Karuna Singh Ankita Kumari

Mycotoxins and Mycotoxicoses



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Preface

Filamentous fungi such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* are common contaminants of food, feed, and indoor environment. During unfavourable conditions, they produce toxic secondary metabolites termed mycotoxins. These mycotoxigenic moulds are producers of both "traditional mycotoxins" such as aflatoxins, ochratoxins, fumonisins, trichothecenes, zearalenone, alternariol monomethyl ether, alternariol, and patulin and "emerging mycotoxins" such as penicillic acid, cyclopiazonic acid, gliotoxin, sterigmatocystin, tenuazonic acid, beauvericin, enniatin, fusaproliferin, moniliformin, culmorin, fusaric acid, sterigmatocystin, butenolide, and emodin.

Mushrooms mostly belong to the subdivision Basidiomycotina; however, few of them are included in Ascomycotina. They are healthy food options, but all of them are not edible. Consumption of toxic species of mushrooms may lead to poisoning syndrome, affecting mainly the gastrointestinal tract and the nervous system. Gyromitrin, amanitin, muscarine, isoxazole derivatives and indole derivatives are well-documented mushroom toxins while others like allenic norleucine are not.

Aflatoxins are major contaminants of corn and corn by-products, peanuts, cotton, pistachios, hazelnuts, Brazil nuts, almonds, and dried figs whilst fumonisins are mostly found in maize but are also prevalent in sorghum, wheat, millet and their products, complete and complementary feeding materials for fishes, hens, calves, pigs, rabbits, horses, lambs, ruminants, and pet animals. *Alternaria* toxins AOH and AME have been found to contaminate wheat, sorghum, barley, sunflower seeds, oats, tomatoes, olives, melons, pecans, and peppers. Ochratoxins produced by *Aspergillus* spp., *Penicillium verrucosum*, and *Fusarium* spp. are general contaminants of wheat, barley, oats, rye, coffee beans, and other plant products including beer wines, spices, and chocolate and feed of pigs, poultry birds, and dairy animals. Zearalenone is a naturally occurring mycotoxin of crops, especially maize infected by *Fusarium* spp. ZEA and trichothecenes persist in the products of wheat, rice, maize, barley, oats, soybeans, and sorghum. A high level of patulin occurs in cereals such as barley, corn, wheat and their processed products, fruits like apple and its

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products, bananas, grapes, pears, strawberries, apricots, cherries, blueberries, peaches, and plums.

Many fungal metabolites with suspected or known toxicity are designated "emerging mycotoxins". They are known to affect vegetables, cereals and cereal products (bread, beer, and baby food), fruits, processed fruit products, oilseeds, dried fish, naturally fermented sausages, foodstuffs (cheese, miso, sake, and soy sauce), and animal feed. The emerging mycotoxins having less toxic precursors are called T "masked toxins". This category of toxins usually remains undetected during analysis. Despite high incidences of toxicity, they are not legislatively regulated in the food and feed.

Direct or indirect exposure to mycotoxins leads to mycotoxicoses in humans and animals. Aflatoxins are potent carcinogens. Aflatoxicosis together with hepatitis B virus is accountable for thousands of human deaths per year, especially in tropical regions. In chickens, pigs, and cattle, effects of aflatoxins include liver and kidney damage. Ochratoxin A, however, is responsible for urinary tract cancer and kidney damage. Fumonisins cause oesophageal cancer and DNA damage in humans and birth defects in mice. Trichothecenes are highly immunosuppressive while zearalenone has oestrogenic effects in animals and men. Patulin, however, is geno-, hepato-, and nephrotoxic. The inhalation of aflatoxins, ochratoxins, stachybotryotoxins, and *Fusarium* mycotoxins has ill health effects in the occupants of damp indoor environments and mouldy buildings. A cocktail of mycotoxins and indoor contaminants (like smoke) induce respiratory disorders and general intoxication as well. The additive effect of mycotoxins is more hazardous than that of a single one and often leads to more severe co-occurrence poisoning.

Although overlapping symptoms make the diagnosis of mycotoxicoses difficult, some specific clinical signs like weight loss and feed refusal in animals, tremors, hepatic and renal necroses, and lung scarring can be helpful in the differential diagnosis of mycotoxicological effects in humans and animals.

A single method for the control of mycotoxins would not be suitable for all agricultural commodities owing to their different chemical structures. Since very low concentrations of mycotoxins are responsible for toxicity, sensitive and reliable techniques are required for detection. TLC, GC, HPLC, LC-MS, and LC-MS/MS have been established as confirmatory techniques in mycotoxin analysis. Apart from this, ELISA dipstick assay, lateral flow tests NIR, MIR using infrared spectroscopy, molecularly imprinted polymers (MIPs), capillary electrophoresis, fluorescence polarization, labelled optical-read dipstick assays, biosensors based on surface plasmon resonance (SPRs) or fibre-optic probes, and immunological array and nanomaterial-based biosensors are rapid screening methods for detecting the presence of a single or multiple mycotoxins.

The easiest strategy to prevent the formation of mycotoxins is to reduce the growth of moulds. Fertilizers, opportune crop rotation tillage, date of the plantation, selection of genetically modified crops, control of infestation by biological or chemical treatments, removal of the crop, and control of insect and weed are widely discussed aspects to prevent fungal infestation and, thus, mycotoxin production in the fields. Since the discovery of aflatoxins in the 1960s, regulations regarding the

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intake of mycotoxins have been implemented by several countries. Generally, the establishment of mycotoxin limits is based on factors such as mycotoxin level in the articles, food consumption and toxicological data, food security issues, and methods of analysis. Due to multi-mycotoxin contamination, the complete destruction of mycotoxins is not possible. Therefore, hazard analysis and critical control point (HACCP) based approach is a control programme for processed foods and feeds including strategies for prevention control, good manufacturing practices, and quality control at all stages of production from field to the final consumer. Uses of additives such as propionic acid, ammonia, and silage additives of microbial and enzymatic origin can turn out to be helpful in controlling mould growth and, consequently, mycotoxin production.

Toxicodynamics and toxicokinetics are two important aspects of mycotoxicological studies explaining the relationship between a toxicant and its biological targets. The kinetics of a mycotoxin can be determined by its route/s of entry and ADMET (Absorption, Distribution, Metabolism, Elimination, and Toxicity) properties. Nonetheless, toxicodynamic methods evaluate the mechanism of action and toxicological effects of the toxicant. In silico methods for mycotoxin risk assessment have also been used to obtain supporting data.

This book has ten chapters. The first chapter covers the historical perspective of mycotoxins along with the timeline while the second one provides an overview including the classification of mycotoxins and mycotoxicoses. Comprehensive information on traditional, emerging, and mushroom mycotoxins is given in Chaps. 3, 4, and 5, respectively. Chapter 6 deals with mycotoxins co-occurrence poisoning whereas new and masked mycotoxins are described in Chap. 7. Mycotoxin detection methods are explained in Chap. 8. The ninth chapter includes mycotoxin management strategies. The last (tenth) chapter of the book covers recent developments in mycotoxicokinetics and mycotoxicodynamics.

The book addresses a wide range of readers including mycologists, clinicians, agricultural scientists, chemists, veterinarians, environmentalists, and food scientists, providing all-around knowledge about mycotoxins to date in a simple manner.

Varanasi, Uttar Pradesh, India

Karuna Singh Ankita Kumari

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Abbreviations

¹H NMR Proton nuclear magnetic resonance

3H-FX Radioactive Fusarenon-X
3-NPA 3-Nitropropionic acid
AAL toxins Alternaria alternata toxins
AAL-TA Alternaria alternata toxins TA
AAL-TB Alternaria alternata toxins TA

ACROs Acromelic acids

Af Aflatoxin

afIJAflatoxin biosynthetic geneafIRRegulatory gene afIRAHDAAmino hexadienoic acid

ALARA As low as reasonably achievable

ALTCH Alteichin ALTs Altertoxins

AME Alternariol monomethyl ether

AMV RT Avian myeloblastosis virus (AMV) reverse transcriptase

AOH Alternariol

AQA Analytical quality assurance ATA Alimentary toxic aleukia

ATX-I Altertoxins I
ATX-II Altertoxins II
ATX-III Altertoxins III
ATXs Altertoxins

AUC_{n_t} Area under the concentration-time curve from time zero to

the last measurable concentration

 $a_{\rm w}$ Water activity BBB Blood-brain barrier

BEA Beauvericin
BUT Butenolide
bw Body weight

xx Abbreviations

C-4 Acetyl residues

CaCo-2 Colorectal adenocarcinoma cells-2

CCPs Critical control points

CEC Commission of the European Communities

CFCF Cell-free culture filtrate

CIT Citrinin
Cl Clearance

Cmax Maximum (or peak) serum concentration

CNS Central nervous system
CPA Cyclopiazonic acid
CT Computed tomography

CTN Citrinin
CUL Culmorin

CYP 450 Cytochromes P450

CYP1A2 Cytochrome P450 family 1 subfamily A member 2
CYP3A4 Cytochrome P450 family 3 subfamily A member 4
CYP3A5 Cytochrome P450 family 3 subfamily A member 5

D3G Deoxynivalenol-3-glucoside

DAS Diacetoxyscirpenol

DDGS Dried distillers' grains with soluble DESI Desorption electrospray ionization

DMSO Dimethyl sulfoxide
DNA Deoxyribonucleic acid
DOM Deepoxy-DON

DOM-1 Deepoxy metabolite of DON

DON Deoxynivalenol EA Ergot alkaloids EC Oesophageal cancer

EFSA European Food Safety Authority
ELEM Equine leukoencephalomalacia
ELISA Enzyme-linked immunosorbent assay

EMOEmodinENNEnniatinsENNsEnniatinsEUEuropean Union

F Bioavailability
F. verticillioides MRC 826
FA Fusaric acid

FAO Food and Agriculture Organization of the United Nations

FAPAS Food Analysis Performance Assessment Scheme

FB Fumonisins B FB1 Fumonisin B₁

FLD Fluorescence detection

FMEA Failure mode and effect analysis

Abbreviations xxi

FPIA Fluorescence polarization immunoassay FTIR Fourier transform infrared spectroscopy

Fum5 gene Fumonisin synthase gene

FUS Fusaproliferin FX Fusarenon-X

GABA Gamma-aminobutyric acid Good Agricultural Practices **GAP**

GC Gas chromatography

Gas chromatography-mass spectrometry GC-MS

Guanosine diphosphate **GDP** Good Hygienic Practices **GHP** Gastrointestinal tract GI

GMP Good Manufacturing Practices

GSP Good Storage Practices

GT Gliotoxin

Hazard analysis and critical control point **HACCP HPLC** High performance liquid chromatography International Agency for Research on Cancer **IARC**

IBO Ibotenic acid

IDH Isoepoxydon dehydrogenase **IEC** Intestinal epithelial cell

IG Intra-gastric ΙP Intra-peritoneally iv Intra-venous

JECFA Joint FAO/WHO Expert Committee on Food Additives

KDa Kilodalton

distribution, **LADME** Liberation. absorption, metabolism and

excretion

LC Liquid chromatography

LC-MS Liquid chromatography-mass spectrometry

Liquid chromatography-mass spectrometry/mass spectrometry LC-MS/MS

Lateral flow devices LFD

Epithelial cell line originally derived from porcine kidneys LLC-PK1 renal cells

LOD Limit of detection LOO Limit of quantification Lysergic acid diethylamide LSD N-methyl-N-formylhydrazine **MFH** Monomethylhydrazine

MH

MON Moniliformin **MPA** Mycophenolic acid

Marginal rate of transformation **MRT**

Mass spectrometry MS

Muscimol MUS

MW Molecular weight xxii Abbreviations

Na-K ATPase Sodium potassium pump adenosine triphosphatase enzyme

NASBA Nucleic acid sequence-based amplification

NIH/3T3 3-day transfer, inoculum 3×10^5 cells from NIH mouse

embryonic fibroblast cells

NIR Near-infrared NIV Nivalenol

nor-1 gene Neuron-derived orphan receptor-1NRPSs Non-ribosomal peptide synthetases

OTA Ochratoxin A OTB Ochratoxin B

OT-GSH OTA-glutathione conjugate

OTHQ OTA-hydroquinone
OTQ OTA-quinone
PA Penicillic acid

PAT Patulin

PCR Polymerase chain reaction
PIDI Provisional tolerable daily intake
PIWI Provisional tolerable weekly intake

pks-A Polyketide synthases

po Per os

PPE Porcine pulmonary oedema

ppm Parts per million RNA Ribonucleic acid Rnase H Ribonuclease H

RP-HPLC Reversed phase high performance liquid chromatography

RT PCR Real-time polymerase chain reaction

RT Room temperature SH Sulfhydryl groups

SLUDGE Salivation, lacrimation, urination, defecation, gastrointestinal

distress, and emesis

SPE CartridgesSolid phase extraction cartridgeSPSRSurface plasmon resonance sensorsSSRSelf-sustained sequence replication

STCSterigmatocystinSTE IIIStemphyltoxins IIISTEStemphyltoxins

 $t_{1/2}$ Half-life T-2 toxin

T7 RNA T7 bacteriophage that catalyses the formation of RNA from

DNA

T7 RNA polymerase RNA polymerase from the T7 bacteriophage

TCs Trichothecenes

TDI (Maximum) tolerable daily intake

TeA Tenuazonic acid