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Review Article

Overview on Mycotoxins Health Hazard to Humans and Animals

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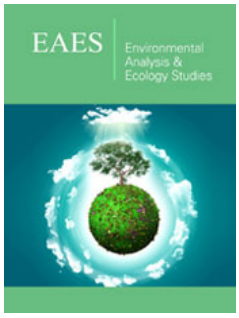

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Abstract

Mycotoxins are toxic secondary metabolites produced by various species of fungi, particularly those belonging to the genera *Aspergillus, Penicillium, Fusarium*, and *Alternaria*. Secondary metabolites are chemicals of a fungus that produce toxic results in another organism. These compounds can contaminate agricultural products such as grains, nuts, and fruits, posing significant health risks to humans and animals. Cytotoxic: disrupt cell membranes, and processes such as protein, Deoxyribonucleic Acid (DNA), and Ribonucleic Acid (RNA) synthesis. Lack of visible appearance of fungus does not negate presence of mycotoxins. Toxins can remain in the organism even after fungus has been removed. Less selective in special organism selection, can cross plant species barrier. It can be heat stable, not destroyed by canning or other processes. Mycotoxins are known to be toxic compounds that are naturally produced by certain types of (fungi). fungi that can produce mycotoxins grow on food such as cereals, dried fruits, nuts and spices. Mould growth can occur before or after harvest, during storage, on/in the food itself especially under warm, damp and humid conditions. Most mycotoxins are stable and survive even during food processing. Here is a comprehensive review of mycotoxins health risks to humans and animals

Keywords: Mycotoxins; Fungi toxic; Patulin; Aflatoxins; Ochratoxins; Fumonisins and Zearalenone

Introduction

Mycotoxins are secondary metabolites produced by some fungi that can evoke a toxic response in humans and animals after exposure e.g. via ingestion of contaminated food or feed [1]. Mycotoxins can enter the food supply chain via fungi that infect plants during their growth or development, and subsequently contaminate derived agricultural products with mycotoxins. In addition, cereals and other different agricultural commodities can be infected by fungi and contaminated by mycotoxins during harvest, storage, processing and shipment [2]. *Fusarium* genera are most important with respect to contamination in the field. *Aspergillus* is an opportunistic fungus, infecting peanuts and maize during the growing period in climate zones with a highly temperature. Both *Aspergillus* and *Penicillium* fungi are commonly associated with contamination during storage. While some fungal metabolites are used as pharmaceuticals or industrial chemicals due to their anti-microbial effects, others can evoke carcinogenic, immunotoxic, hepatotoxic or other adverse health effects in humans or animals after intake of contaminated food or feed [3]. Generally, pigs are particularly sensitive to the effects of mycotoxins, whereas ruminants have a lower sensitivity as a result of an extensive rumen metabolism and microbiota [4]. To ensure food and feed safety, maximum and guidance limits for mycotoxins in specific food and feed products have been established internationally by e.g. the European Union and the Codex Alimentarius Commission. The European Food Safety Authority and Joint World Health Organization Expert Committee on Food Additives reported risk assessments on the most common mycotoxins found in food and feed to support the development of effective measures for risk management [5-6]. Most of the secondary metabolites produced by fungi are unknown and only a few of these metabolites have been subjected to a hazard assessment or have been regulated in food or feed. Due to the development of novel analytical methods, the total list of fungal metabolites is ever expanding and has been estimated to be over 3000 metabolites [7]. The toxic potential of many of these

fungal metabolites is unknown. So, the fungal metabolites may not all qualify as mycotoxins. In this review, i will further refer to all fungal metabolites as mycotoxins. State-of-the-art methods greatly aid to the discovery of new mycotoxins, as well as of modified forms of known mycotoxins. In this respect, modified forms of mycotoxins comprise all conjugates of the parent molecule which are formed in the infested plant and mammalian organism [8]. For example, plants can metabolize the mycotoxins that are polluted with by forming glucoside or sulfate conjugates. Most of these modified mycotoxins are not monitored in food or feed. This can result in an underestimated (risk of the) exposure to the basic compound, as the modified mycotoxins occurring in the plants can be deconjugated to the parent mycotoxin during metabolism in humans and animals [6].

Modified mycotoxins can also add to the exposure and effects of the parent mycotoxin. The occurrence and toxicity effects of newly discovered or modified metabolites are largely unknown. In addition to the unknown effects of new mycotoxins, it appears that the occurrence of 'traditional' mycotoxins sometimes shifts to untypical products, or unusual different regions possibly partially as a result of global warming [9]. As the demand of plant based food and feed increases [10], it is of importance to have an overview of the mycotoxins that can occur in the food and feed supply chain. An overview that includes previously evaluated and newly identified mycotoxins, unevaluated mycotoxins can help researchers to identify possible relevant mycotoxins in the food and feed supply chain. Upon request of the Dutch Food and Consumer Product Safety Authority (NVWA), such an overview of the general information (e.g. maximum limits, health based guidance values and transfer information), the occurrence (e.g. type of products or country of origin) and the toxicological properties of the evaluated and unevaluated mycotoxins was compiled from available literature. A new method was used to gather large quantities of occurrence data, without detailed qualitative assessment of the studies. The gathered information was compiled in a database to be able to create various overviews. The overviews can e.g. be used to identify the possible presence of mycotoxins in specific food or feed products, the maximum limits that have been set for the respective mycotoxins or the health based guidance values and toxicological effects of mycotoxins. The methods that were used to obtain the information, as well as the main observations from this information are described in a study of Althagafi et al. [11].

Mycotoxins are toxic compounds that are produced naturally by certain types of moulds (fungi). Moulds that can produce mycotoxins are able to grow on numerous foodstuffs such as cereals, dried fruits, nuts and spices. Mould growth can occur before or after harvest, during storage, on foodstuff often under warm, damp and humid or bad conditions. Most mycotoxins are surviving and chemically stable in food processing.

Several hundreds of mycotoxins have been identified, but the most commonly observed mycotoxins that present a concern to human health and livestock include ochratoxin A, patulin, zearalenone, aflatoxins, fumonisins and nivalenol/deoxynivalenol (Table 1). Mycotoxins appear in the food chain as a result of mould infection of crops both before and after harvest [12]. Exposure to mycotoxins can happen to human directly by eating infected foodstuff or indirectly from different animals that are fed contaminated feed, in particular from milk.

Table 1: Common specific toxins affecting human health.

Fungus	Mycotoxin
Aspergillus niger	Aflatoxin (A)
Aspergillus ochraceus	Ochratoxin (A)
Claviceps spp.	Lysergic Acid diemthled
Fusarium graminearium	T-2 toxin
Fusarium graminearium	DON
Fusarium moniliforme	ZEN
Penicillium citrinum	Citrinin
Penicillium cyclopi	CPA
Trichoderma sp.	Trichothecene

Mycotoxins Commonly Found in Food and Why They are of Concern

The effects of some food-borne mycotoxins are acute with symptoms of severe diseases appearing quickly after consumption of food contaminated with mycotoxins. Other mycotoxins occurring in food has long-term effects on health, including the induction of cancers and immune deficiency. Of the several hundred mycotoxins identified, about a dozen that have gained the most attention due to their acute effects on human health and their occurrences in food [13].

Aflatoxins are the most poisonous mycotoxins that are produced by certain fungi (*Aspergillus parasiticus* and *Aspergillus flavus*) which grow in hay, grains, decaying vegetation and soil. Crops that are frequently affected by *Aspergillus spp*. include cereals (rice, wheat, sorghum and corn), oilseeds (cotton seeds, sunflower, peanut, and soybean), spices (ginger, black pepper, turmeric, coriander and chili peppers) and tree nuts (Brazil nut, almond, coconut, walnut and pistachio). The toxins can also be found in the milk of animals that are eating contaminated feed, in such of aflatoxin M1. Large doses of aflatoxins can lead to illness poisoning (aflatoxicosis) also can be health threatening, usually through damage to the liver. Aflatoxins are also genotoxic, meaning they can damage DNA and cause cancer in some animal. There is also evidence that they can cause liver cancer in humans [14].

Ochratoxin A is produced by several species of *Aspergillus* and *Penicillium* and is a common food-contaminating mycotoxin. Contamination of food commodities, such as cereals and cereal products, dry vine fruits, coffee beans, spices and liquorice, wine and grape juice, occurs worldwide. Ochratoxin A is formed during the storage of crops and is known to cause a different of toxic effects in animal species. The most sensitive and highly effect is kidney damage, while the toxin may also have effects on fetal development and on the immune system. Contrary to the clear evidence of kidney toxicity and kidney cancer caused by ochratoxin A exposure in animals, this association in humans is unclear, however effects on kidney have been demonstrated [15]. Patulin is a mycotoxin

produced by a variety of different moulds, particularly *Aspergillus*, *Penicillium* and *Byssochlamys*. Often found in rotting apples and apple products, patulin can also occur in various mouldy fruits, grains and other foods [14]. Major human dietary sources of patulin are apples and apple juice made from affected fruit. The acute symptoms of mycotoxins in animals include kidney damage, liver, spleen and toxicity to the immune system. For humans, vomiting, gastrointestinal disturbances and nausea have been reported. Patulin is considered to be genotoxic while a carcinogenic potential has not been illustrated yet.

Fusarium are common soil fungi and produce a different toxin, including Nivalenol (NIV), trichothecenes such as Deoxynivalenol (DON) and T-2 and HT-2 toxins, in addition to Zearalenone (ZEN) and fumonisins. The formation of the moulds and toxins reported on so many different cereal crops. Different *fusarium* toxins are associated with special types of cereal. For example, both DON and ZEN are associated with wheat, T-2 and HT-2 are often toxins with oats, and fumonisins with maize (corn). Trichothecenes are acutely toxic to humans, causing quick irritation to the intestinal mucosa or to the skin and lead to diarrhea [13]. Demonstrated chronic effects in animals which include suppression of the immune system. ZEN has been shown to have hormonal, estrogenic effects and may cause infertility at high intake levels, particularly in pigs. Fumonisins have been related to esophageal cancer disease in humans, and to kidney toxicity and liver in animals.

Mycotoxins Production Under Different Food Right-Conditions

Mycotoxins are having a considerable impact on the health of consumers. There has been plentiful research into the different effects of mycotoxins, fungi, and bacteria on the safety and quality of food and feed. The occurrence and health impacts of foodborne mycotoxins has been studded in different countries, also the researcher are studying the presence of mycotoxins in herbs, nuts, cereals, dried fruits and vegetables, dairy products, infant formulas and child foods. Applying, mycotoxin analysis methods. using different techniques of analysis such as the HPLC method, dilution plate method, thin layer chromatography, total plate count method, and seed-plate method to detect, identify, and isolate mycotoxins [15]. Certain types of mycotoxins (zearalenone, patulin, aflatoxins) and fungi (*Aspergillus flavus*, *Penicillium chrysogenum*, and *Aspergillus niger*) used to be examined in the samples (isolates) of products tested in the Saudi regions. Furthermore, mycotoxins have serious health impacts on consumers and most of the contamination cases are caused by improper storage conditions and/or inappropriate handling and harvesting practices. Each type of mycotoxins is produced by some fungi species that grow in contaminated products and has health impact as showing in Table 2. Because of that strict regulation is very important. So stringent limits in food products worldwide are made due to their potential toxicity.

Table 2: Some types of mycotoxins and their fungi, food sources and health impact.

Mycotoxins	Fungi Produced	Products	Health Impact
Aflatoxins	*Aspergillus flavus* and *Aspergillus parasiticus.*	Corn, peanuts, cottonseed, tree nuts.	Highly carcinogenic, causing liver cancer. Also linked to immune suppression and stunted growth in children.
Ochratoxins	*Aspergillus ochraceus* and *Penicillium verrucosum*	Cereals, coffee, dried fruits, wine.	Nephrotoxic, hepatotoxic, immunotoxic, and potentially carcinogenic, particularly affecting the kidneys.
Fumonisins	*Fusarium verticillioides* and *Fusarium proliferatum*	Corn and corn-based products.	Associated with esophageal cancer and neural tube defects. Also linked to liver and kidney toxicity.
Trichothecenes	*Fusarium* species	Wheat, barley, maize, oats.	Inhibit protein synthesis, causing immunosuppression, nausea, diarrhea, and hemorrhaging. Deoxynivalenol (DON), also known as vomitoxin, is a common trichothecene.
Zearalenone	*Fusarium* species	Corn, wheat, barley, sorghum.	Estrogenic effects, leading to reproductive disorders in livestock. Potential endocrine disruptor in humans.
Patulin	*Penicillium expansum*	Apples and apple products.	Potentially genotoxic and carcinogenic. Mainly a concern in fruit juices and apple-based products

How Mycotoxin Becomes a Health Hazard?

Mycotoxin can affect humans by economic loss due to impaired health of stock animals. Disease: symptoms can include immune suppression, cold/flu-like symptoms, fatigue, headaches, nose bleeds, diarrhea, dermatitis, and sore throats, and vary by species. At the end it might cause death [16].

A. Generally lower risk in well developed countries due to improved standards of living.

B. High intake of different affected product, usually in conjunction with limited amounts of other food sources.

C. Greatest threat caused by long term exposure due to eating spoiled food or meat from animals fed contaminated feed.

D. Mycotoxins are secondary metabolites produced by fungi which contaminate a large fraction of the world's food, mainly staple foods such as eggs, groundnuts, cereals, and tree nuts, besides milk, corn and meat. This worldwide contamination of foods is an enormous problem to human health, principally in less industrialized regions and in the rural areas of some developed countries. The adverse effects of mycotoxins on human health can be both acute and chronic, disturbing problems such as reduction of immunity, liver cancer, alterations in the protein metabolism, gangrene, convulsions, and respiratory problems, among others.

The Symptoms of Mycotoxicosis

Mycotoxicosis is the term that been used for poisoning related with exposures to mycotoxins. Mycotoxins have the potential for acute and chronic illness effects via ingestion, inhalation, entering the blood stream, skin contact and lymphatic system. They inhibit protein synthesis, inhibit particle clearance of the lung, damage macrophage systems, and increase sensitivity to bacterial endotoxin [17].

The symptoms of mycotoxicosis depend on the concentration, length of exposure and the type of mycotoxin; as well as health, age and sex of the exposed individual. The synergistic effects that are related to several other factors such as diet, genetics, and interactions with other toxins have been poorly studied. Therefore, it is possible that alcohol abuse, caloric deprivation, vitamin deficiency and infectious disease status can all have compounded effects with mycotoxins. The symptoms of mycotoxicosis are including:

a) Antibiotics and drugs are not effective in treatment.

b) The symptoms can be traced to different feed or foodstuffs.

c) Testing of mentioned foodstuffs or feed reveals fungal contamination.

d) The symptoms are not transmissable person to person.

e) The degree of toxicity is depend on nutritional status, sex (more often in females than males) and the persons age (often in very young and very old).

f) Outbreaks of symptoms appear seasonally.

Signs vary with the mycotoxin, the dose ingested, the period of exposure and the species affected. Diarrhea paralysis or in coordination, reduced feed efficiency, reduced weight gain or egg production/hatchability, increased condemnations and pale shanks, combs, bone marrows.

The Effect of Mycotoxin on Animals

There are several different effects of Mycotoxin on animals (Figure 1) that has been described in details in a study presented by Zaki et al. [12] as follow: Different mycotoxins are affecting several organ system as show in details (Table 3).

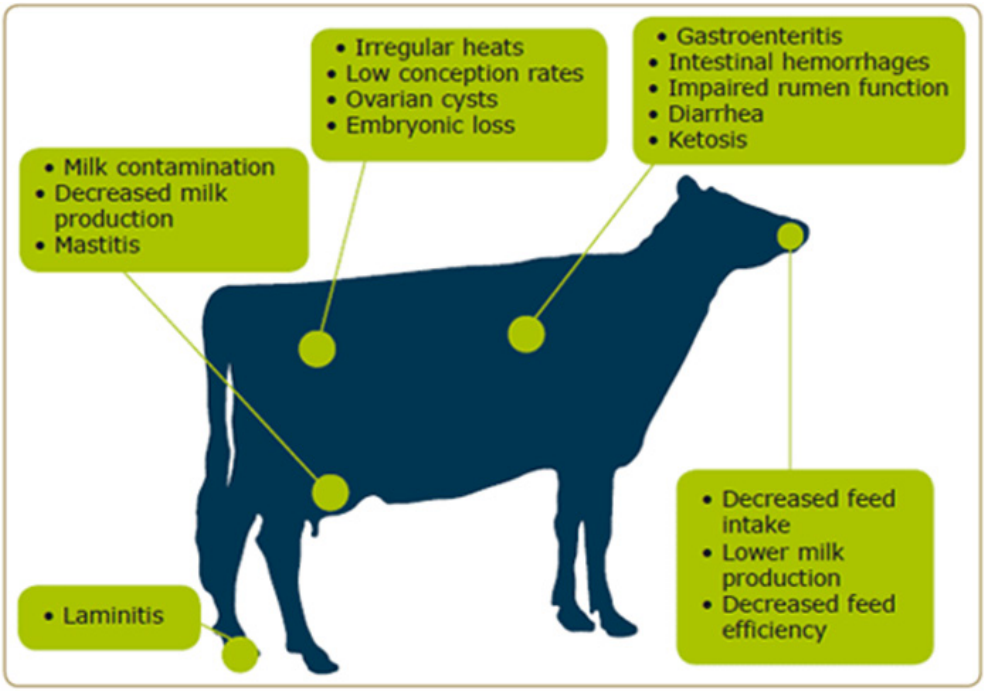

Figure 1: The effect of mycotoxin on animals (Zaki et al. [12]).
A. Feed refusal.
B. Impaired animal health, resulting in reduced production of eggs, milk, weight gain, etc.
C. Metabolites are passed through the milk in cheese, dry milk, and yogurt.
D. Disease.
E. Death in animals.

Table 3: Toxin and the organ system affected [12].

Organ System Affected	Toxins(s)
Vascular	Aflatoxin
Digestive	Aflatoxin, T-2toxin, Vomitotoxin
Respiratory	Trichothecenes

Nervous	Trichothecenes
Cutaneous	Trichothecenes
Urinary	Ochratoxin A, Citrinin
Reproductive	Zearalenone, T-2 toxin
Immune	Many

Mycotoxicosis Laboratory Tests

The mycotoxicosis laboratory tests are differ from each other. These are including immunoassays, TLC, confirmatory test and HPLC. For detection methods and control chromatography High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are standard methods for mycotoxin detection [15]. While immunoassays Enzyme-Linked Immunosorbent Assay (ELISA) provides rapid screening. Mass spectrometry offers high sensitivity and specificity for mycotoxin analysis [18]. So each type of these tests has specific characters and different procedures according to instruments and equipment's that been used as follow:

Lab 1: Confocal microscopy

Confocal Laser Scanning Microscopy (CLSM) or Laser Scanning Confocal Microscopy (LSCM), is an optical imaging technique for increasing optical resolution and contrast of a micrograph by using definite pinhole to block out-of-focus light in image formation. Capturing multiple two-dimensional images at different depths in a sample helps the reconstruction of three-dimensional structures (this process known as optical sectioning) within an object. This technique is usually used extensively in the scientific and industrial fields and typical applications are in biological sciences, semiconductor inspection and materials science [19].

Light goes through the sample below a conventional microscope as far into the specimen as it can penetrate, while a confocal microscope only focuses a smaller beam of light at one narrow depth level at a time. The CLSM achieves a highly limited depth of field and controlled. Confocal microscope is using point illumination and a pinhole in an optically conjugate plane in front of the detector in order to eliminate out-of-focus signal - a name "confocal" stems from this configuration [19]. As only light that produced by fluorescence very close to the focal plane can be discovered, the image's optical resolution, especially in the sample depth direction, is better than that of the wide-field microscopes. However, as much of the light comes from sample fluorescence is sealed at the pinhole, this increased resolution is at the cost of declined signal intensity - so long exposures are often required.

To offset this drop in signal after the pinhole, light density can be detected by a sensitive detector, the avalanche photodiode or Photomultiplier Tube (PMT), transforming the light signal into an electrical one. As only specific point in the sample is illuminated at a time, 2D or 3D imaging demand scanning over a regular raster in the specimen. The beam is scanned through tested sample in the horizontal plane by using one or more oscillating mirrors. This method of scanning usually has a minimum reaction latency and the scan speed can be diverse. Usually slower scans give much better signal-to-noise ratio, resulting in better contrast. Thickness of the focal plane is illustrated generally by the wavelength of the used light divided by the numerical aperture of the objective lens, but also by the optical properties of the specimen [20]. Thin optical sectioning may make these types of microscopes especially good at 3D imaging and surface profiling of samples.

Confocal microscopy supplies the capacity for direct, thick, serial optical sectioning of intact, noninvasive, living specimens with a minimum of sample preparation as well as a marginal improvement in lateral resolution compared to wide-field microscopy. Biological samples are often treated with fluorescent dyes to make selected objects visible. However, the actual dye concentration can be low to minimize the disturbance of biological systems: some instruments can track single fluorescent molecules. In addition, transgenic techniques can create organisms such as a fusion GFP that produce their own fluorescent chimeric molecules. Confocal microscopes (Figure 2) work on the principle of point excitation in the specimen (diffraction limited spot) and point detection of the resulting fluorescent signal. A pinhole at the detector supply a physical barrier that prevents out-of-focus fluorescence. Only the central spot or in-focus of the Airy disk, is recorded.

Figure 2: The confocal microscope.

Uses of Confocal Laser Scanning Microscopy (CLSM) is widely used in various biological science disciplines, from cell biology and genetics to microbiology and developmental biology. Also it can be used in quantum optics, spectroscopy and nano-crystal imaging. Clinically, CLSM is used in the examination of different eye diseases, particularly for qualitative analysis, imaging, and quantification of endothelial cells of the cornea. So it is used for identifying and localizing the presence of filamentary fungal in the corneal stroma especially in cases of keratomycosis, by rapid diagnosis and thereby

early institution of ultimate therapy. Also confocal microscopy can be used in studying biofilms-complex porous structures that are the preferred environment of microorganisms. Some of temporal and spatial function of biofilms can be understood only by studying their structure on micro and meso-scales. The study of microscale is needed to find the activity and organization of single microorganisms.

Lab 2: Agarose gel preparation and electrophoresis

a) Agarose gel was prepared by 1g of dissolving agarose powder in 100ml of Diluted 1X TBE buffer [21,22].

b) Heated by a hot magnetic stirrer until the solution was completely dissolve. 1% agarose gel was used for the visualization DNA.

c) Agarose was left to cool at 45 °C then 2-5µl of red safe dye was added and mixed gently to enable the visualization under ultraviolet UV light.

d) Poured on gel preparing tray with combs.

e) After the gel was solidified, the comb was carefully removed leaving wells for adding samples inside it and the gel tray was placed horizontally in the electrophoresis tank immersed with the same 1X TBE buffer.

f) 5µl of DNA ladder (100-150bp) was loaded and placed in the first hole for electrophoresis and running PCR product at 80 volts for 45min for DNA migration.

g) Dyed gel was visualized and photographed under UV light in a dark room to investigate the presence of extracted DNA and PCR amplification products.

Lab 3: Sensitivity analysis

Sensitivity analysis, or susceptibility testing, helps to detect the most effective antibiotic to kill an infecting microorganism. Infecting microorganisms such as bacteria or fungi that invade human body and cause an infection. A sensitivity analysis is a test that determines the "sensitivity" of bacteria or fungi to an antibiotic or an extract. It also defined the ability of the drug to kill the bacteria [23]. The bacteria (*Psuedomonus*, *E. coli*, *Bucillus subtilis* and MRSA) will spread on a special growing surface then the chemicals (methanol, ethanol acetone, and controls) placed on. Grown bacteria is a culture and bacteria in the medium that will grow and multiply in population.

The bacteria will form large groups of bacteria or colonies, that will be exposed to different antibiotics or extract. These colonies can be susceptible, resistant, or intermediate in response to the antibiotics [24]:

A. Susceptible means that they can't grow if the drug is present. This means the antibiotic is germicide towards the bacteria.

B. Resistant means that the microorganism can grow and survive even if the drug is present. This is a sign of an ineffective antibiotic.

C. Intermediate means a higher dose of the antibiotic is needed to prevent growth.

Lab 4: High-performance liquid chromatography

High-performance liquid chromatography or commonly known as HPLC (Figure 3), is an analytical technique used to identify, quantify or separate each component in a mixture. The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy [25]. HPLC is a highly advanced form of column liquid chromatography. So a solvent forced through under high pressures of up to 400 atmospheres instead of being allowed to drip through a column under gravity, it is. Advantages of HPLC can be summarized in speed, efficiency, accuracy, versatile and extremely precise when it comes to identifying and quantifying chemical components [26].

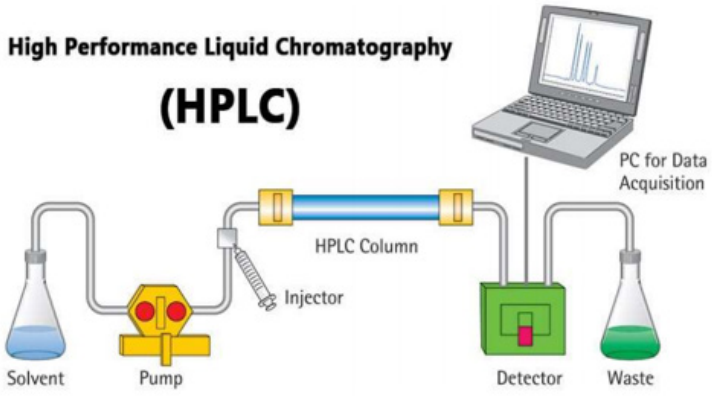

Figure 3: The principal of HPLC.

HPLC Principle:

a) In a separation column the purification takes place between a mobile and a stationary phase.

b) The phase of stationary is a granular material having very small porous particles in a column of separation.

c) On the other hand, the mobile phase is a solvent mixture or a solvent which is forced through the separation column at high pressure.

d) Via a valve with a connected sample loop, i.e. a small tube, the sample is injected into the mobile phase flow from the pump to the column of separation by using a syringe.

e) Afterward, the individual components of the sample migrate through the column at various degree because they are retained to a different rate by stationary phase interactions.

f) After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.

g) Subsequently, a chromatogram is obtained in the HPLC software on the computer.

h) The chromatogram allows the quantification and identification of several substances.

Applications of HPLC: The HPLC has developed into a spread wide method so that it finds its use in all areas of biochemistry, chemistry and pharmacy. Analysis of drugs, analysis of synthetic polymers and analysis of pollutants in environmental analytics. Determination of drugs in biological matrices, isolation of valuable products, quality control of industrial products, product purity and fine chemicals. Water purification and separation of biopolymers such as nucleic acids or enzymes. In addition to pre-concentration of trace components, ligand-exchange chromatography, ion-exchange chromatography of proteins and high-pH anion-exchange chromatography of carbohydrates and oligosaccharides.

Lab 5: Protein gel electrophoresis

Protein gel electrophoresis is used to analyze protein samples, and under denaturing conditions can be used to purify specific components of a mixture that contains more than one protein [27]. Like nucleic acid electrophoresis, the charge to mass ratio of each protein determines its migration rate through the gel. Because the carbon backbone of protein molecules is not negatively charged, negative charge is provided by the inclusion of Sodium Dodecyl Sulfate (SDS) in the loading, gel, and electrophoresis buffers. The negatively charged SDS binds to the specific protein backbone and causes unfolding of the protein. The amount of SDS bound to each protein is proportional to its molecular weight, and the rate of migration through the gel is proportional to the molecular weight by a log-linear relationship [28]. Since, small proteins are more difficult to resolve, because they do not bind SDS well, therefore require modified electrophoresis conditions.

Electrophoresis of covalently joined protein-nucleic acid fusions can also require modified conditions for optimal resolution of different species. This arises from the fact that the nucleic acid component often contributes the vast majority of both the molecular weight and negative charge of fusion molecules, there by altering the electrophoretic properties of these molecules [29]. Standard protein gels are usually composed of two layers. The top-most layer is referred to as the stacking gel, and it comprises about 10-20% of the gel height. The stacking layer contains a low percentage of acylamide, typically 3.5-4.0%, and is buffered at pH 6.8. The lower layer of acrylamide, which comprises the remaining portion of the gel, is the separating or resolving gel. The acrylamide concentration of these parating gel varies according to the samples to be run. Commonly, values of 8-15% acrylamide are used [30]. The pH of the separating gel is 8.8. The difference in pH and acrylamide concentration at the stacking and separating gel interface functions to compress the sample at the interface and provides better resolution and sharper bands in the separating gel.

Lab 6: National center for biotechnology information

The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health (NIH). It is approved and funded by the government of the United States. The NCBI is located in Bethesda, Maryland, and was founded in 1988 through legislation sponsored by US Congressman Claude Pepper [31]. The NCBI houses a series of databases relevant to biotechnology and biomedicine is an important reference for bioinformatics tools and services. Major databases are having Pub Med and Gen Bank for DNA sequences, a bibliographic database for biomedical publication.

The NCBI Bookshelf is a collection of open access, freely downloadable, online versions of different biomedical books. The Bookshelf covers cell biology, molecular biology, microbiology, biochemistry, virology, genetics, disease states from a molecular and cellular point of view and research methods [32]. Basic Local Alignment Search Tool (BLAST) is an algorithm used for calculating sequence similarity between biological sequences, such as nucleotide sequences of DNA and amino acid sequences of proteins. BLAST is a strong tool for having sequences similar to the query sequence within the same organism or in various organisms. The Entrez Global Query Cross-Database Search System is used at NCBI for all the major databases such as protein sequences, nucleotide, PubMed, protein Structures, complete genomes, taxonomy and several others [33].

Mechanisms of Toxicity

In order to know the mechanisms of mycotoxins we have to know how these toxins such as aflatoxins B1 affects humans and animals? In general, there are some effects such as [13,14]:

A. Definite link to cancer in animals.

B. Possible link to cancer in humans. Studies done in Africa and Asia show a correlative link, but not a causative one.

C. Primarily attacks the organ liver, in cases of necrosis, cirrhosis and carcinomas with a secondary affect of immune suppression.

D. Risk factor for neonatal jaundice, especially in areas of maternal consumption.

E. Does not stay in the body for long periods of time, usually the mycotoxins excreted within 96 hours, in animals.

F. In milk, for human consumption, the advisory level of concentration is 5 ppb.

Some mycotoxins are forming DNA adducts, leading to mutations and cancer. Certain mycotoxins are causing neurotoxicity which is crucial for neural tissue function. Many mycotoxins suppress immune function, making the body more susceptible to infections (Table 4). While mycotoxins like zearalenone mimic estrogen, disrupting hormonal balance and reproductive toxicity [34].

Table 4: Mycotoxins are causing carcinogenicity, neurotoxicity, immunotoxicity and reproductive toxicity.

Disease	Mycotoxins
Carcinogenicity	Some mycotoxins, such as aflatoxins, form DNA adducts, leading to mutations and cancer.
Neurotoxicity	Certain mycotoxins, including fumonisins, disrupt sphingolipid metabolism, which is crucial for neural tissue function.
Immunotoxicity	Many mycotoxins suppress immune function, making the body more susceptible to infections.
Reproductive toxicity	Mycotoxins like zearalenone mimic estrogen, disrupting hormonal balance and reproductive health.

For control strategies it should be including pre-harvest and post-harvest for all agricultural products. Pre-Harvest: use of resistant crop varieties, proper crop rotation, and fungicides. Post-Harvest: proper drying and storage conditions to prevent fungal growth. In addition to that decontamination method should be followed: Physical (sorting, washing), chemical (ozone treatment), and biological methods by using mycotoxin-degrading microorganisms. The globally and locally regulatory framework is very effective solution to avoid mycotoxins hazards. Global Standards: Organizations like the Codex Alimentarius Commission set international standards. Country-Specific Regulations: Different countries have varying permissible levels of mycotoxins in food and feed, with the European Union and the United States having some of the most stringent regulations.

How Can We Minimize the Risk from Mycotoxins?

It is important to note that fungi that produces mycotoxins are able to grow on a different crops and foodstuff and also they can penetrate deep into food not just grow on the surface. Mould usually does not grow in properly dried and stored foods, so efficient drying of commodities and maintenance of the dry state, or proper storage, is an effective method against fungal growth and the production of mycotoxins.

To minimize the health risk from mycotoxins, people are advised to [5,17]:

a) Inspect whole grains (especially rice, corn, wheat and *sorghum*), dried figs and nuts such as, pistachio, almond, walnut, coconut, hazelnuts, Brazil nuts and peanuts which are all regularly contaminated with aflatoxins for evidence of mould, and discard any that look mouldy, shriveled or discolored.

b) Avoid damage to grains before and during drying, and in storage, as damaged grain is more prone to invasion of fungi and therefore mycotoxin contamination.

c) Buy fresh grains and nuts as much as possible; make sure that goods are stored properly-kept free of insects, dry, and not too warm; don't keep foods for extended periods of time before being used; and ensure a diverse diet -this not only helps us to reduce mycotoxins hazards, but also improves nutrition.

World Health Organization (WHO) Response

WHO, in collaboration with FAO, is working for assessing the risks to humans of mycotoxins - through contamination in food - and for recommending adequate protection regulations. Risk assessments of mould toxicity in food done by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) are used by governments and by the Codex Alimentarius Commission (the intergovernmental standards-setting body for food) to determines the maximum levels in food or provide other risk management recommendations to control or prevent contamination [5]. Codex standards are the international reference for national food supplies and for trade in food, so that all people in the worldwide can be confident that the food they eat meets the agreed standards levels for safety and quality, no matter where it was produced.

JECFA reported the tolerable intake concentration for many mycotoxins. JECFA or ad hoc FAO/WHO scientific expert groups consist of independent, international experts who conduct regular scientific reviews of all available studies and other relevant data on specific mycotoxins. The outcome of such health risk assessments can either be a maximum tolerable intake (exposure) level, or other guidance to indicate the level of health concern (such as the Margin of Exposure), including recommendation on risk management determinations to control and prevent contamination, and on the analytical methods and monitoring and control activities [5]. These tolerable daily intakes are used by countries and international risk managers, such as the Codex Alimentarius Commission, to estimate maximum levels for mycotoxins in food. The standard levels for mycotoxins in food are very low due to their highly toxicity. So the maximum levels for aflatoxins set by the Codex in various nuts, grains, dried figs and milk are in the range of 0.5 to 15µg/kg (a µg is one billionth of a kilogram). The Codex maximum intake for patulin level in apple juice is 50µg/L. Exposure to mycotoxins should be kept as low as possible to protect the people [35-38]. Mycotoxins not only pose a hazard to both human and animal health, but also effect food security and nutrition by decreasing people's access to healthy food. WHO encourages countries to monitor and ensure that levels of mycotoxins in foodstuff on their market are as low as possible and comply with the both national and international maximum levels, conditions and legislation [39].

Mycotoxins Occurrence in Saudi Arabia

The literature reported that mycotoxins tempt many of scientific interest in Saudi Arabia. Certain types of mycotoxins (zearalenone, aflatoxins) and fungi (*Aspergillus flavus*, *Aspergillus niger* and *Penicillium chrysogenum*) were common in the samples (isolates) of products tested in the Saudi regions. Mycotoxins are

showed to have a considerable hazard on the health of consumers. There has been plentiful research into the effects of mycotoxins, bacteria and fungi on the quality and safety of food and feed. In a review by Althagafi et al. [11] focused on mycotoxins in food and feed produced in Saudi Arabia showed that mycotoxins attract a lot of scientific interest in Saudi Arabia. Some mycotoxins (aflatoxins and zearalenone) and fungi (*Aspergillus flavus*, *Aspergillus niger* and *Penicillium chrysogenum*) were founded to be common in the samples (isolates) of products tested in the Saudi regions as shown in Table 5. The researchers used different method of analysis such as the seed-plate method, dilution plate method, thin layer chromatography, total plate count method, and HPLC method to detect, identify, and isolate mycotoxins. The results showed that mycotoxins have deep health effects on consumers and most of the contamination cases are caused by improper storage conditions and/or inappropriate harvesting and handling practices.

Table 5: Ranges of mycotoxins in food and feed in Saudi Arabia (Althagafi AM et al. [11]).

Mycotoxins	Food and Feed	Range
Aflatoxin B1	Peanuts	8.μg·mL^{-1}
Aflatoxin B2	Sunflower	1.6μg·mL^{-1}
Aflatoxin G1	white cheese	8 to 14ppm
AFT	Nuts	1.0 to 109.0μg/kg
Fusarium sp.	maize and rice	2000μg/kg
Pb and Cd	baby foods & infant formulas	5μg cd/L and 5ppm
Sb and Sn	baby foods & infant formulas	0.04 and 0.054ppm
Fusarium sp.	banana fruits	over 1μg·mL^{-1}
Aflatoxin	Wheat	1.7μg·mL^{-1}

In a study of mycoflora and aflatoxins producing fungi of cotton seed cake in Saudi Arabia [14]. They reported that aflatoxins, produced by fungi like *Aspergillus*, are toxic and carcinogenic compounds found in oilseed crops like cotton. Aflatoxin B1 (AFB1) is the most harmful among the four main types. Nations regulate aflatoxin levels in food and feed due to their toxicity to humans and animals. *A. flavus* is more commonly isolated from crops than *A. parasiticus* and *A. nomius*. Cotton seed cake, imported for dairy feed in Saudi Arabia, may contain aflatoxins, but data on its prevalence in the country are currently unavailable. This study aims to assess fungal flora and aflatoxin presence in cotton seed cake used for dairy feed in Saudi Arabia. Al-Julaifi & Al-Falih [13] studded the commercial animal feed and foodstuff samples that were collected from various sites of Kingdom of Saudi Arabia in 1997-2000. They were analyzed for type A and type B trichothecenes (deoxynivalenol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, nivalenol, fusarenon-x, neosolaniol). The levels of mycotoxins ranged from <2 to 4000μg/kg deoxynivalenol, 3.25 to 500μg/kg fusarenon-x, 3.13 to 600μg/kg nivalenol, 3.13 to 50μg/kg diacetoxyscirpenol, 6.25 to 200μg/kg neosolaniol, 3.13 to 18.75μg/kg HT-2 toxin, and 6.25μg/kg T-2 toxin [40,41].

Surveys conducted in many countries have shown that contamination of food and feed crops by *Fusarium* fungus is an essential agricultural problem. They can occur on a wide spectrum of foods and feeds either in the field or during storage conditions [11,42]. The trichothecenes are an important group of mycotoxins associated with a variety of health hazard in animals and some humans that consume contaminated grains or grain products [33]. Trichothecenes have been classified into four groups. Group A toxins have a functional group other than a ketone at the C-8 position, and are represented by Diacetoxyscirpenol (DAS), Neosolaniol (NEO), HT-2 toxin, and T-2 toxin. Group A is the largest group and includes the most highly toxic members. The second type (group B) is characterized by a carbonyl group at the C-8 position, and includes), Nivalenol (NIV), fusarenon-x (F-X) (18, 22) and deoxynivalenol (DON. Group C has a second epoxide at C-7,8 or C-9,10, and group D contains a macrocyclic ring between C-4 and C-15 with two ester linkages [43].

Mycological, toxicological and chemical studies reported that some of these mycotoxins are often associated with an alimentary disease in both humans and animals. On the molecular level trichothecenes may cause inhibition of DNA and protein synthesis [34]. From 1997 to 2000, different symptoms of toxicosis occurred in animals in different farms of Saudi Arabia. Sheep and cattle showed hemorrhage, skin lesions, respiratory failure, anemia and vomiting associated with feed refusal. In poultry, decreased feed conversion efficiency, weight gain, decreased egg production, and thinner egg shell were observed [13,44].

Conclusion

Mycotoxins represent a significant food safety challenge due to their widespread occurrence and severe health impacts. Ongoing research and technological advancements in detection and control are critical to mitigate their risks. Regulatory frameworks must adapt to new findings to ensure public health protection. The review has shown that mycotoxins can be detected in all kinds of products with various differences between the samples examined. Attention should be paid to storage conditions, harvesting techniques, and handling processes. Also, the regulatory agencies should monitor standard specifications that include maximum permissible limits for toxins in foods in micrograms/kilograms. Strict monitoring of mycotoxins should be regularly implemented to ensure that their amounts in foods are within the allowed limits. There are many factors that increase the production of mycotoxins in foods. One of them is poor storage since storing food at high temperatures and humidity leads to the release of many mycotoxins into food.

Postharvest stages, such as drying and storage, are among the most important stages of production. Food can become vulnerable to mycotoxins if storage are not improper conditions. Usually, the presence of *Aspergillus*, *Fusarium* and *Penicillium*, as well as their fungal toxins, can lead to foodstuff contamination during storage and handling under inappropriate conditions.

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