

Introduction

- Mycotoxins are toxic secondary metabolites produced under appropriate environmental conditions by filamentous fungi species, mainly *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* etc (Zinedine et al., 2007; Oancea and Stoia, 2008 and Turner et al., 2009).
- Contamination with mycotoxins has been reported in a large number of commodities, such as cereals, legumes, fruits, vegetables, wine and beer. Mycotoxins exert a broad range of toxic properties (Table 1) and represent an economic and health risk.
- Diseases produced by mycotoxins are difficult to diagnose:
 - ✓ Very few mycotoxins produce overt signs of poisoning or other symptoms.
 - ✓ They are bizarre molecules with molecular weight 50 - >500.

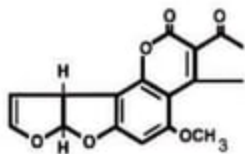
Introduction...

come from:

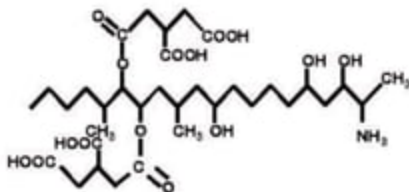
1. Moisture in crops – inadequate drying
 2. Contamination during handling, storage and processing of foods
- **Such small molecules induce no response in human immune system !**
 - Major danger of mycotoxin in diet is our inability to detect them biologically

Types of mycotoxins

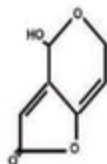
- *Produced by: Aspergillus, Penicillium, Fusarium, Alternaria* etc.
- **Common Members of the Mycotoxin Family are:**
 - ◆ Aflatoxins
 - ◆ Fumonisin
 - ◆ Ochratoxins
 - ◆ Patulin
- Three major genera of molds; *Aspergillus*, *Penicillium*, and *Fusarium* are of significant interest in food safety for production of mycotoxins.
- Mold contamination can occur in the field as well as during harvest, processing, transportation and storage.
- Mycotoxins are highly stable and are difficult to destroy by traditional food processing conditions



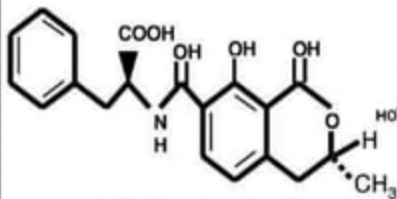
Aflatoxin B1



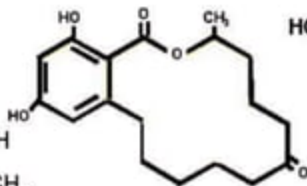
Fumonisin B1



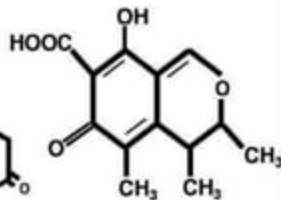
Patulin



Ochratoxin A

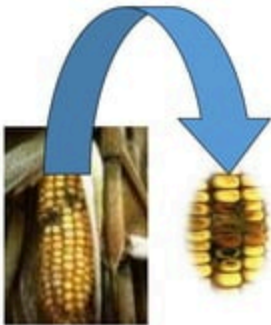


Zearalenone



Citrinin

Fungi	Substrate	Mycotoxin
Aspergillus flavus	Maize, groundnut, oilseed, cotton seed	Aflatoxin
Aspergillus parasiticus	Maize, groundnut, oilseed, cotton seed	Aflatoxin
Aspergillus nomius	Maize, groundnut, oilseed, cotton seed	Aflatoxin
Aspergillus ochraceus	Barkey wheat	Ochratoxin
Aspergillus carbonarius	Grapes wine coffee	Ochratoxin
Fusarium oxysporum	Wheat barley maize	Fumonisin
Fusarium sp.	Wheat barley maize	T-2 toxin
Penicillium verrucosum	Wheat barley maize	Ochratoxin
Claviceps purpurea	Rye	Ergot alkaloids
Stachybotrys	hay	satratoxins



Mycotoxins and their physiological effect

Mycotoxins	Producing organism	Chemical structure	Effect on mammalian cells
Aflatoxins (B1, B2, G1, G2, M1, M2)	<i>Aspergillus</i>	Difuranocoumarin derivatives	Carcinogenic
Citrinin	<i>Penicillium</i>	Benzopyran derivate	Nephrotoxic
Fumonisin	<i>Fusarium</i> <i>Alternaria</i>	Isoflavonoid compounds	Carcinogenic Hepatotoxic
Trichothecenes	<i>Fusarium</i> <i>Trichoderma</i>	Sesquiterpenoid compounds	Cytotoxic Immunosuppressive
Ochratoxin	<i>Aspergillus</i> <i>Penicillium</i>	Dihydroisocoumarin derivatives linked to phenylalanine	Carcinogenic Nephrotoxic Hepatotoxic Teratogenic
Patulin	<i>Penicillium</i> <i>Aspergillus</i>	Unsaturated heterocyclic lactones	Carcinogenic Immunotoxic Genetoxic
Zearalenone	<i>Fusarium</i>	Phenol resorcylic acid lactone	Estrogenic activity Potential carcinogenic and teratogenic

Pathogenesis

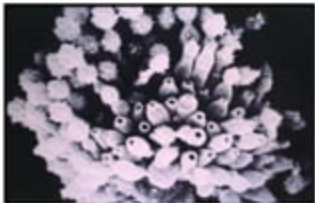
- ✓ Three general mechanisms of mycotoxin action are described as mutagenic, teratogenic, or carcinogenic
- ✓ During the mutagenic action, toxin binds to DNA, especially the liver
- ✓ mitochondrial DNA resulting in point mutation addition or substitution in DNA and affect liver function (hence hepatotoxic).
- ✓ Teratogenic action leads to birth defects
- ✓ the carcinogenic effect cause irreversible defects in cell physiology resulting in abnormal cell growth and metastasis.
- ✓ In recent years, the importance of mycotoxins has been highlighted for their potential use as weapon for bioterrorism.

Signs and Symptoms

- Mycotoxins can cause acute disease manifested by kidney or liver failure or chronic disease including carcinoma, birth defects, skin irritation, neurotoxicity, and death.
 - Edema of legs and feet
 - Abdominal pain
 - Vomit
 - Acute hepatitis
 - Convulsion
 - Cirrhosis
 - Carcinoma of liver
 - Fever
 - Jaundice
 - Acute necrosis
 - Malaise

1. Aflatoxin

Aflatoxin is the name for a group of toxins (poisonous chemical compounds) that are produced by two fungi called *Aspergillus flavus* and *Aspergillus parasiticus*.



Aflatoxin cont..

Four primary aflatoxins, named **B1**, **B2**, **G1** and **G2** plus two additional metabolic products, **M1** and **M2**. The case with aflatoxin toxicity and carcinogenicity has been established for aflatoxin - induced mutagenic activity and DNA damage.

Aflatoxin (*Aspergillus flavus* toxin) is produced by *Aspergillus flavus* and *A. parasiticus*

Aflatoxins occur in different chemical forms; B1, B2, G1, G2, and M1.

The allowable toxin limits are 20 ppb in nuts .

Allowable limit in meats, corn, and wheat is also 0.5 ppb.

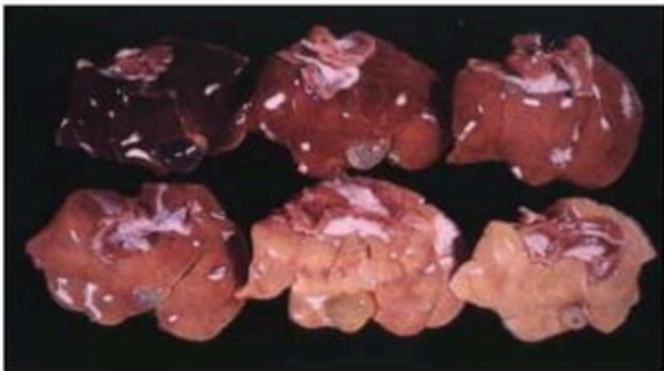
The acute lethal dose for adult human is thought to be 10–20 mg.

The primary target organ for aflatoxin is the liver.. Aflatoxin causes gross liver damage, resulting in liver cancer (hepatocarcinogen).

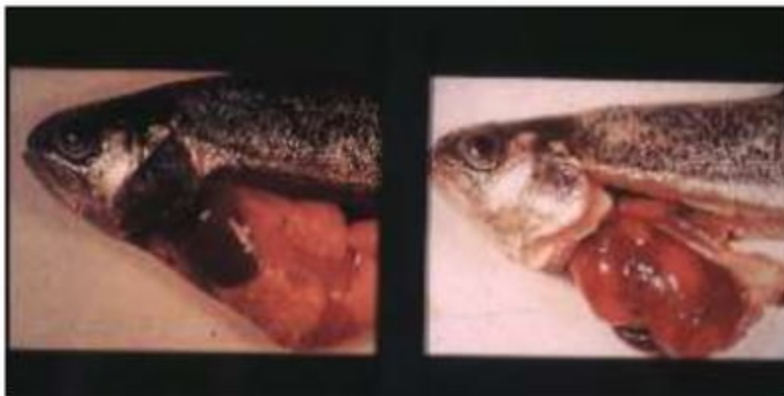
It can also cause colon and lung cancer. classified aflatoxin B1 as a group I carcinogen.

Aflatoxicosis

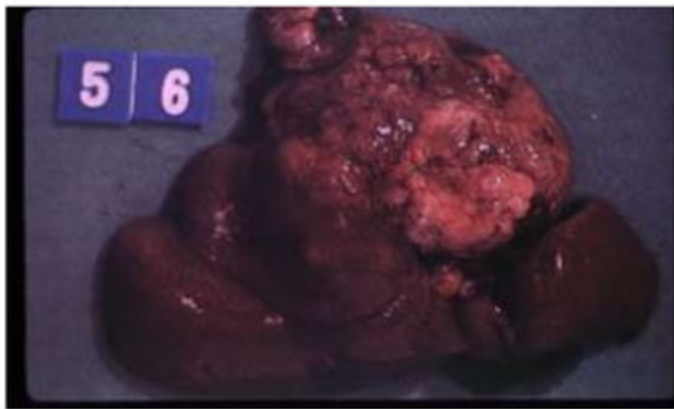
Aflatoxicosis is primarily a hepatic disease.



Aflatoxin B1 and Tumor Induction



Aflatoxin B1 and Tumor Induction



2. Ochratoxin

Aspergillus ochraceus and several other species including *Penicillium* spp. produce seven structurally related secondary metabolites called ochratoxin

Ochratoxin is found in a large variety of foods including wheat, corn, soybeans, oats, barley, coffee beans, meats and cheese. Barley is thought to be the predominant source.

Ochratoxin is hepatotoxic and nephrotoxic and a potent carcinogen.

3. Fumonossins

Fumonossins are produced by *Fusarium verticillioides*, *F. proliferatum*, and *F. nygamai*. *Fusarium verticillioides* under ideal conditions can infect corn

Corns, tomatoes, asparagus, and garlic are the major source of fumonossins.
Fumonossins are highly water soluble and they do not have any aromatic

Fumonossins are highly stable to a variety of heat and chemical processing treatments.
The toxins are reported to cause esophageal cancers in humans.

4. Patulin

Patulin is produced by *Penicillium clariform*, *P. expansum*, *P. patulum* and by *Aspergillus* spp.

Bread, sausage, fruits (apricots, grapes, peaches, pears, and apples), and apple juice are the major source for this toxin.

Patulin is needed in high dosage to show pathogenesis. It is a carcinogenic toxin and is reported to be responsible for subcutaneous sarcoma.

The allowable daily intake limit is 0.4 mg kg^{-1} body weight.

Mycotoxins Severity

1. Type of mycotoxin.
2. Exposure duration and dose.
3. Age.
4. Nutritional status and health of individual.
5. Synergistic effect with other chemicals or mycotoxins.
6. Primary target organs.
 - liver, lungs, kidney, and nervous, endocrine, immune systems

Methods of mycotoxin detection in food and feed



Analytical procedure for mycotoxin determination

Methods of mycotoxin detection in food and feed cont....

- The vital step for a right choice of detection procedure is the extraction and clean-up methods to remove the mycotoxins from the type of matrices.
- 1.Pretreatment methods used:
- depend on chemical structures of the mycotoxins and the biological matrix. These include:
 - ✓ liquid-liquid extraction (LLE),
 - ✓ supercritical fluid extraction (SFE),
 - ✓ solid phase extraction (SPE),
 - ✓ solid phase microextraction (SIME) etc.
- SPE, based on chromatographic columns, is by far the most popular technique currently used for analysis of fumonisin, aflatoxin B1, patulin, ochratoxin in food and feed.

Methods of detection in food and feed cont...

- 2. Detection methods:

➤ **CHROMATOGRAPHIC TECHNIQUES**

- *Thin layer chromatography (TLC)*
- *Gas chromatography (GC)*
- *High performance liquid chromatography (HPLC)*

➤ **PHYSICO-CHEMICAL METHODS**

- Capillary electrophoresis (CE)

➤ **BIOLOGICAL METHODS**

- Biosensors

➤ **IMMUNOLOGICAL METHODS**

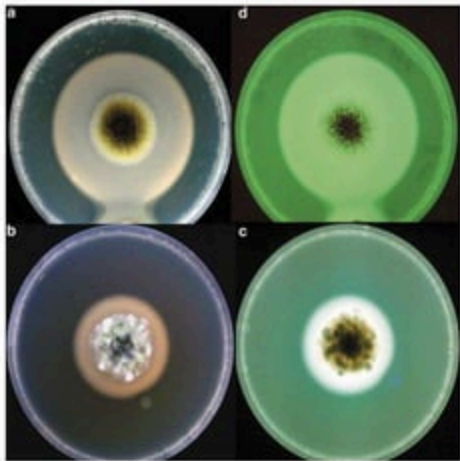
- Immunoaffinity column-based analysis (IAC) or
- enzyme-linked immunosorbent assay (ELISA)

Detection of mycotoxin producing fungi

- **1. Conventional methods**

- Aspergillus, Penicillium, Fusarium and Alternaria, species that often contaminate foodstuffs and feedstuffs.
- Each genus comprises many species.
- Identification and enumeration of aflatoxigenic Aspergillus (Bothast and Fennel 1974).
- Aspergillus can grow in czapek, sabouraud dextrose or yeast extract sucrose (Difco).
- Addition of methyl- β -cyclodextrin (Wacker, Munich) (Fente et al. 2002) or of a combination of methyl- β -cyclodextrin plus bile salts (0.6% Na-deoxycholate) (Rojas-Dura'n et al. 2007) enhances the natural fluorescence of aflatoxins, allowing detection of aflatoxigenic colonies after 3 days (Fente et al. 2002) or 36 h (Rojas-Dura'n et al. 2007a, b) of incubation.

Detection of mycotoxin producing fungi cont....



A non-aflatoxigenic strain (a, b) and aflatoxigenic strain (b, c) of *A. niger* visualized under visible light (a, b) and under 365 nm UV light (c, d). The rim of the white ring around the colony of the aflatoxigenic strain displays faint blue fluorescence (Rojas-Dura'n et al. 2007). With permission of TR Rojas-Dura'n

Detection of Mycotoxigenic Fungi by PCR

- 1. Aflatoxins.
- At least 25 genes are involved in the biosynthesis of AFs and its regulation (Bhatnagar et al. 2006). Primers pertaining to sequences of afl-2, aflD, aflM and aflP, (apa-2, nor-2, ver-2, omt-2, respectively) (Shapira et al. 1996; Geisen 1996; Chen 2002) have been used to detect and identify aflatoxigenic strains of *A. flavus* and *A. parasiticus* among isolated colonies, or in DNA extracts from in foodstuff and feedstuff.

Gene	Synonym	Enzyme	Step in AF biosynthesis pathway
<i>apa-2</i>	<i>afl-2</i>		Polyketide to norsolorinic acid? Regulator of AFB2 biosynthesis
<i>aflD</i>	<i>nor-2</i>	Norsolorinic acid reductase	Norsolorinic acid to averantin
<i>aflI</i>	<i>avfA</i>	Averufin oxidase	Averufin to versiconal hemiacetal acetate
<i>aflM</i>	<i>ver-2</i>	Versicolorin A dehydrogenase	Versicolorin A to demethylstrigmatocystin
<i>aflO</i>	<i>omtB</i>	<i>O</i> -methyltransferase	Demethylstrigmatocystin to sterigmatocystin
<i>aflP</i>	<i>omtA</i>	<i>O</i> -methyltransferase	Sterigmatocystin to <i>O</i> -methylsterigmatocystin
<i>aflQ</i>	<i>ordA</i>	Oxidoreductase	<i>O</i> -methylsterigmatocystin to aflatoxin B2
<i>aflR</i>		Transcription factor containing a zinc cluster DNA binding motif	Positive regulator of AFB2 biosynthesis
<i>aflS</i>	<i>aflJ</i>	Transcription factor	Positive regulator of AFB2 biosynthesis

- **Other Mycotoxins:**

- A real-time PCR assay for ochratoxigenic *Aspergillus* includes primers pertaining to the b-ketosynthase domain of a polyketide synthase from *A. carbonarius* (Selma et al. 2007).
- The PSK4 gene of *Fusarium graminearum* is involved in the synthesis of fumonisins and can be used to detect *Fusaria* that produce zearalenone (Lysøe et al. 2006).
- PCR methods for the detection of fungi that produce aflatoxins, T2 toxin and DON, fumonisins and patulin (Niessen, 2007) are also available.

References

- M. Rai and A. Varma (eds.), *Mycotoxins in Food, Feed and Bioweapons*. Pp21-37.Springer-Verlag Berlin Heidelberg 2010.
- Anca Roseanu, Luiza Jecu, Mihaela Badea, Robert W. Evans. *Mycotoxins: An Overview On Their Quantification Methods*. *ROM. J. BIOCHEM.*, **47**, 1, 79–86 (2010).
- Sarah De Saeger(ed). *Determining mycotoxins and mycotoxigenic Fungi in food and feed*. Pp.427.Wood Head Publishing Limited, Cambrige. 2011.
- Hans P. vanEgmond & Walter H. Paulsch. Determination of mycotoxins. *Pure &Appl. Chem.*, Vol. 58, No. 2, pp. 315—326, 1986.
- Ludwig Niessen. PCR-based diagnosis and quantification of mycotoxin producing fungi. *International Journal of Food Microbiology* 119 (2007) 38–46