INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Present status, prevalence and seasonal variations of aflaxtoxin in cattle feed, Bihar, India

Ajoy Kumar Choudhary[1]*, Smita Tudu[2], Priyanka Kumari[2], Ashish Ranjan[2]

1 Associate Professor, Mycotoxin Research Laboratory, University Department of Botany, Tilka Manjhi Bhagalpur University, Bhagalpur, 812007, Bihar, India. Tel.: +91-9470034344
2 Senior Research Fellow, Mycotoxin laboratory, University Department of Botany, Tilka Manjhi Bhagalpur University, Bhagalpur, 812007, Bihar, India

OPEN ACCESS

Received: 23-04-2020
Accepted: 15-05-2020
Published: 12-06-2020

Editor: Dr. Natarajan Gajendran

Citation: Choudhary AK, Tudu S, Kumari P, Ranjan A (2020) Present status, prevalence and seasonal variations of aflaxtoxin in cattle feed, Bihar, India. Indian Journal of Science and Technology 13(17): 1738-1745. https://doi.org/10.17485/IJST/v13i17.288

***Corresponding author**.
Ajoy Kumar Choudhary

Associate Professor, Mycotoxin Research Laboratory, University Department of Botany, Tilka Manjhi Bhagalpur University, Bhagalpur, 812007, Bihar, India.
Tel.: +91-9470034344
choudharyakbot@gmail.com

Funding: UGC, Regional office, Kolkata for financial assistance (Sanction no. — F.PSB-34/13-14 ERO)

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

Abstract

Background/ Objectives: Aflatoxin B_1, a potent carcinogen is produced by *Aspergillus flavus* Link ex Fries. Natural contamination of aflatoxin in Bihar is fairly high due to socio-economic backwardness and also outdated agricultural and storages practices. **Methods/ Statistical analysis:** In the present investigation cattle feeds collected in different seasons (Winter, Summer and Monsoon) from three different localities (Bhagalpur, Banka and Naugachia) were screened for aflatoxin B_1. Analysis of Variance has been done with the help of Microsoft Excel, 2013 (USA). CD has been calculated at 5%. **Findings:** The amount of aflatoxin B_1 was significantly high (P<0.05%) in household cattle feeds followed by commercial cattle feeds, where the mean value of AFB_1 was $5.02 \pm 2.46 \mu g/kg$ and $4.19 \pm 1.05 \mu g/kg$, respectively. Freshly harvested cattle feeds (maize, paddy husk, paddy straw, maize straw and green grasses) were comparatively safe. Seasons had marked influence on aflatoxin B_1 contamination on various types of cattle feeds. Maximum levels of aflatoxin B_1 was detected during wet seasons in household cattle feeds (Mean value = $7.79 \pm 2.07 \mu g/kg$). Maize grains because of high nutritional value and production, it is one of the major ingredients of cattle feeds. In the locality of Naugachia, flood and frequent rains delay the natural drying of maize kernels during harvesting in monsoons resulting thereby, high mould infestation and aflatoxin synthesis. **Novelty/ Applications:** In order to check the risk of aflatoxin M_1 toxin entrance in food chain and subsequent human/ cattle health and economic losses, frequent evaluation of AFB_1 in cattle feeds be monitored.

Keywords: mycotoxin; aflatoxin B_1; cattle feed; seasonal variation; ANOVA test; Bihar

1 Introduction

Mycotoxins are secondary metabolites of fungi which contaminate food and feed and can cause toxic effects in cattle and human beings [1–5]. FAO and WHO have estimated that 25% of the world's crops (maize, cereals, rice, nuts, cattle feeds etc.) are contaminated by moulds and mycotoxins [6–8]. Aflatoxin synthesized by *Aspergillus flavus*,

A. parasiticus and *A. nominus* [9] is most potent human carcinogen, classified as Class I carcinogen (IARC 2002). It is also mutagenic, teratogenic and immunosuppressive. *A. flavus* infestation and aflatoxin synthesis on food and feed commodities are a global problem especially in tropical and subtropical countries like India, where the climatic conditions are quite congenial for its growth. Food and feed safety and security are the major concern in the current scenario of population growth. Aflatoxin contamination in cereal grains and other agricultural commodities being utilized as ingredients of animal feed can occur at different stages throughout the food chain. The presence of aflatoxin producing fungi could be influenced by different factors such as plant genotype, availability of inoculum, insect activities, cultural practices, climatic and weather conditions during planting, growing, harvesting, processing, transport and storage periods [10–14]. However, in finished animal feed any contaminated ingredient could cause contamination of the entire feed lot. When aflatoxin contaminated feedstuffs are consumed by cattle, it may cause liver toxicity, mutation, alteration in metabolism, decreased disease resistance and reduced milk production [15,16]. Aflatoxin B_1 is biotransformed in cattle liver and is transferred into the milk in the form of aflatoxin M_1 (also called milk toxin). Several reports have confirmed that the levels of aflatoxin M_1 in milk is directly correlated to the dietary intake of aflatoxin B_1 in cattle feed [17,18]. Milk being the complete food, is mostly prescribed to infants, children and old aged people [19,20]. However, those people in turn become more susceptible to the adverse effects of milk toxin (AFM_1) as they have comparatively low immunity. Due to the genotoxic nature of aflatoxin M_1, it has been categorised as class 2 B human carcinogens (IARC, 2002). Considering the significant health risk, stringent regulations have been imposed by most of the countries of the world. European commission (EU) has set a maximum limit of 5μg/kg and 20 μg/kg for aflatoxin B_1 and total aflatoxins (aflatoxin B_1, B_2, G_1 and G_2), respectively.

Cattle rearing are one of the major sources of income in rural as well as semi-urban areas of Bihar. Various agricultural produce such as maize, wheat, paddy (grains, husk or straw) and green grasses are the main dietary food of cattle. Natural contamination of mycotoxins on agricultural produce of Bihar is fairly high because of the socio-economic backwardness and faulty, unhygienic, outdated agricultural and storage practices, prevalence of high temperature and fairly high humidity. In addition to those conditions, flood in the river of Ganga, Kosi and their tributaries is the regular feature of this terrain. This situation leads to excessive moistening of the grains resulting in substantially intense mould growth and mycotoxin elaboration [21,22]. Previous studies have revealed that in Bihar no edible commodities are absolutely safe from the attack of toxigenic fungal strains and/ or aflatoxin contamination [12,23–25]. However, there is no comprehensive research on the occurrence of aflatoxin in different types of cattle feeds of this locality.

The present study was undertaken to observe the incidence of aflatoxin associated with various types of cattle feeds of Bhagalpur and surrounding areas of Bihar, India. In the selected region, variation in temperature and humidity is notable with the change of seasons. Hence, the effect of seasons on the management of feedstock was necessary to be monitored. In the selected localities (Bhagalpur and Banka districts) of Bihar, farmers utilize cattle feeds in three different ways viz. purchase from market (commercial feed), feedstock stacked in farmyard (household storage) and freshly harvested feeds (fresh fodder). Survey and surveillance of the different types of cattle feeds collected during different seasons of the year were monitored for the prevalence of aflatoxin B_1 and the associated health risk.

2 Materials & Methods

2.1 Sampling

A total of 1130 cattle feed samples were collected randomly (following RND methods) from different localities of Bhagalpur, Banka, and Naugachia during three different seasons i.e. Winters (November – January), Summers (April – June) and Monsoons (July – September) of the year 2017-18. Source of collections were categorized as commercial cattle feed (from shops), household stored feed (cattle feed stored in mud houses or covered with hay stacks or stacked open in farmyard) and fresh feed (seasonally available fresh grasses, freshly harvested crop residues/ grains or crop straw from field, however, collected within 24 - 48 hours). Samples of commercial feed included grains of maize, crushed maize, wheat bran, wheat grains and mustard oil cake. Household stock of cattle feed comprised of maize grains, maize straw, paddy husk, paddy straw and wheat bran. Fresh fodder, however, included freshly harvested wheat grain, maize/ paddy straw and husk, crushed maize, maize grain and green grasses. Approximately 250 gms of each samples were collected in sterilized (already dried) polythene bags and were subsequently taken to the laboratory for further analysis within 1-2 days of collections. After monitoring the moisture contents, samples were dried in the laboratory at 60^{o}C for removal of moisture. Further, those samples were stored in deep freezer to restrict the fungal growth. Each dried sample was ground and mixed to obtain 60 gms sub-sample for aflatoxin analysis.

For the isolation of *Aspergillus flavus*, the samples were subjected to blotter test and on sterilized PDA medium and also through dilution plating technique, depending on the nature of the substrates.

2.2 Aflatoxin extraction

Samples were dried (at 60°C) and were separately ground to obtain 50gms of sub samples. Extraction of aflatoxin was made [26]. The residue was finally extracted using chloroform which was further dried on water bath. The extract was then stored in screw tight glass container for thin layer chromatography.

2.3 Qualitative and Quantitative analysis

The determination of aflatoxin was done through TLC technique. Standard aflatoxin solution (obtained from Sigma Company, St. Louis, USA) diluted in benzene-acetonitrile (9:1) at the concentration of 0.5μg aflatoxin B_1 was used as reference spot on HPTLC. The extracts as well as standard aflatoxin B_1 were spotted on HPTC and the chromatographs were developed. The HPTLC plates were further developed by using solvent mixtures of toulene, isoamyl alcohol and methanol in ratio of 90:32:2 [27]. Triflouroacetic acid was used for the chemical confirmation of aflatoxin. The quantity of aflatoxin B_1 was determined through CAMAG TLC scanner (Camag, Muttenz, Switzerland), using a D_2 lamp and K-400 filter along with a Sklar integrator.

2.3.1 Statistical analysis

The mean $\pm$ standard deviation values were calculated using Microsoft Office Excel (ver. 2013, Microsoft, USA). The data was obtained from the factorial analysis of variance (ANOVA), performed in order to estimate variation among feed types and seasons. The significance level was determined at $P<0.05$.

3 Results and Discussion

Eight genera of moulds were isolated from cattle feed samples. Mould spectrum comprised of *Aspergillus, Fusarium, Penicillium, Rhizopus, Alternaria, Curvularia, Cladosporium* and *Mucor* spp [Table 1]. The incidence and diversity of fungi varied with the change in the climatic conditions of various seasons. The incidence of *Aspergillus flavus* was maximum (87%) during the wet seasons (monsoons) followed by Summers (76%) and cold seasons (19%). The moisture contents of the samples ranged from 16 to 46% depending upon the seasons and nature of the samples.

Out of 1130 cattle feed samples (381 commercial feeds, 390 fresh feeds, 359 household stored feeds) tested, 753 (66.6 %) were contaminated with aflatoxin B_1. Altogether 390 cattle feed samples (143 commercial feeds, 117 fresh feeds and 130 household stored feeds) were collected during the winters. Out of 383 samples of summers 132, 136 and 115 were collected from market (commercial feeds), fresh feeds and household stored feeds, respectively. During the wet seasons, 357 samples (106 commercial feeds, 137 fresh feeds and 114 household stored feeds) were collected and screened for aflatoxin contamination. The amount of aflatoxin B_1 was high (5.02 ± 2.46 μg/kg) in the household cattle feed samples [Table 2]. This amount of toxin was significantly high ($p<0.05\%$) compared to the level of toxin found in the samples of commercial and fresh feeds. 33% of the household cattle samples contained aflatoxin B_1 level exceeding the European Union (EU) limit. While considering different types of samples and the different seasons together, it was observed that maximum level of aflatoxin B_1 was detected in monsoons of household cattle feed samples (Mean 7.79 ± 2.07 μg/kg) [Table 1 Figure 1] followed by commercial feed (5.17 ± 0.75 μg/kg). However, in fresh cattle feed mean value of aflatoxin level was 3.04 ± 0.68 μg/kg during this period. Low level (1.1 ± 0.34 μg/kg) of aflatoxin was found during winters in freshly harvested cattle feed. During the dry weather (Summers) aflatoxin contamination was maximum (4.34 ± 0.37 μg/kg) in commercial feed followed by household feed (4.22 ± 0.6 μg/kg). Considering the level of toxins in different cattle feeds, it was obvious that the freshly harvested feedstuffs were comparatively safe for consumption as because 83-95% of the samples contained aflatoxin below the EU limit (5μg/kg). During the present climatic changes, seasonal assessment of aflatoxin B_1 level in different types of cattle feeds can be an important indicator of aflatoxicoses risk. In earlier studies also an interaction between seasons and cattle feed types in relation to aflatoxin contamination have been observed [5,28–31]. Choudhary and Kumari (2014) in an investigation made in Bihar during 2006 - 2010 found that the climatic conditions had correlated well with the incidence of aflatoxin in field crops. Per cent of viable conidia of toxigenic *A. flavus* in wet months (monsoons) was comparatively greater than in cold or dry months. High temperature (25 - 45°C), high humidity (>80%) and improper storage systems prevailing in this area provide congenial condition for *A. flavus* growth and aflatoxin synthesis. Further, seasonal variation in aflatoxin level might be due to the variation in the fungal community as the co-inhabiting mycoflora may have antagonistic or synergistic effects [32–34].

3.1 Aflatoxin B_1 in different types of cattle feeds

The samples collected from the commercial centres had 4.19 ± 1.05 μg/kg of mean aflatoxin B_1 content, however, the range of aflatoxin B_1 was from 0.85μg/kg to 7.56μg/kg. 23.3 % of commercial feed samples were above the acceptable limit (5μg/kg) of

Table 1. Season wise survey of mycoflora associated in cattle feed samples

Season	Moisture content	Fungi isolated	% of positive incidence
Winter	24-32%	*Fusarium monoliforme*	72
		Aspergillus flavus	19
		A. ochraceus	17
		Rhizopus stolonifer	14
		A. candidus	11
		F. oxysporum	27
		F. equisetti	46
		Cladosporium herbarum	7
Summer	16-21%	*Aspergillus flavus*	76
		A. niger	21
		A. fumigatus	4
		Fusarium equisetti	17
		Penicillium citrinum	6
		P. chrysogenum	5
		Rhizopus nigricans	13
		R. stolonifer	12
Monsoon	34-46%	*Aspergillus flavus*	87
		A. niger	27
		A. candidus	3
		A. sydowii	2
		Penicillium islandicum	3
		Curvularia lunata	19
		Alternaria tenuis	18
		Fusarium monoliforme	15
		F. acuminatum	4
		Mucor sp.	6

European Union. The risk of aflatoxin in commercial feeds increases as because maize is being utilized as the major ingredients of cattle feed [13,35]. Maize grains have high nutritional value and also it has high quantity of production in Bihar. In this state, maize is one of the most important staple crops, which is consumed as food and feed in various ways [22]. It is cultivated throughout the year (summer, monsoon and winter), covering an area of 0.6 million hectares with the yearly production of 2.0 million tonnes. The harvested crop is stored for future consumption as food and feedstuffs, however, the storage condition is mostly traditional, poor and unhealthy. In finished animal feed if contamination of an ingredient occur at any stage of crop development, it may cause contamination of the entire feed lot. In household stored cattle feed mean level of aflatoxin was 5.02 ± 2.46 μg/kg and the range of contamination varied from 1.2 μg/ kg to 11.2 μg/ kg. In household stores, it was observed that the feeds were mostly stacked in open places in farmyard, in which later were used before those stacked earlier. Thus earlier stacked feeds were left undisturbed throughout the year till being utilized for cattle consumption. Poor and unhealthy management of drying of feeds were observed in household storage of cattle feeds. Unseasonal rain was the recurrent feature of this locality, which resulted high moistening of the feed and thereby, providing optimal condition for mould infestation and aflatoxin contamination. Freshly harvested feeds showed the least contents of aflatoxin while considering the three sources of feeds. The mean total of the aflatoxin B_1 content in fresh feeds was 2.06 ± 0.97 μg/kg (ranging from 1.5 μg/ kg to 4.4 μg/ kg). About 12.1 % of samples contained aflatoxin B_1 above EU acceptable limit. Cattles fed with fresh fodder had comparatively reduced chance of aflatoxin poisoning. It is evident [Figure 1] that fresh feed was comparatively safe for the consumption, however, even in wet seasons where the maximum (83%) samples were within the limit of acceptance.

CD value for types of cattle feed at 5% = 2.08.

Table 2. Aflatoxin B_1 contamination of cattle feeds in different seasons.

Season	Number of Samples		% contaminated samples	Range of Aflatoxin B_1	Mean value of Aflatoxin B_1	Above EU limit (%)
	Screened for aflatoxin	**Aflatoxin B1 contaminated Samples**				
Commercial feed						
Winter	143	86	60.14	0.85 - 6.1	3.08 ± 1.75	15 (17.44)
Summer	132	91	68.94	3.4 - 4.6	4.34 ± 0.37	21 (23.07)
Monsoon	106	78	73.58	3.6 - 7.56	5.17 ± 0.75	23 (29.5)
Total	381	255	66.93	0.85 - 7.56	4.19 ± 1.05	59 (23.33)
Fresh Feed						
Winter	117	70	59.83	1.5 - 2.6	1.1 ± 0.34	5 (7.14)
Summer	136	82	60.29	2.8 - 3.8	2.04 ± 0.73	9 (10.97)
Monsoon	137	94	68.61	3.4 - 4.4	3.04 ± 0.68	17 (18.1)
Total	390	246	63.08	1.5 - 4.4	2.06 ± 0.97	31 (12.1)
Household feed						
Winter	130	88	67.69	1.28 - 3.9	3.07 ± 0.82	18 (20.45)
Summer	115	79	68.70	2.6 - 4.6	4.22 ± 0.6	26 (32.9)
Monsoon	114	85	74.56	5.4 - 11.2	7.79 ± 2.07	39 (45.9)
Total	359	252	70.19	1.2 - 11.2	5.02 ± 2.46	83 (33.08)

At p value < 0.05, for season,the mean data for incidence of aflatoxin showed significant difference betweenwinters and monsoons for all three districts under observation. For statistical analysis of Season X Feed, CD is 2.08 (at 5%) which clearly show that during summerand monsoon the mean value of aflatoxin is significantly higher compared towinter. In case of feeds procured from household and commercial centres showedsignificantly higher level of aflatoxin B_1 compared to fresh feed.

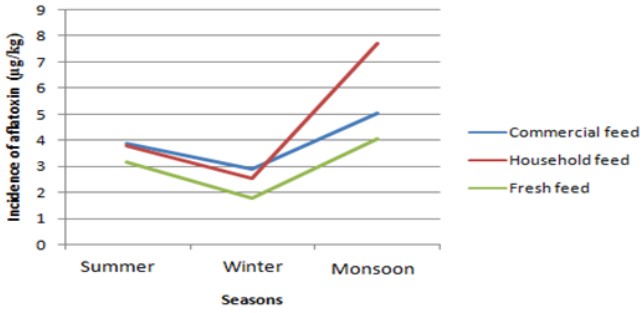

Fig 1. Aflatoxin contamination in various cattle feeds

At p<0.05, the mean value of aflatoxin B_1 in household and commercial feed is significantly high compared to fresh feed.

3.2 Aflatoxin B_1 in different seasons

Season has marked influence on aflatoxin B_1 contamination on various types of cattle feeds. In all the three localities under observation and also considering the types of cattle feeds, wet period showed significantly high levels (p<0.05) of aflatoxin contamination [Figure 2].

CD value for season at 5% is 2.08

At p<0.05, the mean value of aflatoxin B_1 incidence during monsoon is significantly higher compared to winter. However, the mean value of AFB_1 incidence during summer is at par with the winter considering any type of cattle feed.

During the summers and winters the mean value of aflatoxin contamination was 3.53 μg/kg and 2.55 μg/kg, respectively. However, the differences between the levels of toxin during dry and the winter seasons were non-significant [Figure 3].

CD value for season at 5% is 2.08

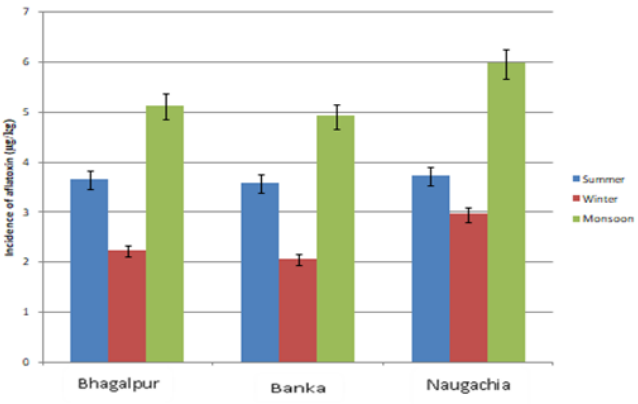

Fig 2. Aflatoxin B_1 levels in cattle feed of different seasons

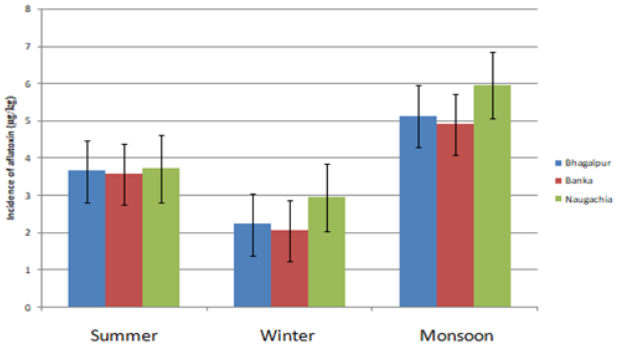

Fig 3. Levels of aflatoxin B_1 contamination at different localities

At $p<0.05$, the mean value of aflatoxin B_1 incidence during monsoon is significantly higher compared to winter. However, the mean value of AFB_1 incidence during summer is at par with the winter considering any type of cattle feed.

3.3 Aflatoxin B_1 contamination at different districts

Feed samples of Naugachia had the highest level of aflatoxin in all the seasons. Mean aflatoxin level was 4.2 ± 1.56 μg/kg in cattle feed of Naugachia which was followed by Bhagalpur (3.7 ± 1.45 μg/kg) and Banka (3.5 ± 1.43) [Figure 2]. Cattle feed of Naugachia contained high levels of aflatoxin during monsoons (11.14μg/kg).

Flood is the recurrent feature at Naugachia and some parts of Bhagalpur where maize (a major ingredients of cattle feed) is harvested in late August/ early September during which crops get submerged with flood water [36]. Maize crops are harvested before full maturity and thereby, kernels contained high moisture contents. Farmers have no option but they further process (peeling, shelling and drying) the crops on nearby National Highway. Drying is delayed because of frequent rains. Such situations provide ideal conditions for *A. flavus* infestation and aflatoxin synthesis. Once the feed ingredients (maize) get contaminated, it enters into the entire food chain.

4 Conclusion

Aflatoxin in cattle feed is an inevitable contaminants produced by *Aspergillus* group of fungi. The present study revealed that aflatoxin B_1 remained present in all types of feedstuffs obtained from the observed localities. *Aspergillus flavus* was most prevalent fungus especially during monsoons and summer seasons. The level of afl B_1 was comparatively high in the household feeds of farmers. Samples collected from commercial feeds also showed the alarming incidence of aflatoxin B_1 (23.3% above EU limit). However, the fresh feeds were comparatively safe for consumption. With respect to seasons, in wet seasons there was the highest incidence of aflatoxin contamination due to prevailing conducive environmental conditions for *A. flavus* growth and

aflatoxin synthesis. Maize (a major ingredient of cattle feed) is harvested during monsoons/ onset of floods, which resulted in excessive moistening of the kernels and thereby, high aflatoxins contamination.

Acknowledgment

Authors are thankful to UGC, Regional office, Kolkata for financial assistance (Sanction no. — F.PSB-34/13-14 ERO).

Conflict of interest

Authors have no conflict of interest.

References

1) Williams JH. Aflatoxin as a public health factor in developing countries and its influence on HIV and other diseases. *World Bank Report*. 2011;p. 1–95.
2) Gnonlonfin GJB, Hell K, Adjovi Y, Fandohan P, Koudande DO, Mensah GA, et al. A Review on Aflatoxin Contamination and Its Implications in the Developing World: A Sub-Saharan African Perspective. *Critical Reviews in Food Science and Nutrition*. 2013;53(4):349–365. doi:10.1080/10408398.2010.535718.
3) Wu F, Groopman JD, Pestka JJ. Public Health Impacts of Foodborne Mycotoxins. *Annual Review of Food Science and Technology*. 2014;5:351–372. doi:10.1146/annurev-food-030713-092431.
4) Hoffmann V, Jones K, Leroy J. Mitigating aflatoxin exposure to improve child growth in Eastern Kenya: study protocol for a randomized controlled trial. *Trials*. 2015;16(1):552. doi:10.1186/s13063-015-1064-8.
5) Obonyo MA, Salano EN. Perennial and seasonal contamination of maize by aflatoxins in eastern Kenya. *International Journal of Food Contamination*. 2018;5(1). doi:10.1186/s40550-018-0069-y.
6) Wu F. Measuring the economic impacts of Fusarium toxins in animal feeds. *Animal Feed Science Technology*. 2007;137:363–374. doi:10.1016/j.anifeedsci.2007.06.010.
7) Pandya J, Arade P. Mycotoxin: a devil of human, animal and crop health. *Advanced Life Sciences*. 2016;5:3937–3941. Available from: https://europepmc.org/article/agr/ind43961403.
8) Lee H, Ryu D. Worldwide occurrence of mycotoxins in cereals and cereal-derived food products: public health perspectives of their co-occurrence. *Journal of Agricultural and Food Chemistry*. 2017;65(33):7034–7051. doi:10.1021/acs.jafc.6b04847.
9) Kurtzman CP, Horn BW, Hesseltine CW. Aspergillus nomius, a new aflatoxin-producing species related to Aspergillus flavus and Aspergillus tamarii. *Antonie van Leeuwenhoek*. 1987;53(3):147–158. doi:10.1007/bf00393843.
10) Bilgrami KS, Choudhary AK, Masood A. Aflatoxin contamination in mustard in relation to agronomic practices. *Journal of the science 221 of Food and Agriculture*. 1991;54(2):221–228. doi:10.1002/jsfa.2740540207.
11) Bilgrami K, Choudhary A. Mycotoxin as pre - harvest contamination of agricultural crops. Bhatnagar D, dekker pub KKSEM, editors;New York. 1997. Available from: https://www.taylorfrancis.com/books/e/9780429079702/chapters/10.1201/9781482270044-3.
12) Choudhary A, Kumari P. Management of mycotoxin contamination in preharvest and post harvest crops: present status and future prospects. *Journal of Phytology*. 2010;2(7):37–52.
13) Pleadin J, Vulić A, Perši N, Škrivanko M, Capek B, Željko Cvetnić. Annual and regional variations of aflatoxin B1 levels seen in grains and feed coming from Croatian dairy farms over a 5-year period. *Food Control*. 2015;47:221–225. Available from: https://dx.doi.org/10.1016/j.foodcont.2014.07.017. doi:10.1016/j.foodcont.2014.07.017.
14) Kumari P. Studies on characterization and micro-sequencing of aflatoxin resistance associated protein of aflatoxin resistance associated protein (RAP) variability of *Aspergillus favus*. 2018.
15) Sinha K, Bhatnagar D. Mycotoxins in Agricultural and Food Safety. 1997. Available from: https://books.google.co.in/books?id=F2K1DwAAQBAJ&printsec=frontcover#v=onepage&q&f=false.
16) Mycotoxins in food: detection and control. Woodhead Publishing. 2004. Available from: https://www.elsevier.com/books/mycotoxins-in-food/magan/978-1-85573-733-4.
17) Prandini A, Tansini G, Sigolo S, Filippi L, Laporta M, Piva G. On the occurrence of aflatoxin M1 in milk and dairy products. *Food and Chemical Toxicology*. 2009;47(5):984–991. doi:10.1016/j.fct.2007.10.005.
18) Mozafari S, Mohsenzadeh M, Mehrzad J. Seasonally Feed-Related Aflatoxins B1 and M1 Spread in Semiarid Industrial Dairy Herd and Its Deteriorating Impacts on Food and Immunity. *Journal of Food Quality*. 2017;2017:1–7. doi:10.1155/2017/4067989.
19) Bahrami R, Shahbazi Y, Nikousefat Z. Aflatoxin M1 in milk and traditional dairy products from west part of Iran: Occurrence and seasonal variation with an emphasis on risk assessment of human exposure. *Food Control*. 2016;62:250–256. doi:10.1016/j.foodcont.2015.10.039.
20) Costamagna D, Gaggiotti M, Chiericatti CA, Costabel L, Audero GML, Taverna M, et al. Quantification of aflatoxin M1 carry-over rate from feed to soft cheese. *Toxicology Reports*. 2019;6:782–787. doi:10.1016/j.toxrep.2019.07.004.
21) Bilgrami KS, Choudhary A. Incidence of *Aspergillus favus* in the aerosphere of maize fields of Bhagalpur. *Indian Phytopathology*. 1990;43(11):38–42. Available from: https://www.cabdirect.org/cabdirect/abstract/19922314439.
22) Choudhary A, Kumari P, Aggrawal R. RAPD based DNA fingerprinting of toxigenic and non-toxigenic strains of Aspergillus flavus isolated from different habitats. *Indian Phytopathology*. 2014;67(3):291–297.
23) Sinha K. Aflatoxin problem in storage and standing maize crops. Mycotoxins in food and feed. India. Allied Press. 1983;p. 23–36.
24) Ranjan KS, Sinha AK. Occurrence of mycotoxigenic fungi and mycotoxins in animal feed from Bihar, India. *Journal of the Science of Food and Agriculture*. 1991;56(1):39–47. doi:10.1002/jsfa.2740560105.
25) Bilgrami KS. Investigation on elimination and inactivation of aflatoxin and other mycotoxins from cereals and oilseeds with special reference to maize and mustard. 1990. Final Technical Report. Indo-US project, Allied Press, p 1-69. .
26) Thomas F, Eppley RM, Trucksess MW. Rapid Screening Method for Aflatoxins and Zearalenone in Corn. *Journal of AOAC INTERNATIONAL*. 1975;58(1):114–116. doi:10.1093/jaoac/58.1.114.
27) Reddy TV, Viswanathan L, Venkitasubramanian TA. Thin-layer chromatography of aflatoxins. *Analytical Biochemistry*. 1970;38(2):568–571.

doi:10.1016/0003-2697(70)90487-2.
28) Wu F, Bhatnagar D, Bui-Klimke T, Carbone I, Hellmich R, Munkvold G, et al. Climate change impacts on mycotoxin risks in US maize. *World Mycotoxin Journal*. 2011;4(1):79–93. doi:10.3920/wmj2010.1246.
29) Bryden W. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. *Animal Feed Science and Technology*. 2012;173(1-2):134–158. doi:10.1016/j.anifeedsci.2011.12.014.
30) Ruscito A, Smith M, Goudreau DN, DeRosa MC. Current Status and Future Prospects for Aptamer-Based Mycotoxin Detection. *Journal of AOAC International*. 2016;99(4):865–877. doi:10.5740/jaoacint.16-0114.
31) Ismail A, Riaz M, Akhtar S, Yoo SH, Park S, Abid M, et al. Seasonal variation of aflatoxin B₁ content in dairy feed. *Journal of Animal and Feed Sciences*. 2017;26(1):33–37. doi:10.22358/jafs/69008/2017.
32) Choudhary AK, Sinha KK. Competition between a toxigenic Aspergillus flavus strain and other fungi on stored maize kernels. *Journal of Stored Products Research*. 1993;29(1):75–80. doi:10.1016/0022-474x(93)90025-y.
33) Choudhary AK. Influence of microbial co-inhabitants on aflatoxin synthesis of *Aspergillus favus* on maize kernels. *Letters in Applied Microbiology*. 1992;14(4):143–147. doi:10.1111/j.1472-765x.1992.tb00670.x.
34) Sinha KK, Choudhary AK. Mycotoxins: Toxicity, Diagnosis Regulation and control through. *Biotechnology Annual Review of Mycology & plant pathology*. 2008.
35) Omeiza G. Aflatoxin Risk in Dairy Production: Assessment of Dairy Cattle Feed Contamination Level by Aspergillus Flavus and A. Parasiticus in both Conventional and Traditional Dairies. *Global Journal of Medical Research*. 2019;1(3). doi:10.21106/ijtmrph.V3.N1s.
36) Sinha KK. Aflatoxin contamination of maize in flooded areas of Bhagalpur, India. *Applied and Environmental Microbiology*. 1987;53(6):1391–1393. doi:10.1128/aem.53.6.1391-1393.1987.