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The implications of some mycotoxins synthesized by microscopic fungi in human pathology

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ABSTRACT
Mycotoxins are toxic substances with small molecule produced as secondary metabolites by filamentous fungi. Mycotoxins produced in high concentrations could produce toxic effects in humans and animals, known as mycotoxicoses. The source of contamination with fungal toxins is represented by the consumed food in which toxin-producing mycelia have developed. The carcinogenic, mutagenic, teratogenic and immunosuppressive effects of some of the mycotoxins have been confirmed in the last years, and among these, the most common is represented by the decrease in resistance to infection due to changes in immune response.
Keywords: *mycotoxins, mycotoxicosis, fungi, cereals.*

1. INTRODUCTION

Mycotoxins are secondary metabolites which can contaminate the food and are mainly produced by filamentous fungi. The Fungi category includes macroscopic mushrooms, console mushrooms, *Lycoperdon* and *Calvatia* spherical mushrooms, rust, thorns and mites, filamentous microscopic fungi and yeasts, as well as lesser-known organisms [1].

About 70,000 species of fungi have been described, but, according to some estimates, the number of fungal species is much higher, and can reach 1.5 million. There are at least 350 species of toxigenic fungi, to which humans are exposed by eating contaminated food or occasionally by inhalation, causing a myriad of pathological conditions, some of them with severe consequences on the human health, such as aflatoxin-induced neoplasms [2;3].

Mycotoxins are primarily synthesized by non-pathogenic fungi which can develop in the organic matter. The potential toxigenic effects of different fungal strains vary, such that the same mycotoxin can be produced by different strains, or a single species of filamentous microfungi can synthesize more mycotoxins [4].

In the last years, the carcinogenic [5], mutagenic, teratogenic and immunosuppressive action of some mycotoxins has been confirmed. The ability of mycotoxins to modify the immune responses and thereby to reduce the immunoresistance to infection is considered the most important and frequent harmful effect on the human health.

Depending on the degree of toxicity, mycotoxins are divided into three major groups:

- ***mycotoxins with high toxicity***: aflatoxins, patulin, zearalenone, T2 toxin, ochratoxin, deoxynivalenol;
- ***medium-grade mycotoxins***: aspergillus acid, penicillinic acid, citrinine, gliotoxin, ipomearin, rugulosin;
- ***mycotoxins with low toxicity***: oxalic acid, fusaric acid, chemothene, trichotecin, tardine.

1.1. Trichotecenes. Trichotecenes represent a family of 148 toxins produced by species of *Fusarium, Trichoderma, Cephalosporium, Myrothecium, Stachybotrys* genera, which grow on cereal seeds (rice, corn, rye, etc.). Toxins have been isolated from a variety of substrates: decomposing vegetable matter, seeds and cereals. The name "*trichotecenes*" comes from trichotecin, one of the first toxins identified as representative for this family [6].

Each species of fungi can synthesize more than one trichothecene, for example *Fusarium tricinctum* produces toxin T-2, HT-2 and diacetoxyscirpenol. Trichothecenes are toxic for humans and other mammals, birds, fish, invertebrates or plants (generally eukaryotic cells) [1].

One of the most famous trichothecenes is T2 toxin, which is the most toxic and has the most severe effects. It is produced by species of the *Fusarium* genus, i.e.: *F. tricinctum, F. solani, F. sporotrichioides, F. equiseti.*

Deoxynivalenol, also known as vomitoxin, is the most common toxin, although with the lowest toxicity. This toxin is synthesized by *F. graminearum* and *F. culmorum* [7].

1.2. Aflatoxins. The most common mycotoxins are aflatoxins, which are found mainly in corn used for animal feed, peanuts and rarely in grains intended for human consumption. There are over 20 aflatoxin-producing micelles, of which the most well-known are *Aspergillus flavus, A. parasiticus, A. nomus, A. ochraceus, Rhizopus sp.* Among the 18 different types of aflatoxins identified, the most important are B1, B2, G1 and G2. The most common and also the most toxic, is aflatoxin B1, while B2 has the lowest toxicity among the members of this family [8].

1.3. **Ochratoxins.** Ochratoxins are a group of chemical compounds in whose L-phenylalanine is coupled *via* an amide linkage to an isocyanurate derivative. These toxins are produced by *Aspergillus sp*. and *Penicillium sp*. The most important and the most toxic ochratoxin that is naturally found in food is ochratoxin A [9]. Other structurally related ochratoxins include ochratoxin B, ochratoxin C, ochratoxin α and ochratoxin β. These types of ochratoxins have been isolated from fungal crops, but are seldom found in food.

1.4. Zearalenone. Zearalenone, also known as mycotoxin F2, is a potent estrogenic metabolite produced by *F. graminearum*, *F. culmorum*, *F. equiseti*, and species of the genus *Gibberella sp.*, which infects the grains before harvest during the flowering period [10;11].

1.5. Patulin. Patulin is a toxic antibiotic produced by several fungal species of the *Penicillium*, i.e.: *P. urticae, P. expansum, P. griseofulvum, P. cyclopum, P. divergens, P. roqueforti, Aspergillus,* i.e.: *A.clavatus, A.giganteus, A.terreus*, as well as *Byssochlamysniveae* species. These mycotoxins mainly are produced in grains, but also in other products, especially fruits, such as apples. It was first isolated as an antibiotic in 1943, by Birkinsaw and his collaborators, from *P. patulus*. A few years later, this substance was isolated by Chain and his collaborators from *P. clavatum* fungus, and was named claviformin.

2. MYCOTOXIN BIOSYNTHESIS

Particular climatic conditions (heavy rain before harvesting), plant blooming (especially maize), insect or parasite attack, mechanical grains harvesting, inadequate storage and drying conditions can favor the production of mycotoxins. These factors were divided into two large groups, although, under natural conditions, their biofeedback may be cumulative or synergistic: i) extrinsic, exogenous factors of the natural/industrial environment - are represented by temperature, light, humidity, osmotic pressure, oxygen concentration; ii) intrinsic factors – are dependent on the nature of the food that can influence the growth and the activity of starter cultures, but also the specific nature of food alteration process.

In order to establish the optimal conditions for the storage of cereals, it is good to know how these factors influence qualitatively and quantitatively the development of microorganisms and the production of toxins [12].

3. INFLUENCE OF EXTRINSIC FACTORS ON MYCOTOXINS PRODUCTION

Temperature does not only influence the growth of mold species, but also the production of their metabolites. Mycotoxins are synthesized at temperatures lowers than those favorable for the optimal growth of fungi, ranging from 20-30ºC [1]. In the development of microorganisms, the effect of temperature is due to its influence on the state of water aggregation, the rate of enzymatic reactions and the plasticity of the cell membrane. When the optimal development temperature is exceeded, most molds become mesophilic.

Moisture, or the degree of the presence of water in the environment, as well as in the substrate, is an important factor for the development of molds and the synthesis of mycotoxins.

As myocetes develop in darkness or in dimly lit spaces, the exposure of nutrients to light, UV rays or sunlight limits mycotic contamination, and under continuous illumination the synthesis completely ceases.

All mold species are aerobic and therefore need oxygen to grow and to carry out their metabolic reactions. However, some species have the ability to grow in the canned foods or in an oxygen-free atmosphere by acquiring it from the substrate.

Osmotic pressure can cause changes in mycelium morphology and cell structure. Molds are osmophilic organisms, growing in environments with high osmotic pressure, and for this reason, they can alter foods containing a large amount of carbohydrate.

Fungicides are chemicals used to prevent the growth of fungi, and therefore, the accumulation of mycotoxins in different products.

4. INFLUENCE OF INTRINSIC FACTORS ON MYCOTOXINS PRODUCTION

The substrate is influencing the mycotoxins production, which is synthesized primarily by non-pathogenic fungi that grow on organic matter. The optimal substrate for the development of mycotoxins should be rich in carbohydrates.

Although microscopic fungi can develop in a wide pH spectrum, from 2 to over 9, toxin synthesis is however favored by an acid pH rather than alkaline. It must be mentioned that molds can modify the pH using organic food sources [12]. In the absence of a strong buffer, most of these microorganisms can modify the pH, so as to favor their growth, typically at a value of 4-6, 5.

Crop rotation is an effective way to reduce the risk of contamination depending on the fungal source and the cultivated species. It is very effective to reduce especially the contamination of winter cereals.

Although interactions between mycotoxigenic fungi and insects have not been studied extensively, some insects have been shown to disseminate mycotoxic species, others use them as a source of food, while others avoid them.

5. INFLUENCE OF MYCOTOXINS ON THE ANIMAL AND HUMAN HEALTH

The structural, chemical, biological and toxicological properties of mycotoxins are different. Toxicity levels are extremely variable, depending on the assimilated amount, exposure period, animal species, sex, age, race, psychological status, nutritional standards, environmental conditions (including hygiene, temperature, air conditioning, humidity, production density) and the possible synergisms between mycotoxins present in food. Studies have shown that the use of mycotoxin-contaminated food presents the potential risk of inducing a wide variety of human diseases.

Approximately 25% of mankind's food supplies are contaminated with mycotoxins. Fungi producing dangerous mycotoxins are rarely present in the environment but are most commonly found on and in stored seeds, as well as in products derived from them (food and feed). The ingestion of contaminated food with a mycotoxin threshold has immediate effect and causes acute toxicity (partial or complete loss of function of a certain organ), and after long exposure to low doses of mycotoxins, the chronic toxicity associated with the delay somatic development and weight loss, increases susceptibility to infections, reproductive malfunctions, carcinogenesis appears. Some mycotoxins cause reversible disturbances, such that affected organisms can recover if the toxic food is removed [13].

Mycotoxins like aflatoxin, ochratoxin, patulin, deoxynivalenol, zearalenone, T2 toxin, produce important economic losses through the qualitative impairment of animal and plant products in which mycotoxins are present as residues that endanger human and animal health [14]. Mycotoxins have a wide range of activities and target species (Table 1).

Depending on the interaction of mycotoxins with the substrate molecules, several mechanisms of action are distinguished: i) the toxin reacts with the enzymes in the substrate and modifies and blocks its action at this level; ii) the toxin interacts with a substrate causing its alteration, thereby modifying the enzymatic reaction; iii) the toxin reacts with an enzyme substrate by blocking its interaction with the enzyme; iv) the toxin reacts with enzyme co-factors, altering the functions of the enzyme.

The biochemical mechanisms of action of mycotoxins are diverse, i.e.: i) protein synthesis and functions inhibition; ii) mutagenic effects; iii) endocrine disruptors (e.g., zearalenone). Some mycotoxins could exhibit all these mechanisms simultaneously (e.g., patulin) [13; 15].

Table 1. Toxigenic fungi, their metabolites and their effects on the target species**.** (adapted after Mills, 1990)

Fungi	Toxin produced	Toxic effects	Species affected
Fusarium graminearum	Trichotecenes Toxin T2 Deoxynivalenol	✓ Gastrointestinal acute syndrome ✓ Severe leukopenia ✓ Immunological effects ✓ Skin damage ✓ Intestinal necrosis ✓ Death	Man Pig Horse Rabbit Cat
Aspergillus flavus	Aflatoxins	✓ Hepatocellular carcinoma ✓ Hepatotoxicity	Man Horse, Rabbit, Dog
A. ochraceusg	Ochratoxin A	✓ Nephrotoxicity ✓ Teratogenic effect ✓ Necrosis in the lymphoid organs(thymus)	Pig Rabbit Dog
F. graminearum	Zearalenone	✓ Reproductive dysfunctions	Pig, Sheep, Rabbit, Horse, Dog

Fungi	Toxin produced	Toxic effects	Species affected
P. expansum	Patulin	✓ Neurotoxic effects, vomiting, anxiety ✓ Pulmonary oedema ✓ Hepatic congestion ✓ Heart failure	Man Mouse Cat

6. MAIN MYCOTOXINS INTOXICATION SYMPTOMS

Depending on the type of intoxication, acute or chronic, the symptoms and clinical manifestations of trichothecenic intoxication vary from acute toxicity, which causes gastric and intestinal, skin and respiratory lesion to chronic toxicity due to repeated exposure at low doses of trichothecene, evolving in 4 stages: i) inflammation of the gastrointestinal mucosa, with emesis, diarrhea, abdominal pain, excessive salivation, headache, tachycardia; ii) leukopenia (latent stage) characterized by a decrease in white blood counts, granulopenia (decreased platelet number) and progressive lymphocytosis; iii) localized skin rash, with secondary expanding on larger areas and iv) recovery stage characterized by the healing of skin lesions [16; 17;18].

The effects of eating food infused with aflatoxin on humans may be also acute or chronic. The acute toxicity causes acute food toxicity, fever, vomiting, jaundice, hemorrhage, pulmonary edema. In severe cases, serious liver damage, convulsions, coma and even death may occur. Chronic toxicity may cause decreased growth and development of organisms, the appearance of neoplasia, especially of hepatocellular carcinoma [19].

In animals and humans, ochratoxin A toxicity involves a number of clinical symptoms and manifestations in relation to the ingested dose [20; 21]. The acute toxicity leads to anorexia, polyuria, polydipsia, digestive hemorrhage, dehydration that can cause death a few weeks after dosing [22; 23]. Chronic toxicity causes effects on lipid and carbohydrate metabolism, congestive and hemorrhagic effects, immunotoxic, neurotoxic, nephrotoxic, teratogenic, genotoxic and carcinogenic effects [24; 25].

The specific toxic effect of the most known zearalenone product is on the reproductive system: redness of the genital organ, nipple secretion, the presence of numerous vesicular and chicory follicles filled with fluid in the ovary [26].

The specific toxic effect of the most common patulin product is that it can alter the immune response of the host. Numerous *in vitro* and *in vivo* studies have shown that patulin exhibits its immune suppression effect through the inhibition of several functions of macrophages [27].

In addition, some molds that produce these substances are themselves toxic, for example, *Aspergillus flavus* and *A. parasiticus* for which three types of human symptoms have been diagnosed: infection, allergy and toxicosis. The infection is an invasion of living tissues while allergy is a manifestation of hypersensitivity to a fungal antigen equipment [28].

7. METHODS FOR THE DETECTION OF MYCOTOXINS

Analytical methods for the rapid, sensitive and correct determination of the presence of mycotoxins in unprocessed cereal and grain products are very necessary in order to properly assess both the relevant risk of exposure and the toxicological relevance for humans and animals, as well as to ensure that maximum levels set by the EU or other international regulations are met [29]. Techniques for determining mycotoxins are laborious and require sophisticated equipment [28]. Determination of mycotoxins involves sampling, extraction with organic solvents or water, chromatographic column purification and quantitative detection. In the quantitative and qualitative determination of mycotoxins, different analytical methods are used, e.g.: chromatographic methods (thin layer chromatography, gas chromatography, high performance liquid chromatography); electrophoretic; immunochemical; radio-immunochemical methods or immunoaffinity methods [30].

Laboratory risk management must include: identification of the hazardous source and possible health effects in the event of the use of appropriate equipment; developing methods for the manipulation of toxins and producing microorganisms; understanding the meaning of responsibility in order to allow for the application of decongestion measures [31].

8. CONCLUSIONS

Fungi have extremely diverse roles in nature as decomposers of organic matter, have economic importance through their use in the mycological bioindustry (e.g., industrial production of alcoholic fermentation drinks and in the bakery industry). Fungi consistently associated with food contamination belong to *Aspergillus*, *Fusarium* and *Penicillium* genera and the penetration of mycotoxins into the human body through ingestion, inhalation, dermal absorption can cause acute or chronic poisoning, manifested through diverse symptoms affecting the digestive, cardiovascular, respiratory, excretory and reproductive systems.

Mycotoxins present a real danger for both animals and humans, and it is imperative that mycotoxin producing species manipulation to be done with care and work under conditions that prevent contamination of materials and atmosphere.

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