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Mycotoxin Strategies: Impact on Global Health and Wealth

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Abstract

Mycotoxins are toxic secondary metabolites of molds show antagonistic possessions on human beings, wildlife, and agro sector that result in mystery disorders and economic disturbances. The mycotoxins contaminated foods and fodder are omnipresent and become a global concern. Due to the consumption of contaminated food and fodder, epidemic out breaks are a frequent and a common phenomenon. The most important agriculture oriented mycotoxins are aflatoxins, possible for hepatic cancer, childhood impairment and also causes acute toxicosis; fumonisins, are linked with esophageal cancer and neural tube defects (NTDs); while immunotoxic deoxynivalenol (DON) and other trichothecenes, cause gastroenteritis; and ochratoxin A (OTA), has been connected with kidney diseases. This review primarily describes each group of mycotoxins in detail, and their adverse effects on global population health and wealth.

Introduction

Mycotoxin is a combination of the 'mykes'fungus and the Latin word 'toxicum' poison. The mycotoxin' is usually chemical products formed as secondary metabolites by a few fungi that readily colonize crops in the field or after harvest found at trace levels reserved for the relatively small (MW700), toxic, which hardly identifiable. As per the opinion of Food and Agriculture Organization (FAO) it is assessed that nearly 25% of the global fodder crops are adulterated with mycotoxins [1]. It is predicted that 200,000 people are added to the global food demand daily and by the year 2050, the world population will surpass 9 billion [2].

Mycotoxin Diversity

Mycotoxins are made of vastly diverse organic structures categorized by a range of heteroatom-containing organic chemicals found in mold-polluted food sources and may be harmful if ingested in high enough quantities or over a long enough period of time. While plentiful reviews have extensively enclosed mycotoxins biological nature, production and sound effects in human and animal health [3-5]. Fruits are highly susceptible to fungal invasion during the stage of ripening because, the pH of the tissue rises, softening of skin layers, accumulation of soluble carbohydrates, and deterioration of defense system happens [6]. A small number of dried fruits contamination by mycotoxins has been regulated in laws are in great demand in the food markets [7]. The Commission of the European Communities (2006, 2010) has executed strong regulations to minimize the contamination of ochratoxin 10 μg kg^{-1} (OTA), aflatoxin 2 μg kg^{-1} (AFB1), and over-all aflatoxins (4 μg kg^{-1} for the total of AFB1+AFB2+AFG1+AFG2) in dried vine fruit (currants, raisins, and sultanas).

Major Mycotoxins

The majority clusters of mycotoxins occur quite often in food commodities are released by the following five fungal origin: aflatoxins, aflatoxin-producing *Aspergillus* spp.; ochratoxin A produced by significant members of *Aspergillus* and *Penicillium*; trichothecenes (T-2 and HT-2 toxins are type-A trichothecene mycotoxins, and type B: deoxynivalenol (DON) zearalenone (ZON)), fumonisin B1 and B2 produced by *Fusarium proliferatum*, *F. moniliforme*, fusaproliferin, beauvericin, enniatins and moniliformin are included as emerging *Fusarium* mycotoxins,; ergot alkaloids produced by ergot fungi *Claviceps purpurea*; and toxins produced by *Alternaria* sp. are alternariol monomethyl ether (AME), alternariol (AOH), altenuene, altertoxin, and tenuazonic acid (TA), a tetramic acid derivative.

Classification of Mycotoxins

There are more than 400 known mycotoxins out of which are six major classes of mycotoxins are frequently occurring: aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin ergot alkaloids and Patulin (CAST Report (2003)(Table:1).

Though less stated, there are some fungal toxins, such as enniatins (ENs), alternaria toxins, moniliformin (MON), citrinin (CTN), beauvericin (BEA), cyclopiazonic acid, roquefortin C, mycophenolic acid, penitrems, verruculogen, griseofulvin, citreoviridin, produces acute and chronic toxicity.

S.No	Mycotoxin	Organism	Remarks
1	Aflatoxins	*Aspergillus spp.*	Aflatoxin B1a heptocarcinogen, strongly impair Liver to become yellowish in color, fatty highly fibrous in appearance.
2	Fumonisin	*F. verticilloides*	Leukoencephalomalacia a disease commonly seen in horses that promotes softening of the white matter in the brain leads to tumor.
3	Sterigmatocystin	*A. versicolor* *A. flavus* *A. parasiticus*	Sterigmatocystin is a carcinogen, mostly contaminated with aflatoxin producing species in fodder.
4	Ochratoxin	*A. ohcraceus* *P. viridicatum*	Ochratoxin A (OA) linked with a disease called Balkan nephropathy, human carcinogen. Target organ are nephrons.
5	Cyclopiazonicacid (CPA)	*P. cyclopium* *A. flavus* *A. oryzae*	Chemically, an indole tetrameric acid, often seen in association with aflatoxin an inhibitor of calcium-dependent ATPases causes hemorrhagic lesions.
6	Alternariols	*Alternaria* spp.	Most common mold spore encountered growing on cement, wall paper, tile grout, leaves, onions, tomatoes, corn, peanuts, fruits, vegetables, grain, etc.
7	Patulin	*P. patulinum*	Patulin is genotoxic polyketide lactone antiviral agent causing gastrointestinal lesions. It relatively temperature stable even during pasteurization. It is come across in apples and grapes, corn, and many berries.
8	Tremorgens	*P. crustosum Aspergillus* spp.	These mycotoxins cause "staggers" in man and animals. toxin-induced gastroenteritis and neurotoxicity is commonly found in tremoragens contaminant food like in animal feed, millets, corn, wheat, and nuts.
9	Trichothecenes	*Fusarium spp.*	T-2 toxins (T-2) are sesquiterpene compounds exhibiting anti-leukemic properties causes cellular death, and toxicosis in a short life span. T-2 is too toxic to generally be reflected to be a warfare agent.
10	Deoxynivalenol (DON)	*F. graminearum*	DON (vomitoxin) responsible for emesis vomiting, because it causes gastro-intestinal inflammation. DON infection leads to decreased weight loss, anorexia, mal function nutritional efficiency and diminished immune system.
11	Zearalenone	*Fusarium spp.*	Zearalenone is a non-steroidal estrogenic potent mycotoxicoses causing reproductive disorders in animals fed with contaminated fodder. Zearalenone is seen in various parts of the globe (warm and cold climates) in grain crops.

Table 1: Types of Mycotoxins [8].

Aflatoxins: Aflatoxins are highly polyketide-derived, toxic and carcinogenic metabolites mainly produced by members of *Aspergillus* section [9,10]. There are nearly 20 various types of aflatoxins of which the four major aflatoxins consist of aflatoxins B1, B2, G1 and G2 produced by selected toxigenic strains isolates (not all isolates are toxigenic) of either *Aspergillus flavus* or *A. parasiticus* and often by *Aspergillus nomius* grow on various kinds of foods, and beverages. The aflatoxins were named from *Aspergillus flavus* (+toxin) as it emits blue colour when tested under UV light, aflatoxin G1 was the first green defined-spot). Aflatoxin B1 is about 3 times more toxigenic compared with AFG1. Aflatoxins are most prevalent in oil producing seed crops although it can occur any unfavorable condition. AFB1 and AFG1 are considered as Class I human heptocarcinogen, strongly impair Liver to become yellowish in color, fatty highly fibrous in appearance, functions are strongly impaired (Figure 1).

## Permissible limits of aflatoxins

During 1960s, aflatoxins were listed under carcinogenic to laboratory test animals. FDA in 1969, (CAST, 2003) fixed 20 ppb as level for aflatoxins at for all foodstuffs, in order to include animal feeds, based on FDA's agency and analytical capability.

Globally 50 countries formulated their own legislation regarding maximum permitted levels from 0 to 50 µg/kg of AF in food and feeds IARC (2012) [3,11], in food stuffs is 1-20 ppb and in the feed the permissible limit range is 0-50 ppb. The limits for the aflatoxin M1 in the milk for human consumption are 0.05-0.5 ppb [1]. Maximum limits of 2 µg/kg for AFB1 and 4 µg/kg for total aflatoxins in herbal drugs have been set by the European Pharmacopoeia (EP, 2011).

Environmental factors affecting aflatoxin production: The growth of saprophytic species grown under water logging and humidity are favorable for asexual spores carried by wind or insects to the growing crop. When cereal crops such as wheat, barley, corn and oats growing moisture/humidity (N14%) at warm temperatures (N20°C), are liable for the crops to aflatoxin contamination.

Trichothecenes: Trichothecenes constitute a very vast group of mycotoxins having a tricyclic 12,13-epoxytrichothec-9-ene (EPT) ring system in collectively classified into four groups constructed on pattern of EPT (Types A, B, C and D) [12]. (T-2, HT-2, DON, DAS, NIV, and ZEN) produced by various species of moulds, in particular those belonging to the genus *F. graminearum*, *F. culmorum*, *F. sporotrichioides*, *F. poae* and *F. equiseti* are the most important

trichothecene producing species. The main toxic effect of trichothecenes, at cellular level appears to be a primary inhibition of protein synthesis by action on peptidyl transferase activity interfering 60S subunit of the ribosome.

Figure 1: Structure of aflatoxin B (AFB1& AFB2), G (AFG1& AFG2), and M (AFM1& AFM2).
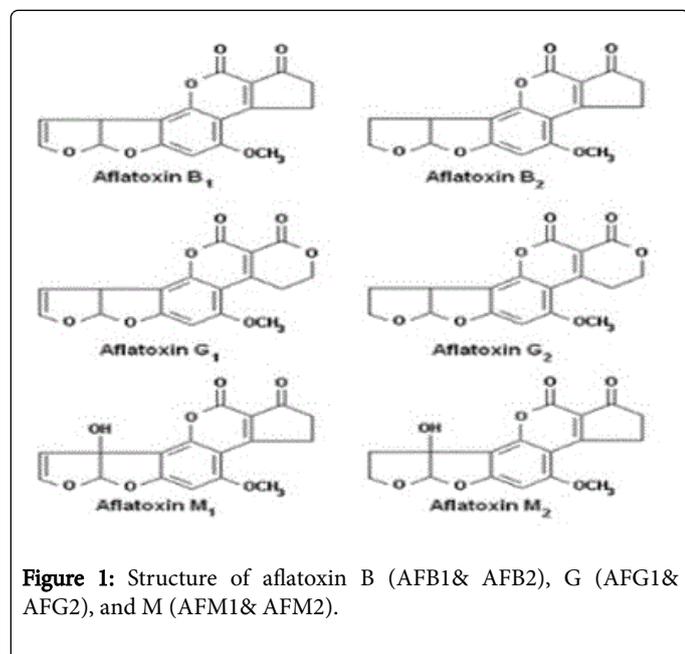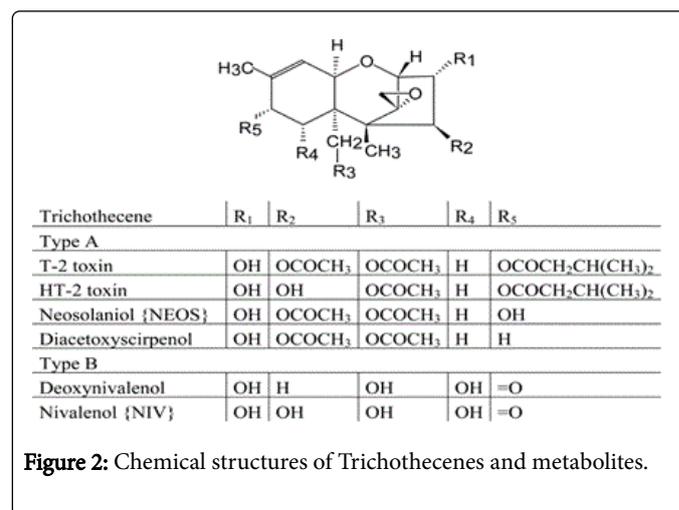
Types of Trichothecenes

Trichothecenes are sesquiterpenoid mycotoxins with emetogenic properties, and harmful to hematopoietic organ and to immune function as they are effective inhibitors of eukaryotic protein pathway, with impact on global health and wealth. They are synthesized by a wide variety of mycogenic species comprising, *Myrothecium, Stachybotrys, Fusarium, Cephalosporium, Trichoderma* and *Trichothecium*.

The Type A trichothecenes (T-2 toxin (T-2) and HT-2 toxin (HT-2), and diacetoxyscirpenol (DAS)), produced by soil fungi and plant pathogens *Fusarium acuminatum, F. poae*, and *F. sporotrichiodes*, include T-2 toxin and HT-2 toxin, are the utmost toxigenic members of this mycotoxin family. Mostly type B tricho toxins, producers of *F. graminearum* and *F. culmorum* (Fusarenone-X (FUX), deoxynivalenol (DON), and nivalenol (NIV)), are fewer toxic compared to the afore Type A members, but are found at greater concentrations in cereal grains and foods. Based on the presence or absence of a carbonyl group at the C8 position trichothecene are differentiated in to types A and B [13]. Deoxynivalenol (DON, vomitoxin), is most prevalent worldwide acutely toxic trichothecene, because it is a very common contaminant.

Trichothecene heavy content dosages exposures in lab animals lead anorexia, gastro intestinal failures, leukocytosis, cardiac failure, which may ultimately cause death. Whereas moderate doses impairs food intake, reduces weight gain, disrupts immune function, hemostatic derangements [14], and can cause developmental effects.

T-2 Toxin: Trichothecene (T-2) also called as fusariotoxin T2, insariotoxin which have a common skeleton that includes a double bond between C9 and C10 and an epoxide group at C12 and C13inhibits DNA and RNA synthesis in vivo and in vitro [15], stress induce phosphokinase pathways, typically activate pro inflammatory

gene expression, disrupt gastrointestinal function, and growth hormone tolerance, and cause cell death [16]. Topical application of T-2 toxin in mice [17] alters brain barrier permeability of blood. Tissue distribution studies suggest that liver is the major organ for metabolism of T-2 toxin. Once the toxin enters systemic circulation, regardless of route of exposure, it affects rapidly proliferating tissues. T-2 toxin exposure by oral, parenteral, and cutaneous cause lesions in lymphoid, gastrointestinal tissues [18]. The involvement of oxidative stress and activation of various signaling pathways like MAP kinases, caspases have been shown in T-2 toxin induced apoptosis in vitro [19]. The main biotransformation pathway of T-2 toxin is deacetylation of C-4 acetyl groups to form HT-2 toxin [20] (Figure 2).

Trichothecene	R_1	R_2	R_3	R_4	R_5
Type A					
T-2 toxin	OH	OCOCH₃	OCOCH₃	H	OCOCH₂CH(CH₃)₂
HT-2 toxin	OH	OH	OCOCH₃	H	OCOCH₂CH(CH₃)₂
Neosolaniol {NEOS}	OH	OCOCH₃	OCOCH₃	H	OH
Diacetoxyscirpenol	OH	OCOCH₃	OCOCH₃	H	H
Type B					
Deoxynivalenol	OH	H	OH	OH	=O
Nivalenol {NIV}	OH	OH	OH	OH	=O

Figure 2: Chemical structures of Trichothecenes and metabolites.

Deoxynivalenol (DON): Deoxynivalenol (DON, non-fluorescent), an immunosuppressive the most common trichothecene, is non-classifiable as carcinogen to humans, however, it can cause deleterious health effects like anorexia, weight loss, malnutrition, endocrine dysfunction and immune alterations [16]. DON intake by human's ranges per day from 0.77 to 2.4 µg/kg body weight/day (FAO/WHO, 2001). In this regard, the provisional maximum tolerable daily intake (PMTDI) set for this mycotoxin and its acetylated derivatives is 1 µg/kg body weight (b.w.) for general exposure, and 8 µg/kg b.w. for acute exposure (JECFA, 2001). DON-contaminated food intake may exhibit vomition syndrome in Humans [21] (Figure 3).
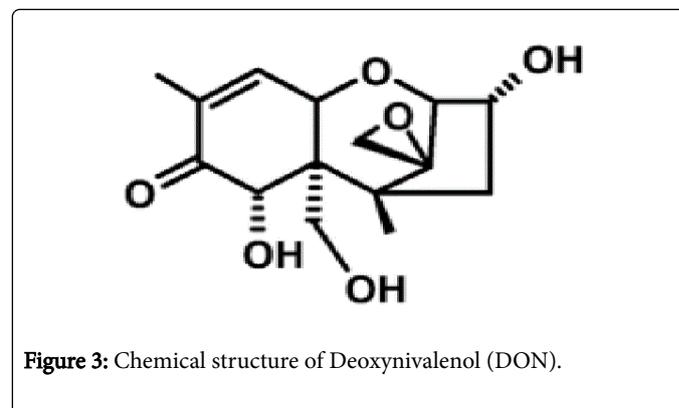
Figure 3: Chemical structure of Deoxynivalenol (DON).

Control of insect pests and good climatic storage conditions (14% moisture), minimize contamination of will minimize toxigenic molds. Again, if grains have matured and are stored under appropriate conditions, DON does not further accumulate in storage. Baking and

malting using contaminated wheat and barley are adversely affected [8]. European commission (EC, 2006) sets Permissible of limits Deoxynivalenol was 200 µg/kg for processed cereals and 1250 µg/kg for unprocessed cereals.

Ochratoxin: A fungal metabolite Ochratoxin was first discovered in 1965 as produced by different species of *Penicillium verrucosum, P. nordicum, Aspergillus niger, A.* quadrupole and *A. carbonarius* that showed toxic behavior towards animals. The family of ochratoxins consists of three members, A, B, and C.

Ochratoxin A (OTA) is the most abundant and the most toxic of the three while ochratoxin B (OTB) and ochratoxin C (OTC) are less important and less common [22]. Frequent spoilage of cereals, vegetables, dried fruits, spices, coffee, fermented beverages was observed mainly because of OTA [23]. Environmental factors like temperature, moisture, incubation time, light effects the production of OTA [24]. Of the ochratoxin has equal importance as aflatoxins among *Aspergillus* toxins. OTA shown to be hepatotoxic, nephrotoxic, teratogenic, immunotoxic, and carcinogenic in experimental model. The mechanism of action seems to be related to the formation of DNA adducts [25]. Ochratoxin is a carcinogen in rats and mice and is suspect as the causative agent of human disease. Balkan Endemic Nephropathy is one such kidney disease, often with associated tumors, of humans that is considered by some to be caused by ochratoxin [26] (Figure 4).
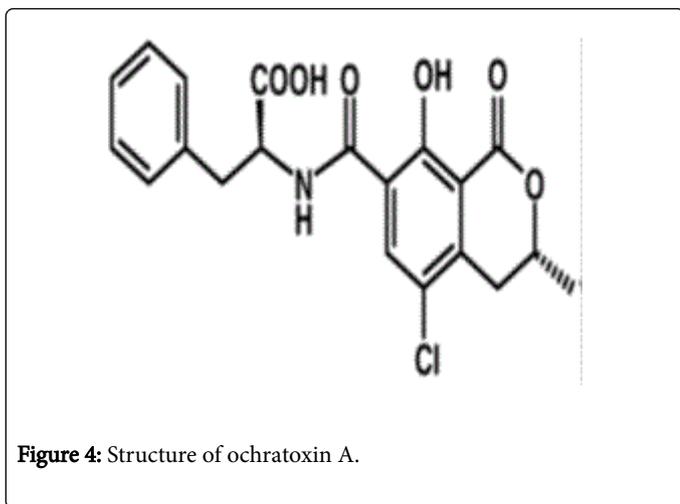

Figure 4: Structure of ochratoxin A.

Bui-Klimke et al. [27] calculated unadjusted odds ratios from epidemiological studies that correlated OTA exposure (measured by urinary OTA) with various adverse health endpoints.

Gilbert et al. [28] concluded urinary OTA levels with dietary OTA exposure shows adverse health endpoint significantly associated with nephritic syndrome. The review of the epidemiological data suggests that, there are no statistically considerable evidence for human health risks associated with OTA exposure [29]. Populations in which OTA exposures are extremely high (such as those studied in Egypt and Sierra Leone) may experience a significantly increased risk of nephritic syndrome. However, it is evident that extreme OTA contamination was not seen in most parts of the world from the tests of urinary OTA levels collected in multiple regions around the globe, hence the risk of OTA- nephritic syndrome is not expected to be significant on global scale.

The tolerable levels of OTA contamination for food items was formulated by European commission has described below: for dried fruits and soluble coffee 10.0 µg·kg^{-1}, for raw cereal grains and roasted coffee 5.0 µg·kg^{-1}, 3.0 µg·kg^{-1} for cereals intended for human consumption, 2.0 µg·kg^{-1} for wine and grape juice and 0.5 µg·kg^{-1} for baby foods and cereal based foods intended for young children (European Commission) [30]. The provisional tolerable intake of OTA weekly set up by The World Health Organization has established as 100 ng·kg^{-1} of body weight (equivalent to 14 ng·kg^{-1} body weight/day) [31]. Thus sensitive, fast and selective detection methodologies are warranted in the field of human health care and for diagnostics of OTA.

Zearalenone: Zearalenone (ZEN) is a non-esteroidal estrogenic macrocyclic β-resorcylic acid lactone is produced by *Fusarium graminearum, Fusarium culmorum, Fusarium crokwellense, Fusarium sambucinum* and *Fusarium equiseti*, in various parts of world [32]. The *Fusarium* sp. grow generally in moist cool conditions and similarly invade crops under these more favorable conditions. In wheat, sorghum and corn, zearalenone occurs in pre-harvest grain but in other commodities it is a bit insufficient to determine pre-or postharvest crops [33].

The structures of ZEN and its derivatives resemble 17 β-estradiol and the potential for endocrine disruption has been evaluated using several models [34]. Early findings reported that ZEN induces genotoxic effects by induction of DNA-adducts, fragmentation, apoptosis [35], micronuclei, and chromosome aberrations, neurotoxic effects, proliferation and macronucleus synthesis in different cell lines [36] (Figure 5).
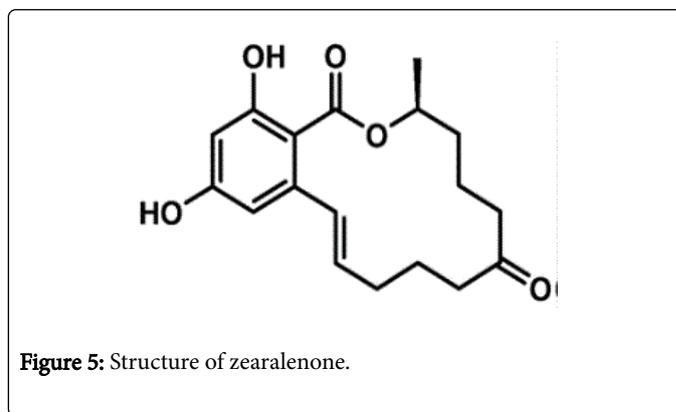

Figure 5: Structure of zearalenone.

The effects of ZEN on the central nervous system have received limited attention and therefore, a limited number of reports are available on their neurotoxicity [37]. The toxin also exerted its effects on astrocytes by regulating the plasma membrane reporters responsible for glutamate uptake. But no evaluation has been carried out on key regulators of brain function such as brain derived neurotrophic factor (BDNF); tyrosine hydroxylase (TH) and amino acid decarboxylase (AADC) which play a major role in brain functioning as well as neurotransmitter synthesis. Tyrosine hydroxylase or tyrosine 3-monooxygenase is the enzyme responsible for catalyzing the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) leads to neurotransmitter metabolism. The genes coding aromatic L-amino-acid decarboxylase deficiency (AADCD) is responsible for deficiency in neurotransmitter metabolism [38]. The decarboxylation of L-3,4-dihydroxyphenylalanine (DOPA) is catalyzed by AADC encoded

protein to dopamine, L-5-hydroxytryptophan to serotonin and L-tryptophan to tryptamine.

ZEN and a-zearalenol (ZOL) are potential endocrine disruptors at picomolar levels influenced negatively the chromatin structural stability and viability of swine spermatic cells [39] by altering hormone signaling, production as measured by the H295R steroidogenesis assay [40].

ZEN and its derivatives induce an estrogen effect like proliferation of ER-positive human breast cells (MCF-7) [41]. Considering the capacity of these mycotoxins to interfere with the homeostasis [42], residues in agricultural products are a significant human health concern.

Gas chromatography triple quadrupole mass spectrometry (GC–QqQ MS) substantially improves the selectivity and sensitivity compared to single-stage MS by eliminating chemical noise and isobaric interference [43]. Compared with ion-trap gas chromatography-tandem mass spectrometry, which has been applied for residue detection in agriculture products and foods [44], GC–QqQ MS is more stable and provides better quantitation results, especially for low levels in complex matrices.

Fumonisins: The fumonisins are heat-stable up to (150°C) non-fluorescent group of mycotoxins derived from *Fusarium*, Liseola section, particularly grown in warmer regions. *F. proliferatum*, *Fusarium verticillioides* are the main producer of fumonisins around the globe at high temperate zones along with relatively high water activities. Mainly in maize under this extreme conditions *F. napiforme*, *F. dlamini*, *F. nygamai*, *F. verticillioides* and *F. proliferatum* grow well during pre and post-harvest storage stages [45]. B series (fumonisins B1, B2, and B3) analogues are having vast importance among 12 fumonisin series, among this fumonisin B1 is pertaining importance (CAST, 2003). During the process of fermentation, a little degradation of FBs is observed [45] (Figure 6).
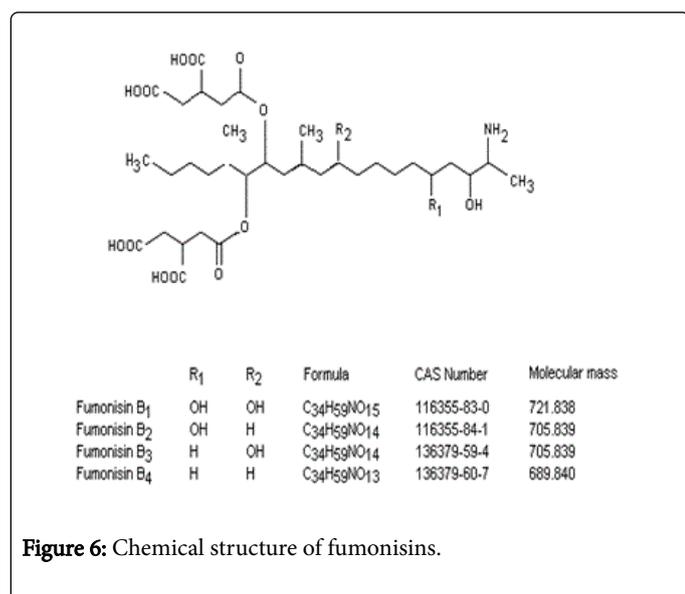

	R_1	R_2	Formula	CAS Number	Molecular mass
Fumonisin B$_1$	OH	OH	$C_{34}H_{59}NO_{15}$	116355-83-0	721.838
Fumonisin B$_2$	OH	H	$C_{34}H_{59}NO_{14}$	116355-84-1	705.839
Fumonisin B$_3$	H	OH	$C_{34}H_{59}NO_{14}$	136379-59-4	705.839
Fumonisin B$_4$	H	H	$C_{34}H_{59}NO_{13}$	136379-60-7	689.840

Figure 6: Chemical structure of fumonisins.

Norred et al. [46] states that FB1 group, inhibits the catalysis of sphinganine (Sa) and recycling of sphingosine (So) which is mediated by enzyme ceramide (CER) synthase. Samples from animal species like tissues, urine, and blood are used as potential biomarkers of FBs (Sa to So ratio) exposure, but studies have not allowed an accurate validation [47] (Figure 7).
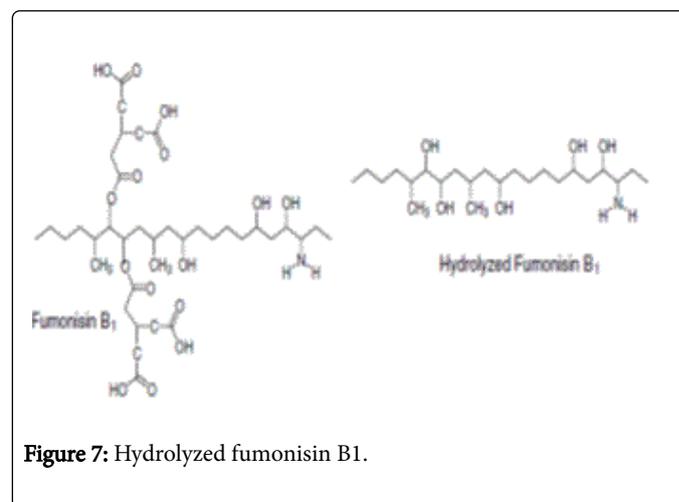

Figure 7: Hydrolyzed fumonisin B1.

The monotoxicity of FB1 on innate, humoral immunity, and cell response [48] changes in the expression of cell surface molecules, which are important in immune cell communication and NTDs [49] may be due to changes in various cytokines and chemokines in the immune system. The mechanism of action of FB1 induced NTDs [50] is the inhibition of uptake and metabolism of folic acid [51]. The fumonisins disturbs cellular signal pathways resulting from ceramide synthases inhibition which leads to carcinogenic effects in animals (JECFA, 2012). Fumonisins (FB1+FB2) levels in maize and maize-based foods for direct human consumption are regulated with a maximum level of 1000 µg/kg (EC, 2007). Fumonisins are stable at neutral pH and up to 175°C, but as pH decreases and temperature increases above 175°C stability also decreases over 90%. The exposure of FB1 on liver and esophageal [52] causes cancers in majority populations of liver cancer in China and South Africa [53]. Fumonisin B1 exposure to rodents above (P50 ppm) is hepato, nephron carcinogenic in male and female rats, [55] the major target organs are intestine, liver and kidney. Vander Westhuizen et al. [56] reported urinary FB1 excretion of 0.075 % in humans. Although no hydrolyzed product has been found in urine or bile, it is assumed that hydrolysis occurs in the gut and is probably performed by microorganisms.

In horses and related species, a fatal disease known as Equine Leuko Encephalo Malacia syndrome characterized by the presence of liquefy active necrotic lesions in the white and grey matter of the cerebrum are observed which leads to blindness, and decreased feed intake followed by death after several hours or days [57].

The current analytical techniques available for FB1 primarily include instrumental analysis like high performance liquid chromatography (HPLC) [58], liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) [59], Gas chromatography (GC) and Fourier transform near infrared spectroscopy (FT-NIR) [60], and immunoassays like Enzyme-linked immunosorbent assay (ELISA) [61], and Gold immune chromatographic assay (GICA) [62]. The former techniques rely on expensive, sophisticated instrumentation and trained personnel, and require complicated sample preparation processes including extraction, cleanup, and/or derivatization. The latter methods are easier to be operated, but they cannot do without anti-FB1 antibodies. False positive or negative outcomes of immunoassays result from the characteristics of antibodies that they

are sensitive to temperature and pH variation. Since its discovery by both Gold's group [63], aptamer technology has emerged as a novel approach to evolve nucleic acid recognition molecules alternative to antibodies for bioassay [64].

In 2007 European Union (EU) Commission finalized regulatory limits for sum of FB1 and FB2 in corn used food stuff with a maximum level 1000 ng g^{-1}, in cereals 800 ng g^{-1} for human consumption. For infants and young children, the limits of exposure are (200 ng g^{-1}). If these recommended levels are maintained in food stuffs the exposure of fumonisins can be regulated (FDA, 2001).

Ergot Alkaloids: Mycotoxins derived from prenylated tryptophan compounds characterized by a tetracyclic ergoline ring system released by fungal species *Claviceps purpurea* infect mainly cereals. There are four main groups of ergot alkaloids: the clavines, the lysergic acids, the lysergic acid amides, and the ergopeptides. Several Ascomycota fungi, representing two distantly related orders (Eurotiales and Hypocreales), produce ergot alkaloids.

The physiological effects of Ergot Alkaloids act as partial agonists [65] or antagonists at different adrenaline, noradrenaline, dopamine, and serotonin receptors they affect nervous, circulatory, reproductive, and immune systems [66]. Symptoms include reduced fertility, changes in blood pressure, lowered immune response, hallucinations, disturbances in sleep–wake cycles, and gangrene of the limbs [67] (Figure 8).
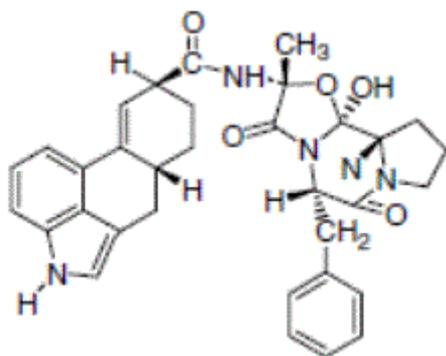

Figure 8: Chemical structure of Ergotamine.

Recent studies suggest that EAs contamination in fodder results in disturbance of gastrointestinal tract, neurotransmitter receptors, particularly adrenergic, dopaminergic, and serotonergic receptors subjected to oxidative biotransformation primarily by cytochrome P450 3A4, conjugated with glucuronic acid. Repeated dosing of EAs, to lab animals like rats results in decreased body weight, hormonal changes (EFSA, 2012). In more recent years, outbreaks have occurred in human populations and the effects included gangrene and loss of limbs and nervous signs including giddiness, drowsiness, nausea and vomiting [68,69].

Patulin: Patulin, 4-hydroxy-4H-furo [3,2c] pyran-2(6H)-one, is mycotoxin released by variety of molds *Penicillium, Aspergillus* and *Byssochlamys* genera of which *Penicillium expansum* is a common contaminant of damaged fruits [70].

PAT has greater affinity towards sulfhydryl groups, which shows inhibitory activity on many enzymes which alters the intestinal barrier function and cause [71] gastric ulcers, vomiting and kidney damage [72]. Hence, PAT is concluded as genotoxic, mutagenic, and neurotoxic, in laboratory exposed rodents. PAT alters the immune system. The action of PAT on macrophage functions shows increasing number of splenic T lymphocytes and depressed serum immunoglobulin concentrations, hence increase in number of neutrophilsers [73].

JECFA - Joint FAO/WHO Expert Committee finalized maximum tolerable daily intake for patulin in Food Additives is 0.4 mg/kg of body weight per day [7] (Figure 9).
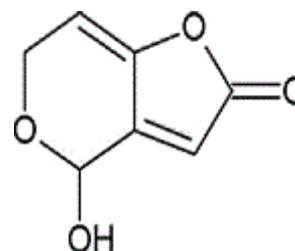

Figure 9: Chemical structure of patulin.

Other important mycotoxins with fewer occurrences: Other important mycotoxins that can be found as contaminants of foods include citreoviridin, gliotoxin, griseofulvin, mycophenolic acid, Kojic acid, wallemenols, β-nitropropionic acid, penitrems, penicillic acid, viomellein, vioxantin and xanthomegnin.

The term "emerging mycotoxins" has been assigned recently, to toxins for which little knowledge was available in the routinely determined nor legislatively regulated past but now more research is focused to determine their occurrences and potential health effects. Mycotoxins in this group include fusaproliferin, beauvericin, enniatins and moniliformin. Recent findings about contamination of emerging toxins in feed ingredients were revealed, beauvericin was placed first followed by enniatins with the highest prevalence [74]. Due to the lack of research currently there are no regulations implemented to limit the presence of these mycotoxins in food additives (CAST, 2003).

Global trends of mycotoxin regulation: Majority countries of globe have implemented regulations to limit mycotoxin exposure that affect the consumer health and world wealth. In 2012 as per the annual report of the Rapid Alert System for Food and Feed (RASFF), border rejection notifications in the European Union is main hazard of mycotoxins. For instance, reviewed report compared the regulatory management of feed ingredients among the seven nations including approval and risk assessments entitled 'Comparison of Regulatory Management of Authorized Ingredients, Approval Processes, and Risk-Assessment Procedures for Feed Ingredients' was written on behalf of the International Feed Industry Federation for the following jurisdiction that included European Union, Canada, Brazil, China, Japan, South Africa, and United States [75]. These steps involve the documentation and evaluation of regulatory procedures for feed ingredients (Table 2).

Hazard Category	Alert	Border Rejection	Information for attention	Information for follow up
Allergens	64	3	17	1
Biocont aminants	6	9	26	2
Food additives and flavourings	10	59	23	47
Foreign bodies	24	61	26	47
GMO/Novel food	2	52	14	22
Heavy metals	57	108	79	24
Industrial contaminants	16	9	18	14
Mycotoxins	38	425	53	9
Parasitic infestation	4	13	13	25
Pathogenic microorganisms	162	159	168	103
Pestiside residues	19	320	90	18
Residues of Veterinary medical products	12	18	16	14

Table 2: 2012 Notification by Hazard Category in the EU. Rapid alert system for food and feed (RASFF) Annual report 2012.

Mycotoxin	2008	2009	2010	2011	2012	Total
Aflotoxins	902	638	649	585	484	3258
Deoxynivalenol (DON)	4	3	2	11	4	24
Fumonisins	2	1	3	4	4	14
Ocharatoxin	20	27	34	35	32	148
Patulin	3					3
Zearalenone	2				4	6
Total	933	699	688	635	525	3450

Table 3: Mycotoxin Notification in EU during 2008-2012. Rapid alert system for food and feed (RASFF) Annual report 2012.

Several countries around globe have set regulations to limit international trade due to mycotoxins contamination of food items, because of this activity best quality foods are sold to foreign nations and the pestiferous is made available for animals and in some regions for humans. Multi-exposure to various mycotoxins is bit complicated to evaluate the adverse health effects after consumption [76] (Table 3). About 20% of the global supply of food is produced by impoverished people [77] and they mostly use as food to support themselves or their families. About 15% of the food supply to the U.S. comes from producers from other countries are refusals due to mycotoxins. The recent introduction of the FDA regulations for imported foods cover major steps in the assurance of food safety in this nation that already has the most secure food in the world. New proposed rules include the Foreign Supplier Verification Programs for Importers of Food for Humans and Animals and the Accreditation of Third-Party Auditors/Certification. Bodies to Conduct Food Safety Audits and to Issue Certifications [78,79]. The purposes of the rules are to strengthen import verifications in order to procure best free food and food security in the U.S.

Strategies to prevent fungal toxins exposure: Avoidance of fungal-contaminated foods is a good way to prevent mycotoxin exposure, as they are microscopic and invisible to the naked eye. Even if visibly moldy, some populations are forced to consume contaminated foodstuffs in view of food security. Populations heavily reliant on singular staple crops prone to mold contamination are at highest risk for mycotoxin exposure.

Economy of various countries is related to the dietary diversity. Chen et al. [80] agricultural reforms demonstrate in China in 1980s led to diminished maize consumption resulted in a drastic reduction of AF exposure, which, independent of HBV vaccination, reduced the risk of primary liver cancer. This powerful evidence shows the astounding effect economics and policy has on cancer prevention. Nevertheless, economic change may be slow and unmanageable in certain parts of the world. Thus, multiple intervention strategies have been proposed to prevent and/or mitigate mycotoxin exposure. These include primary prevention strategies, which aim to avoid exposure and secondary intervention strategies, which modulate metabolism, thereby reducing the internal dose. Kensler et al. [11] recently reviewed primary and secondary intervention strategies that address AF exposure. Authors describe examples of primary prevention strategies, including the use of community education in post-harvest intervention trials [81] to reduce AF in groundnut crop and the use of NovaSil clay as an AF enterosorbent [82] and chlorophyllin as a trapping agent [83] to reduce the uptake of AF from the gastrointestinal tract.

Additionally, intervention trials using chemopreventive agents, oltipraz [84], sulforaphane, and green tea polyphenols [85] are described. In both primary and secondary strategies, AF biomarkers served to evaluate the effectiveness of intervention.

Economic impact of mycotoxins: Economic losses due to mycotoxins are diverse and can be associated with reduction of quality foods for humans and animals, reduction in animal production due to feed refusal or diseases, increasing medical cost for toxicosis treatments, increased costs to find alternative foods, to design adequate

management to contain contaminated supplies, to improve detection and quantification methods and to develop strategies that reduce toxin exposure. A study by Robens and Cardwell in 2003, loss due to AF contaminated corn and peanuts, as well as FB contaminated corn and DON contaminated wheat resulted in estimated loss ranging from $0.5 million to over $1.5 billion for the U.S. [86]. Specifically for aflatoxins, Chinese studies aimed to evaluate the cost benefit of setting standard limits in different foods suggests that their applications might have significant effects in reducing liver cancer risk and food loss in that country [14]. It was concluded that following the CODEX Alimentarius standard at that time had less impacts in trade flow among the nations than if stringent E.U. rules were followed [87]. Global paddy production according to FAO for 2013 has been set at 746.4 million tonnes (497.6 million tonnes milled basis). Price for rice ranged from roughly 400 to 650 U.S. dollars for 5 different varieties just in July 2013 (FAO, 2013). It is evident that loss due to mycotoxins in this widely consumed cereal may have disastrous monetary impacts not just for the producers but for all of the world population.

Mycotoxins control: A control program for mycotoxins from field to table should involve the criteria of a HACCP approach which will require an understanding of the important aspects of the interactions of the toxigenic fungi with crop plants, the on-farm production and harvest methods for crops, the production of livestock using grains and processed feeds, including diagnostic capabilities for mycotoxicoses, and to the development of processed foods for human consumption as well as understanding the marketing and trade channels including storage and delivery of foods to the consumer's table. Common diagnostic and surveillance methods include culture, biochemical tests, and antibody based methods (e.g., agglutination and enzyme-linked assays) [88]. Definitive identification involves the amplification-based methods of regular and real-time PCR (quantitative PCR, or qPCR) [89]. Although fast and sensitive, PCR and qPCR are too complex to carry out in a basic clinical laboratory setting lacking highly-trained personnel. A good testing protocol for mycotoxins is necessary to manage all of the control points for finally being able to ensure a food supply free of toxic levels of mycotoxins for the consumer. There is continued need for point-of-care assays that are simple, sensitive, and economical.

Methodological issues: The contamination data often provided by researchers and consumption data from national dietary surveys are a common approach to evaluate the dietary exposure. These investigations are mainly useful to evaluate the nutritional status of the populations regarding energy and/or nutrients. From this survey we can estimate several food categories susceptible to contamination by mycotoxins. EFSA's Scientific Committee collects the large food consumption data over 5 years at an increasing level of detail. In 2008, EFSA formed the "Concise European Food Consumption Database" which is useful to improve Comprehensive European Food Consumption Database to be used for detailed exposure calculations in 22 different Member States (EFSA, 2011d). At individual level (73%) and 27% at household level, a gold standard method 24 h duplicate diet was performed increasing precision at the expense of loss of representativeness [90]. This 24 h duplicate diet methodology was used to find out the exposure of children and adults to several mycotoxins [91-104].

Conclusion

Monitoring of food commodities for the presence of microbial hazards is a primary step in ensuring food safety. At international level,

many countries have opted for regulation of mycotoxins in food commodities but in India no such efforts were made against Food-borne mycotoxins. Regulations on these toxins can be useful to comply with global market regulations in the near future. To control mycotoxins from contaminating food products there is a need for monitoring and control at different critical steps of the food chain to ensure food safety. These include food supply, monitoring during food processing monitoring of final products and also during storage. To find out the presence of mycotoxins in wide range of foodstuffs and their harmful effects to human health, research groups have devoted much effort to finding suitable mycotoxin detection techniques regarding food safety and quality requires a multidisciplinary approach based on a new generation of innovative and advanced technologies and tools to be used along the food chain for contaminants monitoring. In this review, we discuss the effects of mycotoxins on humans or animals some important aspects of toxicology and control have still resided in the realm of the unknown and unexplored. Only with continued research on understanding the effects and modes of mycotoxin action in various species, have regulations and control strategies been forthcoming.

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