



## Mini Review

# Mycotoxins in Foods: Occurrence, Challenges and Management in Context of Nepal

Prateek Joshi<sup>1,2\*</sup> , Thanapoom Maneeboon<sup>3</sup> , Chayanee Cheerakupt<sup>4</sup> 

<sup>1</sup>Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

<sup>2</sup>Department of Food Technology and Quality Control, Babarmahal, Kathmandu, Nepal

<sup>3</sup>Scientific Equipment and Research Division, Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Bangkok, Thailand

<sup>4</sup>Ramkhamhang University, Khet Bang Kapi, Krung Thep Maha Nakhon, Bangkok, Thailand

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#### \*Corresponding author

Prateek Joshi,

Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand.  
Email: [prateeklokanthali@gmail.com](mailto:prateeklokanthali@gmail.com)

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### Abstract

Incidence of mycotoxins in principal foods and grains is a major threat to achieving food safety but still remains one of the most underrated and ignored sources for food borne diseases, particularly in less developed countries. Furthermore, food insecurity together with ineffective government regulations and environmental conditions that favor fungal proliferation and toxin production merge together to make the people's life even harder in countries like Nepal. Apart from wasting huge quantities of food every year, mycotoxins are associated with various acute and chronic health disorders including carcinogenic, mutagenic, estrogenic, digestive, vascular and nervous defects. Staple diets in less developed countries like Nepal are largely based on crops like maize, susceptible to mycotoxins which may ultimately lead to chronic health problems in large population. Although there is an immediate need to address the food safety challenges caused by mycotoxin contamination in cereal grains, studies so far in Nepal has been conducted mainly in aflatoxins in limited commodities only and very less efforts have been made to manage and mitigate the problems caused by mycotoxins in Nepal. Therefore, a thorough control of mycotoxins in overall food chain is essential to safeguard the health of the population which could be achieved by implementing stricter regulations, modern and scientific post-harvest operations, effective monitoring programs and raising necessary awareness among stakeholders.

## Introduction

Mycotoxins are toxic secondary metabolites produced by various molds that are able to contaminate wide range of staple foods and cause several kinds of detrimental health effects in humans and animals through chronic exposure or acute toxicity (Wielogorska et al., 2019). The entry of toxigenic fungi and mycotoxins in human food supplies initiated in mankind when human began to cultivate crops

and to store them from one season to the next, possibly 10,000 years ago. The storage process of crops probably initiated providing an optimum environment for fungi to grow and produce mycotoxins. Grains have always been the chief source of mycotoxins in the diet of human (Pitt and Miller, 2017). Mycotoxins can easily mount up in maturing corn, cereals, soybeans, sorghum, peanuts, and other food

and feed crops in the field and in grain during transportation and their entrance to the food chain can be either directly from plant-based food components contaminated with mycotoxins or by indirect contamination from the growth of toxigenic fungi on food commodities (Alshannaq and Yu, 2017). The incidence of mycotoxins in food chain has massive public health significance because of its nephrotoxic, immunotoxic, teratogenic and mutagenic effects in human and animals ranging from death to disorder of central nervous system, cardiovascular, pulmonary systems and digestive tract (Bhat and Vasanthi, 2003).

The co-occurrence of different mycotoxins in the same food commodity may change the nature of toxicity to animals and human beings due to possible antagonistic, additive or synergistic effects (Alassane-Kpembi et al., 2017). Despite the fact that synergism of mycotoxins could intensify the health risks, study on co-occurrence of mycotoxins has not received much attention till now (Silva et al., 2002). Basically, traditional and inappropriate practices of post-harvest operations along with open-air storage arrangements support insect infestation, fungal growth and multi-mycotoxin formation. Although Government of Nepal has endorsed Nepal GAP Implementation Directives on 15<sup>th</sup> of October 2018, most of the farmers are unaware of it and have not adopted the practices since Good Agricultural Practices (GAP) is still a new concept for them (Joshi et al., 2019). Apart from that, very little and scattered surveys have been done till now regarding co-occurrence of mycotoxins and fungal profile in Nepal (Karki et al., 2003). In order to control and reduce the impact of mycotoxins in overall food chain, deep understanding of the fungal ecology is critical for the development of efficient mitigation strategies for the competent authority which not only reduces the post-harvest losses but also plays a great role in ensuring food safety by reducing the risks posed by the presence of mycotoxins in food (Bryden, 2009; Magan and Aldred, 2007).

### Major Mycotoxins and Molds in Foods

Most of the mycotoxins in food are produced by three fungal genera: *Aspergillus*, *Penicillium* and *Fusarium* and

the syndromes caused by the consumption of mycotoxins are known as mycotoxicosis. Toxigenic fungi can produce mycotoxins during both pre harvest and post-harvest periods (Gnonlonfin et al., 2013). Mycotoxins cannot be completely removed by physical and chemical treatments. So, once they occur in food they continue to persist even when the food is processed or stored (Scott, 1991). Apparently, mycotoxins can be divided into five major types that can be seen quite often in food: deoxynivalenol/nivalenol, zearalenone, ochratoxin, fumonisins and aflatoxins. (Tola and Kebede, 2016). International Agency of Research on Cancer (IARC) has categorized different mycotoxins based on their health effects on humans and animals (Table 1).

Aflatoxins are the most potent carcinogens produced mainly by *Aspergillus flavus* and *A. parasiticus* that are present normally in soil and various organic materials (Liu and Wu, 2010). As reported in India, first outbreak of aflatoxicosis killed more than 100 people due to contaminated food. Aflatoxin-producing fungi grow on a wide variety of foods such as cereals (maize, rice, barley, oats, and sorghum), peanuts, ground nuts, pistachio nuts, almonds, walnuts and cottonseeds (Yabe et al., 2003). Ochratoxins are another important group of mycotoxins mostly produced by *Aspergillus ochraceus*, *A. carbonarius*, *A. sclerotiorum* and *Penicillium verrucosum* (Wagacha and Muthomi, 2008). *Penicillium verrucosum* is more common in temperate regions of the world at temperatures below 30 °C, while *A. ochraceus* is more prevalent in tropical regions of the world (Magan and Aldred, 2007). This toxin mainly contaminates cereals, coffee, wine, beer and grape juice (Milićević et al., 2010). Similarly, another toxin zearalenone is mainly produced by *F. graminearum* and *F. culmorum* and usually contaminates wheat (*Triticum aestivum*), barley (*Hordeum vulgare* L), maize (*Zea mays* L) oats (*Avena sativa*), rice (*Oryza sativa*) and sorghum (*Sorghum Bicolor*) (Milićević et al., 2010). Zearalenone is often found associated with reproductive disorders of farm animals and sometimes in hyper estrogenic syndromes in humans.

**Table 1:** Classification of major mycotoxins by IARC and associated health effects

Mycotoxin	Associated health effects in human	Classification (IARC)
Aflatoxins (B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> )	Carcinogenic, hepatotoxic and immuno-suppressive	Group 1
Vomitoxin/ DON	Vomiting, nausea, diarrhea, toxicosis and reproductive disorder	Group 3
Fumonisin	Nephrotoxic, carcinogenic, immuno-suppressive	Group 2B
Ochratoxin A	Genotoxic, carcinogenic, immunosuppressive	Group 2B
Zearalenone	Carcinogenic, reproductive disorder	Group 3
Patulin	Neurologic and GI disorders	Group 3

Group 1: Carcinogenic to humans, Group 2A: Probably carcinogenic to humans, Group 2B: Possibly carcinogenic to humans, and Group 3: Not classifiable as to its carcinogenicity to humans (IARC, 2012)

Source: Channaiah (2019)

Other important *Fusarium* toxins encountered in food and feed are fumonisins and deoxynivalenol. Fumonisin is one of the most commonly occurring mycotoxins in maize globally which are generally regarded as *Fusarium* toxins and produced by *F. verticillioides* and *F. proliferatum* (Logrieco et al., 2003). Various climatic factors and insect infestation facilitates the growth of *Fusarium* which ultimately leads to fumonisins production (Santiago et al., 2015). Naturally, fumonisin B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub> occur in maize with fumonisin B<sub>1</sub> being the most toxic carcinogen of them all (Milićević et al., 2010). Additionally, consumption of fumonisin B<sub>1</sub> contaminated maize is linked to esophageal cancer in humans (Logrieco et al., 2003). Another toxin Deoxynivalenol (DON), also known as vomitoxin belongs to the trichothecenes group of mycotoxins produced by *Fusarium*, mainly by *F. graminearum*, *F. crookwellense* and *F. culmorum* responsible for contaminating cereals like maize (Flannery et al., 2011). DON if consumed for a prolonged period of time can have various lethal effects in humans such as anorexia, decreased weight gain, altered immune function and decreased nutritional efficiency (Sobrova et al., 2010).

### Occurrence History of Mycotoxins in Nepal

Many studies have been carried out since 1980 s in Nepal focusing on aflatoxin in different foods and feed ingredients from different parts of Nepal especially aflatoxin B<sub>1</sub> and B<sub>2</sub> (Karki et al., 2003). Most of the studies regarding aflatoxin occurrence have been carried out in cereal products especially maize and they have revealed that the occurrence of aflatoxin in maize is high and average incidence is about 50%. Similarly, a governmental body for assuring food safety, Department of Food Technology and Quality Control (DFTQC), reports that about one fifth of the maize samples contain aflatoxin greater than the (MPL) maximum permitted limit (20 µg kg<sup>-1</sup>) as established by the Government of Nepal (Pokhrel, 2016).

Studies regarding mycotoxins in Nepal, so far, have been conducted mainly in the aflatoxin. As a result, the data accumulated at present are confined principally of the level of aflatoxin in certain commodities only. Little or nothing is known about levels of other mycotoxins like ochratoxins, zearalenone, DON and fumonisins with regards to the maize and other staple diets in many regions of Nepal. Similarly, not much study is done about the mycobiota and fungal profile in Nepalese staple foods. In a previous study by (Gautam et al., 2008), 42.5% of maize samples collected from Kathmandu valley were contaminated with aflatoxin B<sub>1</sub> with an average value of 50.17 µg kg<sup>-1</sup>. Likewise, when maize and maize product samples were collected from eastern Nepal, one third samples were contaminated with aflatoxin of which one fifth of samples contained aflatoxin greater than 30 µg kg<sup>-1</sup> (Koirala et al., 2005). Similarly, in a study carried out on 141 maize samples from different areas of Nepal, occurrence of aflatoxin was 70% of which 15.7% of samples contained total aflatoxin content more than 20 µg kg<sup>-1</sup> (Rai et al., 2013). These results clearly indicate that, considerable percentage of maize available in Nepal contains aflatoxin which could be a health risk. Likewise, in 2004, 40% of the Nepalese maize samples (N=46) contained fumonisin B<sub>1</sub> above the level of 1000 µg kg<sup>-1</sup> of which 11% of samples had contamination level more than 10000 µg kg<sup>-1</sup> (Desjardins and Busman, 2006). Very less research has been carried out regarding DON occurrence in Nepalese grains, however, according to a study by Desjardins et al. (2000), out of 74 maize samples collected from foothills of Nepal 16% of the samples were contaminated with nivalenol and DON having value more than 1000 µg kg<sup>-1</sup>. Some of the important studies conducted in recent times regarding aflatoxins in Nepal and some neighboring countries can be summarized as in Table 2.

**Table 2:** Study reports of aflatoxin surveillance in Nepalese maize and in other countries

Country	Year	N	%P	CL	%E	References
Nepal	1995-2003	288	32	64-859	20	(Koirala et al., 2005)
Nepal	2008	120	42.5	50.17	NA	(Gautam et al., 2008)
Nepal	2013	141	70	NA	15.7	(Rai et al., 2013)
Nepal	2016	NA	NA	NA	20	(Pokhrel, 2016)
India	2014	150	18.6	48-58	100	(Mudili et al., 2014)
India	2014	25	76	20.6-402.4	100	(Mohana et al., 2014)
China	2014	622	29	3.1	NA	(Cheng et al., 2014)

N = Total number of samples; %P = Percentage prevalence of aflatoxin in maize; CL = Contamination level in µg kg<sup>-1</sup> (average or range); %E = Percentage of samples exceeding 20 µg kg<sup>-1</sup>; NA = Not available

### Regulatory Aspects of Mycotoxins

In order to safeguard consumers from health risks arising from mycotoxins, lots of countries have implemented regulations or guidelines to limit exposure but still, the regulatory status lacks harmony and consensus (Duarte et al., 2010). In order to protect the animal health and human consumers, The Joint Expert Committee on Food Additives (JECFA), a scientific advisory body of the World Health Organization (WHO) and the FAO, evaluates the risks posed by various mycotoxins. In the United States and the European Union, regulatory and recommended guidance for mycotoxins are issued by the FDA and the European Commission (EC) advised by the European Food Safety Authority (EFSA), respectively (Smith et al., 2016).

Regarding the regulatory aspect, the establishment of government-authorized regulatory guidelines and laws is essential. Continuing support from national governments or regional communities to encourage and fund activities that contribute to reliable exposure risk assessment and risk management of mycotoxins in their respective regions is also important to protect consumers from the health threat posed by mycotoxin contamination (Anukul et al., 2013). Regulations have been established in many countries to protect the consumer from the harmful effects of mycotoxins. Current regulations mostly concern the

aflatoxins, but regulations for other mycotoxins are now rapidly developing. Various factors play a role in the decision-making process of setting limits for mycotoxins. These include scientific factors such as the availability of toxicological data and survey data, knowledge about the distribution of mycotoxins in commodities, and analytical methodology. Economic and political factors such as commercial interests and sufficiency of food supply have their impact as well (Van Egmond and Jonker, 2004). So far, Government of Nepal has set MPL only for total aflatoxins (20ppb) only and not for other hazardous mycotoxins like ochratoxins, zearalenone, fumonisins and DON. Major mycotoxins and their US and EU limits on food and animal feed are shown in the Table 3.

### Mycotoxin Related Challenges in Nepal

Cereal grains can get contaminated with mycotoxins in field, during harvest and post-harvest conditions by various fungi. Fungal contamination of grains is not only considered the second most important cause of grain yield loss but it also decreases the processing and nutritional quality of the grain (Miller, 2008). Apart from the economic losses that occur due to fungal development, the growth of fungus in grain is also a major problem for animal and public health due to the probable production and accumulation of mycotoxins (Golob, 2007).

**Table 3:** Major mycotoxins and their US and EU limits on food and animal feed

Mycotoxin	Fungal Species	Food Commodity	US FDA (µg kg <sup>-1</sup> )	EU (EC 2006) (µg kg <sup>-1</sup> )
Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	<i>Aspergillus flavus</i> <i>Aspergillus parasiticus</i>	Maize, wheat, rice, peanut, sorghum, pistachio, almond, ground nuts, tree nuts, figs, cottonseed, spices	20 for total	2–12 for B <sub>1</sub> 4–15 for total
Aflatoxin M <sub>1</sub>	Metabolite of aflatoxin B <sub>1</sub>	Ground nuts, tree nuts, figs, cottonseed, spices	0.5	0.05 in milk 0.025 in infant formulae and infant milk
Ochratoxin A	<i>Aspergillus ochraceus</i> <i>Penicillium verrucosum</i> , <i>Aspergillus carbonarius</i>	Cereals, dried vine fruit, wine, grapes, coffee, cocoa, cheese	Not set	2–10
Fumonisins B <sub>1</sub> , B <sub>2</sub> , B <sub>3</sub>	<i>Fusarium verticillioides</i> <i>Fusarium proliferatum</i>	Maize, maize, products, sorghum, asparagus	2000-4000	200–1000
Zearalenone	<i>Fusarium graminearum</i> <i>Fusarium culmorum</i>	Cereals, cereal products, maize, wheat, barley	Not set	20–100
Deoxynivalenol	<i>Fusarium graminearum</i> <i>Fusarium culmorum</i>	Cereals, cereal products	1000	50–200
Patulin	<i>Penicillium expansum</i>	Apples, apple juice, and concentrate	50	10–50

Source: Alshannaq and Yu, (2017)

Mycotoxin contamination of grain particularly aflatoxin has been a great problem in Nepal in the field of food safety. Numerous studies have shown that the incidence of aflatoxin contamination in maize is high and average prevalence is about 50% (Pokhrel, 2016). Several other studies have shown that cereal and cereal products of Nepal are heavily contaminated with aflatoxins. In Nepal inappropriate drying of grains is one of the main problems during post-harvest operations. For instance, harvesting time of the summer maize is late monsoon when the cobs have quite high moisture content (23-28%). Rural farmers do not have proper knowledge of moisture measurement and hence simply dry maize in sun for 4 to 5 days before storage which favors the growth of fungus in stored maize (Thapaliya et al., 2010).

Preferably cereal grains like maize should be dried to 13-14% before being stored to avoid mycotoxin development but such is not the case in Nepal. Less modern techniques like modification in cultural practices, use of chemical fungicides and improvement to resistant cultivars are also not producing satisfactory results in the reduction of mycotoxin levels in grains in developing countries (Palumbo et al., 2008). In Nepal, less use of modern storage techniques, structures and lack of knowledge regarding proper harvesting technology has made the situation more worse by increasing the chances of mold contamination (Thapaliya et al., 2010). Nepalese farmers lack sufficient information on proper harvesting and handling which results in significant damage by insects and fungi during storage and marketing. Likewise, old processing practices results in mechanically damaged grain which are more prone to fungal growth and mycotoxin production. To summarize, due to lack of efficient farming system, timely harvesting, proper handling and processing, use of modern storage facilities and regular inspection during storage, cereal grains in Nepal are more prone to fungal attack and subsequent production of mycotoxins.

According to Desjardins and Busman (2006), apart from aflatoxin in grains, surveys in maize of Nepal has found that *Fusarium* species dominate among many other ear rotting fungi of which the predominant species are *F. verticillioides* and *F. proliferatum* which produce fumonisins, and *F. graminearum* which produces trichothecenes like nivalenol and 4-deoxynivalenol. In Nepal, various studies on mycotoxins are carried out focusing on aflatoxins but a very little work has been done on fumonisins, nivalenol (NIV) and deoxynivalenol (DON) so far. So, a comprehensive investigation on the occurrence of other types of mycotoxins has not yet been covered (Karki et al., 2003).

To reduce the impact of mycotoxins on public health and to limit the access of mycotoxin contamination in different markets, the regulations set by the country always should be based on sound risk assessment processes combined with

the development of adequate sampling and analysis methods (López-García, 2010). But in case of Nepal, only narrow and scattered survey reports on aflatoxins and other mycotoxins are available till now. Apart from setting maximum permitted limit (MPL) for total aflatoxins (i.e. 20 ppb), Government of Nepal is yet to set MPL for other important mycotoxins like ochratoxins, fumonisin, zearalenone, DON etc.

## **Mycotoxin Management in Food and Way Forward**

Apart from health risks, mycotoxin contamination in agricultural commodities has significant economic implications. Losses due to rejected shipments and low price for the inferior quality products can be devastating for less developed countries. Similarly, its impact on livestock production includes mortality as well as reductions in productivity, weight gain, feed efficiency, fertility, and ability to resist disease. Ultimately, there is the indefinable cost of pain, suffering, anxiety, and reduction of the quality of life. Since toxigenic fungi can contaminate cereal grains with mycotoxins in field as well as during harvest and post-harvest conditions, a multi-disciplinary approach is required to manage the mycotoxins and its associated risks in overall food chain. Likewise, in order to develop the strategies for effective control of mycotoxins, it is crucial to have proper information on the prevailing climatic conditions in the agricultural areas where the crops are being produced (Bhat and Vasanthi, 2003).

In case of mycotoxins, prevention is always better than cure because once the crop is contaminated with mycotoxins, it is extremely difficult and expensive to remove it. There are several strategies that can be employed for the management of mycotoxins in agricultural commodities, some of which can be discussed under the following sub-headings.

### **Primary Prevention**

Prevention of mycotoxin contamination at field or pre-harvest condition is impressively cheaper and effective method than decontamination after harvesting at the final stages of food chain in context of countries like Nepal. Some practical primary prevention techniques could be using fungal resistant varieties of plants, crop rotation and sensible cultivation practices, using chemical fungicides, preservatives and biological control methods, controlling insect infestation in the field, harvesting at appropriate maturity, storing crops at low temperature whenever possible, using improved and rapid drying methods to lower the moisture content of grains after harvesting and during storage, applying good agricultural practices etc. (Wagacha and Muthomi, 2008).

### **Inhibition of Fungal Growth**

It is obvious that suppressing the growth of toxigenic fungi in earlier stages of food chain is an economic way to control mycotoxins. Fungal growth inhibition methods could be

using chemical fungicides, insecticides, biological treatments, use of bio-pesticides, removing damaged grains early on which restricts the fungi to grow, storing crops at appropriate temperature, use of pest and rodent proof storage etc (Bryden, 2009; Neme and Mohammed, 2017).

### **Good Post-Harvest Management**

Apart from earlier mentioned primary prevention methods, postharvest mitigation strategies like proper transportation and packaging, post-harvest insect control are some important and cost-effective method to control mycotoxins in grains. Similarly, grain processing methods like sorting, cleaning, milling, fermentation, baking, roasting, flaking, nixtamalization and extrusion cooking are also found to reduce mycotoxin concentration (Neme and Mohammed, 2017). Other post-harvest interventions include education programs and awareness campaigns among stakeholders, decontamination of grains by physical, chemical or biological methods, irradiation etc. Furthermore, mycotoxin control should be incorporated in HACCP plans as an important aspect of an overall management approach which should include strategies for prevention, control, and quality from farm-to-fork (Murphy et al., 2006).

### **Regulations and Policies**

Regarding the regulatory aspect, the establishment of government-authorized regulatory guidelines and laws is essential. Continuing support from national governments or regional communities to encourage fund activities that contribute to reliable exposure risk assessment and risk management of mycotoxins in their respective regions is also important to protect consumers from the health threat posed by mycotoxin contamination (Anukul et al., 2013). In case of Nepal, MPL for other mycotoxins (ochratoxins, zearalenone, fumonisins and DON) needs to be established as soon as possible. At the same time, it is also important that policy makers should ensure that mycotoxin management regulations are simple, effective and easy to understand by all responsible stakeholders and harmonized with international laws and regulations.

### **Conclusion**

Mycotoxin, which is also regarded as a hidden danger in food and feed is likely to become a bigger issue in overall food chain in the future. Once contaminated, they are extremely difficult to remove from food chain. When consumed mycotoxins not only cause health hazards in humans and animals but also has long term impact on overall economy and quality of life. It is therefore important to raise awareness and improve traditional post-harvest practices to control the progression and colonization of toxigenic fungi in crops. Focus should be on preventing them from entering the food chain. Apart from that, regular monitoring of grains, risk management activities and mycotoxin mitigation measures across the whole country

should be prioritized in order to understand the extent of the problem and minimize the risk.

### **Authors' Contribution**

All authors conceived and designed the paper. All authors wrote and revised the manuscript. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

### **Conflict of Interest**

The authors declare that there is no conflict of interest.

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