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Technical Note

Mold, Fungi and Mycotoxins

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Mold and fungi are ubiquitous in the world. Their size and the mobility of their spores cause rapid spread through agricultural products under favorable environmental conditions. Significant crop losses and foodborne illness can be attributed to mold and fungi when secondary metabolites, called mycotoxins, develop. The botanicals, cannabis and food industries battle continuously with such contaminants. In the United States, the CDC estimates that 48 million people get sick from foodborne illnesses, and up to 3,000 die from foodborne diseases each year. More than 250 agents are known to cause foodborne illness and are introduced through contamination, improper handling practices and sanitation. These agents can be chemical, physical or biological.

Biological contaminants in the form of microbes are by far one of the greatest concerns for illness. The five types of microbes are bacteria, viruses, parasites, protozoa, and fungi. Fungi are a very diverse kingdom of single and multicellular organisms, (they were once considered plants). We now know that fungi are more closely related genetically to animals than plants.

Fungi (which are nonphotosynthetic) derive their nutrients from decaying or dead matter (saprophytes), or from living organisms (parasites). Scientists have identified a hundred thousand known species of fungi. This is a fraction of the over 1 million likely species present on Earth.

The classification of Kingdom Fungi is constantly being debated with the influx of DNA data. Currently the kingdom contains seven phyla which span the different forms of fungal organisms from single-celled yeasts to multicellular mushrooms. Some species of fungi produce biologically active compounds which are used in food production and medicine; however, there are also many toxic compounds produced by fungi.

The typical toxic mushrooms most of us think about are from the phylum Basidiomycota, or club mushrooms. The most infamous members are the Death Cap and Skull Cap mushrooms which produce secondary metabolites like amatatoxins, phallotoxins, and ergotamines. The more insidious toxic fungi are from the phylum Ascomycota, which include the molds, yeasts, mildews, etc. The most common secondary metabolites produced by these fungi are called mycotoxins and are associated with food contamination. Secondary metabolites are not needed for the normal life cycle of the organism and, in many cases, the reason for their production is unknown. One species of fungi may produce different mycotoxins and some mycotoxins are produced by multiple types of fungi. Most of the major mycotoxins of concern to human beings come from a few dozen species from the phylum Ascomycota or the sac fungi.

Table 1. Major Types of Mycotoxins

Mycotoxins	Fungus	Health Effect	Source
Aflatoxins: B1, B2, G1, G2 (M1)	Aspergillus and Penicillium Species	Hepatotoxin Carcinogen Immunosuppressant	Cereals, Maize/Corn, Tree Nuts, Ground Nuts
Ochratoxins: A, B, C, α, β, γ	Aspergillus and Penicillium Species	Nephrotoxin, Immunosuppressant, Renal and Hepatic Carcinogens	Vegetables, Cereals, Vine Fruits, Wine, Beer, Coffee

Trichothecenes Toxins: NT-1, NT-2, T-2, HT-2, Diacetoxyscirpenol (DAS), Nivalenol (NIS), Deoxynivalenol (DON), Fusarenon-X	Fusarium and Gilerallazeac Species	Hepatotoxin Carcinogen Immunosuppressant, Neurotoxin, Necrosis, Malabsorption, Skin Issues	Grains, Rice, Oats, Cereals, Maize/Corn
Fumonisins: B1, B2, B3	Fusarium Species	Esophagus Cancer, Liver Cancer, Neurotoxin	Maize/Corn, Cereals, Grains, Beer, Garlic, Beans, Asparagus
Zearalenone	Fusarium Species	Reproductive Issues, Infertility, Early Development	Corn, Wheat, Oats, Sorghum, Sesame Seeds, Hay, Silage
Cyclopiazonic Acid	Aspergillus and Penicillium Species	Convulsions	Cheese, Maize/Corn, Ground Nuts
Citrinin	Aspergillus and Penicillium Species	Neurotoxin, Nephrotoxic Carcinogen	Rice, Cheese, Soy, Maize/Corn, Cereals, Grains
Patulin	Aspergillus and Penicillium Species	Neurotoxin, Mutagenic, Hepatotoxin Immunosuppressant	Fruits, Juices, Vegetables
Ergot Alkaloids	Claviceps Species	Digestive Disorders, Nervous Disorders, Seizures, Vomiting, Headaches	Cereals, Grains, Maize/Corn

Many crops, like botanicals and cannabis, are prone to fungal growth due to agricultural conditions and moisture content. Plant materials with over 14% moisture can encourage mold growth. Some mycotoxins, especially aflatoxins and ochratoxins, need oxygen to grow so the reduction of the oxygen in the storage areas can retard growth.

Toxic and lethal dosages of mycotoxins can be quite low for acute poisonings. Ochratoxin A has a tolerable daily intake designated by the World Health Organization (WHO) of 5 ng/kg of body weight per day. Ochratoxin A is very toxic with an LD50 of 20-25 mg/kg of body weight. WHO recognizes products containing more than 1 mg/kg of aflatoxins as potentially dangerous or life threatening. The Food and Drug Administration (FDA) has limits for mycotoxins in human and animal feed up to 20 µg/kg for direct human exposure.

Mycotoxin Analysis

Mycotoxin analysis is challenging due to the ubiquitous nature of molds and the varied agricultural conditions for products. Molds and other toxins can be distributed heterogeneously in a batch or harvest and the location and concentration of the toxins can vary greatly. The analytical targets tend to be in the very low parts per billion ranges.

The simples types of tests are qualitative tests which provide positive/negative screens for the presence of a toxin. These tests involve simple colorimetric chemical reactions that are carried out in a test tube, or container with reactant or lateral flow material, and a color change indicates in the presence of target analytes.

More detailed tests are needed for more quantitative results. These tests range from quantitative test strips to more complex and sensitive instrumental analysis. In many cases, testing laboratories prefer higher throughput analysis and employ more advanced techniques such as fluorometry, chromatography and mass spectroscopy.

Fluorometry is the study of the visible spectrum of fluorescence. The fluorimeter measures the intensity and wavelength distribution of an emission spectrum after excitation by light. Molecules that undergo fluorescence can be measured accurately and down to the parts per trillion range using a fluorimeter. Many mycotoxins (B1, B2, G1, G2, etc.) used to be detected by black light under which they would glow due to their fluorescence. In some mycotoxins, they can be treated with a binding agent to become fluorescent and measurable fluorometry. The first step of testing for mycotoxins with a fluorimeter is to isolate the mycotoxins from the rest of the extracted material using some method of separation like an immunoaffinity or other chromatography columns. Immunoaffinity columns use monoclonal antibodies to isolate target analytes by containing them in the column until they are washed with the proper solvent and concentrated into an extract. Other chromatography columns are substrates that select for size, chemistry and polarity to retain analytes until the time they are washed into an extract.

Mycotoxin testing is often conducted in conjunction with other types of cannabis testing such as for terpenes, pesticides and potency. In these multiple target methods, more complex analysis methods are used such as liquid chromatography-mass spectroscopy (LC-MS). These systems can test for multiple targets but need a high level of expertise to run and are often costly to purchase and maintain unlike simpler test methods.

Agricultural products are plagued by a variety of pests and contaminants. The agricultural practices and processes can lead to increased exposure to mycotoxins. There are significant health concerns regarding mycotoxin contamination of products which are not only processed into edible foods but also used as botanicals and nutraceuticals. It therefore becomes critical to understand the nature of fungi and mycotoxins and how to reduce them in agricultural and cannabis products.

Further Reading

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