Mycotoxin Problem in Nepal: A Review

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Abstract

Mycotoxins are a particular group of toxic secondary metabolites produced by certain fungi on plant and animal products. Their effects on human and animal systems may be cytotoxic, teratogenic, carcinogenic, mutagenic and/or oestrogenic, etc. The problem of mycotoxins is worldwide. Practically all the food and feed commodities are prone to fungal attack and subsequent production of mycotoxins. In Nepal, studies on mycotoxin problem is limited to aflatoxins particularly on aflatoxin B1 and aflatoxin B2, recently fumonisins, nivalenol (NIV) and deoxynivalenol (DON) have also been reported in maize. But successful investigation on the occurrence of other types of mycotoxins has not yet been covered. In this paper an attempt has been made to review the works on mycotoxin problem in Nepal taking account of future prospective of mycotoxin research in the country.

Key words: aflatoxins, fumonisins, toxigenic fungi

Introduction

The term "Mycotoxin" is derived from the Greek word "Mykes" meaning fungus and the Latin word "toxicum" meaning poison. Indeed, mycotoxins are a particular group of toxic secondary metabolites produced by certain fungi growing on agricultural products in the field and/or during storage. The toxic syndromes produced in human beings and animals due to ingestion of mycotoxin contaminated foods/feeds are termed as Mycotoxicoses (Bilgrami 1996). Mycotoxins are elaborated on cereals, pulses, oilseeds, dry fruits and vegetables, dried fish and shrimps, milk and milk products, different types of meat as well as on a wide variety of other consumable articles (Bilgrami 1984). Because there is a relatively high intake of cereal and oilseed crops and their products in the diet of farmed animals such as poultry, pigs and cattle, mycotoxin contaminated feed poses serious safety implications for crop, livestock, poultry producers, grain handlers, food and feed processors; consumers worldwide and eventually affect national economies (Brown et al. 1998).

Mycotoxins are mainly produced by four important genera of fungi viz. Aspergillus, Fusarium, Penicillium and Alternaria. Production of mycotoxins depends on species or strain of the fungus and on the ecological conditions for its development, in particular food source, temperature and humidity (Neegaard 1979).

Although about 300 mycotoxins are described (Cole & Cox 1981) the most important ones from economical and toxicological points of view are aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, citrinin and patulin.

Aflatoxins are most important of all the mycotoxins, because they are acutely toxic and highly carcinogenic and most widespread (Bilgrami et. al '992). They are produced by the toxigenic strains of three closely related species of the genus Aspergillus: A. flavus, A. parasiticus and A. nomius (Kurtzman et al. 1987).

Ochratoxin is produced by certain species of fungi of the Aspergillus ochraceus group as well as by several Penicillium species particularly P. viridicatum (Steyn 1971). The toxin causes nephropathy, a disease of the kidney, found in pigs, chickens, ducks, dogs and also in human beings (Coker & Jones 1988).

Trichothecenes mainly nivalenol (NIV) and deoxynivalenols (DON) are potent protein inhibitors. They are produced by various Fusarum species. Consumption of grain contaminated with trichothecene can cause anemia and immunosuppression, haemorrhage, nausea, diarrhoea and emesis (Marasas et al. 1984).

Fumonisins are produced by F. moniliforme. They can cause equine leukoencephalomalacia, porcine pulmonary edema and experimental liver cancer in rats.
(Desjardins et al. 2000a). Epidemiological studies have associated consumption of maize containing high levels of Gibberella fujikuroi MP- A and fumonisins with the occurrence of high rates of human oesophageal cancer in certain regions of South Africa (Marasas et al. 1984, Rheeder et al. 1992).

Citrinin is a nephrotoxin produced by certain species of Penicillium: P. citrinum, P. viridicatum, P. notatum, P. expansum (Sinha 1996). It can cause kidney damage in animals.

Zearalenone or F2 toxin, a potent estrogenic mycotoxin (Gupta 1998), is produced by Fusarium species especially F. graminearum, F. roseum and F. nivale. This mycotoxin causes an estrogenic syndrome in swine.

Patulin, a carcinogenic and mutagenic mycotoxin, is produced by Penicillium patulum. It can cause death of the cattle (Sinha 1996).

**Mycotoxin Incidence in Nepal**

I. Aflatoxins. Aflatoxins are probably the most common mycotoxins and most widely investigated of all the mycotoxins. These are basically heterocyclic bisdifteranocoumarin compounds which include Aflatoxin B1, B2, B2a, B3, G1, GM1, G2, G2a, M1, M2, M2a, GM2, P, Q, R0, RB1, RB2, AFL, AFLH, AFLM and methoxy, ethoxy and aceto-derivatives (Bilgrami & Choudhary 1998). Practically all the food and feed commodities are prone to invasion by aflatoxin producing fungi and subsequent production of aflatoxins. Aflatoxins have been detected in almost all the cereals and pulses as well as groundnuts, mustard, sunflower, cottonseed, copra, cassava, herbs and spices, cattle feed and poultry feed, milk and milk products from different parts of the world.

Natural occurrence of aflatoxins in food and feed commodities has also been reported from different parts of India, which have been reviewed by Bhat et al. (1978), Bilgrami (1984, 1996) and Bilgrami and Sinha (1984).

In Nepal, studies on mycotoxins are mainly focused on aflatoxins but they have not been reviewed yet. So, the present attempt has been made to do so.

Since 1980, most of the survey works on aflatoxins have been carried out as routine works by Central Food Research Laboratory (CFRL) HMG, Kathmandu (Karmacharya 1988) and have been published in its Annual Bulletins. Karki et al. (1979) concluded that corn might be a risk commodity for aflatoxin production during storage and movement of grain from plain Terai to the deficit hill areas. Karmacharya (1984) collected 465 samples of various food commodities and 94 samples of different feeds and feed ingredients from different parts of Nepal and analysed for aflatoxin contamination. In her study maize and groundnut were mostly contaminated with aflatoxin B1 and B2. Among feed samples, 25 of 58 poultry feed samples were highly contaminated with aflatoxins.

Karmacharya (1988), again, analysed 764 samples of various food commodities collected from hills and Terai regions and 148 samples of different feed ingredients collected from different feed industries of Kathmandu at the Central Food Research Laboratory, HMG, during 1980 to 1986 for aflatoxin contamination. She reported that of the total number of respective samples analysed, 19% of maize, 29.5% of maize flour, 17.9% to 33.8% of groundnuts, 31.7% of peanut butter and 9.4% of wheat flour were contaminated with aflatoxins. In case of feed, about 50% of poultry feed, 26.7% of cattle feed and 20% of pig feed samples were contaminated with aflatoxins. Analytical results showed that maize and peanuts were the most susceptible commodities.

Joshi et al. (1987) found that Aspergillus flavus strain isolated from corn grown at plain Terai produced higher amount of aflatoxin B1 (6334 ppb) followed by Kathmandu valley, hilly area and Himalayan regions. Joshi and Karki (1988) again reported that in Terai plain area, where temperature and humidity were relatively high, infection of Aspergillus flavus predominated from the onset of storage and increased along with the increase of storage period, while in mountainous area and Kathmandu valley, no A. flavus was detected until nine months of storage. They observed that Thangro seems to be an appropriate storage system for avoiding aflatoxin hazards in the Kathmandu valley. Also, Bhakari type of storage can be improved for preventing mold infection and subsequent toxin production.

According to a study for the period 1986-1987 carried out by CFRL, HMG, 206 samples of food, food ingredients, feed and feed ingredients were analysed for aflatoxin contamination (CFRL 1988). In this study, 66 samples of corn collected from different storage structures (piled stack, hanging in the caves of the house or inside Varandah or window, Kunyu construction, Thangro) of farmers’ houses from different places of Nepal (Rampur, Hetauda, Nijgadh, Sarlahi, Kathmandu, Khumaltar and Godawari) from 2043 Shrawan to 2044 Ashadh (August 1986 to July
Aflatoxins were not detected in any of the samples. Also 36 samples of shelled grains of corn and 16 samples of corn flour collected from Kathmandu valley Rampur and Nijgadh were analysed for aflatoxin contamination. 36 samples of shelled grains of corn

12 were contaminated with aflatoxins (AFB1 = trace to 70.5 ppb; AFB2 = 2 to 4.2 ppb). Again 60 samples of food and food ingredients collected from markets of different parts of Nepal (Kathmandu, Pokhara, Biratnagar, Nepalgunj, Kaliaya) were analysed and results of the analysis are shown in Table 1.

Table 1. Aflatoxin content in various food and feed ingredients collected from markets of various parts of Nepal (1986–1987)

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Commodities</th>
<th>Total samples</th>
<th>Contaminated samples</th>
<th>Aflatoxin level (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corn grains</td>
<td>23</td>
<td>6</td>
<td>tr - 241</td>
</tr>
<tr>
<td>2</td>
<td>Corn flour</td>
<td>18</td>
<td>5</td>
<td>14.1-42.3</td>
</tr>
<tr>
<td>3</td>
<td>Peanut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>Shelled peanuts</td>
<td>11</td>
<td>6</td>
<td>5.6-141</td>
</tr>
<tr>
<td>(b)</td>
<td>Unshelled peanuts</td>
<td>1</td>
<td>1</td>
<td>634.6</td>
</tr>
<tr>
<td>4</td>
<td>Peanut butter</td>
<td>7</td>
<td>5</td>
<td>tr - 330</td>
</tr>
</tbody>
</table>

Source: CFRL (1988)

In the same report, 28 samples of food, food ingredients and feed commodities (wheat, wheat flour, wheat soya milk powder, Suji, barley, red gram, lentil, betel nut, poultry feed and pig feed) were collected from different parts of Kathmandu valley and analysed for aflatoxin contamination. Only poultry and pig feeds were contaminated with aflatoxins.

In another report as shown in Table 2, out of 379 samples of food, feed and feed ingredients collected from various parts of Nepal, 182 samples were contaminated with aflatoxins (CFRL 1989). As shown in the Table, 24% of corn grain, 35% of corn flour, 13% of roasted unshelled peanut, 35% of fried salty peanut, 82% of poultry feed, 77% of feed and 2% of feed ingredient samples were contaminated with aflatoxins.

Table 2. Aflatoxin contamination in various food, feed and feed ingredients of Nepal (1988 – 1989)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Types and source of commodities (hills and Terai)</th>
<th>Total no. of samples</th>
<th>No. of samples contaminated with AFB1</th>
<th>Level of aflatoxin</th>
<th>% of total contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Food commodities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Corn grains</td>
<td>66</td>
<td>16</td>
<td>tr - 63.4</td>
<td>24.00</td>
</tr>
<tr>
<td>2</td>
<td>Corn flour</td>
<td>34</td>
<td>12</td>
<td>tr - 93.3</td>
<td>35.00</td>
</tr>
<tr>
<td>3</td>
<td>Roasted unshelled peanuts</td>
<td>15</td>
<td>2</td>
<td>9.3 - 933.5</td>
<td>13.00</td>
</tr>
<tr>
<td>4</td>
<td>Fried salty peanuts</td>
<td>20</td>
<td>7</td>
<td>3.7 - 373.4</td>
<td>35.00</td>
</tr>
<tr>
<td>B</td>
<td>Poultry feeds and eggs (a poultry farm, Lalitpur)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Poultry feed</td>
<td>50</td>
<td>41</td>
<td>tr - 112</td>
<td>82.00</td>
</tr>
<tr>
<td>2</td>
<td>Eggs</td>
<td>50</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C</td>
<td>Feeds and feed ingredients (Kathmandu valley)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Feed samples</td>
<td>125</td>
<td>96</td>
<td>tr - 190</td>
<td>77.00</td>
</tr>
<tr>
<td>2</td>
<td>Feed ingredients</td>
<td>19</td>
<td>8</td>
<td>2 to 94</td>
<td>42.00</td>
</tr>
</tbody>
</table>


Note: ppb = parts per billion; AFB1 = Aflatoxin B1; AFB2 = Aflatoxin B2; tr = Traces, ND = Not detected; CFRL = Central Food Research Laboratory
Aflatoxins were in high level in peanuts (9.3 to 9333 ppb) and feed samples (trace to 190 ppb). Aflatoxins were not detected in egg samples.

Shrestha and Amatya (1996) analysed 73 samples of food, feed and feed ingredients for the detection and estimation of aflatoxin. The levels of aflatoxin contamination were higher than the prescribed level of HMG/N in 8 feed samples, 2 corn feed ingredients and 1 flour sample.

Shrestha and Amatya (1997) again analysed 273 samples of food, feed and feed ingredients for detection and estimation of aflatoxin B1 and B2. Out of the total samples, they found 43 samples heavily contaminated with aflatoxin. Aflatoxins were in high level in raw peanut, butter nut, feed, corn and corn flour. Three contaminated samples of feed and 9 samples of food were found above the prescribed permissible level of HMG/N. The prescribed permissible level of aflatoxin of HMG/N for food and feed are 30 ppb and 50 ppb respectively (Nepal Gagette 1988).

Shrestha (1997) analysed 200 samples of food commodities which included maize and maize products and peanut and peanut products collected from Kathmandu valley. 16% of the raw peanut samples were contaminated with aflatoxins. Twelve percent were having high risk level ranging from 30 to 1610 ppb (B1) and 175 ppb (B2). Similarly, 16.12% of the fried roasted peanuts were contaminated with aflatoxin B1 and B2.

Among other peanut products, 80% of peanut butter samples were contaminated with aflatoxin. 6.67% of corn samples were contaminated with aflatoxin. 17.39% of the corn products (grits and flower) were found to be contaminated with aflatoxin. In all, the contamination level was less than 30 ppb.

Shrestha and Amatya (1998) again analysed 149 samples of food, feed and feed ingredients for detection and estimation of aflatoxin B1 and B2. Fifty two out of 149 samples of food, feed and feed ingredients were found to be contaminated with aflatoxin B1 and B2. Aflatoxin levels in 9 samples of peanut and 5 samples of feed were found above the prescribed level of HMG/N.

In another survey, out of 183 samples of different varieties of foods, 77 samples were contaminated with aflatoxin B1 and B2 which were from traces to the level of 1736 ppb, and 294 ppb, respectively (CFRL 1999). Sixty samples were contaminated above the prescribed limit of HMG/N.

Joshi and Karki (1999) and Joshi (2000) isolated mycotoxigenic fungi from 165 samples of Nepalese corn that were grown in different elevations ranging from 72-2300 meters above sea level, for determining their toxigen potential. Fisularium species and A. flavus were found to be the most predominant mold flora in the hills and Kathmandu valley (up to 50%) whereas A. flavus ranked high in Terai and Inner Terai (upto 100%). The amount of toxin produced by A. flavus strains from the corn grown at different altitudes varied appreciably. A. flavus strains isolated from corn grown in the plains of Terai produced high concentration of aflatoxin B1 (6334 ppb), followed by Kathmandu valley, hill areas and Himalayan Regions. Aflatoxin produced by different representative strains of A. flavus from the various ecological regions showed a distinct altitude bias.

Shrestha et al. (1999) and Shrestha (1999) carried out a random screening program concerning the aflatoxin producing fungi A. flavus at different places of Nepal. About 258 fungal isolates were obtained from 11 corn samples. Out of 258 isolates, 37 (14.37%) isolates were A. flavus and only 17 (46.0%) A. flavus isolates could produce aflatoxins. A. flavus could produce maximum aflatoxin B1 of 0.22 µg in 5 ml yeast extract sucrose broth when it was incubated at 30°C for 7 days and 0.17 µg in 5 ml yeast extract broth when it was incubated at 30°C for 24 hours. Aflatoxin B1 was found from trace to 171 ppb and B2 from trace to 5 ppb in 12 corn samples out of 41, collected from different places of Nepal (Biratnagar, Birtamod, Dharan, Dhulikhel, Kathmandu, Lalitpur and Rupandehi). Also different chemical agents (Lemon grass oil, Mentha arvensis oil, Timur oil, Citric acid, Lactic acid, Tartaric acid, Ethanol, Sodium chloride and Neem leaf) were used to study the growth suppression of A. flavus. Study of the growth suppression of A. flavus showed that it could not grow at concentration of 0.1% lemon grass oil, M. arvensis oil and Timur oil and also at concentration of 5% citric acid, lactic acid and tartaric acid but slowly grew at concentration of 5% ethanol. There was no effect in the growth of the A. flavus when using sodium chloride and neem leaf.

Acharya (1999) screened 15 isolates of A. flavus obtained from different edible foodstuffs of Kathmandu for their potentiality to produce aflatoxin. She also studied the level of aflatoxin contamination in 30 samples of different edible foodstuffs (corn, peanut and cheese) taken from different retail markets of Kathmandu. Out of 15 isolates, 4 isolates of A. flavus were aflatoxigenic. Analysis of aflatoxins in the edible foodstuff showed that 23.33% samples were positive to aflatoxin B1 and B2 contamination. Higher contamination was observed in corn (AFB1 = 139.98-194.17 ppb; AFB2 = 2.87-
16.37 ppb) followed by peanut (AFB$_1$ = 62.8-78.83 ppb; AFB$_2$ = 12.10-18.16 ppb).

Khadka et al. (2000) studied aflatoxin contamination in livestock feed and feed ingredients of Nepal. Altogether 100 samples comprising of 25 livestock feeds, 50 poultry feed and 25 feed ingredients were analysed. The percentage of samples with aflatoxin contamination was 80%, 74% and 72%, respectively, for livestock feed, poultry feed and feed ingredients. Aflatoxin B$_1$ was detected in aflatoxin positive samples either alone or in combination with aflatoxin B$_2$.

2. Fumonisin, Nivalenol and Deoxynivalenol Very limited studies have been carried out on mycotoxins other than aflatoxins in agricultural commodities of Nepal (Ueno et al. 1993, Desjardins et al. 2000a, 2000b. These studies were mainly focussed on fumonisin, nivalenol (NIV) and deoxynivalenol (DON). Desjardins et al. (2000b) analysed 48 rice samples collected from small holder farm and from Nepal Agricultural Research Station in the foot hill region of Central Nepal and in the Kathmandu valley and adjoining regions of eastern-central Nepal. Though the fumonisin producing species Gibberella fujikuroi MD D. and trichothecene producing species G. zeae were isolated from them, they were not contaminated with these mycotoxins.

Desjardins et al. (2000a) again analysed 78 samples of maize and maize based foods and 27 samples of wheat seeds collected from the foot hills of the central and eastern Himalayas. In their study they found strains of G. fujikuroi mating population produced fumonisins and strains of F. graminearum produced NIV or DON. Fumonisins were >1000 ng/g in 22% of 74 maize samples. NIV and DON were >1000 ng/g in 16% of maize samples but were not detected in wheat.

Future Prospects of Mycotoxin Research in Nepal

Studies on mycotoxin in Nepal are centered mainly on the general survey of aflatoxin contamination in some agricultural commodities. However, pertinent studies on mycotoxins other than aflatoxins and their associated problem should never be ignored. Again informations regarding the toxic effects of mycotoxins in animals and human beings are not available in Nepal. It is therefore, felt need that the impact of mycotoxin exposure and risk assessment should be prioritised in initiating future work. Acute and chronic effects of mycotoxin in human beings can only be estimated by epidemiological observation of suspected cases of mycotoxicoses including the diet associated with such incidences.

Since only limited and scattered survey reports on aflatoxins and other mycotoxins are available in Nepal, there is need of systematic surveillance of different food items including agricultural commodities for assessing the risk of mycotoxin contamination in those items. The surveillance can be made in various agro-ecological zones of Nepal which can help in identifying high -, low- or no-risk areas and commodities.

Emphasis should also be given towards preventive methods for the control of mycotoxin contamination. Various approaches such as host–plant resistance, cultural, biological and chemical control practices can be adopted for the purpose. Priority should be given to the research on genetic resistance to fungal invasion and toxin production especially in local cultivars, resistant varieties should be multiplied and popularized in risk prone-areas. Efficient drying, storage, aeration system can control aflatoxin contamination in agricultural products such as cereals, pulses, oilseeds.

Recent studies have shown that many naturally occurring chemicals can inhibit fungal infection and toxin production (Bilgrami et al. 1979, 1992, Sinha 1985, 1990, Sinha et al. 1993, Shrestha 1999, Joshi 2000). So priority should also be given, to exploit the use of locally available, non-toxic plant extracts, constituents to control the production of mycotoxins in agricultural crops.

In Nepal, CFRL, HMG, presently Department of Food Technology, presently Department of Food Technology and Quality Control (DFTQC), Kathmandu has been involved in identifying the fungal contamination particularly aflatoxins and the extent of toxin production in food commodities in view of reducing toxic hazard to human beings. Some research works on mycotoxin especially fumonisin have been carried out by Plant Pathology Division, NARC, Khumaltar, Lalitpur. Also work on aflatoxins has been carried out by Central Department of Microbiology, Tribhuvan University in collaboration with DFTQC.

Taking view of the diverse nature of Nepalese agro-food system and its impact in the food chain, concerted efforts from different research organizations is vitally essential to tackle multi-prong problem of fungal infection and mycotoxins.

The toxic effect of combined doses of aflatoxin and fumonisin seems to be the area of future investigation, as the agro-ecological niches of the country is favourable for the harbour of causative fungi.
The other promising area of future intervention on curbing incidence of aflatoxin production on peanuts and corn is to develop practicable approach on preventing growth and development of toxigenic fungi in the food system. The other area of focus should concentrate on developing predictive models for basic information on fungal growth and toxin production from the standpoint of tolerances of commodities to adverse storage conditions.

References


