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Jutimala Phookan
Department of Plant Pathology, Faculty of Agricultural Sciences, Arunachal University of Studies, Namsai, Arunachal Pradesh, India

Gompi Padu
Department of Entomology, Faculty of Agricultural Sciences, Arunachal University of Studies, Namsai, Arunachal Pradesh, India

Huirem Diana Devi
Department of Entomology, Faculty of Agricultural Sciences, Arunachal University of Studies, Namsai, Arunachal Pradesh, India

Corresponding Author:
Jutimala Phookan
Department of Plant Pathology, Faculty of Agricultural Sciences, Arunachal University of Studies, Namsai, Arunachal Pradesh, India

Qualitative and quantitative studies on mycotoxins

Jutimala Phookan, Gompi Padu and Huirem Diana Devi

Abstract
Mycotoxins have low molecular weight with various chemical structures and bio- activities which can be toxic to plants, animals or other microorganisms. These are fungal metabolites are structurally diverse which also contaminate animal feed, human food etc. After the discovery of aflatoxins in 1960s, the research for mycotoxins in food increased. This led to the detection of over 100 toxic fungi and also about 300 mycotoxins. These mycotoxins have created lot of health hazards to human as well as animals since time immemorial, ergot alkaloids and aflatoxins being the most important of all. India is one such country in which the food products are affected by mycotoxins. Mycotoxins have different utilities *viz.*, these are used as bactericide, weedicide, insecticide, pharmaceuticals etc. Mycotoxins are also used in bio-warfare. Analysis of these mycotoxins is also an important aspect to protect public health. Regulations and control strategies of mycotoxins and their related fungus are being taken up for the betterment of human and animal health. Inspite of all these it is nearly impossible to reduce mycotoxins from food and feed although various the regulatory efforts are being applied. Numerous approaches can be used to reduce the harmful impact of the toxins on agriculture industry as well as on human and animal health. Further utilization of mycotoxins in various fields is also extremely important.

Keywords: mycotoxins, aflatoxin, food, health

Introduction
Mycotoxins are produced as secondary metabolites by filamentous fungi which are categorized as low-molecular-weight natural products. They have unfavourable effects on humans, animals, and crops which can cause diseases as well as economic losses. This has resulted as a significant problem worldwide. The mycotoxins of agro– economic value include aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins and ergot alkaloids. Some fungi produce more than one mycotoxin and some of the mycotoxins are produced by more than one fungus. Occurrrance of mycotoxins are much higher in areas where the climate is hot and humid, though they are also found in temperate climates.

The word 'mycotoxin' has been derived from two Greek words '*mykes*' which means fungus and '*toxikon*' which means toxins. Bennett (1987) [3] defined mycotoxins as the products which are produced by fungi and can evoke a toxic response in higher vertebrates and other animals even when fed in low concentrations. The term mycotoxin was coined in the year 1962 after an unusual veterinary crisis occurred near London, England.

The chemical structures as well as the biological activities are diverse as there are different mycotoxins recognized. These can also be known as "chemical defense system" of fungi as they defend the fungi against other microorganisms, nematodes, insects and even vertebrates. Till date about 300-400 mycotoxins have been reported from about 100 genera of fungi. Among different genera producing mycotoxins *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. are the major producers. Exposure of mycotoxins to human mainly occurs by three different ways *viz.*, inhalation, ingestion and dermal contact which may lead from acute to chronic heath disorders in human and animals. Mycotoxins mainly affect the plants in field conditions and stored food items. Ingestion of these

The most important event related to mycotoxin in terms of history is the Turkey X disease. After the outbreak of this disease in turkey in England in the 1960s, the term mycotoxin was being coined. It occurred due to contamination of *Aspergillus flavus* in groundnut meal which produced the mycotoxin aflatoxin B1. About 1,00,000 young turkeys on poultry farms died. Soon, the mycotoxin rubric got to include as fungal toxins (e.g., the ergot alkaloids), as antibiotics (e.g., patulin), and some secondary metabolites. The period from 1960 to 1975 has been depicted as 'the mycotoxin gold rush' as many scientists started working on the research for these toxic agents.

Ergot alkaloids are an important group of mycotoxins causing the disease ergotism in human. Ergotism has been reported since 857 A.D. in the Rhine valley of Europe. Ergotism occurs when there is the intake of alkaloids produced by the fungus *Claviceps purpurea* that infects rye and other cereals. It is also known as St. Anthony's fire as Gaston de la Valloire dedicated a hospital to St. Anthony who was one of the earliest monks in France, after the outbreak of ergotism in France in 1039. The word fire is due to the reason that the people suffering from this disease experience a burning sensation in their limbs. The latest repot of ergotism was in 2001 in Ethiopia.

In India as well, deaths due to mycotoxins occurred in 1974 in west India due to aflatoxicosis. Similarly gastrointestinal disorders were reported in 1987 in Srinagar due to contamination of *Aspergillus* and *Fusarium* in the food. In North East India in 1986, pigs in Meghalaya and ducklings in Tripura were affected due to aflatoxicosis.

Economic Importance

Mycotoxins have a negative impact on economics as it causes loss in farm animals and crops as there are difficulties in their management as well. It also pictures a pessimistic opinion in international as well as national trade as it does not obey the rules with national criteria being laid down for highest tolerated levels of the mycotoxins. The mycotoxins in human and animal food can be observed when the postharvest materials stored badly and also when the pre-harvest crops are invaded by a mycotoxigenic mold which may have a pathogenic relationship with the plant.

Mycotoxins cause about 25% of loss in food items throughout the world. Biomin an international company related to mycotoxins in Austria carries out a survey annually. In 2016 they carried out the survey in some of the countries of the world and collected 304 samples. The results can be summarized as.

- On an average 30 mycotoxins and metabolites were present in each sample
- 9.5 of 10 samples were contaminated with *Aspergillus* and *Fusarium* toxins or both
- 96% of 10 or more mycotoxins and metabolites

Emerging mycotoxins were also reported in corn and wheat *viz*, culmorin (61%), moniliformin (93%), beauvaricin (80%) and eniatins (83%).

India is one of the top ranking countries where the economy gets affected by the contamination of mycotoxins in human food. In a study it has been reported that nearly 51% of the 387 stored grain samples were tested in Bihar from 1985 to 1987, and it showed that it has been contaminated with molds. Researchers also reported that the farms of Ludhiana and Punjab have been contaminated by mycotoxins at a range of 50 to 400 µg/kg in 21 feed samples out of 28 samples. A loss of about 20 million dollars within a decade in India has been recorded due to groundnut contamination with mycotoxins.

Toxicity of mycotoxins

Table 1: Fungal species producing mycotoxins with their major food and LD50 values

Mycotoxin	Major Foods	Species	LD50 (mg/kg)
Aflatoxins	Maize, groundnuts, figs, tree nuts, milk, milk products	*Aspergillus flavus, Aspergillus parasiticus*	0.5 (dog) 9.0 (mouse)
Deoxynivalenol	Cereals	*Fusarium graminearum*	70 (mouse)
T-2 toxin	Cereals	*Fusarium sporotrichioides*	4 (rat)
Ergotamine	Rye	*Claviceps purpurea*	62 (mouse)
Fumonisin	Maize	*Fusarium moniliforme*	-
Ochratoxin	Maize, cereals, coffee beans	*Penicillium verrucosum, Aspergillus ochraceus*	20-30 (rat)
Patulin	Apple juice, damaged apples	*Penicillium expansum*	35 (mouse)
Sterigmatocystin	Cereals, coffee beans, cheese	*Aspergillus versicolor*	166 (rat)
Zearalenone	Maize, barley, wheat	*Fusarium graminearum*	Not acutely toxic

Occurrence of mycotoxins in food

Table 2: Type of mycotoxins occurring in food commodities with their maximum concentration

Commodity	Type of mycotoxin	Maximum concentration(ppb)
Wheat	Aflatoxin B1	49.2
	Zearalenone	69.8
	Ochratoxin A	1.4
	Deoxynivenol	100
Rice	Aflatoxin B1	16.65
	Aflatoxin B2	10.4
	Aflatoxin G1	8.4
	Aflatoxin G2	12.0
Maize	Aflatoxin B1	409.3
	Aflatoxin B2	17.2
	Ochratoxin A	7.32
	Deoxynivenol	>1500
Milk	Aflatoxin M1	0.98
Milk products	Aflatoxin M1	1.52
Peanuts	Aflatoxins	98.8
Processed peanut	Aflatoxin B1	1.24
	Afatoxin G2	1.74
Peanut products	Aflatoxin B1	183

	Aflatoxin B2	46.7
Nuts	Aflatoxins	17
Nuts and dried fruits	Aflatoxins	14
Chillies	Aflatoxin B1	67.33
	Aflatoxin B2	41.6
	Ochratoxin A	94

Factors affecting the production of mycotoxins

There are several factors such as physical, chemical and biological factors that affect its production. It may either stimulate or inhibit the production of mycotoxins.

Physical factors

1. **Temperature:** A temperature range of (4.5-32) ºC is favourable for production of mycotoxins. *Pithomyces chartarum* which causes facial eczema disease in ruminants is killed by a period of frost or drought but a temperature of >18 ºC is ideal for growth and toxin production.
2. **Relative humidity:** A relative humidity of more than 70% favors the growth of fungi and the production of mycotoxins in most of the fungi.
3. **Moisture:** The growth of field fungi and advanced decay fungi requires high moisture content *i.e.,* 22-25% wet weight, whereas storage fungi are able to grow on substrates in which the moisture content has been reduced to 13-18%.
4. **Oxygen:** Atmospheric oxygen concentration < 20% lowers the toxin production.
5. **Mechanical damage:** damaged shells of nuts or damaged apples are more likely to be affected by the contamination of mycotoxins.

Chemical factors

1. **Nutritional factors:** Presence of carbohydrates such as glucose, fructose, xylose and also some inorganic ions such as cadmium, iron, lead *etc.* stimulates the production of mycotoxins.
2. **Use of fungistats:** Fungistats causes the growth of fungi to be static. The mainly include food preservatives, when used in moderate amounts (≥0.3%) stimute the production of mycotoxins.

Biological factors

1. **Strain of the organism-** The toxicity of mycotoxins produced by different strains of the same fungus differs.
2. **Competitive growth of the fungus-** Presence of a particular fungus may inhibit the production of mycotoxin by another fungus. For example- presence of *Nocardia* and *Aspergillus niger* inhibits the production of aflatoxin in food.

Utilization of Mycotoxins

Mycotoxins can also be intelligently utilized as insecticides, bactericides, weedicides, pharmaceuticals, growth promoters etc.

- **Bactericides:** Beauvaricin from *Beauveria bassiana* is used against bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Staphyllococcus aureus*, *Sarcina lutea*.
- **Insecticides:** Destruxins from *Metarhizium anisopliae* is used as insecticides against insects such as Lepidopteran pests, spurce bud worm. Neuropeptides are also used insecticides and miticides.
- **Weedicides:** Colletotrichin is widely used against velvet leaf weed and also the weeds found with Solanaceous crops. Cercosporin is used against *Convolvulus arvensis*, *Cyperus* sp. etc. Alternarin is used against water hyacinth.
- **Growth Promoter:** Ergot alkaloids produced by endophytic fungi of the genus Epichloë appear to enhance growth, disease resistance and drought tolerance of their grass hosts. Derivatives of zearalenone *i.e.*, α-zearalenone and β-zearalenone are used as growth promoter in sheep and cattle.
- **Pharmaceuticals:** Penicillin from *Penicillium notatum* has been widely used as antibiotics. Pure ergotamine is used in the treatment of migraine headaches. Similarly mycophenolic acid extracted from *Penicillium brevicompactum* is used as immuno-suppressive pharmaceutical.
- **Bio-Weapons:** Aflatoxins and T-2 toxins have been widely used in the preparation of bio-weapons. These bio-weapons were being used in World War II and Iraq-Iran War (1983-84).
- **Virus Suppressor:** Antiviral activity of destruxin B was found to suppresses the expression of the hepatitis B viral surface antigen (HBsAg) gene in human hepatoma Hep3B cells which carry an integrated viral gene in its chromosome. Furthermore, it was shown that destruxin B can reversibly suppress HBsAg production.

Regulation of mycotoxins

Although there has been an alarming raise of mycotoxins in human food, the laws and regulations imposing the limits of mycotoxins are observed to be alarmed mostly in the developed countries. The developed countries have set some limitations for the control of these mycotoxins which our country lacks. The limitations of different mycotoxins depends on different factors such as occurrence of mycotoxins in various products, availability of toxicological data, methods of sampling and analysis, implications for inter-country trade and the presence of food supply. Contamination of mycotoxins in foodstuffs has become a top concern for human health. In case of regulatory aspects, many countries have established regulations to guard the consumers from ingesting of harmful mycotoxin- contaminated foodstuffs, and to make sure about the fair practices in the trade. It is observed that several conditions play role in management practices and to set limits for mycotoxins. The regulatory philosophy also changes in different regions and countries of the world. In developing countries, ample amount of protection must be taken into consideration for the amount of food available. Thus, a wide range of standards are present among different national or multilateral agencies. Different countries have the lawful right to look after their consumers from the toxic effects of these mycotoxins. However, setting up of mycotoxin regulations is a multifaceted and complicate activity that involves many factors and interested parties. The first limit for mycotoxins was set in the late 1960s for

aflatoxins. Until the late 1990s, setting of mycotoxin regulations was mostly a national affair. In 2003, about 100 countries developed definite limits for mycotoxins in food and feedstuffs. Of all the mycotoxins, aflatoxins are most regulated worldwide. Almost all countries with mycotoxin regulations in Asia have regulatory limits for aflatoxin B1 or total aflatoxin (the sum of aflatoxins B1, B2, G1, and G2) in food, followed by aflatoxin M1, patulin, ochratoxin, deoxynivalenol, and zearalenone.

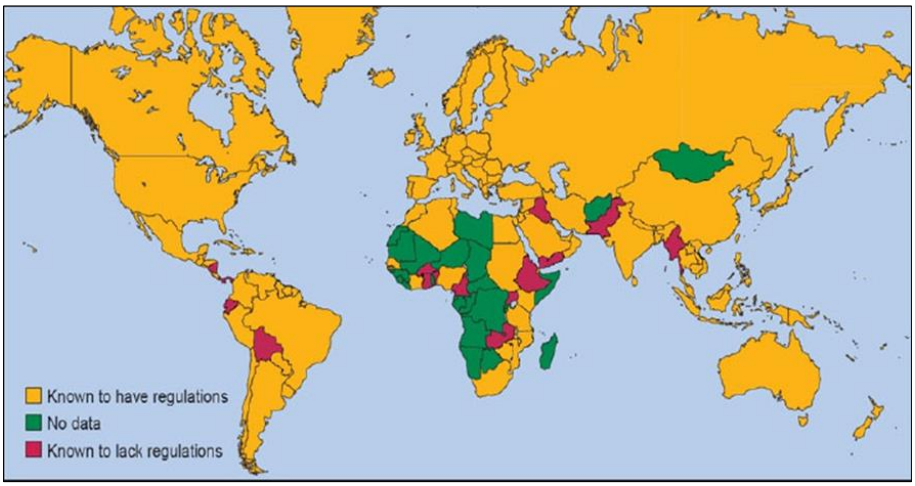

Fig 1: World map showing countries with and without regulations of mycotoxins

Table 3: Regulatory values of mycotoxins according to EU and US FDA

Mycotoxins	European Union (µg/kg)	US FDA (µg/kg)
Aflatoxin B1	2-8	20
Aflatoxin M1	0.05	0.5
Deoxynivenol	200-700	1000
Fumonisin	200-1000	2000-4000
Ochratoxin	3-10	-
Patulin	10-50	-
Zearalenone	20-200	-

Designing of strategies for reducing or eliminating mycotoxins and also the fungal sources is very much needed. Fungal growth in crops and Agri-products is the main source of mycotoxin formation. Many factors are implicated in enhancing the production of mycotoxins in food products. They may include susceptibility of plant to fungal infection, suitability for fungal growth, climate, presence of moisture content and physical damage to the seeds due to various insect pests. The fungi producing mycotoxins may infect the crop at different stages like, pre-harvesting, harvest-time, and also during post-harvest handling and storage. Contamination of various food items with mycotoxins is a hazard that has always existed since ages. The health of animals as well as human gets affected to a great extent due to its contamination. The toxicity of the mycotoxins differs with the type of toxin, the animal species exposed to it, and the extent of exposure, age, and nutritional status. It is almost not possible to get rid of mycotoxins from food and feed in spite of the regulatory efforts as these molds are ubiquitous. Also there are a various ways by which these mycotoxins are harnessed for the betterment of humankind. Some of them are also industrially produced. Multiple and integrated approaches will be needed to minimize to manage the negative impacts of the mycotoxins on the agriculture industry as well as to human and animal health.

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