of 37.9 %, 39.8 %, 37.4 % and 40.4 % in AFs B_1, B_2, G_1 and G_2 respectively, when the initial

Table 1. Critical factors influencing the efficiency of AF degradation methods

Method	Key factors affecting efficiency
Concentrated solar radiation	Radiation intensity and temperature control
Digestion & thermal processing	Heat treatment and stability of AFB_1 complex
X-ray irradiation	Irradiation dose and time of exposure
Gamma radiation	Radiation dose and precision control
Ammoniation	Ammonia concentration and handling safety
Ammonia-treated diets for livestock	Ammonia concentration and diet composition
Biodegradation	Fungal activity and AF concentration
Lignolytic fungi	Fungal species and environmental conditions
Recombinant oxidase	Specific crop and oxidase activity
S. acidaminiphila & BSF larvae	Substrate type and microbial/larval interaction
Enzymes from *Trichoderma* spp.	Enzyme activity and environmental control
Black seed oil	Oil composition and food matrix
Physical & chemical methods	Method choice, treatment conditions and AF type

Table 2. Comparative overview of physical and microbial AF detoxification strategies and their applications

Methods	Objectives	Applications across domains	Reference
Concentrated solar radiation	Reduction of AF contamination in coconut oil	Food safety in coconut oil production	(16)
Thermal processing and digestion analysis	The effect of thermal processing on AFB_1 stability	Food industry - safe cooking processes	(17)
X-ray irradiation	Degradation of AFB1 through X-ray exposure	Post-harvest food sterilization	(18)
Gamma radiation	Preserve hemp flour and inactivate AF	Grain and spice storage treatments	(20)
Ammoniation treatment	Detoxification of groundnut press cake to remove AFs	Animal feed detoxification	(32)
Ammonia treatment in diet	Evaluate the impact of ammonia-treated AF-contaminated diets in sheep	Livestock industry feed safety	(57)
Biodegradation using *T. harzianum* AYM3	Reduce AFB1 in maize grains using endophytic fungi	Bioremediation of crops	(46)
Lignolytic phenoloxidase from *T. hirsuta*	The biodegradation of AFB_1	Agricultural waste biotreatment	(58)
Recombinant oxidase from *A. tabescens*	Biodegradation of AFB1 in rice and peanut seeds	Targeted enzymatic detoxification	(22)
S. acidaminiphila combined with BSF larvae	AFB1 degradation in a novel combination method	Waste management, feed production	(58)
Trichoderma enzymes	Evaluate *Trichoderma* enzymes for AFB_1 and ochratoxin A degradation	Industrial enzyme detoxification	(27)
Black seed oil detoxification process	Detoxification of AFs in spices using black seed oil	Spice detoxification in food industry	(28)
Physical and chemical methods (e.g. heat, UV light)	Reduction of AFs in food and feed	Food regulation, technology development	(21)
HPTLC	Assessment of total AF in fish feed and feedstuffs	Fish feed safety, quality control in animal feed production	(29)
Ultraviolet-LED cold-light irradiation	Degradation of AFB_1 in peanut oil	Food safety, oil industry	(74)
Enzymatic extract from *Pleurotus eryngii*	Degradation of AFB1 using sustainable enzymatic extract	Bioremediation, food industry	(80)
Gamma-ray irradiation	Inactivation of AFs in almonds	Food preservation, grain storage	(46)
Gamma radiation	Reduction of ZEA in grains	Grain safety, food processing	(47)
Green antioxidants	Reduce AFs in peanut oil seeds during storage	Food industry, agriculture	(89)
Physicochemical analysis	Evaluation of AF levels in industrial and non-industrial sesame oil	Food industry, oil production	(73)

AF concentration was 10 ng/g (35). Similarly, when peanuts contaminated with AFs (initial level: 85 µg/kg) were roasted at 160 °C, 180 °C and 200 °C for 5 to 25 min, AF levels were reduced by 61.6 %, 83.6 % and 89.7 % respectively (36). Mild heat treatment combined with gaseous ozonation has also proven effective. In peanut kernels, ozonation at 75 °C for 10 min resulted in 77 % degradation of AFB_1 and 80 % degradation of aflatoxin G_1 (AFG_1) (37). Another emerging technique is microwave heating, which offers benefits such as rapid drying, reduced energy consumption and minimal quality loss. For example, artificially contaminated peanuts treated with microwave radiation at 360 W, 480 W and 600 W showed AF degradation ranging from 59 % to 67 % (38).

Chemical detoxification of AFs using ammoniation

Ammoniation, a chemical treatment process that utilizes ammonia gas, has been investigated for the detoxification of AFs in groundnut press cake. According to studies, ammoniation can successfully bring AF levels, especially AFB_1 down to safe levels when used under carefully monitored circumstances. These conditions include maintaining ideal moisture content (12 % - 16 %), temperature (80 °C - 100 °C) and exposure time (30 - 60 min) followed by sufficient aeration to eliminate any remaining ammonia (39). Due to its efficiency and scalability, this method is already employed in some regions for the detoxification of animal feed. However, several limitations hinder its broader application in the human food sector, particularly within the European Union (EU).

Framework for regulation of AF detoxification in oilseeds

AF contaminated oilseeds detoxification particularly using chemical, physical or microbiological methods, present serious regulatory issues. While only a handful specifically address detoxification procedures, numerous international organizations and national authorities have established standards or maximum allowable limits for AFs in food and feed products.

Codex Alimentarius (WHO/FAO)

The Codex Alimentarius Commission establishes global guidelines for food safety. There are currently no approved chemical detoxification procedures for AFs in human-consumed food, despite the fact it sets maximum limits for AFs (e.g. 10 - 15 µg/kg for total AFs in peanuts). Instead, Codex places a strong emphasis on good agricultural practices and preventive measures (40).

The European Union

Strict EU regulations forbid the use of chemical detoxification methods for food. Commission Regulation (EU) No. 2015/786 on other hand, establishes standards for authorized detoxification techniques for animal feed, including controlled microbiological and physical processes. Before being approved for sale, detoxified feed ingredients must be evaluated for efficacy and safety (41).

The Food and Drug Administration (FDA) in the United States (US)

Chemical detoxification of AFs in human food has not been approved by FDA in US. Ammoniation has however, been provisionally permitted for AF contaminated feed under certain conditions, especially for maize and cottonseed used for animal consumption. For safety, the FDA mandates appropriate record keeping and residue analysis (42).

African nations

Following the guidelines of the EU and Codex Alimentarius, many African nations have adopted regional regulations specifying maximum permissible levels of AFs in food commodities. Under its Bureau of Standards, Kenya has instituted AF regulation, but Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) mandates that all detoxified food and feed undergo a pre-market safety evaluation (43).

These include concerns about nutritional degradation, the potential formation of harmful byproducts and low consumer acceptance. As a result, while ammoniation remains a viable option for feed-grade detoxification, its adoption for food-grade oilseeds is limited and subject to stringent regulatory oversight.

Irradiation-based decontamination of AFs

X-ray irradiation has emerged as a promising method for the degradation of AFB_1. In this process, electrons accelerated by high voltage strike an anode metal target, leading to energy loss and deceleration. While most of the energy is dissipated as heat, a portion is emitted as X-ray photons (44). X-ray irradiation at a dose of 10 kGy achieved an AFB_1 degradation rate of up to 81 %. AFB_1 samples with initial concentrations of 5, 10 and 20 µg/mL showed degradation rates of 81 %, 77 % and 38 % respectively, demonstrating dose-dependent efficacy (45).

AFB_1, B_2, G_1, G_2 have also been successfully broken down by gamma ray irradiation in solution and in spiked almonds, however research has indicated that high doses may alter sensory characteristics like texture, flavour, colour depending on product type and irradiation level. The treatment led to significant reductions in toxin levels and microbial populations (46). The key advantages of gamma irradiation include high effectiveness, microbial control and extension of product shelf life. However, potential drawbacks include alterations in food quality and regulatory restrictions, especially in products intended for human consumption. In another application, hemp flour exposed to gamma radiation showed successful decontamination. A dose of 4 kGy effectively reduced aflatoxin levels, while 8 kGy eliminated both molds and microorganisms. Additionally, 3 kGy was sufficient for complete bacterial elimination. Importantly, the treatment preserved the nutritional value of hemp flour, making it a viable method for safe decontamination. However, precise dosage control is essential, as different contaminants require varying dose levels for effective removal (20).

Gamma irradiation has also been successfully applied to Zearalenone (ZEA) decontamination in grains. Using High-Performance Liquid Chromatography (HPLC), ZEA residues were measured in grain samples collected from Egyptian markets. ZEA was successfully removed by gamma doses between 5 and 20 kGy without negatively impacting the grain's nutritional value, as shown by the negligible changes in protein, crude fat, carbohydrate and vital minerals levels (46). Despite its effectiveness, the cost of equipment, facility setup and strict process management are important considerations when scaling this method (47). In peanuts, one study reported an initial AFB_1 level of 158.68 µg/kg at 16 % moisture content, which was reduced by 96 % following a 45 min gamma irradiation treatment (48). These findings reinforce irradiation technologies, both X-ray and gamma as viable and powerful tools for AF mitigation, provided they are implemented with regulatory compliance and process precision.

Pulsed light (PL) treatment for AF degradation

PL treatment is emerging as a promising non-thermal method for the removal of spoilage organisms and chemical contaminants, including AFs. This technique works by delivering a sequence of short, high-intensity light pulses, which can effectively inactivate bacteria, yeasts, molds and even viruses. In PL systems, a gas-discharge lamp releases electricity in a fraction of a sec, storing the energy in a capacitor. A xenon lamp is commonly used as the light source due to its ability to produce broad-spectrum radiation ranging from ultraviolet to near-infrared (200—1100 nm). Food products subjected to PL experience a combination of photophysical, photochemical and photothermal effects, which contribute to the degradation of microbial and chemical contaminants (49). In groundnut, studies have demonstrated the efficacy of PL treatment for AF reduction. A previous study reported degradation rates of 82 % in peanuts with skin and 91 % in peanuts without skin (50).

Microbial degradation of AFs in oilseeds

In this study, microbial degradation of AFs in oilseeds is explored through the application of specific bacteria, fungi and microbial enzymes that are capable of transforming AFs into less toxic or non-toxic compounds. One particularly promising strategy is microbial detoxification technology, which includes both adsorption and enzymatic biodegradation.

One biological mechanism for AF reduction is microbial adsorption, wherein microbial cells bind to AFs and form stable complexes. However, this process is reversible and influenced by factors such as temperature and cell concentration, making optimization essential (51). Over the past few decades, various microorganisms isolated from different environments have demonstrated efficiency in AF removal, including *B. subtilis*, *S. cerevisiae*, *Enterococcus faecium* and other *Bacillus* species (52). In addition to adsorption, enzyme-mediated degradation is another effective biological approach. Enzymes and secondary metabolites produced by microorganisms such as *Staphylococcus warneri*, *Mycobacterium fluoranthenivorans* and *Trametes versicolor* have shown the ability to degrade AFB_1 (53). Comparative overview of physical, microbial and chemical detoxification is shown in Table 3.

Different fungal strains also exhibit varying AFB_1 degradation capacities. Notably, *Aspergillus niger*, *A. parasiticus*, *Trichoderma viride*, *Mucor ambiguus* and others have been reported to significantly reduce AFB_1 levels under controlled conditions (53). Additionally, several microorganisms including actinomycetes, bacteria, yeasts and non-toxigenic strains of *Aspergillus* can inhibit the growth of toxigenic *Aspergillus* species and suppress AF production (54). Fermentative bacteria are considered excellent candidates for biological detoxification because of their ability to degrade various chemical bonds in mycotoxin structures using enzymes (55). This enzyme-mediated breakdown of mycotoxins, often referred to as biological detoxification, presents a viable alternative to conventional chemical or physical methods.

For e.g. *Trichoderma* spp. produces a variety of defence related enzymes including phenylalanine ammonia-lyase, β-1,3-glucanase, peroxidase and chitinase. These enzymes are essential for the breakdown of mycotoxin and inhibition of fungal infections in addition to improving microbial survival in stressful environments (56). Comparative analysis of the microorganism's efficacy, safety and industrial feasibility are shown in Table 4.

Fungal biodegradation of AFs

Table 3. Comparative overview of physical, microbial and chemical detoxification

Detoxification method	Effectiveness	Safety	Cost	Scalability	Regulatory status	Nutritionl impact	Reference
Physical	Moderate (40 %-80 %)	High	Low-moderate	High	Verified in numerous areas	Minimal: heat may deplete certain nutrients	(93)
Chemical	High (up to 99 %)	Moderate	Low-High	Moderate	Approved for feed; limited food use	Possible loss of proteins/ vitamins	(94)
Microbial	Variable-High	High	Low-Moderate	Low-Moderate	Not yet approved	Minimal: may enhance probiotic value	(95)

Table 4. Comparative analysis of the microorganism's efficacy, safety and industrial feasibility

Microorganisms	Mechanism	AF reduction	Safety profile	Industrial aspects	Reference
S. cerevisiae	AF binds physically to the cell wall through adsorption, involving attachment rather than actual breakdown or degradation	The binding efficiency ranges from 60 %- 85 %, contingent on cell concentration, pH, strain	Widely used for probiotics, baking and brewing	It is highly effective, easily adaptable to processing systems and already in industrial use	(96)
T. versicolor	Produces the laccases, peroxidases that break down AFB_1 enzymatically into harmless byproducts	In liquid cultures, deterioration can reach 95 %	Biotechnologically safe and not harmful	Moderate: needs systems for enzyme extraction	(97)
B. subtilis	It exhibits adsorption through cell wall components and produces oxidoreductase enzymes that are capable of degrading aflatoxins	90 % reduction under ideal circumstances	Widely used in feed industries	Stability in formulations	(98)
A. niger	Releases enzymes that can hydrolyze and oxidize AFB_1	Degradation under controlled lab ranges from 80 %-90 %	Ochratoxins may be produced by some strains although others have mixed safety record with GRAS certification	Moderate: Needs a risk assessment tailored to strain	(99)
E. faecium	AF is bound by components of cell surface; enzymatic degradation potential is restricted	There has been reported 70 % drop in *in vitro* experiments	Certain strains are opportunistic infections	Moderate: requires safety testing prior to use in food and feed	(100)
T. viride	Utilizing hydrolases and peroxidases, enzyme mediated biodegradation involves some competitive exclusion	AFB_1 reduction of 75 %- 85 % were observed	Usually nonpathogenic, it is regarded as safe for biocontrol	High: often used as biopesticides and in agriculture	(101)

Fungi have shown great potential in the biodegradation of AFB_1 through both direct enzymatic activity and gene-level suppression of toxin biosynthesis pathways. *Trichoderma harzianum* AYM3, an endophytic fungus isolated from maize, has demonstrated significant biodegradation potential. When applied to AFB_1-contaminated maize grains, the fungus not only significantly reduced AFB_1 content but also suppressed the expression of key genes involved in AF biosynthesis. This method is environmentally friendly, safe for food and feed applications and exhibits dual functionality, both detoxification and gene inhibition. However, its effectiveness can vary depending on crop type and environmental conditions. In another study, laccase, a ligninolytic phenol oxidase enzyme from the white-rot fungus *Trametes hirsuta*, degraded AFB_1 (57). The enzyme was effective in both model systems and actual food matrices, converting AFB_1 into non-toxic derivatives via oxidative breakdown. This enzymatic method is efficient, has a broad substrate range and is highly suitable for environmental and food safety applications. However, limitations include the sensitivity of laccase to environmental factors and the high cost associated with enzyme production and purification (58).

Post-harvest biodegradation of AFB_1 in rice and peanut seeds was achieved using a recombinant oxidase enzyme derived from *Armillaria tabescens*. The study demonstrated that the enzyme efficiently degraded AFB_1 under storage conditions, making it a promising post-harvest detoxification tool. Strengths include high specificity, effectiveness in storage environments and applicability to widely consumed crops. However, challenges remain in the form of complex recombinant enzyme production, cost constraints and the need for validation at commercial scales.

Additionally, the cell-free supernatant of *Pantoea* sp. achieved 68.3 % degradation of AFB_1, demonstrating the potential of microbial metabolites in AF detoxification (59). Further highlighted the synergistic effect of co-culturing *Pleurotus ostreatus* and *A. niger*, which achieved a maximum AFB_1 degradation of 93.4 %. Individually, *P. ostreatus* and *A. niger* improved degradation by 65.9 % and 37.6 % respectively, suggesting enhanced performance through microbial combinations (60).

Bacterial degradation of AFs

Bacteria play a significant role in the biodegradation of AFB_1 through enzymatic activity and microbial metabolism (61). An integrated biological system involving *Stenotrophomonas acidaminiphila* and black soldier fly (BSF) larvae showed efficient degradation of AFB_1 and its combination with BSF larvae further enhanced the detoxification process. This synergistic approach has potential applications in feed bioconversion systems, offering an eco-friendly, cost-effective and sustainable strategy for managing agricultural waste and contaminated feed. However, variability in microbial activity, the need for optimized environmental conditions and regulatory concerns related to insect-based feed remain significant challenges. Another study reported that *Bacillus amyloliquefaciens* WF2020 could effectively degrade AFB_1 in the concentration range of 1 to 8 µg/mL (62). Similarly, *Bacillus licheniformis* (BL010) reduced AFB_1 levels by 89.1 % and after three incubation cycles, its crude enzyme solution achieved a maximum degradation of 97.3 %. A number of additional bacterial strains have also been found to be effective aflatoxin degraders. *E. faecium* HB2-2 and *Myroides odoratimimus* strain have both shown significant degrading activity. The reductions of AFB_1, AFB_2, AFM_1 by *Pseudomonas aeruginosa* N17-1 were 82.8 %, 46.8 % and 31.9 %. Similarly, it was found that 89.5 % of AFB_1 was degraded by *Lactobacillus plantarum*. *Bacillus velezensis* DY3108 reduced AFB_1 levels by 91.5 % (63-67).

Enzymatic degradation of AFs

Enzymes from *Trichoderma* spp. have been applied to degrade AFB_1 and ochratoxin A, two of the most toxic mycotoxins affecting food and feed safety. This study proved that certain extracellular enzymes, such as oxidoreductases and hydrolases from *Trichoderma*, could efficiently degrade the toxins under gentle conditions. This enzyme degradation process has the advantages of high specificity, environmental safety and use in food systems without generating toxic residues. However, efficacy depends on the purity and stability of the enzymes and scaling up for industrial applications remains challenging. Generally, the study supports using enzymes in detoxification as a valid and natural solution for mycotoxin control (26).

AFs: Characteristics and Impacts

Chemical structure and properties

The chemical nature of AFs, particularly the difuran ring structure of AFB_1, contributes to its exceptional thermal stability. This makes it highly persistent in food and feed systems. This structural resilience necessitates rigorous physical or chemical treatments for effective breakdown (28). A clear understanding of the molecular structure enables the development of targeted detoxification strategies such as ozonation and acidification, which act on specific chemical bonds. However, due to the chemical stability of aflatoxin, such methods often require harsh processing conditions that may compromise the nutritional quality and safety of treated products. The occurrence of AFB_1, AFB_2, AFG_1 and AFG_2 in fish feed has been studied using High-Performance Thin Layer Chromatography (HPTLC), which relies on the chemical stability and fluorescence characteristics of AFs (29). The difuran ring structure in aflatoxin allows detection via UV-induced fluorescence without prior breakdown. Additionally, structural variations such as the C8 - C9 bond saturation in B_2 and G_2 enable clear differentiation between AF types. While HPTLC is a high-throughput, cost-effective screening tool, it does not neutralize or degrade AFs. The remarkable chemical stability of AFB_1 continues to hinder effective detoxification, as reflected in its intricate molecular structure and the structural variations observed among different aflatoxins (AFs). These classifications and distinct chemical configurations underline the resilience of AFs against degradation by conventional physical and chemical methods in Fig. 2.

Toxicity and health effects

Thermal treatment may influence aflatoxin stability; however, prolonged exposure can deteriorate food quality (68). X-ray irradiation has also been investigated for AF degradation, though cost and radiation safety remain concerns (69). Gamma radiation has proven effective in inactivating AFs in hemp flour, but requires specialized equipment and facilities. Ammoniation is another detoxification process widely used for animal feed, although its efficacy depends heavily on processing conditions. While ammonia treatment has shown potential for improving livestock health, its long-term effects on feed quality need further

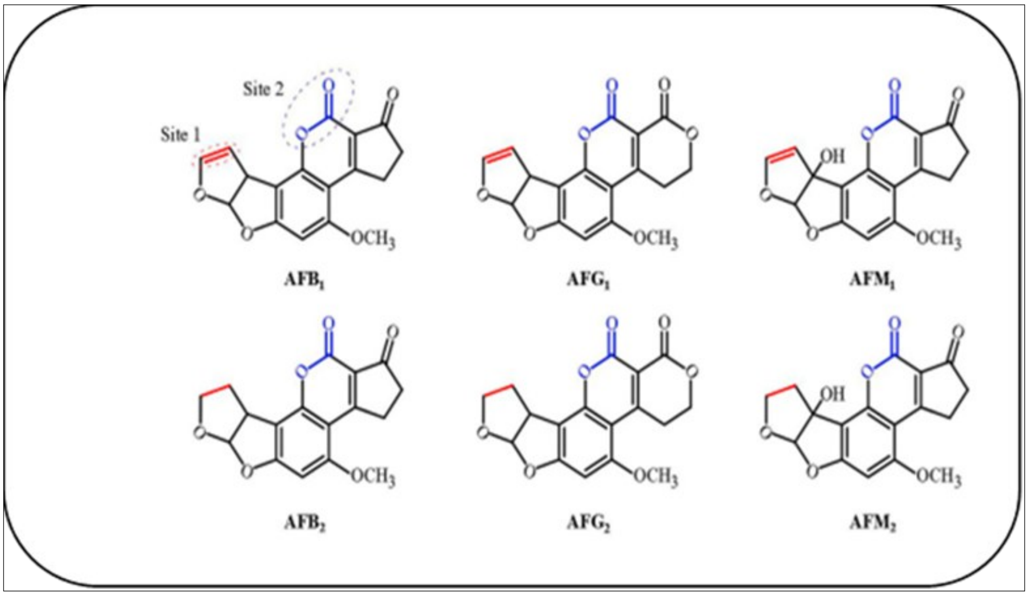

Fig. 2. Chemical structures and classification of major AFs.

evaluation (70). The use of phenol oxidase enzymes to degrade AFs has shown promise, but its effectiveness is limited to specific food matrices. Similarly, the recombinant oxidase enzyme from *A. tabescens* can degrade AFs efficiently, but issues related to cost and potential allergenicity must be addressed.

Microbial degradation provides another promising route, although environmental concerns and ethical considerations, such as the use of insect larvae, may hinder widespread adoption (71). Enzymatic detoxification using *Trichoderma*-derived enzymes is also promising, but possible allergenic responses require caution. Natural products like black seed oil have been found to detoxify AFs in spices, though scaling up this approach for industrial application remains a challenge (72). Finally, physical and chemical treatments such as heat treatment and adsorption can reduce AF levels but tend to be energy-intensive and may alter the sensory and nutritional qualities of food products (73).

Factors affecting AF degradation efficiency

The efficiency of aflatoxin degradation is influenced by multiple factors, including treatment intensity, method type, food matrix and processing conditions. For example, concentrated solar radiation has proven effective in reducing AFs in coconut oil; increased radiation intensity and controlled temperature enhance degradation efficiency. However, overexposure may deteriorate the quality of the oil, presenting a key limitation (74). Digestion and heat treatment also play a significant role, particularly affecting the stability of the AFB_1 complex. While thermal processes offer rapid degradation, they may also reduce the nutritional value of the treated food or feed (75).

X-ray irradiation has been shown to degrade AFB_1 effectively, with higher doses resulting in better degradation rates. However, excessive irradiation can damage food quality, limiting its use to specific applications. Similarly, gamma radiation is another potent inactivation method, but it requires sophisticated equipment and precise dose control to avoid compromising food safety and quality (76). Ammoniation has been applied for detoxifying AFs in groundnut press cake, though challenges remain in handling ammonia safely and avoiding chemical residues in the final product. While ammonia-treated feed has shown benefits for animal health, the process can alter the nutritional composition, which is a notable drawback (77).

Biodegradation using endophytic fungi offers an eco-friendly solution, but its effectiveness decreases at higher aflatoxin concentrations. Lignolytic fungi such as *Trametes* species spp. can degrade aflatoxin using oxidative enzymes, although the process is slow and not suitable for large-scale industrial use (78). The use of recombinant oxidase enzymes has shown promise in degrading AFB_1 in peanuts and rice, but its effectiveness is crop-specific and may not extend to other food systems. While enzymes have demonstrated excellent degradation efficiency in controlled environments, production costs and scalability issues remain key limitations for industrial application (79). As a low-cost biological alternative, black seed oil has been explored for detoxifying AFs in spices. However, its application is limited by processing challenges and variability across different food matrices (80).

In summary, the efficacy of AF degradation treatments is highly dependent on multiple factors, including the type of AF, the nature of the food or feed matrix and the treatment conditions. Although chemical treatments are generally effective, concerns regarding safety, toxicity and residual chemicals must be addressed, as summarized in Table 1.

Current trends in microbial degradation of AFs

Utilization of specific microbial strains

Various microorganisms such as *B. subtilis*, *Pseudomonas* spp. and *Rhodococcus erythropolis* can enzymatically degrade AFs into less harmful compounds, while fungi like *A. niger* and *Trichoderma* spp. reduce AF levels by breaking them down into smaller, less toxic molecules. In competitive exclusion, non-toxigenic strains of *A. flavus* can suppress the growth and toxin production of toxigenic strains. These microbes are being extensively studied for their detoxification potential and stability across various oilseed environments (81).

Enzymatic degradation

Enzymatic degradation involves the use of specific enzymes such as laccases, peroxidases and aflatoxin oxidases (AFOs) to break down AFs into non-toxic compounds. Researchers are actively purifying these enzymes from microorganisms and exploring their application in oilseed processing, with enzyme immobilization techniques enabling their reuse. This method offers high specificity, a low environmental footprint and minimal impact on the nutritional quality of oilseeds (82).

Probiotic and fermentation approaches

Probiotic-mediated degradation by strains such as *Lactobacillus* and *S. cerevisiae* offers an environmentally friendly approach to reduce AF levels, particularly in animal feed. These microorganisms can adsorb and degrade AFs during fermentation, while also enhancing the digestibility and nutritional quality of oilseed byproducts. This method shows promise for developing safer feed ingredients and potential applications in food-grade products (83).

Genetically Modified Microorganisms (GMMs)

GMMs are being developed to improve AF degradation by introducing genes for more effective enzymes. Such "super strains" are being tested for efficacy, safety and regulatory approval, with potential applications in the food and feed industries. Commercialization is restricted, but ongoing studies intend to develop microbial agents for safe and efficient AF removal in oilseed processing, storage and fermentation (84).

Challenges in physical degradation

Solar radiation used for thermal processing is highly dependent on weather conditions, which limits its reliability and scalability for industrial applications (85). A major drawback observed in heat-treated systems is the potential for microorganisms to release secondary toxins, even when adsorption capacities are improved (86). Likewise, while ammoniation has proven effective in reducing AF levels, especially in animal feed—its application in human food systems remains controversial. Nutritional alterations and the possible formation of toxic byproducts are notable concerns. As a result, regions such as the EU prohibit ammoniation as a food additive for human consumption, despite its successful use in livestock feed (39).

The adoption of irradiation techniques such as X-ray, UV-LED and gamma rays presents further challenges due to the need for sophisticated and costly equipment. These treatments, while effective in degrading AFs, may cause undesirable changes in food texture, taste and nutritional composition. Scaling up these methods for large-scale commercial use remains difficult because of the complexity and financial burden involved (87). Furthermore, gamma radiation treatments require highly accurate dosage control to maintain food quality while ensuring detoxification. Improper dosing can compromise both efficacy and product integrity. Despite its potential, widespread implementation of radiation technology is still constrained by high operational costs and the need for substantial infrastructure investment (88).

Challenges in microbial degradation

The effectiveness of fungal biodegradation methods is highly influenced by environmental parameters such as temperature, pH and humidity. These factors must be maintained at optimal levels for consistent results. However, fungal treatments often show variability in performance across different crops and environmental conditions, which limits their widespread adoption (89). Additionally, the high cost of enzyme production and purification presents a major barrier to the commercial application of enzymatic detoxification. In particular, recombinant enzyme technologies, while effective, remain economically unfeasible for large-scale post-harvest use due to their complex and costly production processes (90).

Another significant limitation lies in the application of combined biological systems. Maintaining synergistic activity between multiple microorganisms or enzymes requires precisely optimized conditions, which complicates their implementation in practical settings (91). Furthermore, the reliability of extracellular enzyme-mediated degradation of mycotoxins depends on factors such as enzyme purity and stability. These limitations make it challenging to develop robust enzymatic detoxification systems for industrial-scale use in food and feed processing (92). The comparative data supporting the discussed detoxification strategies and relevant studies cited exclusively in Table 3 & 4.

Future scope

1. Ensuring the safety of thermally processed food requires integrated systems capable of neutralizing microbial toxins generated during heat treatment. Affordable UV-LED or gamma irradiation devices can be introduced at small and medium scales, offering a viable solution for local food processors.
2. To improve fungal strains' environmental adaptability for more efficient detoxification, bioengineering breakthroughs are required. Strains with increased resistance to abiotic stresses and greater mycotoxin degrading ability can be engineered using tools like CRISPR-Cas9 for targeted gene editing, transcriptome profiling for stress response research and synthetic biology techniques for pathway optimization. Field validation of enzyme-based methods under diverse agro-ecological conditions is crucial to assess performance and cost -efficiency. Future research should focus on developing stable enzyme variants through protein engineering to ensure functionality across varying conditions.
3. Enzymatic detoxification in the food and feed industry can be made more commercially viable by developing straightforward recombinant expression systems such as yeast-based platforms or bacterial hosts like *Escherichia coli* for the production of enzymes like laccase and manganese peroxidase. Meanwhile, improving fermentation and bioprocessing technologies can lower the cost of producing enzymes.
4. Developing combined biological models under optimal conditions may unlock new detoxification synergies. Broader industrial adoption also requires harmonized regulatory frameworks, particularly for insect-based feed products and affordable purification strategies for extracellular enzymes such as those from *Trichoderma* spp. Integrating these biological methods into existing food and feed systems is essential for widespread application.

Conclusion

The field of aflatoxin degradation in oilseeds is rapidly advancing through the integration of innovative approaches such as solar radiation, thermal processing, gamma irradiation and microbial biodegradation. When combined with emerging technologies like recombinant enzyme applications, these strategies hold significant promise for enhancing food safety and promoting sustainable agricultural practices. However, several challenges remain particularly in terms of scalability, cost-effectiveness and the need for method optimization across various oilseed types and processing environments. Future research should prioritize improving the efficiency and field applicability of these techniques, exploring synergistic hybrid methods and minimizing their environmental impact. Continued innovation and interdisciplinary collaboration in aflatoxin detoxification will play a critical role in strengthening global food security, protecting public health and fostering environmentally responsible agricultural systems. The integration of cutting-edge techniques like solar radiation, thermal processing, gamma irradiation and microbial biodegradation is accelerating the field of AF degradation in oilseeds together with cutting edge technologies such as applications of recombinant enzymes and bioengineered microbial strains. However, despite promising results at the laboratory scale, significant challenges still persist.

- Varying effectiveness in various oilseeds matrices.
- Standardized protocols are lacking for commercial use.
- Byproducts may be harmful, necessitating careful safety testing

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Authors' contributions

KN and KNN developed the concept for this manuscript. KN carried out the literature review and wrote the initial draft. VM, SM, PM and VKM contributed essential feedback and revisions. KN and KNN finalized the manuscript. All authors reviewed and approved the final version of the manuscript.

Compliance with ethical standards

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