

THE PREVALENCE AND IMPACT OF AFLATOXIN CONTAMINATION IN NUTS AND DRY FRUITS

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Abstract:

Aflatoxins are toxic, carcinogenic secondary metabolites produced by *Aspergillus flavus*, and they are responsible for the contamination of nuts and dried fruits. The aim of the study was to determine the prevalence of aflatoxin contamination in nuts and dried fruits sold in different countries. Dried fruits could contain harmful mycotoxins, posing a risk to health. Fungi, which are integral components of the soil, cause contamination of many food products, including dried fruits and nuts. This fungal burden not only leads to the deterioration of these nutrients but is also responsible for mycoses and mycotoxicoses in consumers, especially immunocompromised individuals. The high burden of aflatoxigenic and drug-resistant fungi in edible products such as dried fruits and nuts poses an immediate threat to the human population and therefore requires rapid control. Aflatoxins were found in food and feed globally, and their economic impact was extensively studied. This review examined the risks of consuming moldy produce and the potential for infection by aflatoxigenic fungi. It also discussed the previous state of aflatoxin contamination in various countries worldwide. Aflatoxins were detected in a range of foods and feeds, with dried fruits (almonds, raisins) and nuts (peanuts, pistachios, walnuts, cashews, etc.) being the primary sources. This review also focuses on the impact of aflatoxin on the health.

Keywords: Aflatoxins, *Aspergillus flavus*, mycotoxins, mold.

Introduction:

Aflatoxin is a group of toxin produced by certain type of mold called *Aspergillus flavus*, *Aspergillus paraciticus*. The presence of mold in agricultural products can pose a significant risk to human health as it can produce toxic substances known as mycotoxins (Set and Erkmén, 2010) These mycotoxins are naturally occurring secondary metabolites of fungi and can be harmful when consumed. Moulds can contaminate agricultural crops at various stages such as growth, harvest, storage, or processing. Dried fruits are particularly vulnerable to mould and mycotoxin formation due to their high sugar content, harvesting method, and drying conditions (Trucksess and Scott, 2008). Raisins, sultanas, figs, apricots, and dates are among the most consumed dried fruits, and their mycotoxin contamination can begin on the trees, worsen during harvesting and sun drying, and persist

during storage, especially in warm climates where these fruits are grown. Fungi such as *Aspergillus*, *Penicillium*, and *Alternaria* are infectious agent of fruit spoilage and can also produce harmful mycotoxins, leading to considerable financial losses in the food industry, including the drying process (Jackson and Al-Taher, 2008). Inadequate storage, improper transportation and marketing conditions, and fungal development can potentially be factors in the generation of mycotoxin in animal feed.

Aflatoxin (AF) and *fumonisin* (FB) are two main mycotoxins found in food and feed products in Sub-Saharan Africa (SSA) (Ochieng *et al.*, 2021; Okoth *et al.*, 2012). While *A. parasiticus* naturally creates the four main kinds of aflatoxin (AFB1, AFB2, AFG1, and AFG2), *A. flavus* naturally produces AFB1 and AFB2 (Norlia *et al.*, 2019). While commercially manufactured pure AFs or FBs are readily available, they may not fully represent the naturally occurring mycotoxins that farm animals consume in their natural habitat (Ochieng *et al.*, 2022). The traditional technique for detecting aflatoxin is crushing kernel tissue in an organic solvent and then applying certain techniques like thin-layer chromatography (TLC), enzyme-linked immunosorbent test (ELISA), or high-performance liquid chromatography (HPLC). Reliable and simple techniques for detecting the formation of aflatoxin have been devised, utilizing the PCR technique (Shapira *et al.*, 1996; Sweeney *et al.*, 2000) and fluorescing on a medium containing cyclodextrin to identify the isolates (Fente *et al.*, 2001; Rahimi *et al.*, 2007). For long-term preservation, nuts and dried fruits are the best option because they have a longer shelf life than "fresh fruits" (Masood *et al.*, 2015). Fungal strikes in nuts and dried fruits primarily cause the generation of hazardous secondary metabolites, especially aflatoxin. "The International Agency for Research on Cancer (IARC) classifies aflatoxins (AFs) as Group I cancer-causing compounds" produced by *Aspergillus flavus* and *A. parasiticus*.

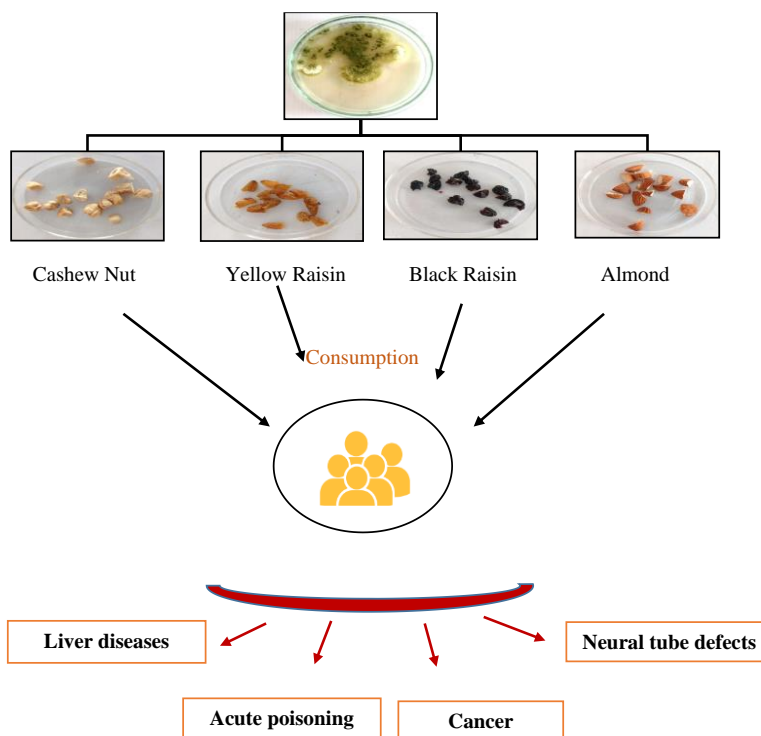


Figure 1. Aflatoxin contamination routes

Aflatoxin producing fungi:

Many *Aspergillus* species as well as species belonging to other fungal genera have falsely been implicated in the generation of aflatoxin. It has been determined that thirteen species belonging to three sections of the genus *Aspergillus* produce aflatoxins: section *Flavi* (*A. flavus*, *A. pseudotamarii*, *A. parasiticus*, *A. nomius*, *A. bombycis*, *A. parvisclerotigenus*, *A. minisclerotigenes*, *A. arachidicola*), section *Nidulantes* (*Emericella astellata*, *E. venezuelensis*, *E. olivicola*), as well as section *Ochraceorosei* (*A. ochraceoroseus*, *A. rambellii*) (Varga *et al.*, 2011).

The studies has demonstrated a close relationship between fungal development and the generation of aflatoxins by creating fungi as hazardous secondary metabolites (Jamali *et al.*, 2011; Shams-Ghahfarokhi, 2013; Jahanshiri *et al.*, 2015; Varga *et al.*, 2011). *Aspergillus* species may grow in temperatures ranging from 6 to 54°C. The ideal temperature range for growth is 35 to 37°C. For the fungus to produce toxins, the ideal temperature range is 28 to 33°C. Water activity of 0.78 to 1 is ideal for *Aspergillus* species growth, with an optimum of 0.95. The ideal water activity range for the formation of toxins is 0.90 to 0.95.

Table 1. Aflatoxigenic fungi and their type

Dried fruits / Nuts	Aflatoxin type	Aflatoxigenic fungi	References
Almond	AFB ₁ , B ₂ , G ₁ , G ₂	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Gholami-Shabani <i>et al.</i> , 2017
Cashew nut	AFB ₁ , B ₂	<i>Aspergillus flavus</i>	Adetunji <i>et al.</i> , 2019
Raisin	AFB ₂ , G ₂	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Alghalibi <i>et al.</i> , 2008
Pistachio	AFB ₁ , B ₂ , G ₁ , G ₂	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Mirabolafathy <i>et al.</i> , 2006
Peanut	AFB ₁ , B ₂ , G ₁ , G ₂	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Adeyeye, 2019
Walnut	AFB ₁ , B ₂ , G ₁ , G ₂	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	Habibipour <i>et al.</i> , 2016
Figs	AFB ₂ , G ₂	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	Gholami-Shabani <i>et al.</i> , 2017

Aflatoxin related diseases:

The primary sources of vital nutrients, including protein, fatty acids, potassium, dietary fiber, and bioactive substances, are thought to be dried fruits and nuts. Additionally, they improve human health by lowering the risks associated with diabetes, obesity, and cardiovascular illnesses (Carughi *et al.*, 2015). Compared to fresh fruits, dried fruits and nuts have a longer shelf life. Exports play a major role in a nation's economy, and the majority of developing nations export food products, which are regarded as the foundation

of their economies (Alghalibi and Shater, 2004). In this sense, dried fruits and nuts are particularly significant because they bring in more than \$3 billion in earnings for the US each year through exports (Johnson *et al.*, 2009). Mild allergic reactions to serious, invasive infections that pose a serious risk to life are examples of these disorders. Among the 600 million food-borne illnesses that affect people globally each year are mycoses linked to contaminated food.

Aflatoxin in nuts & dried fruits:

Pistachio nut:

Aflatoxin was found in pistachio nut samples collected from Iranian orchards at different phases of growth, suggesting that toxigenic fungal contamination usually happened in the later stages of nut development. The report by Mirabolfathy *et al.*, (2006) states that pistachio nut aflatoxin contamination is definitely a significant economic issue for Iran. Aflatoxin levels in Iranian pistachios have led to a restriction on imports by the European Union. Aflatoxin-producing fungus and aflatoxins were found in pistachios in Iran in 1970 (Houshyarfard *et al.*, 2013).

In the Pistachio Research Institute, Arjmand *et al.*, (2006) used a completely randomized design with six replicates to compare aflatoxin contamination in various phases of pistachio processing between 2003 and 2004. Using HPLC, the aflatoxin content of pistachio samples was determined. An analysis of variance showed that the various processing technique components differed significantly from one another. Rehulled pistachio nuts, stained nuts, tiny screened nuts, and final pistachio nuts had mean contamination values of 286, 210, 140, and 1.04 ppb, in that order. The outcomes showed that in order to reduce aflatoxin in the final pistachio, it is essential to separate stained and very small nuts and avoid mixing rehulled pistachios with pistachios that hulled readily on the initial stage.

Walnut:

Walnuts (*Juglans regia* L.) are a notable source of proteins, minerals, vitamins, and omega-3 fatty acids. Iran is the fourth-largest producer of walnuts globally, with a variety of cultivating regions (Habibipour *et al.*, 2016). The European Food Safety Authority (EFSA 2017) established maximum limits (MLs) for AFB1 (2 µg/kg) and the total amount of aflatoxins B1, B2, G1, and G2 (4 µg/kg) in walnuts. It also introduced acceptable exposure limits for other components contained in foods. Their results showed that 280 out of 450 samples had AFB1 levels above the 2 µg/kg MLs reported by the EFSA Scientific Committee. According to Singh and Shukla's, (2008) investigation, AFB1 was found in 21% of Indian walnut samples, with values ranging from 140 to 1220 µg/kg. When Aflatoxins in nuts were surveyed in Pakistan, walnuts showed the highest amounts of Aflatoxins. Three of the seven samples had Aflatoxin levels (6.0 to 10.8 µg/kg) that were higher than the 4 µg/kg limit imposed by the European Commission (Luttfullah and Hussain, 2011).

Cashew nut:

Because of their delicious flavor, cashew nuts are prized and expensive food delicacies in many cultures and customs around the world (Irtwange and Oshodi, 2009;

Abdulla, 2013). The cashew nuts from South Africa, Nigeria are more polluted with both mild and significant mycotoxins. In terms of mycotoxin exposure level, cashew nuts may be regarded as a safe snack. When separate standards were used for each metabolite, as opposed to multimix standards in the LC-MS/MS method, the HPLC-FLD method was able to detect the two major mycotoxins (aflatoxins and zearalenone) in the nut, proving to be a more effective method for detecting mycotoxins in nuts than the LC-MS/MS method. They studied project, matrix effects had a limiting effect on the quantitative outcomes of LC-MS/MS analysis (Adetunji *et al.*, 2019).

Almond:

A fruit that is popular due to its great nutritional content and taste is the almond (*Prunus amygdalus*), a member of the *Rosaceae* family. Almonds are highly vulnerable to a variety of illnesses brought on by bacteria, fungi, nematodes, insects, and mites. Almonds should ideally be determined between the middle of August and October to avoid pest exposure, fungus growth, and ultimately Aflatoxin production. Toxic fungal contamination in almonds originates from the soil where almond fruits and navel orangeworm (NOW: *Amyloides trans-itella*) and other pests were once afflicted (Schade *et al.*, 1975). There is no recognized limit for daily consumption (TDI) of these genotoxic human carcinogens. However, maximum levels (MLs) of 8 µg kg AFB and 10 µg kg total AFs (sum of AFs) have been set for almonds in the European Union (EU) (European Commission, 2010).

Raisin:

Being one of the healthiest dried fruits, raisins have a very high calorie content. Pakistan is one of the Asian nations where grapes are grown on 13,000 hectares of land, producing 49.0 MT of grapes year (PAR, 2015). Aside from their high nutritional content, raisins are vulnerable to fungal infestation, which can result in the production of toxic byproducts known as mycotoxins. Raisins with high moisture and sugar content can promote fungal growth and mycotoxin production (Alghalibi *et al.*, 2008). The main cause of aflatoxin production in raisins is *A.flavus*, with concentrations in Egyptian raisins ranging from 300 to 550 µg/kg aflatoxin B1 (Abdel-Sater and Saber, 1999).

According to Pitt (2000), mycotoxins are secondary fungal metabolites that are formed during the harvesting and storage of agricultural commodities by fungal species such *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. *A. flavus* and *A. parasiticus* are the most common genera isolated from raisins and are in charge of producing aflatoxins (AFs) contamination. According to surveys conducted around the world, raisins are far more susceptible to Ochratoxin -A (OTA) than AFs (Alghalibi *et al.*, 2008; Rahimi & Shakerian, 2013; Perrone *et al.*, 2013). Numerous investigations have indicated that raisins are especially vulnerable to Aflatoxins contamination. Less research, nevertheless, has been done on the contamination of raisins with Aflatoxins.

Apricot:

The Mediterranean and Middle East are the primary regions where apricots (*Prunus armeniaca*) are produced. According to FAOSTAT, Turkey (811,609 tonnes), Iran (457,308 tonnes), Uzbekistan (430,000 tonnes), Algeria (319,784 tonnes), and Italy (198,290 tonnes) were the top five producers of fresh fruit, or apricots, in 2013. Although apricots, especially

apricot kernels, are understudied, they are highly susceptible to aflatoxin contamination. In 1999, first alert on aflatoxins in apricot kernels was published in the European Rapid Alert System for Food and Feed (RASFF) network. Aflatoxin B1 (AFB1) and total aflatoxins (AFs) maximum limits in European apricot kernels meant for further processing (12 µg/kg for AFB1 and 15 µg/kg for AFs) and ready-to-eat (8 µg/kg for AFB1 and 10 µg/kg for AFs) have been in line since 2010 (Zivoli *et al.*, 2016).

Prevention and control of contamination of Nuts & Dried fruits:

Mycotoxin contamination is typically not linked to a single production step because to the wide range of elements that influence it; however, depending on the crop, fungus, mycotoxin, and environmental circumstances, one stage may be more significant than the others. Dried fruit that has been contaminated with mycotoxin may have been contaminated by fungal infection on the trees, increased during harvesting and sun drying, and allowed to continue accumulating during storage due to unsuitable conditions and restoring (Drusch & Ragab, 2003). Early harvesting significantly reduced both post-harvest contamination of the harvested produce and fungal infection of crops in the field. It was discovered that early peanut harvesting and threshing lowered aflatoxin levels and increased gross returns compared to delayed harvesting (Adeyeye, 2019). According to Adejumo and Adejoro (2014), dehydrating produce quickly to a lower moisture content is essential to lowering the amount of mycotoxin contamination in agricultural produce. This is because it creates an environment that is less conducive to fungus growth and reproduction, insect attack, and longer food preservation. It has been discovered that quick drying following harvest can reduce the risk of mycotoxin release from fungus in crops. Postharvest measures that reduced mycotoxin contaminations included keeping crops in a dried state and utilizing rapid mechanical methods of dehydration. Adeyeye, (2019), revealed that mycotoxins in produce could be reduced by physically separating infected food based on color and giving it a thorough water wash. By implementing good agricultural practices such as crop rotation, farm irrigation, effective weed control, adoption of mold-resistant crop varieties, and use of bio-control such as non-mycotoxigenic fungal strains, aflatoxigenic fungal infestation on crops and the subsequent formation of mycotoxin in agricultural produce can be minimized during production and before harvest.

Conclusion:

Fungi play an invaluable role in nature, but many produce harmful metabolites, such as aflatoxins, which pose significant health risks to humans and animals. Aflatoxins, commonly found in food and feed, especially dried fruits (e.g., almonds, raisins) and nuts (e.g., peanuts, pistachios, walnuts, cashews), primarily contaminate products during storage. Their economic and health impacts have been widely studied, but public awareness in underdeveloped nations about the dangers of moldy produce and aflatoxigenic fungi remains low. The scarcity of clinical studies on human exposure to aflatoxins highlights the need for further research. Addressing the aflatoxin issue requires comprehensive evaluations of contamination in each country, the development of sensitive detection methods, effective prevention strategies, and safe decontamination techniques. National

and international collaboration is essential for gathering data and implementing action plans to reduce aflatoxin exposure and mitigate its risks in the future.

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