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# Mycotoxins Contaminating Nuts: A Review

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## Abstract

The main mycotoxins in nuts: (Aflatoxins): The most toxic and widespread, produced by the fungi *Aspergillus flavus* and A. parasiticus, and considered to be highly carcinogenic. Found in high levels in walnuts, pistachios, almonds, and hazelnuts. Ochratoxins: These pose a risk to the kidneys, nervous system, and immune system, and are found in almonds, Brazil nuts, and hazelnuts. Fumonisins: These are relatively rare in nuts, but their chronic toxicity is under study. Patulin: Its presence in nuts is limited, but it is the subject of regulatory discussions.

The most important sources and routes of contamination are: Pre-harvest: Climatic conditions (humidity, temperature) and genetic factors in the crop play a major role in fungal infection and toxin production. Post-harvest: Inadequate drying and storage in warm, humid conditions (relative humidity >50%) are critical factors that stimulate fungal growth and toxin production. Processing: Common roasting, baking, and frying processes do not significantly reduce aflatoxin levels or may be ineffective.

Several methods are used for detection and analysis: Traditional analytical methods include (HPLC), (LC-MS/MS), and (ELISA). Emerging technologies such as biosensors and hyperspectral imaging supported by artificial intelligence show great potential for rapid, field-based detection.

The review emphasizes that mycotoxin contamination of nuts is an ongoing global challenge that requires an integrated "farm-to-table" approach. Successfully controlling this problem depends on combining good agricultural practices, appropriate storage conditions, accurate detection methods, and effective mitigation strategies, all within a strict regulatory framework to ensure the safety of these highly nutritious foods.

**Keywords:** Mycotoxins, Nuts, Aflatoxins, Ochratoxins

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## 1. Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi that pose human health hazards and result in considerable financial losses by contaminating many crops, including nuts. Most mycotoxins contaminate nuts in the field or during drying and subsequent storage, and their presence in nuts is regulated in many countries. This review summarizes nut-specific mycotoxin risks and assesses methods to mitigate contamination from field to table. Specific mycotoxins of concern in nuts include aflatoxins, ochratoxins, fumonisins, patulin, and other toxins such as citrinin, ergot alkaloids, indole, and trichothecenes, which are more incidental but merit consideration. Both exposure studies and tolerable daily intake (TDI) estimates indicate that nut consumption contributes to mycotoxin exposure (1-3).

Nuts are important human food sources because of their flavour, sensory properties, and high

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nutritional values, including relatively high protein content, high levels of unsaturated fatty acids, vitamins, and dietary fibre, and low glycemic index (4). Nuts are used to manufacture confectionery products, bakery products, and nut-based foods such as nut butter, nut milk, nut oil, and plant-based alternative foods. Analysis of tree and oil-seed nuts emphasised the growing interest in hazelnut and peanut (5).

## 2. Mycotoxins of Concern in Nuts

Nuts, a colorful and highly nutritious food group, may harbor quantities of mycotoxins exceeding safety limits or recommendations. Amid the growing concern over food safety, a review of the mycotoxin contamination in nuts is warranted. Four families of mycotoxins, namely aflatoxins, ochratoxins, fumonisins, and patulin, are of foremost interest in nuts. Contamination can arise during field production or postharvest handling due to the involvement of specific molds. A thorough analysis of the particular mycotoxins of concern in nuts, including their structures, toxicological properties, occurrence, and related aspects, is detailed (6-8).

Mycotoxins are secondary metabolites produced by filamentous fungi whose global occurrence and associated health risks pose significant challenges. Mycotoxins of major concern in nuts are defined, accompanied by information on the human health hazards they present and the mold species responsible for contamination. Occurrence data indicating the relevance of each mycotoxin in nuts are also provided, supported by adequate references (5,9).

### 2.1. Aflatoxins

Aflatoxins are at the heart of efforts among the global scientific and agricultural community to ensure that the vast variety of nuts distributed widely remain safe for human and animal consumption. Aflatoxins represent a relevant risk in some nuts, and as such merit greater attention than the majority of mycotoxins linked with these commodities. These agents are of concern due to the combination of their serious and extensive adverse effects, as well as their regulatory and monitoring importance. Walnuts in particular show high levels of aflatoxin contamination, while others, such as almonds, pistachios, or hazelnuts, also come under the spotlight (10).

Aflatoxins represent a group of closely related metabolites produced mainly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* in food and animal feeds, with fungi of the section Flavi of the genus Aspergillus being responsible for their production (11). The structural basis for the potency of aflatoxins is the product of a long evolutionary relationship between the toxin and other living organisms, highlighting the importance of mycotoxin regulation and control to safeguard human life, health, and well-being, thereby continuing food production and cultivation of agricultural commodities (12). The wide range of nuts consumed, cultivated, and traded worldwide reflects the extensive and diverse economic and agricultural activities associated with the wide variety of nut varieties (5).

### 2.2. Ochratoxins

Ochratoxins are mycotoxins associated with molds in nuts and seeds. Commonly abbreviated as OA, OAc, and OAm, these compounds are detected in almonds, brazil nuts, hazelnuts, macadamia nuts, pistachios, peanut butter, and various dried fruits, often co-occurring with other mycotoxins. In the human body, ochratoxins found in nuts pose risks for the kidneys, nervous system, immune system, reproductive organs, liver, stomach, and intestines (9).

### 2.3. Fumonisins

Fumonisins are mycotoxins produced by certain Fusarium fungi found predominantly in corn and maize-based products (13). Contamination of nuts appears to be rare, yet consideration of their health implications nonetheless warrants attention (14). The structural similarity between fumonisins and sphingolipids highlights an important commonality with the aflatoxins and ochratoxins. Although fumonisins exhibit markedly lower acute toxicity than the other mycotoxins considered here, their comparative chronic toxicities and potential for immunotoxic effects remain open questions (15).

### 2.4. Patulin and Other Toxins

Patulin is a toxic compound that has been detected in some nuts and is now included in regulatory discussions. However, documented occurrences of this mycotoxin in nuts are still few (16). Other toxins produced by Aspergillus, Penicillium, Fusarium, and Claviceps species may also contaminate kernels, but few data are available on their presence and incidence.

## 3. Sources and Pathways of Contamination

Contamination of edible nuts by mycotoxins is a major issue that affects human health and economic development in many countries. Contamination of nuts and edible seeds takes place from field to table, during growth, harvesting, transporting, processing, and distribution (17). This review examines the sources and pathways of contamination concerning the four mycotoxins of greatest concern in nuts, namely aflatoxins, ochratoxins, fumonisins, and patulin. It highlights the significant pre-harvest factors, such as climate conditions and crop genetics, that may govern the pre-harvest introduction of mycotoxins in selected nut crops. Furthermore, it describes post-harvest handling practices that are crucial for preventing nuts from infection by aflatoxin-producing fungi (5).

Because nuts are either consumed whole or added as a food ingredient without further heat treatment, their culinary-processing conditions only slightly reduce the initial contamination level and may concern nutritional and toxicological aspects. Although roasting is widely adopted in the nut-processing industry, studies show that the current roasting conditions imposed in the industry do not significantly affect aflatoxin stability. On the other hand, matrix-upgrading changes during roasting appear to present some opportunities to mitigate aflatoxin contamination. Moreover, several processing practices are usually adopted by the nut-processing industry, such as baking, frying, and blanching, according to the current scientific data available (18,19).

### 3.1. Pre-harvest Factors

Revised instructions acknowledge a complex structure, non-sequential content, and a broad range rather than specific details. Restructured content to focus solely on mycotoxins, nuts, and fungal species attacking nuts, aligning precisely with added insights on susceptible nuts (8).

Nuts, being rich in lipid and carbohydrate content, are frequently invaded by fungi that produce mycotoxins during cultivation and harvesting (5). Those of utmost concern are aflatoxins, ochratoxins, fumonisins, patulin, and Alternaria toxins, due to their toxic effects and global regulation. Aflatoxins have been best studied, with A. flavus and A. parasiticus being the major contaminating species in nut crops (20).

Nuts, due to their high lipid and carbohydrate content, are prone to fungal invasion that leads to toxin accumulation during cultivation and harvesting. The fungal species that are known to attack nuts and produce mycotoxins are diverse. Aflatoxins, ochratoxins, fumonisins, patulin, and toxins produced by Alternaria species rank among the most significant toxins from a worldwide perspective. These mycotoxins exhibit harmful effects and are regulated in many countries (20).

Aflatoxins are arguably the best-known mycotoxins, having been discovered more than 60 years ago. Aflatoxin-producing Aspergillus species comprise a highly diverse group of fungi capable of causing significant contamination in a broad range of food matrices. Among nuts, A. flavus and A. parasiticus are the predominant aflatoxin-producing species. A. flavus is particularly much more widely distributed than A. parasiticus and contaminates various crops in the field as well as during storage. Food heavily contaminated by these two species is still often found in many areas of the world (21,22).

### 3.2. Post-harvest Handling and Storage

Several practices carried out from harvest to consumption may influence contamination levels in nuts and nut products. Inadequate drying or storage of nuts with high moisture content in poorly ventilated and warm places may enhance the development of fungi responsible for aflatoxins, ochratoxins, and fumonisins, whereas specific conditions during roasting may either degrade mycotoxins or, in the case of patulin, promote its formation (2,23).

Post-harvest handling operations comprise relaxation of the harvest-induced physiological stress of the nuts, drying to moisture levels that prevent microbial growth, sorting to remove damaged and pest-infested nuts, and storage in controlled conditions to minimize further deterioration. Relative humidity (RH) is critical for nuts and kernels, as moisture contents in excess of 6.5–7.5% at 20–25°C and 9.0–11.0% at 30°C enable fungal growth on whole nuts or kernels. Furthermore, aflatoxin B1 production may occur in whole nuts kept at RH over 50%. Integral to post-harvest management, sanitation operations remove contaminants from equipment and facilities, and water used for rehydration. Surface cleaning, container fumigation, and warehouses, pest control, and in-shell nut and kernel cleaning are recommended tasks (24,25).

### 3.3. Processing and Roasting Effects

Nuts contaminated with fungal toxins are probably a greater threat to human health than any other group of foods. The wide variety of nuts—hard and soft shelled, dried and fresh, sweet and salty, and sweetened and unsweetened—only adds to both consumer appeal and the potential for harmful residues. Various nuts are routinely subject to processing, such as cleaning, grading, roasting, slicing, and peanut butter preparation; residues may also benefit or suffer from these treatments. Evidence of good processing can help build confidence in the safety and quality of food nuts (8).

Processing and roasting can reduce the mycotoxins in nuts, but their relative effects depend on the type of product processed, the type of mycotoxin of concern, and the specific processing conditions employed. In groundnuts and hazelnuts, careful sorting and heating eliminate significant amounts of aflatoxins (26). Temperatures typical of nut roasting further degrade toxins (27). Food safety is significantly affected by mycotoxin contamination of dried fruits, nuts, and spices. Various approaches are available regarding temperature, time, and other parameters (18).

## 4. Analytical Methods for Detection

Nuts represent a major commodity in the global food market, consumed for their rich nutritional value and health benefits. They are susceptible to contamination by mycotoxins that pose health risks and spoil both raw and processed nuts, potentially leading to significant economic losses depending on the level of contamination. Therefore, a thorough understanding of mycotoxin contaminations in nuts—covering their chemical characteristics, occurrence in the supply chain, methods for their analysis, risk assessment, health impacts, and mitigation techniques—is vital to support future research and regulation (8,28).

Four mycotoxins—aflatoxins, ochratoxins, fumonisins, and patulin—are of particular concern in nuts due to their toxic effects and high contamination levels across globally traded nuts. Aflatoxin B1 (AFB1), classified as both a carcinogen and mutagen, is the most toxic mycotoxin and has been detected worldwide in practically all traded nut varieties. Additionally, nuts receive ochratoxin A (OTA) through pre- and post-harvest pathways, with hazardous levels reported in hazelnuts, cashews, groundnuts, and walnuts. Fumonisins are also found in hazelnuts and almonds from infected plant tissues, while patulin levels remain low but still pose health risks. Beyond these four, certain other mycotoxins that occasionally contaminate nuts and their potential health impacts have been described (8,29).

### 4.1. Sampling and Sample Preparation

Mycotoxins are highly toxic secondary metabolites produced by several genera and species of filamentous fungi. Dry nuts are important. Consequently, nut products have a high risk of mycotoxin contamination if contaminated nuts are processed. Only a few studies are available regarding the presence of mycotoxins in nut products, which can be closely associated with either whole nuts or nut paste products. Nevertheless, there is a wide range of analytical methodologies employed for the detection of mycotoxins in different matrices, including nuts and nut products, having varying degrees of complexity. These approaches are valuable in understanding the prevailing mycotoxin loads in food items, and in turn help in regulating contamination levels in food products (30,31)

Sampling is considered the most critical step in analytical procedures, especially for mycotoxins, where inhomogeneous distribution of contaminated kernels may account for up to 75% of total variance. Variability can be reduced by increasing the sample or subsample size and the number of aliquots analysed. A number of sampling strategies have been developed for different products and contaminants, including aflatoxins. Sampling and sample processing methods should be adopted conforming to the prevailing regulatory or standard guidelines. Several sampling recommendations and checklists/sampling tools have been issued by FAO, and many of them have also been incorporated into the Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in food (32,33).

### 4.2. Immunoassays and Chromatography

Regulatory bodies and individual countries have adopted maximum levels for aflatoxins (AFs), and the presence of multiple mycotoxins is increasingly being considered. Monitoring methods must therefore permit the determination of several compounds in a single analysis. The methods that have been used to analyse AFs, ochratoxin A, and other mycotoxins in nuts consist of thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) with fluorescence or ultraviolet detection, enzyme-linked immunosorbent assay (ELISA), and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (34,35).

In immunoassays, a chemical analyte forms an antigen–antibody complex with a specific antibody. The concentration of the analyte is determined based on a measurable signal originating from a label that is bound to the antibody or a secondary molecule. Besides radionuclides and enzymes, other labels that have been used include coloured and fluorescent dyes, chemiluminescent agents, and electrochemical probes. Typically, the detection limit (DL) is in the mid- to low parts-per-billion range for AFs. ELISA formats may be competitive or non-competitive. The former are more common in mycotoxin analysis (36,37). ELISA kits offer several advantages, including the availability of test kits for many mycotoxins, rapid and simple sample preparation, short assay times, and tolerable moisture and fat contents in samples. ELISA formats have been reported for the analysis of aflatoxin B1, ochratoxin A, fumonisin B1, deoxynivalenol, zearalenone, and T-2 toxin in nuts. At the turn of the 21st century, the LC-MS detection of multi-mycotoxin residues, including

AFs, in many food products was established. Multi-analyte methods for AFs, ochratoxins, fumonisins, and zearalenone have also been developed, in which LC coupled to tandem mass spectrometry (LC-MS/MS) was the most prominent technique. ELISA detection of simultaneously present AFs and ochratoxin A has been reported based on the analysis of a single extract. ELISA kits have been available for several mycotoxins, among them AFs, ochratoxin A, zearalenone, deoxynivalenol, and fumonisins (38,39).

### 4.3. Emerging Technologies

Classification methods based on specific criteria make it possible to compare emerging technologies and assess their potential for monitoring mycotoxins in nuts. Sensor technologies remain the most relevant, particularly in areas such as rapid, flexible, simple-to-use, multiplexed detection, and low cost (40).

Mycotoxins pose an increasing threat to food safety due to climate change. Trustees need new, cost-effective methods to reduce mycotoxin content in food chains. Analytic methods intervene at the end of the food chain. Near real-time, on-site monitoring at early production stages is ideal to support preventive approaches and control strategies. Emerging technologies address gaps in conventional analytic techniques. Advances in miniaturization, connectivity, and computing (41).

Three technology domains address these needs: integrated mono and multi-parameter sensor systems, modelling and information systems for data interpretation, and biosensors. Integrated sensor systems detect and quantify environmental characteristics impacting mycotoxin production. Modelling systems help interpret measurements and assess risks. Biosensors detect hazardous mycotoxins directly and quantify hazard levels. Monitoring precursors readily predicts mycotoxin risk without the need for their direct measurement. Effective mycotoxin management combines direct detection systems with those for environmental conditions (16,42).

## 5. Health Implications and Risk Assessment

Tree nuts are widely consumed as healthy snacks or ingredients in many food products, providing great nutritional benefits. Due to their high energy and fat content and being eaten in non-heat-treated forms, they are important commodities at risk of mycotoxin contamination (5). Mycotoxins are secondary metabolites produced by fungi species capable of causing harmful effects in humans and animals (9). Aflatoxins, ochratoxins, fumonisins, and patulin are the most critical mycotoxins found in nuts globally, warranting consideration in commodity risk assessments. Aflatoxins remain the most toxic, with aflatoxin B1 (AFB1) being the most potent carcinogen naturally occurring in food, followed by ochratoxin A (OTA) and fumonisins (FB1) (10).

Contaminated nut sources pose health threats to consumers and economic risks to producers, as many countries impose strict regulations on mycotoxin levels (6,43).

## 6. Mitigation Strategies and Good Manufacturing Practices

Protracted exposure to mycotoxins poses a serious health risk in the nut supply chain. The consumption of contaminated nuts has been linked to several health effects, including liver damage and cancer, both serious endpoints of prolonged mycotoxin exposure. Furthermore, foodstuffs such as nuts, milk, cereals, and edible oils are relatively tolerated, which significantly contributes to the spread of contaminated materials among the population. High prevalence of exposure has been reported in Europe, Asia, and Latin America (44-46).

A multi-hurdle approach offers precautionary controls across the nut supply chain, from agricultural practices to processing and distribution. Within each stage of the supply chain, a combination of good agricultural practices, such as climate monitoring, crop variety selection, harvest techniques, storage, packaging, and transportation have been recommended to minimize contamination and mycotoxin production. Specific agricultural techniques such as irrigation followed by crop rotation, variety selection of high-yield and low-toxin-producing crops, timely harvesting of mature crops, or cutting off infected parts of crops followed by immediate storage have been cited (5,47).

### 6.1. Agricultural Practices and Variety Selection

In many regions of the world, including the Mediterranean, tropical, and subtropical climatic zones, the fungal contaminants A. flavus and A. parasiticus are documented to develop on nuts of various crops. Aflatoxins—potent carcinogens and mutagens—are among the metabolites they produce, posing welfare hazards for agricultural workers as well as consumers. These toxins cause damage and ultimately death in laboratory animals with low concentrations of the amount producing 50% lethal dose (LD50). In shelled nuts, levels greater than the European Union permissible limit of 4 µg/kg would already be expected to breach the stricter OECD guidance value (clearly focused on nuts) of 1.36 µg/kg (5). An aflatoxin B1 contamination value of 20 µg/kg at the point of consumption corresponds to a maximum tolerable daily intake (MTDI) of 0.02 ng per kg body weight per day, at least 200-fold the average European nut exposure of around 0.0001 ng/kg body weight/day. These observations highlight the urgent need for a critical review of aflatoxins in the contemporary nut supply chain (21,22).

Several previous reviews specifically cover fungal contamination in non-tree nuts across various regions and contaminants, yet still lack comprehensive information regarding contamination levels and risk. Considering this context, the specific objectives of a review solely devoted to mycotoxins of concern in edible tree nut crops are to compile up-to-date findings on the mycotoxins actually present in contemporary supplies and to examine the associated impact after assembly of sufficient knowledge on specific nut mycotoxins and risk exposure. Hence, Aflatoxin B1, ochratoxin A, fumonisins, and patulin are listed at the outset as the main mycotoxins of concern for nuts (48,49).

### 6.2. Harvesting and Post-harvest Techniques

Contamination of nuts by various species of fungi is a matter of great public concern worldwide because many fungal species produce mycotoxins, and nuts are a highly consumed food source. Hazelnut is a high-value commodity that is cultivated in large amounts and contributes significantly to agricultural economies. Mycotoxin occurrence and levels have been studied extensively on various commodities, and yet, hazelnut lacks research in this regard (5). Contamination remains a risk in many other commodities and, therefore, knowledge gaps exist (8).

Hazelnuts show mycotoxin contamination in the field and during storage. Pre-harvest and post-harvest safety can help mitigate contamination with mycotoxins and fungal presence in hazelnuts. Pre-harvest factors include climate, crop variety, and pest management. Critical control points involved in post-harvest handling include drying conditions, packaging, and storage. Other critical control points are found in processing and roasting (50).

### 6.3. Storage, Packaging, and Transportation

Nuts and dried fruits, for their convenience and health-promoting bioactive compounds, are highly regarded food items for consumers and thus are good carriers for aflatoxins. Storage conditions and materials directly affect food quality and, consequently, the safety of nuts and dried fruits, and have an impact on bioactive compounds. Workers are commonly exposed to aflatoxins via nuts and dried fruits in work environments far from factories. Desiccation of hazelnut is directly related to mycotoxin contamination, with moisture being one of the key factors facilitating fungal proliferation. Aflatoxins, fungi, and mycotoxins on stored work environment of food production, such as bakeries and oil manufacturing, are still understudied. Furthermore, Hazelnuts and, in general, nuts are not yet banned in Middle East markets where aflatoxin contamination is a severe concern. Aflatoxins may still be a concern on almond thus it is still important to study hazelnuts regarding aflatoxin levels. Current European Regulations for Nuts focus on aflatoxin B2 and total aflatoxins (51,52).

Systems of good practices are an effective way to secure food safety throughout the food supply chain. It is possible to provide countermeasures for prevention, such as agricultural practices, harvesting and post-harvesting practices, storage, packaging and transportation, processing intervention, and decontamination. A survey shows that agricultural practices have been the dominant countermeasure against aflatoxin contamination. Agricultural practices improve farm input sustainability, enhance the production performance of the plants, and consequently offer opportunities to the other good practices countermeasures to further alleviate the aflatoxin contamination (53).

### 6.4. Processing Interventions and Decontamination

Decontamination options for mycotoxins in nuts concern researchers, operators, and consumers. Processing interventions do not completely eliminate contamination. Treatment may thus be limited to lowering amounts while seeking to retain safety, nutritional quality, and sensory acceptability. Several measures apply at the agricultural and harvest stages, post-harvest handling, storage, and transport, as well as processing. Protocols aim to reduce growth, infection, and contamination, mitigate the transfer of mycotoxins, and restrict conditions that favour production by fungi already established. The focus here is on decontamination procedures after nuts arrive at processing plants (26).

Crucial factors across production, processing, and marketing include, but are not limited to, temperature, relative humidity, storage time, sanitation methods, and the type of packaging used. Processing and packaging must comply with regulations from authorities like the European Food Safety Authority. Industrial trials have examined decontamination treatments separately or in various combinations (44).

## 7. Conclusion

Mycotoxins are toxic secondary metabolites produced by fungi contaminating food, leading to health, economic, and food security challenges worldwide. Exposure to mycotoxins through polluted edible nuts is emerging as a significant risk across the globe, drawing attention from academic, industrial, and governmental agencies. Literature shows still pose serious hazards when consumed. However, limited information is available, and nuts were originally overlooked. Therefore, this review aims to consolidate the entire picture regarding mycotoxins in nuts to assess mycotoxin risks in nuts and identify research gaps for future studies.

Aflatoxins (AFs), ochratoxins (OTs), fumonisins (FBs), and patulin (PAT) are among the key mycotoxins of concern in edible nuts worldwide. AFs are potent carcinogenic toxins, and the ingestion of AF-contaminated nut products is regarded as a considerable risk in several regions. OTs and FBs have negative health effects, and extensive health risk assessments have been carried out at various exposure levels. Although all nuts may theoretically be contaminated with PAT, no data have been reported regarding its presence in nut sources.

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