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Postharvest Handling and Prevalence of Aflatoxins Contamination in Nepalese Maize Produce

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In Nepal maize is produced and consumed in significant amount. Summer maize contributes about seventy percent of the total maize production but has high possibility of fungal infection before and during harvest. Traditional practices of post harvest operations and outdoor storage structures are supportive for insect infestation, mold growth and development of mycotoxins. Several studies have shown that the incidence of aflatoxin contamination in maize is high and average prevalence is about 50%. Department of Food Technology and Quality Control (DFTQC), a governmental body reports that about 20% of the maize samples contain aflatoxin greater than the ML (20ppb) as set by the government. On the basis of available data, a simple deterministic exposure assessment for total aflatoxin via maize shows that the situation is alarming and needs immediate attention. In Nepal aflatoxin concern in maize demands a need to further investigation and a risk assessment for revealing the existing situation. This review aims to find out the current situation of aflatoxin contamination in maize produce of Nepal and provide possible ways to reduce the contamination.

Keywords: Maize, Postharvest handling, Aflatoxins, Maximum limit

Introduction

Maize is one of the most important grain crops produced in Nepal. In terms of area and production in Nepal, maize stands as the second important crop. Area under summer maize is about 73.9% whereas spring and winter maize occupied 14.2% and 11.9%, respectively (Gurung *et al*., 2011). The summer maize matures during the monsoon season and, after harvesting, for long term storage, maize is traditionally left with the husk on a platform or rack in the farmyard after drying (Desjardins *et al*., 2000). Sun drying is the only technology available to the farmers. The maize ears with or without husk are stored in a traditional outdoor structure known as *Thangro*, while the maize grains are stored in a variety of indoor structures, including *Bhakari* (woven bamboo structure), *Ghyampo* (an earthen structure) etc. (Paudyal *et al*., 2001).

Maize crop is very important for mid hill people and it contributes to food security and livelihood options in those areas (Gurung *et al*., 2011). Maize accounts for about 15 % of total cereal consumption. For poor people, it accounts for 17-19 % of their cereal consumption (CBS, 2011). According to GoN, (2014) 2283 thousand metric ton of maize was produced in the fiscal year 2013/14. The utilization statistics indicates that the chief use of maize as food accounts for 70%, while the proportion for feed purpose accounts for 20% and other uses mainly as inputs in several industries and seed as 10% (Paudyal *et al*., 2001). Average per capita consumption of maize is 45.5 kg/capita/year, which is even more in the hills, where maize is vital for survival (Gurung *et al*., 2011). As staple food of most foothill populations, it is used to produce a variety of porridges, snack foods, and fermented beverages (Desjardins *et al*., 2000).

Aflatoxins are widely recognized as a major health problem, especially in hot, humid countries. This is a particular serious problem in such crops as maize, rice, peanuts, tree nuts, and dried fruits. Aflatoxin production normally occurs in the field, particularly when stimulated by drought, stress, and high temperatures or during prolonged drying. Aflatoxin-producing molds grow exponentially in conventional multi-month storage as a combination of heat and high humidity (Villers, 2014). *Aspergillus faavus*, *A. parasiticus*, *A. tamari* and *A. nomius* are the fungi that produce aflatoxins B1, B2, G1, and G2 as a secondary metabolite (Rai *et al*., 2013).

The major problem with postharvest handling in Nepal is the difficulty of drying maize. The summer maize harvesting season coincides with the late monsoon when cobs have a

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relatively high moisture content (23- 28%). Farmers have neither the knowledge nor the equipment to measure maize moisture content and therefore they simply dry the maize for 4-5 days before storing. Lack of knowledge regarding to harvesting technology, factors of contamination and lack of proper storage techniques and structures have further worsen this situation (Thapaliya *et al.*, 2010). Aflatoxin producing fungi infests food grains or other food items usually during storage (Koirala *et al.*, 2005).. Ideally maize should be dried to 13-14% before being stored (Paudyal *et al.*, 2001).

According to Karki *et al.*, (1979) corn contaminated with aflatoxins especially during storage and movement of grains from the *Terai* (Southern plains of Nepal) to the deficit northern hilly areas. Various studies done in the past have revealed the presence of aflatoxin in Nepalese maize. Moreover the contamination in the maize is much higher than any other cereal crops. Since, maize is staple food to many people after rice, its consumption represents that significant population is exposed to aflatoxin and thus the occurrence of aflatoxin in maize is a serious issue.

Aflatoxins are of particular public health importance because of their effects on human health (Gautam *et al.*, 2005). Aflatoxins have both carcinogenic and hepatotoxic actions, depending on the duration and level of exposure (Lewis *et al.*, 2005). Low-level, chronic exposure is carcinogenic and has been linked to growth retardation in children (Hoffmann *et al.*, 2013). Ingestion of higher doses of aflatoxin can result in acute aflatoxicosis, which manifests as hepatotoxicity or, in severe cases, fulminant liver failure (Lewis *et al.*, 2005). Aflatoxins of public health importance that occur naturally in cereals exist in four forms, namely Aflatoxin B1, B2, G1and G2 , with aflatoxin B1 being the most potent form of the aflatoxins. These toxins are acutely toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic (Kimanya *et al.*, 2008). According to (Raad *et al.*, 2014) for non-European countries, the ingestion of 1ng/kgbw/day aflatoxin would induce 0.0083 liver cancer cases per year per 100,000 persons. Suleiman and Rosentrater, (2015) have shown that the risk of liver cancer from consumption of 50 g/person (60kg) per day food contaminated with 20ppb AFB1 per 100,000 populations is 1.4, similarly for 100 gram consumption in the same contamination level the number would be 14. This study was carried out on the hypothesis that corn consumed in Nepal is contaminated with aflatoxins in significant amounts. It is also assumed that the rural population is at high risk due to the mycotoxin exposure as corn is part of their daily diet. This review aims to collect relevant information regarding corn consumption pattern and the aflatoxin contamination in corn and suggest feasible ways to reduce the contamination.

Contamination of maize by moulds and production of aflatoxins

Aflatoxin contamination in maize is a worldwide problem. Aflatoxin producing fungi can grow on a wide range of agricultural commodities, the most important of which are maize and peanuts (Rai *et al.*, 2013). FAO estimates that 25% of world food crops are affected by Mycotoxins (Waliyar *et al.*, 2015). Contamination of Aflatoxins can take at any point along the food chain from the field, harvest, handling, shipment and storage (Salim *et al.*, 2011). The following table tries to explain the possible steps where the contamination of mold can occur and the probability of aflatoxin production.

Table 1. Qualitative expression for mold contamination and aflatoxin formation probability for summer maize post-harvest in Nepal

Operations	Average Conditions; (Tempr, RH%), Seasons	Possible explanation	Mold contamination probability	Aflatoxin formation probability
Pre-harvest	(16-24)^{o}C, (61-85): in Hills; (18-29), (63-78) in Terai; Season: Spring-Summer (Paudyal *et al.*, 2001).	In humid area where rainfall is above normal, maize ear are susceptible to fungal rot from silking to harvest (Muduli *et al.*, 2014). *Aspergillus* grows best at 25^{o}C (Mehta *et al.*, 2012).	Low	Low
Harvesting at appropriate time	23-24oC, (85-86) in Hills; Post Monsoon in Hills; (28-29^{o}C, (70-78) Monsoon (Paudyal *et al.*, 2001).	Moisture content of harvest crop is (23-28) % (Manandhar *et al.*, 2000). *A. flavus* and *A. parasiticus* can grow at low water activities (a_w 0.75-0.8) (Mehta *et al.*, 2012). But for aflatoxin production, high aw is needed (Thapaliya *et al.*, 2013).	Medium	Low

Delayed harvesting/ Field drying on Plant		Late harvesting or field drying increases the level of contamination (Hell *et al*., 2000). According to Kaaya *et al*. (2006), Aflatoxin levels increases by about 4 times by the 3rd week and more than 7 times when maize harvest was delayed for 4 weeks.	High	High
-Storage Practices	10-24°C, (60-86): in Hills; (28-29°C, (49-78) Post monsoon Season (Paudyal *et al*., 2001).	Traditional storate structures for maize storage do not provide adequate protection over rodent and insect pests (Manandhar *et al*., 2000). Insects can disseminate spores of *A. flavus* in the field and stored products (Hell *et al*., 2000). Smoking can reduce contamination of aflatoxin when stored indoor.	Medium	Medium
-Hanging indoor		Incidence of fungi of the *Aspergillus* genus and aflatoxin contamination is higher in insect-damaged maize than in insect free samples in hot humid countries (McMillian, 1987).	High	High
-Shelling and Storage in bins/silos		Before storage in bins, grains are dried. According to Thapaliya *et al*. (2010), at 13% moisture, there is no growth of *Aspergillus*.	low	low

Aflatoxin contamination in maize in the world

Numerous studies have reported the contamination of Aflatoxin (AF) in maize. The following table summarizes that there is aflatoxin problem mainly in developing countries. In developed countries, maize is used as a feed rather than food, so it is of lesser concern thought their legislations are more stringent (Table 6) regarding aflatoxin than the developing countries.

Table 2. Aflatoxins contamination in maize in different countries

S.N.	Country	Commodities	Number of Samples	Aflatoxin	Level of presence (ppb)	Reference
1	Karnataka, Andhra Pradesh, Tamilnadu, India	Maize	50	AFB1	58 - 270	Muduli *et al*. (2014)
			50	AFB1	52 - 383	
			50	AFB1	48 - 191	
2	Togo	Maize Grains	100	Total Aflatoxin	0.7 - 108.8	James *et al*. (2007)
3	Ghana		600		0.4 - 490.6	
4	Benin		519		24 - 117.5	
5	Haiti	Maize Grains	11	Total Aflatoxin	<5.9 - 78	Schwartzbord and Brown, (2015)
6	Argentina	Freshly harvested maize	1655	AFB1	0.38 - 2.54	Garrido *et al*. (2011)
		Storage maize	1591	AFB1	0.22 - 4.5	

Previous studies of Aflatoxin contamination in Nepal

Several studies conducted in Nepal have shown the presence of fungal toxins in maize. Reports published by governmental body DFTQC during the interval of (2002-2009) reveals the presence of Aflatoxin contamination in maize samples (Table 3) collected within Nepal.

Table 3. Reports of Aflatoxin contamination in maize in Nepal

Commodity	S1 / S2	AFB1(ppb)	AFB2(ppb)	Reference
Maize	3 / 7	217-549	30-45	DFTQC, 2002/03
Maize	1 / 3	366	ND	DFTQC, 2003/04
Maize	1 / 2	23	ND	DFTQC, 2004/05
Maize	2 / 11	30-137	ND-9	DFTQC, 2005/06
Maize	0 / 8	ND	ND	DFTQC, 2006/07
Maize	5 / 11	293.37	46.67	DFTQC, 2007/08
Maize	0 / 9	ND	ND	DFTQC, 2008/09

S1= number of samples exceeding the ML (20µg/kg); S2 = Total number of Samples; LOD=1 ppb for AFB1 and AFB2 respectively; ND = not detected

Studies conducted by Gautam *et al*., (2005) have demonstrated that 42.5 % of maize samples collected in Kathmandu valley were contaminated with AFB1 with an average value of 50.17µg/kg. Similarly Koirala *et al*. (2005) found that 1/3rd of the maize and maize product samples collected from Eastern Nepal were contaminated with aflatoxin and 20% of maize & maize products contained aflatoxin greater than 30 ppb. While results of study done on aflatoxin contamination in 141 maize samples from different region of Nepal by Rai *et al*., (2013) showed the prevalence of aflatoxin in 70% of the samples while 15.7% of samples contained total aflatoxin content greater than 20µg/kg (Table 4). These results clearly indicate that, considerable percentage of maize available in Nepal contains aflatoxin which could be a health risk.

Table 4. Aflatoxin content in maize samples from 5 different region of Nepal.

Region	Number of samples	Range of total aflatoxin (µg/kg) in maize	Average aflatoxin µg/kg	Reference
Eastern	26	ND to 17.4	3.8	
Central	38	ND to 30	8.0	
Western	18	ND to 30	7.7	Rai *et al*. (2013)
Midwestern	19	ND to 27	8.1	
Far western	13	1 to 30	15.6	

ND = not detected (LOD for total Aflatoxin= 1 ppb)

Maize Consumption and exposure assessment

Maize consumption data for Nepalese population were obtained from various literature sources. According to Ranum *et al*., (2014), the average maize consumption of Nepalese is 98g/day, similarly FAO, (1996) show an amount of 135g/day for Nepal; Rai *et al*., (2013) used 143g/day for calculating the total dietary intake for aflatoxin for Nepalese. Likewise, Gurung *et al*., (2011) have shown that per capita availability of maize per year is 45 kg which would be nearly equal to 125 g/person/day. FAOSTAT, (2015) shows that maize provides 359kcal/capita/day of energy for Nepalese; this value would be equal to 105 g/capita/day according to food composition table for Nepal, 2012. However WHO, (2012) has placed Nepal in G09 cluster of countries and for which the amount of consumption is 25g/day. Based on the above references for consumption, from descriptive statistics, the average amount, maximum amount and 97.5th percentile in kg/kgbw/day would be 0.001753, 0.0023 and 0.002367 kg/kgbw/day respectively. Similarly, taking the mean, maximum content and 97.5th percentile of aflatoxin concentration in the findings of Rai *et al*., (2013), a deterministic exposure assessment (Table 5) could be done for total aflatoxins for maize in Nepal.

The exposure is the multiplication of total aflatoxin concentration and consumption. The exposure value was divided by the provisional maximum tolerable daily intake (PMTDI) to find the risk of exposure. Here the PMTDI for aflatoxin B1 (1ng/kgbw/day) established by Couper-Goodman (WHO, 1988) was considered for total aflatoxin, since B1 is a major component of aflatoxin and according to (EC 1058/2012), ratio of Aflatoxin B1 to total aflatoxin is 0.6. It is considered danger if the risk (ratio of exposure to PMTDI) is >1.

Table 5. Simple deterministic risk assessment for acute exposure via aflatoxin contaminated maize consumed in Nepal

Parameters	Mean	Max	97.5 Percentile	Unit
Total aflatoxin concentration	7	30	24.2	µg/kg
Consumption	0.0017	0.0023	0.0023	kg/kg BW/day
Exposure	0.012	0.069	0.057	µg/kg BW/day
PMTDI	0.001	0.001	0.001	µg/kg BW/day
Risk (exposure/PMTDI)	12	69	57	
Danger	Yes	Yes	Yes	

The findings from Table 5 are highly alarming as the exposure value is higher than the PMTDI value; it shows that Nepalese population is at high risk via consumption of aflatoxin contaminated maize. 97th percentile for contamination is higher (24.2) than the ML set by the Nepalese Government (20 ppb) and the risk is very very larger (57) than the PMTDI. Aflatoxins are considered as genotoxic and carcinogenic and AFB1 is included in the group I carcinogens by IARC, (1993). This demands a further investigation into the problem. Since deterministic risk assessment is done as a preliminary assessment practice; further risk analysis is suggested to finding out the accurate consumption pattern, exposure level and percentage population at risk for better risk management and risk communication.

Reduction of aflatoxin production

In Nepal there is problem with drying after harvest (Paudyal *et al*., 2001) and storage (Thapaliya *et al*., 2010) which is sought to be the critical point aflatoxin reduction. Higher aflatoxin levels were prevalent with maize mono cropping, no use of fertilizer, damage of maize in field, delayed harvesting, field drying, heaping harvested maize and cobs shelled latter, no preparation of the storage structure, no insect control and storage of maize in poorly aerated structures; whereas rotation of crop, mixed-cropping, use of diammonium phosphate fertilizer, farmer awareness of incomplete husk cover, harvesting at crop maturity and with husk, sun drying on platform without husk, immediate removal of damaged cobs, use of clean & aerated storage structures, use of insecticide or smoke caused lower aflatoxin levels (Hell *et al*. 2008; Hell and Mutegi, 2011).

Aflatoxin management policy and practices

At present there is a global concern to minimize and monitor the natural contaminants in agricultural goods and maize is also of high concern (Koirala *et al*., 2005). Several researches have been conducted to determine the optimum temperature & moisture content of grains during storage to prevent the growth of *Aspergillus* species and aflatoxin production. In maize inoculated with *A. flavus* and stored at 27^{o}C for 30 days with varying moisture contents, an association between moisture content and Aflatoxin levels was established. At 16% moisture, aflatoxin levels reach 116µg/kg, while at 22% moisture, 2166 µg/kg aflatoxin were obtained (Morena-Martinez *et al*., 2000). Thapaliya *et al*. (2010) found that maize at 13% moisture content showed no aflatoxin formation and proved that such low moisture content does not support for *A. flavus* growth and toxin formation. Nepal government has set a standard for food grains for moisture content which should not be greater than 16% and should contain less than 10% insect damaged grains (Nepal Gazzette, 2001). Except the food standards, GoN has no policies and programs for aflatoxin management. In practice, Nepalese sort out the infested and moldy grains before cooking. Field study conducted by Desjardins *et al*. (2000) reports that, Nepalese maize is contaminated with multiple fungal toxins and also demonstrated that Nepalese urban and rural women were able to detoxify fuminosin and deoxynivalenol contaminated maize grain by hand-sorting visually diseased kernels to an acceptable level (<100ng/kg).

Aflatoxin analysis facility is limited in Nepal (Koirala *et al*., 2005). Only few institutions are involved in food safety issues. The laboratories require sophisticated equipment, highly trained manpower, and certified reference standard and validated analytical method to carry out the contaminant. No organized national survey has been so far conducted periodically and only few data are available regarding aflatoxin and its toxicity.

Detoxification

Toxic effects of mycotoxins can be limited by natural or synthetic agents such as antioxidants, vitamins, food components, e.g., phenolic compounds chlorophyll, fructose, medicinal herbs and plant extracts, and mineral and biological binding agents, e.g., hydrated sodium calcium aluminosilicate, bentonites, zeolites, activated carbons, bacteria, and yeast. Chemo-prevention (e.g. Pltipraz) can bock, retard or even reverse the carcinogenic effect resulting from mycotoxin exposure. Natural components of fruits and vegetables like chlorophyll interfere with the absorption of potential carcinogens and reduce the toxin that reaches susceptible tissues (Farombi, 2006).

Chemical detoxification involves the use of chemicals like calcium hydroxide, ammonia, methanol, hydrogen peroxide, but their use is limited. Separate treatment by 3% hydrogen peroxide, 75% methanol, 3% perchloric acid can reduce aflatoxin to 20 ppb, but at the cost of loss in weight, protein and lipids (Villers, 2014). Studies have shown that maize can be treated with ammonia for removal of aflatoxin to a greater extent, but such maize is only acceptable for feed purpose (Hell and Mutegi, 2011). Moreno-martinez *et al*. (2004) studied the fate of aflatoxin B1 and B2 during nixtamalization (maize treatment with (1-3% calcium hydroxide solution at boiling temperatures) of contaminated maize for tortilla making process and ended the study with the conclusion that the degradation rate of aflatoxin was greater than 60%. Similarly (Mutangi *et al*., 2007) found that soaking in water for 6 hours nominally reduces aflatoxin content and with dehulling the loss was 2 times in comparison to whole grain maize. Likewise, treatment of whole grains with ammonium persulphate (0.2%) and sodium hypochlorite (0.5%) were found to reduce significant quantity of aflatoxin after 14 hours of treatment. However, as the concern for chemicals and additives is increasing, no chemical or additive method has gained general acceptance for decontamination method (Villers, 2014).

Legislations regarding Aflatoxin

Over 100 nations have set regulatory limits on allowable aflatoxin levels in human food (Wu and Guclu, 2012). According to the Nepalese Food Act (1967) and Nepalese Food Regulation (1970) the Maximum Limits (MLs) of Aflatoxin is 20µg/kg for cereals intended as food (Rai *et al*., 2013). In Nepal, DFTQC is the governmental body responsible for monitoring the residue level in such food and feed on regular basis (Koirala *et al*., 2005). Table 6 shows the ML for aflatoxins in different countries. From the table we can see that ML for AFB1 is stricter in Australia and EU. Though many countries have standards for aflatoxin content, due to their carcinogenicity, the ADI cannot not been allocated.

Table 6. Maximum limit for total aflatoxin allowed in different countries.

Country	Food	Maximum limit (ML)	Reference
India	Cereals	30 µg/kg for total Aflatoxins	PFA, (2004)
US	All foods except milk	20 µg/kg	Rai *et al*. (2013)
EU	Maize	5 µg/kg for AFB1 10 µg/kg for total Aflatoxins	EC No 1881/2006
Australia	All food products	5 µg/kg for AFB1 10 µg/kg for total Aflatoxins	Gautam *et al*. (2005)
Kenya	All food products	20 µg/kg for total Aflatoxins	Lewis *et al*.(2005)

Conclusion

On the basis of available data and research findings done in the past, this study points that the prevalence of aflatoxin in maize in Nepal is high. Considerable finding level of aflatoxin in maize is hazard for the health and as well as the economy of the country. Exposure calculations show that maize contaminated with at average aflatoxin content (8.6 ppb) are a risk and chronic exposure is sure to cause health hazard. The major steps which could support aflatoxin formation could be the traditional post harvest practices and climatic conditions of Nepal ranging from temperate to tropical with high humidity all round the year. However, proper drying and storage in airtight containers prevents aflatoxin production to an unsafe level. Government of Nepal has set ML for total aflatoxin in food. Yet, further research and risk assessment in maize and maize products is sought to be extremely essential for finding out population at risk due to aflatoxin exposure. Similarly it would also help to formulate necessary intervention methods for pre and post-harvest operations so as to alleviate the aflatoxin problem. Aflatoxins are natural contaminants, so they can be present in susceptible food grains like maize but preliminary caution should be taken which do not allow fungal growth and development of mycotoxin. Detoxification by chemicals is still a question to human health due to increasing food safety concerns. But use of resistant variety seeds, GAP, IPM, better storage facilities, better surveillance system and increasing awareness among the farmers are obviously reliable ways that reduce the amount of aflatoxins to a safer level in cereals grains.

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