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Mycotoxin-contaminated food and feed in Saudi Arabia: review of occurrence and toxicity

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Abstract:

Introduction. Mycotoxins are reported to have a considerable impact on the health of consumers. There has been plentiful research into the effects of mycotoxins, fungi, and bacteria on the safety and quality of food and feed. This research paper reviews the literature on mycotoxins.

Study objects and methods. The study focused on mycotoxins in food and feed produced in Saudi Arabia. We reviewed literature on the occurrence and health impacts of foodborne mycotoxins. We also studied the presence of mycotoxins in herbs, nuts, cereals, dried fruits and vegetables, infant formulas and baby foods, as well as dairy products. Finally, the paper offers a review of mycotoxin analysis methods.

Results and discussion. The findings showed that mycotoxins attract a lot of scientific interest in Saudi Arabia. Certain types of mycotoxins (zearalenone, aflatoxins) and fungi (*Aspergillus flavus*, *Penicillium chrysogenum*, and *Aspergillus niger*) were common in the samples (isolates) of products tested in the Saudi regions. Furthermore, the researchers used 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.

Conclusion. Most importantly, the results showed that 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.

Keywords: Toxins, fungi, herbs, nuts, cereals, dried fruits, vegetables, baby foods, dairy products, health, food contamination

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INTRODUCTION

Mycotoxins are toxic substances produced by certain molds (filamentous fungi). The most common of such molds include *Aspergillus*, *Penicillium*, and *Fusarium*. Different molds produce different mycotoxins, with over 400 mycobacterial toxins identified so far and more to come [1]. There are many fungi that grow on foods, especially those stored under certain temperatures and humidity favorable to their growth. Most mycotoxins produced by fungi are carcinogenic to the cells and cause nervous disorders. Mycotoxins have been investigated all over the world and reported to have serious health impacts on humans [2]. These toxins can be produced in the field before or after harvest and during storage of various materials. As a result of growth, fungi perform metabolic processes and produce mycotoxins. The growth of fungi requires specific environmental conditions such as increased humidity, poor storage, abundance of oxygen, contamination with fungi and mechanical damage [3].

Statement of the problem. The storage of grains and products is accompanied by many microorganisms, such as fungi, yeasts and bacteria. They multiply when conditions are appropriate for their growth, causing damage to stored materials. This, in turn, leads to lower product quality, as well as chemical changes in the product. Fungi play a dangerous role, especially during storage, compared to other microorganisms. Mycotoxins have significant economic impacts on many agricultural crops, especially wheat, yellow corn, pistachios, nuts, cotton seeds, and tea. As reported by the Food and Agriculture Organization of the United Nations, 25% of the world's crops are contaminated with fungal toxins, with annual losses of about 1 billion metric tons of foods not suitable for consumption [4].

Mycotoxins can be found in all products that are affected by mold and can be seen with the naked eye where the mold is invisible or long gone. Therefore, a high quality product may contain hazardous substances. To prevent mycotoxins from entering food products, special government programs have been developed to control the production and storage of cereal crops, food raw materials, and animal feed. There are standards for the maximum permissible concentration of mycotoxins per group in different raw materials. During quality certification, each batch is checked for compliance with the standards [5].

The purpose of this article was to review the occurrence and health impacts of food-borne mycotoxins in Saudi Arabia, to elicit the advanced trends in mycotoxin analysis methods in Saudi Arabia, and to identify the effect of mycotoxins on different foods in Saudi Arabia.

STUDY OBJECTS AND METHODS

The work was performed at the Faculty of Medical Science, Department of Toxicology, University of Jeddah, Saudi Arabia. The study objects included scientific and methodological publications, articles in scientific periodicals, conference materials, intellectual property items, regulatory documents, and Internet resources. The methods employed to analyze theoretical data included registration, filing, grouping, classification, comparative analysis, and consolidation of scientific materials.

RESULTS AND DISCUSSION

History and occurrence of mycotoxins. Aflatoxin is the most common of all mycotoxins and one of the most carcinogenic compounds of natural origin. In 1952, the consumption of rotting yellow corn by pigs intended for breeding caused poisoning in the south of the United States. In 1960, so called X-Disease resulted in the death of 100 000 turkeys in England. Aflatoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* fungi. The symptoms of aflatoxin-contaminated food poisoning include loss of appetite, lethargy, muscle weakness, liver bleeding and necrosis, kidney injections, and liver cancer. Aflatoxin of Type B1 is the most dangerous of all mycotoxins. This poison is produced by most types of the *Aspergillus* genus, especially *A. flavus*, which grows on a number of nutrients such as corn and peanut seeds [5].

Mycotoxins are among the strongest known toxins which can cause serious diseases in concentrations of even $< 1\times10^{-6}$. One cause is that they are heat resistant to a degree that is difficult to destroy by traditional thermal treatments used in manufacturing and cooking processes. Another cause is that they spread quickly from mold colonies to food [6].

Mycotoxins can affect several products that consumers eat on a daily basis, including bread, coffee, and spices, as well as milk and its derivatives [6]. In addition to health hazards, they lead to huge material losses when contaminating raw materials [7]. Mycotoxins represent key health risks to consumers. They are commonly present in foods, varying to certain degrees in each type of products.

In Saudi Arabia, mycotoxins occur in many local and imported products due to storage conditions, geographical area, and harvesting techniques [8]. Therefore, it is important that local agencies adhere to the applicable standards to prevent contamination of products. Several studies have examined the toxicity of mycotoxins in different individual food and feed products, focusing on the type and occurrence of mycotoxins in Saudi Arabia. This review aims to identify the occurrence and health impacts of food-borne mycotoxins in food and feed, as well as identify the effect of mycotoxins on different foods in Saudi Arabia.

Occurrence and health impacts of food-borne mycotoxins in Saudi Arabia. Humans and animals can be exposed to, and poisoned by, mycotoxins in direct and indirect ways. Direct exposure can be through contact with the skin, inhalation, or consumption of mycotoxin-contaminated food. Indirectly, humans can be exposed through the consumption of animal products contaminated with residual fungal toxins or their derivatives, such as milk, eggs or meat from animals that were previously fed on toxin-contaminated feed. In general, mycotoxins reach human and animal food through its contamination with the fungus that produces these toxins as the food material encourages fungal growth during different stages of production, transportation or storage [9].

The presence of mycotoxins in foods and tissues is of enormous importance in the field of public health since they are capable of causing acute and chronic effects in humans and animals. These effects range from death to the disruption of the central nervous system, heart, pulmonary and intestinal blood vessels. Mycotoxins are the greatest source of concern as they can cause cancer. Cases of food contamination with aflatoxin were recorded as deaths. Mycotoxins are divided according

Table 1 Ranges of mycotoxins in food and feed in Saudi Arabia

Reference	Mycotoxins	Food & Feed	Range
Alwakeel and Nasser [16]	Aflatoxin B1	peanuts	8.4 μg·mL^{-1}
Alwakeel and Nasser [16]	Aflatoxin B2	sunflower	1.6 μg·mL^{-1}
Aly *et al*. [17]	Aflatoxin G1	white cheese	8 to 14 ppm
El Tawila *et al*. [18]	AFT	nuts	1.0 to 109.0 μg/kg
Abdel-Gawad and Zohri [19]	fungi	nuts	1966–7703 and 1949–7432 colonies/g
Al Husnain and AlKahtani [20]	Fusarium sp	maize and rice	(2000 μg/kg)
Al Khalifa and Ahmad [21]	Pb and Cd	baby foods and infant formulas	5 Ug cd/L and 5 ppm
Al Khalifa and Ahmad [21]	Sb and Sn	baby foods and infant formulas	0.04 and 0.054 ppm
Alghuthaymi and Bahkali [22]	Fusarium	banana fruits	over 1 μg·mL^{-1}
Al-Kahtarii [23]	aflatoxin	Wheat	1.7 μg·mL^{-1}

Prepared based on the findings of this study

to their regions of influence in mammals (hepatotoxins, renal toxins, neurotoxins, muscular toxins, cardiac toxins, skin toxins, reproductive toxins, gastrointestinal toxins), and there is no doubt that higher concentrations of mycotoxins in food increase the resulting damage in the organism [10].

Mycotoxins were reported to constitute a major health risk to consumers. In Saudi Arabia, several studies have been conducted to analyze the risks of food-borne mycotoxins and all of them have confirmed their health risks [2]. In a study by Al-Hindi *et al*., the agar wood was tested in Jeddah city, with mycotoxins isolated and storage conditions analyzed for their impact on the growth of fungi. The researchers used the dilution plate method for the isolation of mycotoxins [7]. Their findings revealed *Rhizopus*, *Fusarium*, and *Penicillium* to be the common types of mycotoxins found in the analyzed samples. The study concluded that the direct exposure to these fungi jeopardizes consumers' health.

Alwakeel, in another study, tested the fungi in fruits growing in Saudi Arabia in order to identify their most common types and health impacts [11]. Out of 34 isolates, 14 were found to be contaminated by *Penicillium chrysogenum*, *Adametzii*, and *Aspergillus oryzae*. According to the author, these fungi are mostly useful and scarcely pathogenic. However, they still cause diseases in people with weak immunity. The study recommended evaluating all products for mycotoxins in order to know the types of fungi present in them and the associated health risks.

In a further study by Bokhari, the fodders of wheat bran, corn, and barley were evaluated from seven Saudi Arabia regions [12]. The analysis showed that most of the samples were contaminated with fungi and toxins. Furthermore, zearalenone, ochratoxin, and aflatoxins were found in the samples and reported to have negative health impacts.

The contamination by mycotoxins can occur both directly or indirectly [13]. Direct contamination is the result of eating food contaminated with these toxins, especially peanuts, peanut butter, and nuts such as pistachios and almonds. Mycotoxin contamination most commonly occurs in the field before harvest and also during some stages of production such as storage and handling, or oil extraction. Equipment can also be a source of contamination, leading to poisoning [5].

Indirect contamination, on the other hand, is caused by using substances (or processed foods) contaminated with mycotoxins. An individual can be exposed to low concentrations of mycotoxins as a result of consuming animal products that contain residues of mycotoxins originating from livestock fed on contaminated feed [9].

Researchers have been able to discover aflatoxin in some types of infected foods such as nuts (peanuts, pistachios, cashews, and almonds), crops (corn, wheat, rice, barley, soybeans, cocoa, and cotton seed), and fodder made from fish, potatoes, and seeds [14]. Olives and legumes can also be contaminated with mycotoxins. Aflatoxins are mostly common in nuts, especially peanuts from which peanut butter is made and used in many food industries, e.g., to make chocolate and biscuits. Also, breakfast cereal flakes prepared from wheat or corn may be contaminated with aflatoxin [15].

Table 1 shows concentration ranges of mycotoxins in food and feed in Saudi Arabia.

Advanced trends in methods of mycotoxin analysis. Analytical chemistry has advanced in recent years and introduced new methods for mycotoxin analysis and titration. Identifying the amount of any chemical substance requires certain steps including sampling, extraction, cleaning up, and analysis [24].

Chromatography is considered one of the most common and effective methods for separating and estimating chemicals in terms of quantity and quality. It is performed in several ways, including thin layer chromatography, gas chromatography, and high performance liquid chromatography [4].

High performance liquid chromatography (HPLC) is among the most important methods for chromatographic separation of liquids. This method uses the static medium in the form of small-sized particles that is pushed by the moving medium (liquid) through the column using a pump at pressures of up to 8000 Psi. The column has velocities between 50 and 50 mL/min

although the particles in the medium are only several micrometers in diameter. It has already been possible to obtain flow velocities of 1–4 mL/min using columns filled with 5 μm particles. In this way, compounds can be separated that are not volatile or those that are not resistant to high temperatures. Also, it is possible to use fine particles of solid material with adsorption properties, materials with ion substitution properties, gels with specific pores, as well as liquid loaded on fine particles of solid material [5].

The relationship between certain materials and a specific mycotoxin contamination profile has motivated the researchers to focus on certain materials to study and investigate. Also, researchers use a variety of methods to detect mycotoxins. Eshelli *et al*. reported that various approaches have been used to assess and control mycotoxin contamination [25]. Critical challenges still exist due to the diverse and complicated nature of food. Different methods, such as genomics, proteomics, transcriptomics, and metabolomics, are used to detect fungal contamination and to identify and isolate mycotoxins, whether before or after harvest. Also, multi-omics techniques are used with developed technical approaches and chemometrics to deliver an explanation of these metabolites yielded before or during crop contamination.

Taheur *et al*. discussed the removal of mycotoxins and detoxification in order to biologically control these subjects [1]. The literature on mycotoxins biotransformation showed the ability of fungi, bacteria, and enzymes to control and minimize the level of mycotoxins. This, in turn, enhances the quality of food and feed, especially meat, eggs, and milk. On the other hand, microbial detoxification was reported to be a trending strategy that does not leave any poisons or less poisonous compounds.

Furthermore, Bokhari used polar solvents such as acetone, water, methanol, and others to investigate the presence of mycotoxins in camel diseases [12]. The results showed that most of the mycotoxins can be dissolved in polar solvents and cannot be solved in non-polar solvents. Also, Bokhari used a similar technique to investigate mycotoxins in anise seeds and black pepper fruits, with *Fusarium*, *Aspergillus* and *Penicillium*, reported as widely spread [6].

Mycotoxin analyses in food and feed in Saudi Arabia. Various techniques of analysis have been employed to identify mycotoxin in different products in Saudi Arabia, as well as in animal feed and food (maize, wheat).

Almoammar *et al*. examined fungal infestation and mycotoxin contamination in camel feedstuffs in Saudi Arabia [26]. The results showed that many samples were contaminated by moulds relating to *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Setosphaeria*, and *Nuersopra*. *Fusarium verticillioides* was the biggest generator of fumonisin, zearalenone, and deoxynivalenol (1050, 245, and 640 μg/kg, respectively). *Alternaria alternata* was the biggest generator of altenuene and alternariol (52 and 29 μg/kg, respectively). The Charm ROSA quantitative lateral flow assay method was employed to measure overall aflatoxin. The aflatoxin B1 was found between 1.0–3.5 ppb in the gathered samples.

A study of sorghum grains conducted by Yassin *et al*. found 31 species from 17 fungal genera [27]. They used an agar plate method to check 24 samples gathered from the markets in Riyadh city, Saudi Arabia. According to the findings, *Rhizopus stolonifer*, *Aspergillus niger*, and *Penicillium funiculesum* were the most common isolated species of fungi. Also, the use of HPLC to examine mycotoxin showed that the mycotoxin-producing isolates differed in kind and concentrations of yielded toxins.

In another study, Alghuthaymi and Bahkali examined the fumonisin B1, deoxynivalenol, and zearalenone in 19 *Fusarium* isolates from bananas brought to Saudi Arabia in 2012 [22]. The trinucleotide repeat analysis was utilized to identify these isolates. The simple matching coefficient was used to determine the similarity degree between the isolates. The fumonisin B1 was reported as common in the imported fruits. Furthermore, Bokhari examined the existence of mycotoxins in camel diseases. In this study, 20 samples of fodders were investigated in seven regions of Saudi Arabia to identify zearalenone, ochratoxin, aflatoxins, and fungi [12]. The analysis of isolates from natural feed showed ten genera and 38 kinds of fungi. The level of toxigenic fungi ranged from 6 to 30 percent in the natural feed and from 5 to 20% in the compound feed.

Effects of mycotoxins on different foods. Mycotoxins are toxic metabolites that are produced by many strains of fungi and are found in food products and livestock feed. These toxins are often invisible, tasteless and chemically stable at high temperatures and during long storage periods. Since some mycotoxins are highly toxic, they may have an adverse effect on health and, thus, products need to be reliably tested. Exposure to mycotoxins occurs either directly, by consuming food contaminated with mycotoxins, or indirectly, by consuming animal-based products, especially milk [9]. The effects of some mycotoxins transmitted by food are severe. Mycotoxins cause serious pathological symptoms that occur immediately after consuming contaminated foods. Other fungal toxins that appear in foods have long-term effects on health, including cancer and immune deficiency [10].

Mold is not the only way for mycotoxins to reach nutrients – they can be found in milk, meat and eggs that were not previously contaminated with mold [13]. Mycotoxins are produced by different groups of molds, namely three genera: *Aspergillus*, *Penicillium*, and *Fusarium*. Despite their harmful effects (plant diseases, food rot and spoilage, production of toxins), fungi play a distinctive role in many food and therapeutic industries such as the production of dairy products,

bread, antibiotics, vitamins, enzymes, cholesterol, fats, and ferments for livestock feed. The growth of fungi, and consequently their production of mycotoxins in agricultural products, is influenced by a set of natural factors, including the fungus strain, nutrient nature, relative humidity, temperature and time, ventilation, spoilage, growth and maturity [14].

The risks caused by mycotoxins poisoning are either through direct ingestion of food contaminated with poison or through the consumption of food such as milk, eggs and liver from animals whose feed was contaminated with poison. There are two types of exposure to aflatoxin, depending on the dose taken. The first is acute exposure – eating a large quantity of poison in food or fodder in a short period of time, which sometimes leads to death. The second is chronic exposure – eating poison in small quantities for a long period of time, leading to tissue changes and then to infection. Several symptoms and conditions caused this way include weakness and lethargy, loss of appetite, weakness of muscles, liver and kidney bleeding, liver necrosis and cirrhosis, bile cell division, liver cancer, acquired immunodeficiency, arteriosclerosis, biliary cirrhosis, diabetes, brain damage, heart failure, hyperactivity syndrome, high blood pressure, infertility, inflammation of the intestine, corneal stiffness, and Alzheimer [15].

Aflatoxin is found in grains such as corn, wheat, barley, rice, and pistachio in addition to its presence in poultry meat and eggs. The toxicity of these substances depends on the nature of poison, its quantity, period of consumption, and food composition. The general effects of aflatoxin on bird health include liver and kidney cancer, hormonal imbalance, lower immunity, proneness to disease, and reduced egg production. In particular, 10 ppb of aflatoxin B1 reduces egg production by half [5].

Also, it is reported that feeding livestock, such as cows, sheep and goats, on feed contaminated with aflatoxin for long periods leads to symptoms and diseases similar to those in birds, negatively affecting milk production, the quality of milk, and muscle tissue [15]. Since aflatoxins are very persistent compounds during the manufacturing process, they cannot be destroyed by normal biochemical methods based on laboratory experiments. However, it is possible to reduce the risks associated with aflatoxin-poisoned food by using special processing procedures to remove the toxins from food. The choice of these procedures depends on the chemical stability of aflatoxin, the nature of food treatment, and the type of their interaction with the contaminated food [9].

Mycotoxins in herbs. In Saudi Arabia, many studies have reported the existence of mycotoxins in herbs [28]. For example, Hashem and Alamri tested the presence of contamination by molds in a number of herbs in Riyadh city, Saudi Arabia [29]. They studied 520 fungal isolates, which represented 57 species, from dried and ground spice samples on three different media using the standard dilution plate method. The study revealed the presence of *Aspergillus*, *Penicillium*, and *Rhizopus* in the species. Also, they found *A. flavus* in ginger. The study indicated that *A. flavus* occurs in herbs due improper storage conditions and, unless they are properly managed, these herbs pose serious health risks.

On the other hand, Bokhari examined mycotoxins in 50 samples of spices such as marjoram, ginger, cumin, pepper, red pepper, and anise. The samples were collected from the retail markets in Jeddah city, Saudi Arabia [6]. The results showed that *Aspergillus*, *Penicillium*, and *Fusarium* were the most widespread fungi in the selected samples. Furthermore, *A. flavus* was found in most of the tested spices. These findings showed that the degree of fungal contamination did not exceed the limits set by the international agencies.

Also, Bokhari tested the existence of aflatoxin and ochratoxin in coffee beans in Jeddah governorate, Saudi Arabia. The researcher used HPLC to test the samples and found that aflatoxin B and ochratoxin were the most common mycotoxins found in the coffee beans [8]. The study indicated improper storage conditions as the key factor of contamination. Also, the coffee beans contamination was found to be a health risk to the consumers and most of the contamination cases occured during the treatment process.

Bokhari and Aly investigated fumonisin B1 in herbal tea consumed by Saudis [30]. Fumonisin B1 (0–266 μg/kg) was detected via HPLC with fluorescence detection. The samples of herbal tea were checked for mycotoxins. The researchers found that the most common mycoflora were shown in 13 types representing 25 species, the most important being *Aspergillus*, *Penicillium*, and *Fusarium*.

Mycotoxins in dried fruits and vegetables. Mycotoxins are naturally occurring contaminates of dried fruits and vegetables. The factors that influence their existence include the type of fruits and vegetables, the geographical region, treatments before and after harvest, harvesting techniques, and storage conditions. Dried fruits are fruits in which most water has been removed naturally by sun-drying or using special dryers. Fruit drying is also a traditional method of long-term preservation and storage [16]. Dried fruits include raisins, peaches, figs, and apricots. About half of their production is sold in the local markets of many countries.

Dried fruits are a source of basic mineral elements (calcium, iron, potassium, copper, boron), vitamins (A, C, K) and plant fibers, which are sometimes low in daily diets. Dried fruits are exposed to many microorganisms, such as bacteria and fungi, which play a large role in the processes of corruption. This condition occurs at different stages before and after harvest, as well as during storage. Several studies have confirmed the

contamination of dried fruits with many species of fungi such as *Aspergillus*, *Rhizopus*, *Alternaria*, *Penicillium Scopulariopsis*, and *Ulocladium*, dominated by *Aspergillus* and *Penicillium* [31].

Dried fruits are food pillars that support the growth of fungi and their production of compounds and metabolic products, including mycotoxins. Thus, mycotoxin contamination is a major concern in many countries of the world. The most common mycotoxins in dried fruits are *Strigmatocystin*, *Patuline*, *Ochratoxin*, and *Aflatoxin cyclopiazonic*. These toxins are produced by certain types of *Aspergillus ochraceus*, *A. parasiticus*, and *A. flavus* [5].

Gherbawy *et al*. analyzed mycotoxins in dried fruits in a number of retail markets in Taif city, Saudi Arabia [32]. The researchers checked the existence of both aflatoxins and ochratoxin A in the dried fruits, isolating 22 fungi from 50 kinds of dates. Using the thin layer chromatography, they found *R. stolonifer*, *A. flavus*, *A. niger*, and *P. chrysogenum* to be the most common mycotoxins in the samples. Their toxicity was measured using Artimia larvae and 9 of 36 isolates were found to contain *A. niger*.

In a study by Alwakeel and Nasser, 40 samples of dried seeds were analyzed in Riyadh city, Saudi Arabia. The researchers used the seed-plate method and the sucrose/glucose-Czapek's agar [16]. Mycotoxins were detected with the thin layer chromatography. Also, the total plate count method was employed to find bacteria. The results showed that *A. flavus* and *A. niger* were dominant in all types of media. Aflatoxin B1 was found in the peanuts (8.4 $\mu g \cdot mL^{-1}$) and aflatoxin B2, in the sunflower (1.6 $\mu g \cdot mL^{-1}$).

In another study, Al-Hazmi analyzed the existence of patulin and ochratoxin A in apple juice produced in Jeddah city, Saudi Arabia [31]. The contamination with fungi was tested on a potato dextrose agar. Also, HPLC was used together with the UV detector in order to determine patulin. The findings showed that the whole samples of apple juice were free of yeasts and fungi. In addition, out of 17 samples, only one contained patulin in a concentration higher than 153 ppb. Also, ochratoxin A was found only in five types. According to Ouf *et al*., patulin is among the most common mycotoxins in fruits, accounting for 84%, especially in the damaged parts of apples, pears, and grapes [33]. The juices and jams produced from these fruits are also infected with fungi secreted by this toxin. The researchers also found aflatoxins B1 and C1 in cherries and carrots, which were then passed on to the juice. Finally, the study revealed concentrations of aflatoxin in dried apricots and figs [33].

Mycotoxins in infant formulas and baby foods. Baby foods are among the key products due to their importance to infants and toddlers. Different studies have examined mycotoxins in baby foods and formulas, especially in the Saudi context. In a study by AlFaris *et al*., the presence of aflatoxins in baby foods was tested using the liquid-liquid extraction method, as well as the immunoaffinity column cleanup [34]. The samples were taken from the markets of Riyadh city, Saudi Arabia. The findings showed that the levels of aflatoxins in baby foods were in line with the European Standards.

Aly *et al*. analyzed the presence of minerals and microbes in white cheese for children and infants in Jeddah city, Saudi Arabia [17]. The researchers found aflatoxin G1 (8–14 ppm) in three samples of cheese using the immune-adsorbent column chromatography. On the other hand, the study showed that yeast and fungi relied on the products that contained Potato Dextrose and Sabouraud. The authors concluded that the cheese products for babies were risk-free as they were not contaminated with a large amount of microbes. However, the removal of affected parts of food does not lead to the complete elimination of fungal toxins formed in these foods.

Therefore, consumers should avoid the growth of fungi responsible for the production of most important toxins. These toxins have a cumulative effect that can manifest 10–20 years after eating contaminated food. Another problem is that the detection of these toxins does not stimulate the immune system and there are no drugs to reduce their impact. As a result, they constitute a health disaster worldwide [10].

Mycotoxins in dairy products. Mycotoxins in dairy products have interested researchers all over the world, including Saudi Arabia, due to the importance of these products to public health, especially to that of children. Dairy products are especially vulnerable to contamination by aflatoxins due to highly complicated processing procedures they undergo. Unless the processing is well controlled, dairy products are more likely to be contaminated, compared to other products. Also, since cow milk is one of the most important dairy products, it has been well studied and analyzed to identify the types of mycotoxins found in it and reasons for contamination [13].

For example, Rahmani *et al*. reviewed some studies that investigated the existence of aflatoxin M1 (AFM1) in milk in a number of countries in the Middle East [35]. They ranked the countries according to the presence of AFM1 in raw milk. The findings showed that ultrahigh temperature milk in Saudi Arabia had lower concentrations of AFM1 than that in Iran and Turkey. The study also indicated that AFM1 in raw milk was higher in Palestine, Lebanon, and Egypt than in Iran, Turkey, and Syria. Finally, the authors concluded that children in the Middle East, compared to other countries, are at a higher risk since they eat raw milk, which may contain high AFM1.

Abdallah *et al*. analyzed ultrahigh cow milk in Najran, Saudi Arabia, to detect the presence of aflatoxin M1 [36]. For the experiment, 96 samples were tested using the enzyme-linked immunosorbent assay. AFM1

was found in about 79 samples. Also, they found that AFM1 was below the permissible levels in the positive samples. This means that the existence of AFM1 in the milk products in Najran does not seem to constitute a severe health problem.

Nasser examined white cheese in Saudi Arabia to detect whether it was contaminated by fungi [3]. For the experiment, 13 kinds of microorganisms belonging to four types of mold and yeast were separated from 20 samples of cheese. The malt extract was utilized. The results showed that most of the cheese samples were free of fungi. Also, *Penicillium* members and *Aspergillus* A were reported to be common in cheese.

Mycotoxins in nuts. There have been studies into the occurrence of aflatoxin contamination in different nuts in Saudi Arabia. For example, El Tawila *et al*. examined 264 samples of nuts gathered from the markets of Makkah city, using the Aflatest immunoaffinity column technique [18]. The findings showed that 70% of the samples were contaminated with aflatoxin (1.0–109.0 μg/kg). In particular, aflatoxin was found in 18% of groundnuts, 18% of almond, 33% of pistachio, 49% of walnuts, 16% of cashew, and 44% of hazelnut samples. About 23% of the samples were within the tolerable limits set by the European Union. Only two products exceeded the limits, namely pistachio and peanut samples.

In another study, Abdel-Gawad and Zohri examined seeds of walnut, pistachio, hazelnut, chestnut, cashew, and almond [19]. The samples were collected from the markets of Ar'Ar city, Saudi Arabia. The study revealed 50 species and three kinds of fungi, namely *A. flavus*, *P. chrysogenum*, and *A. niger*. Their volume ranged between 1966–7703 and 1949–7432 colonies/g of dry seeds.

The permissible ranges of aflatoxins for nuts in Saudi Arabia have been established in line with the standard developed by the GCC Standardization Organization (GSO), as shown in Table 2.

Alhussaini examined 12 samples of edible nuts and dried fruits purchased from the markets in Mekka and Al-Dawadmy regions, Saudi Arabia [38]. Using two methods, dichloran rosebengal chloramphenicol (DRBC) and dichloran 18% glycerol (DG18), the researcher separated 23 fungal species relating to 12 genera. The most important fungi were *A. flavus*, *A. niger*, and *Penicillium citrinum*. Other kinds, found in moderate amounts, included *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus sydowii*, *Eurotium amstelodami*, *Paecilomyces variotii*, and *Trichoderma harzianum*. Out of 40 *Aspergillus* strains, 16 (40%) were reported to generate mycotoxins. Aflatoxins B1 and B2 were generated by eight out of 20 *A. flavus* strains (100–600 μg/L of culture medium). Aflatoxins G1 and G2 were also produced by one isolate of *A. parasiticus* (200 μg/L). Ochratoxin A was produced by *A. niger*, *Aspergillus brasiliensis*, *Aspergillus aculeatus* and *Aspergillus sclerotioniger* strains (100–200 μg/L).

Table 2 Permissible level of aflatoxins in nuts in GCC countries

Commodity/Product Name	Maximum level, μg/kg
Almonds	10
nuts	10
Hazelnuts	10
Peanuts	15
Pistachios	10
Pistachios	15
Dried figs	10

GCC Standardization Organization [37]

In another study, Alwakeel and Nasser analyzed edible nuts using 40 samples of dried seeds picked up from various places in Riyadh city, Saudi Arabia [16]. The seed-plate and dilutions plate approaches were used. Also, the thin layer chromatography was employed to detect mycotoxins. Furthermore, the presence of bacteria was analyzed by the total plate count technique. The study showed that *A. niger* and *A. flavus* existed in all kinds of medium. Also, Aflatoxin B was found in peanuts and sunflower seeds, where it was dominant. Four samples of nuts demonstrated a contamination with bacteria.

Mycotoxins in cereals and cereal-based foods. Cereals have been among the most common objects of study with regard to mycotoxins, especially in the Saudi context. For example, Al-Kahtarii examined mycotoxins in grain and wheat materials [23]. In their study, wheat and other types of grains were analyzed independently for the presence of any types of mycotoxins. About 16 samples were collected from the retail markets in Riyadh city. With the HPLC technique, the study showed that *Alternaria* (69%) and *Aspergillus* (24%) were the most noticeable genera in the samples. Also, the samples contained *Aspergillus* (24%) and *Fusarium* (7%).

In another study, Al Husnain and AlKahtani investigated the presence of fungi in the seeds of maize and rice in certain locations of Saudi Arabia [20]. They also aimed to solve the diversity in 18s rDNA gene found in a number of mycotoxins. The isolated fungi were classified as *Fusarium* sp. and *Alternaria* sp. based on the morphological characters and spores. According to the results, *Fusarium* sp. was the most highly detected fungus, with more than 2000 μg/kg fumonisin, particularly in the 2011 cereal season. The study also showed that the cereals with the highest level of mycotoxins were the ones taken from Dammam.

Mahmoud *et al*. examined four types of cereals, including white and yellow corn and red sorghum seeds, imported to and produced in Saudi Arabia [39]. The experiment was conducted on 80 samples of sorghum and corn grains using the direct plating method. The analysis showed that the white and yellow grains

contained *Aspergillus* spp. and *Fusarium* spp. to a higher degree and *Alternaria* spp. to a lesser degree.

Abudabos *et al.* examined the presence of mycotoxins in dried grains in Saudi Arabia [15]. The study showed that the grains contained higher levels of fumonisin B2, aflatoxin B1, aflatoxin G1, and deoxynivalenol. However, the samples showed a lesser presence of other aflatoxins and ochratoxin A.

Mycotoxins in animal feeds. In Saudi Arabia, natural ingredients such as wheat bran and corn are the most common kinds of animal feed. Bokhary, in his study on camels in Saudi Arabia, reported ochratoxin, zearalenone, and aflatoxins among common fungi found in 20 samples of camel fodder [12]. Some of the tested samples had extra amounts of zearalenone, aflatoxins, and ochratoxin A, as well as other fungi. The study concluded that these fungi can cause health problems to camels.

In a study by Al-Julaifi and Al-Falih, 843 commercial animal feed and foodstuff samples were gathered from all over Saudi Arabia in 1997–2000. They were analyzed for type A and type B trichothecenes (diacetoxyscirpenol, neosolaniol, HT-2 toxin, T-2 toxin, nivalenol, fusarenon-x, deoxyniva-lenol) [40]. The levels of mycotoxins ranged from < 2 to 4000 µg/kg deoxynivalenol, 3.25 to 500 µg/kg fusarenon-x, 3.13 to 600 µg/kg nivalenol, 3.13 to 50 µg/kg diacetoxyscirpenol, 6.25 to 200 µg/kg neosolaniol, 3.13 to 18.75 µg/kg HT-2 toxin, and 6.25 µg/kg T-2 toxin.

All animal feed – whether prepared in the field, imported or stored in silos – may contain mycotoxins. Under certain moisture and heat conditions, these mycotoxins lead to the growth of fungi. Some of these fungi are toxic, affecting animal health and productivity. The symptoms of poisoning with mycotoxins in milk cattle and fattening calves are mostly general and non-specialized, overlapping with other diseases [2]. The diagnosis mainly depends on the exclusion of similar diseases through the examination and analysis of animal feed for mycotoxins. Also, 90% of the problems caused by mycotoxins are subclinical. They include lower productivity and food intake, diarrhea that can be dark or bloody, and increased rates of pathological problems in the herd (placenta retention, displacement of rennet, ketosis, and udder infections) [41].

Mycotoxins accumulate in the tissues of animal flesh or products when animals consume feeds contaminated with aflatoxin. They can pass on to humans and cause liver cancer, even through very small amounts consumed for a period of time. Animal fungal contamination has also been proven harmful to the health of people who eat meat or other animal products, especially children who rely on milk as a staple food.

Some studies have demonstrated a relationship between increased liver cancer and the daily consumption of foods contaminated with aflatoxin. Moreover, laboratory experiments have shown that eating contaminated food may lead to fetal abnormalities, decreased growth and damage to the immune system in animals [40].

The best way to avoid aflatoxins is to eat food from reliable sources (especially peanuts, almonds and nuts). Also, food producers must harvest, manufacture, store, and trade products under appropriate sanitary conditions. The risk of food contamination with aflatoxin increases in countries that suffer from high temperatures and humidity leading to poor storage [14].

CONCLUSION

The review has shown that mycotoxins can occur in all kinds of products with relative differences between the samples tested. 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. Post-harvest stages, such as drying and storage, are among the most important stages of production. Food can become vulnerable to mycotoxins if storage conditions are not subject to strict control. Usually, the presence of *Aspergillus*, *Fusarium* and *Penicillium*, as well as their fungal toxins, can lead to food contamination during storage and handling under inappropriate conditions.

CONTRIBUTION

Alisraa Mohammed Althagafi collected the data, contributed data and analysis tools, performed the analysis and wrote the draft. Hamad Majob Alshegifi collected the data, contributed data, performed the analysis, and wrote the draft. Thamer Salem Qussyier collected the data, contributed data, performed the analysis, and wrote the paper draft. Abdalbasit Mariod contributed data, revised the paper, and submitted the paper. Mansour Tobaiqy performed the analysis and revised the paper.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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