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«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL
AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

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THE SYSTEMATICS
AND BIOLOGICAL PROPERTIES
OF TOXINOGENIC FUNGI



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PROPERTIES OF TOXINOGENIC FUNGI

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Fungi represent a vast heterogeneous group of organisms, differing in their morphology, methods of reproduction, cycles of development, ways of nutrition and habitats.

At present many mycologists and biologists support the conception concerning the three classes of multicellular organisms - plants, animals and fungi, according to which the fungi are given the status of a separate kingdom in the organic world, on a par with the plants and the animals. These classes of multicellular organisms differ, in the main, by the type of their nutrition and metabolism, namely:

1. The plant kingdom is characterized by the presence of chlorophyll and photosynthesis.

2. The animal kingdom is characterized by heterotrophic metabolism according to the type of ingestion the sources of nutrition.

3. The fungal kingdom is characterized by heterotrophic metabolism effected by adsorption.

Fungi display features of both the animal and the plant kingdoms. Their affinity with animals is based on the nature of nitrogen and carbohydrate metabolism, the presence of chitin in the cell membranes of the majority of fungi. Their affinity with the plants is based on the character of nutrition — absorption (not ingestion) of food, and apical growth.

Before going over to an examination of the principles on which the systematics of toxinogenic fungi is based, let us dwell

briefly on their morphological features.

Fungi - Organisms Devoid of Chlorophyll

In most fungi the vegetative body (thallus) consists of a network of hyphae, which make up the mycelium, and only in some of them, it consists of a single cell. The hypha, the main morphological structure of the majority of fungi, represents a filamentous form surrounded by a membrane characterized by apical growth and ramification. Continuously deposited beneath the growing terminus is the membrane of a new cell. A system of intertwinning hyphae makes up the mycelium, which is also one of the characteristic morphological features of the majority of fungi.

The fungal hyphae, of which the mycelium is formed, has a vast surface through which nutritive substances are absorbed by osmosis from solutions. The hyphae of some fungi are divided by transverse septa, whereas the hypha of other mycelian fungi has no transverse septa and in this case the ramified system consists of a single cell.

The hyphae are covered with a rigid membrane, 80 to 90 per cent of which is usually made up of nitrogen-containing and nitrogen-free polysaccharides - chitin, chitosan, carbohydrates, glucuronic acid; low quantities of proteins - lipids and polyphosphates are also present. In most fungi the main component of the cell membrane are chitin and chitosans, and in comycetes - cellulose.

The composition of the cell membrane in individual fungal taxons is diverse, depending on the conditions of cultivation, the age of the organism, its taxonomic position. Microfibrils

of chitin and cellulose usually form the skeleton, with the built - in numerous other components of the cell membrane. Situated beneath the cell membrane is the cytoplasmic membrane which surrounds the inner part of the cell - the protoplast.

The cytoplasm of a fungal cell contains structural proteins and not bound with organelles enzyme-cells, amino acids, carbohydrates, lipids, and other elements. The main organelles of a fungal cell are the mitochondria - the energy centers (basically similar to the mitochondria of higher plants), lysosomes, vacuoles, containing reserve substances - volutin, lipids, glycogen, and also fats (mainly unsaturated fatty acids); there is no starch.

The fungal cell contains from one to several nuclei having a dual membrane, a nucleolus and chromosomes; the structure of the fungal nucleus places fungi among eukaryotic organisms.

In yeasts and yeast-like fungi there are no true hyphae, their vegetative body consists of mononuclear cells; reproduction is by division or budding or by both methods. In the more simply organized fungi the vegetative body is a clump of protoplasm devoid of a membrane or a cell with a membrane and hypha-like processes. Upon maturation the entire cell turns into one or several mobile productive organs - zoospores. This type of vegetative body, in distinction from eukaryotic, is called holokaryotic, and is characterized by a marked differentiation of the vegetative and reproductive mycelium.

Variations of hyphal growth are known, such as the formation of chlamydo-spores, cells with a thickened membrane, strands, rhizomorphs, sclerotia, appressoria, and others, which complicate the morphological structure or help to withstand unfavour-

able conditions.

During cultivation, either on hard or fluid nutrient media, the fungal mycelium grows in colonies, with radial growth of the germinated spores and branching mycelium. When immersed in a substrate the mycelium is called substrate, when growing above the substrate - aerial.

Fungi are known to have sexual, asexual and vegetative reproduction. The typical organ of reproduction is the spore, formed on the branches of the mycelium, within or outside special structures - fruit bodies. The spores of fungi, including toxigenic ones, play an important role in their dissemination and invasion of various substrates. As a rule, they are formed in the air. The number of spores produced by various species on the fruit-bearing organ of one or another type varies widely, from just a few to tens and hundreds of thousands. The duration of the spore-producing period also differs. In most fungi the spores are immobile, while being mobile in the more primitive forms. The morphology of the spores and the mode of their production, the structure of the fungi fruit-bearing organs is one of the leading criteria in their systematics. In many fungi one observes the formation of false tissue of which the fruit bodies, sclerotia and other structures consist. This tissue is formed by a more or less dense interweaving of hyphae and it is called a pseudoparenchyma or plectenchyma.

Some fungi display transformations of the mycelium: strands, rhizomorphs, sclerotia.

With vegetative reproduction even small particles of hyphae with at least one intact cell, are easily regenerated and continue to grow, even under unfavourable conditions. This mode of

reproduction is rather widespread in many fungi under natural conditions. Fragments of mycelium with small particles of soil attached may be carried by the wind, and, upon landing on an appropriate substrate under favourable conditions, may start a new organism. Budding mycelia during growth disintegrate into individual cells which, in turn, keep budding (e.g., in Endomycetales). In a mycelium with typical hyphae one may also occasionally observe disintegration from the tip of the hyphae into individual cells, more frequently of egg or longitudinal-elliptical shape, called oidia. Given favourable conditions, every oidium may start a new fungus.

Spores of asexual reproductive may be of endogenous and exogenous origin. An indefinite, mostly a large number of the former are produced within the more or less swollen and roundish cells - sporangia. The spores formed in the sporangia, having a membrane but devoid of organs of locomotion, are called sporangiospores. The sporangia are formed on special differentiated branches of the mycelium - sporangiophores, from which they are separated by a septum.

Asexual reproductive spores of exogenic origin are called conidia. They are produced on more or less separate branches of hyphae - conidiophores, and sometimes on the serrated prominences of the hyphae. Young conidia usually appear as a small swelling which is then separated by a septum from the conidiophore and, continuing its growth, assumes the shape characteristic of the conidia of the given fungal species, sometimes being divided by septa into several cells. Conidia are rather diverse in shape. They may be unicellular or divided by one or several transverse, and sometimes also longitudinal septa; they are colour-

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less or of different tints.

The conidiophores may be simple or ramified. The ramifications of the conidiophores and modes of conidium production vary and are typical of one or another fungal species. Conidia are usually formed at the apex of a conidiophore or its ramifications. They may be joined into bundles, into so-called coremia, gathered in a continuous layer on the plectenchymatous network of hyphae as a kind of sporodochium, or within the fruit bodies, in pycnia or pseudopycnia.

All the above-described modes of fungal reproduction are asexual ones. The ways of sexual fungal reproduction are also quite diverse. Fertilization results in the production of spores, usually resting ones. In oomycetes fertilization results in the appearance of oospores, in zygomycetes - of zygospores.

The spores developing as a result of sexual reproduction in cup fungi are called ascospores. The ascospores appear endogenously in the maternal cell, the ascus.

In distinction from ascus spores, basidiospores form on so-called basidia. Basidiospores are an attribute of the class of basidial fungi. They are mostly formed on the serrated processes of the basidia - sterigmata (one on each), four on each, more seldom two, but in some species of the basidiomycetes there are one, three, eight and more on a single basidium.

In the higher basidiomycetes the basidia form a hymenial layer on or within the fruit bodies which have varied structures.

The basidiospores are always unicellular, colourless or differently tinted.

During cultivation, particularly on dense media, the fung-

al colonies of some species differ morphologically, and this is of diagnostic significance. Their growth in a multispore culture (or upon the inoculation of mycelian fragments and conidia) proceeds radially. The colonies are smooth, radial or rugose, spreading widely over the substrate (i.e., with intensive linear growth), or the growth is restricted, with copious or slightly developed fluffy, powdery, felt-like, velvety or leathery aerial mycelium.

The colonies are further distinguished by the arrangement and colouring of the sporophore, aerial mycelium and nutritive medium. When cultivated on special media in colonies, saprophyte or facultative parasitic fungi usually form typical organs of reproduction, parasitic fungi - frequently colonies consisting of a transformed, membranous or leathery, strongly rugose mycelium, often of limited growth.

Some kinds of Mucor fungi of the zygomycetes class have pathogenic properties and cause mycoses of the lungs and other organs in man and animals.

These fungi have a well-developed mycelium, usually non-septate. Not infrequently the mycelium sends forth stolons and rhizoids by which the fungus attaches itself to the substrate. The sexual and asexual reproduction known for the majority of species is not of the same biological value. Asexual reproduction is more copious, which determines the dissemination of the fungus and its invasion of food and other products on which they are widespread. After the fusion of the mycelium of two different sex signs a sexual reproductive spore - a zygote is produced, which has a dense membrane and is capable of withstanding unfavourable factors; after a certain period of rest it begins to

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grow by means of the so-called germinal sporangium. The spores produced in the germinal sporangium, when growing, give rise to a mycelium formed on which are sporangia - organs of asexual reproduction, containing a great number of sporangiospores within. When released from the sporangium upon the dissolution of its membrane, sporangiospores are capable of instant growth on a favourable substrate (usually on food or fodder) putting the beginning to a new mycelium by vegetative development.

The class of cup fungi (Ascomycetes) belongs to one of the numerous taxons. Nearly 30,000 species of this class, varied in structure, habitat and mode of existence, include yeasts, truffles and *Morchella* fungi. A characteristic feature is sexual reproduction which results in the formation of asci, usually containing 8 spores. The asci are produced right from the zygote or on ascogenic hyphae developing from the zygote - singly, in groups, or in various spore-bearing structures - cleistothecia, apothecia, perithecia.

The mycelium is haploid, usually well developed, septate. Asexual reproduction usually plays an important role in the development cycle; it is rather varied and frequently concerns individual species of the Deuteromycetes class.

Such cup fungi as the ergot (*Claviceps purpurea*) and (*Cl. paspalum*) produce toxins and cause diseases in man and animals, known under the names of *Claviceps toxicoses*, or ergotism.

During the developmental cycle of these fungi the growing ascospore forms a ramified mycelium from which a conidial stage emerges, called sphaecelia. The sphaecelial conidia, getting into the ovary in the ears of cereals (most frequently rye and millet) grow into a mycelium and, instead of the grain, produce a scler-

rotium (ergot), from which a stroma is formed, inside which the sexual process takes place, asci appear in special fruit bodies - perithecia, containing 8 filamentous ascospores each. When released the latter are disseminated and get onto a substrate where they grow; the cycle is resumed. The sclerotium stage is toxic, since they contain toxins - derivatives of lysergic acid and clavines. They parasitize on many varieties of cultivated and wild-growing cereals (particularly pasture grasses). The number of sclerotia in a single inflorescence varies from 20 to 70, depending on the weather and degree of toxicity.

It is distributed in Europe (apart from the northern areas of the Scandinavian Peninsula and the Far North of the European part of the USSR), in Asia and North America, some areas of Australia and South America. At present it is especially hazardous when affecting fodder grasses.

The greatest number of toxinogenic fungi belong to the Deuteromycetes class (imperfect fungi), which are characterized by the absence of the typical sexual process and reproduce mainly by asexual reproductive spores - conidia. The conidiophore apparatus of the majority of fungi in this class consists of more or less specialized mycelian branches - conidiphores, on which numerous conidia are formed by different spores, which facilitates the considerable dissemination of these fungi on various food and fodder substrates.

The fungi of this class usually have a well-developed separate aerial and underground mycelium. The total number of species in this class comes to 15,000. Many of them are pathogens of diseases in plants, men and animals, and are also of importance in nature and practice. We follow with a description of some

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species of toxmogenic deuteromycetes.

Genus Penicillium Lin

The mycelium is colourless or lightly tinted, sometimes darkening with age. The conidiophores are colourless, usually with transverse septa, upright or rising, deflecting from the hyphae of the substrate or aerial mycelium. Branching at the apex or only at the top, forming a raceme. The racemes differ according to the type of branching: symmetrical or asymmetrical. One-tier racemes (single-verticillate) consist of one sterigmatic verticil, situated at the apex of a conidiophore, which sometimes slightly expands right under the verticil. Two-tier racemes (double-verticillate) consist of sterigmatic verticils situated on the cylindrical branches - metulas, also arranged in a verticil at the top of the conidiophore stalk.

Asymmetric racemules are those in which the metulas' verticils are arranged at the apices of asymmetrically rising branches of the conidiophore (usually one central and lateral). The one-cell conidia are formed in basipetal chains, mostly more or less spherical, more seldom ovate or elliptical, smooth, sometimes rough or verrucous, in mass more or less lightly tinted, seldom white. Some species are known to have ascogenous ones in the form of small cleistothecia, in a somewhat greater number - sclerotial.

The species of this genus are widespread in nature, usually on foodstuffs, raw fodder and other substrates.

Penicillium Urticae Bainier

Colonies with limited growth on Czapek's agar reaching 2-2.5 cm in diameter on the 12th-14th day at room temperature,

radially furrowed; in most strains with a scalloped margin and the central zone frequently greatly elevated, 0.5 to 1 mm thick in the marginal zone, up to 2-3 mm in the center, with a clearly granular surface; most strains have clearout coremia, at least in the marginal zone, with copious and continuous production of spores, greyish-green in colour; in some strains the spore production is less copious (in part downy), pale-bluish-grey. The colonies of strains with an abundant spore-production are dark green with greenish-grey shades. Their reverse side is at first dull-yellow and then from orange-brown to red-brown; the agar is tinted around the margins of the colonies; no exudate is excreted, but if it does form - then in large quantities, light, immersed in the colony; in some strains there is a strong aromatic smell, others are odourless. Conidiophores are arranged partially in coremia, partially singly, tortuous and smooth. The racemules are slightly spread apart, branching, with elements bearing conidia rising from different nodules, with branchlets, spread apart, with secondary branchlets from the metula, usually in groups of two to four; the sterigmata are short, crowded in verticils, usually 8-10 in each; the conidia are elliptical or spherical, with a thin, smooth membrane, in columellar more or less spread apart. They produce the toxin patulin and invade many foodstuffs and fodder.

Genus Aspergillus Mich

The mycelium is white, lightly tinted in some cases slightly brown, occasionally forming spherical or hemispherical sclerotia made up of thick-walled cells. The conidiophores are simple, the apex with a conical or pyriform, hemispherical or spherical swelling bearing sterigmata. The sterigmata are arranged radial-

ly to the surface of the swelling (in its upper part) and rise parallel to the axis of the conidiophore, simple or in two tiers, i.e., forming on the apex of the basal sterigma two or several sterigmata of the second tier. The conidia are unicellular, in simple, not branching chains on the apex of each sterigma, making up the radial head, or adhering in the shape of a column; spherical, ovate or elliptical, smooth, rough or echinulate, colourless or differently coloured. The cleistothecia are thin-walled, usually lightly tinted, forming rounded or elliptical asci with ascospores (known in a few species only). The ascospores are more or less disc-shaped, usually with a more or less distinct furrow, or also surrounded by crests or a membrane.

Aspergillus Fumigatus Fres

Colonies on Czapek's agar spread widely over the substrate; their appearance varies from velvety to deeply fasciculate or loosely fluffy; at first white, as conidial heads form, they turn green, but with time the tint changes to lily-green or even beaver-grey; the reverse and substrate in some strains are colourless, in others they are tinted yellow, green or even brown-red. Conidial heads in the colonies are compact, frequently densely packed. In different strains their sizes vary from 400 to 500 microns, but usually they are much shorter, sometimes very small. Conidiophores are short, smooth, up to 300 microns long, usually more or less green, particularly in the upper part; they grow directly from immersed hyphae or as short branching aerial hyphae. They gradually elongate, changing into apical flask-like swellings 20-30 microns in diameter (frequently of the same colour as the conidiophores). They are usually

spore producing only in the upper half; the sterigmata are arranged in a single row, tinted, packed, with axes not strictly parallel to the axis of the conidiophore. In their mass the conidia are green, echinulate, rounded to hemispherical, usually 2.5-3 microns in diameter.

Grows well at temperatures up to 45-55°C, is one of the most common microorganisms growing in composts and other organic materials and reproducing at high temperatures.

Aspergillus Flavus Link

Colonies on Czapek's agar are distinguished for speedy growth, reaching from 3 to 6-7 centimeters in diameter on the 10th day. The mycelium is usually fine but densely entwined, which in some strains forms an immersed margin (1-1.5 cm); radial or cerebriform colonies occur.

Spore production in most strains is copious, rising right from the mycelium; young conidial heads frequently have yellow shades changing with age into dark yellow-green. The reverse of the colonies is colourless or pinkish-dull-yellowish; the reverse of strains with copious sclerotia formation is dark red to brown. Exudates are usually not conspicuous, but in isolates producing sclerotia they are red-brown. There is no odour, but, if any, it is unpleasant. Many strains occasionally produce sclerotia, particularly in fresh isolates, the predominant type of colony is variable in shape, size and colour; mostly circular or semicircular, from white, red-brown to black, usually about 400-700 microns, more seldom up to one mm in diameter. The conidiophores (conidial heads) are typically radial, indistinctly divided into columns, up to 500-600 microns in diameter, mostly 300-400 microns, with small heads (from 50 to 300 microns); co-

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nidiophores have a dense membrane, are colourless, very rough, usually shorter than 1 mm, but in some strains (particularly, in cultures kept for long under laboratory conditions) - from 2.0 to 2.5 mm long, immediately under the swelling - within 10 microns; the swellings, elongated in young cultures, later semi-circular or circular, are 10-65 microns in diameter, mostly 25-45 microns; sterigmata are arranged in one or two rows on the normal swellings; one head seldom contains sterigmata of both types. Single-tier sterigmata, as a rule, are formed on small swellings; the conidia-producing apices are usually vesicular; conidia of this type are circular or semicircular, distinctly echinulate, varying from 3.0 to 6.0 microns in diameter; they produce aflatoxin.

Genus Stachybotrys Corda

Ramified conidiophores, brown or nearly colourless, with cylindrical or clavate sterigmata at the apex, fused at the base, with verticillate arrangement. The conidia are unicellular, spherical or elongated, smooth or echinulate-verrucose, dark coloured. The mycelium is dark-coloured.

SAPROPHYTES

Stachybotrys Alternans Bon

At first the mycelium is pale-olive, sometimes nearly colourless, later olive-brown. The spore-producing hyphae for the most part have sympodial branching. The conidiophores are pale-olive, then olive-brown, darker at the top, 40-90 x 3.5-5 microns, at the apex with a bundle of sterigmata. The sterigmata appear 5-8 (9), seven in a bundle, elongated-obovate, 10-12 x 4-5.5 microns, fused at the base. The conidia are at first pale-

olive, smooth, later darkening, finely-echinulate-verrucose, then verrucose, elongated-ovate-elliptical or nearly cylindrical, 6.9-14 (17) x 3-7.7, or usually 7.7-11.5 x 4.5-7.2 microns. Mature conidia are black, opaque, torulose, globose-elliptical or globose. Each sterigma forms 3-7 (10) conidia gathered in a head on a sterigmatal bundle. Develops on cellulose-rich vegetable substrates, in soil, on straw, moist paper. Occurs in Western Europe and in the European part of the USSR.

Genus Dendrodochium Bon

The sporodichia are cushion-shaped or verrucose, of different size and shape, white or lightly tinted, without setae. The conidiophores are with nearly verticillate arrangement, or have trimerous branching, colourless, densely packed, forming a continuous layer. The conidia are terminal, ovate or elongated, colourless or lightly tinted, unicellular.

Dendrodochium Toxicum Fidoplet Bil

The mycelium is white, its hyphae 1-4 microns thick. The sporodochia are more or less globose or irregular, superficial, mostly 0.2-1 mm in diameter, at first with a white, downy mycelial margin, with olive-black or black layer of conidia, which become glistening upon drying up, and sometimes fuse. The conidiophores are densely packed on the plectenchymatous network of hyphae with an irregular or dendriform branching, up to 3 microns thick, with terminal branches, 12-40 x 1.5-2 microns, usually in verticillate arrangement. The conidia are elongated-elliptical, acuminate at both tips, 6.5-8 x 2.75-3.5 microns, pale-greenish, nearly colourless, in mass - dark-green or dark-olive-green.

Optimal growth temperature is around 25°C, but a rather intensive growth occurs also within the range of 7 to 35°C. Intensively destroys cellulose. On a wort agar the first to develop is an abundant white mycelium on which a great number of sporodochia soon make their appearance. Great quantities of the latter are also formed on moist straw or chaff, but this requires sufficient humidity and a temperature close to optimal; at lower temperatures and lower straw humidity it develops in the mycelian stage only.

Genus Fusarium Link

Predominant are fusiform-falcate, fusiform, less frequent - fusiform-lanceolate microconidia, tapering towards both tips, with a more or less distinct stalk or papilla at the base, more seldom devoid of a stalk, with a terminal cell (short, conical, key-shaped, sometimes with a more or less rounded or elongated, gradually or abruptly narrowing, occasionally filamentous, usually with 3-5, more seldom with a greater or lesser number of septa). They develop in an aerial mycelium on more or less differentiated simple or ramified conidiophores, sometimes on serrated hyphal processes, not infrequently as a continuous layer on the stroma in the sporodochia or in the pionnotes - accumulations of mucus on the hyphal networks, or immediately on the substrate; in mass - they are of varied light colouring.

Small conidia (microconidia) are formed in the mycelium singly, in false heads or chains, on more or less distinct, simple or ramified conidiophores; oval, ovate, elongated, more seldom nearly spherical (the latter are frequently observed at an older age), pyriform, clavate, fusiform, one-two-cell in mass;

species are usually more abundant than macroconidia.

In addition to the typical microconidia, the cultures of some species, along with macroconidia, give rise to small conidia of a transitional type with three septa.

Chlamydospores in the hyphae appear single, in chains or nodules, intercalary or terminal, sometimes also in macroconidia; colourless or tinted different shades of yellow-brown.

On culture media the mycelium is white, white-pinkish, red, light-cream, straw-yellow, greyish-lilac-purple or brownish. The stroma is mainly of an ochre, pinkish, blood-red or light-cream colour; straw-brown, carmine-pink, wine-purple, brown, more seldom blue-black or untinted. In some cases white, yellow, brown or blue sclerotia are produced.

Fusarium Sporotrichiella Bil

Microconidia are formed in an aerial mycelium, more seldom in the sporodochia and pionnotes; fusiform-falcate, with gradually tapering non-elongated terminal cell and a more or less distinct stalk, sometimes in the shape of a papilla; they appear in the sporodochia usually with septa, 26-48 x 3.8-5 microns; those produced in the aerial mycelium usually have three septa, 17-28 x 2.8-4.5 microns.

The microconidia are pyriform-lemon-shaped (3.8-12.5 x 3.8-6.6 microns) or clavate (9.5-15 x 3.8-6.5 microns). Produced on simple or ramified conidiophores; singly or in small chains; upon ageing the cultures assume a more or less spherical shape. Oval-cylindrical microconidia, unicellular or with a single septum, 5.7-17 x 2-3.5 microns. The quantitative ratio of conidial types in different forms is not the same. The aerial mycelium is fast-growing, tall; upon spore production it becomes powdery.

white, white-pinkish or red in colour. The stroma on a dextrose-potato or wort agar is blood-red, ochre-yellow-brownish of different shades, more seldom colourless. Chlamydo-spores are produced in the substrate mycelium and macroconidia - upon the culture ageing.

Develop on different plants, in the cariopsis of cereals, fruits, vegetables, some insects and fungi in the soil.

It is ubiquitous.

In the process of metabolism they produce substances which are toxic to animals and plant organisms. Some forms are pathogenic to plants. The grains of cereals affected by this fungus which happened to be used for food have caused the disease called alimentary toxic aleukia.

Fusarium Graminearum Schwabe

Macroconidia in sporodochia, pionnotes, in the aerial mycelium, elliptically curved, with a gradually and uniformly narrowing, conical, somewhat elongated apical cell, with a distinct stalk at the base, usually with five septa; in mass it is whitish-pink, golden-yellow, carmine-purple, with three septa - 25-66 x 3-6 microns, with five septa - 35-75 x 2.6 microns, with six septa - 50-75 x 4-6 microns. The aerial mycelium is well developed, fluffy, floccose-downy, white, white-pink, blood-red. The stroma is red-ochre-yellow, dark-green, ochre-olive. The sclerotia are pink to dark-red, not infrequently absent. The chlamydo-spores in the mycelium are not copious, intercalary, frequently absent.

Occurs mostly as a parasite of cereals on the caryopses ("drunken bread"), ears, stalks, roots, and also on some wild-growing cereals, sometimes on plants belonging to other families,

and in soil. Encountered in all parts of the world. Produces toxins with an estrogenic effect.

The ecological aspects of mycotoxicoses were studied by many authors in our country and abroad. In this connection one should primarily point to the competitive capacity of a number of toxinogenic fungi to grow and produce toxic metabolites at low or higher temperatures. *Fusarium sporotrichiella*, which has demonstrated a higher toxin content when cultivated at temperatures of 12-18°C as compared to 26-34°C can serve as an example. On the other hand, in case of toxinogenic species of the genus *Aspergillus*, for instance, *A. flavus* preeminently affecting fodders in the southern zones, it is advisable to study the toxin-producing processes by cultivating them at higher temperatures as well. The geographic distribution of toxinogenic fungal species is largely associated with the distribution of the vegetable substrates affected. The substrate-related occurrence of toxinogenic species is not narrow, yet it can be pointed out that, for example, *Stachybotrys alternans* *Dendrodochium toxicum* preeminently affects coarse fodders (straw, cereals, hay), *Fusarium sporotrichiella* - grain cereals, particularly when stored under unfavourable conditions, *Aspergillus flavus* - cereal crops, whereas in the regions where groundnuts are cultivated *A. fumigatus* affects all kinds of combined fodders, particularly at increased temperatures of cultivation, though these and other species are capable of growing on other substrates as well.

Being organisms devoid of chlorophyll, fungi may use for carbon nutrition most diverse, at times rather complex, compounds, not infrequently causing specific transformations in their molecules. According to late literature data, some fungal species

use as sources of nutrition carbonic compounds which make up part of virtually all classes of organic substances: carbohydrates, alcohols, sugars and organic acids, proteins, amino acids, peptides, aromatic compounds, glucosides, hydrocarbons, etc. Sources of nitrogen nutrition may be proteins, peptides, amino acids, nitrate, and in some cases nitrite or ammoniacal nitrogen (in the form of different ammonium salts), as well as ammonia. There are reports that some fungal species are capable of using elemental nitrogen. No less extensive is the amplitude of mycellial fungi adaptability to other environmental factors, most important of which are humidity, temperature and availability of oxygen.

Needing some ready organic substance, the fungi find it in the living plants and animals, parasitizing on them or developing in diverse organic substances of animal or vegetable origin, including food products and foodstuffs.

The sources of the mass dissemination of toxinogenic fungi, causing flare-ups of diseases among human or farm-animal populations, are the substrates they affect, foodstuffs and raw fodder. A considerable part of microscopic fungi, at times including toxinogenic ones, are found on the surface of vegetating plants, dry remnants of weeds or wild-growing plants, where they make up the complex of so-called "epiphytic" microflora. Typical representatives of "epiphytic" microflora damage plants and grain not during vegetation, but in the period of harvesting and particularly during storage at increased humidity.

Of critical importance for the contamination of grain and coarse fodders is their moisture content. Understood as critical humidity is such a moisture content in a substrate, when

free water appears, steeply enhancing the activity of oxidative and hydrolytic enzymes and creating conditions for grain invasion by microorganisms. Maximum critical humidity values for the grain of wheat, rice and barley - 14,5-15%; for the grain of maize and millet - 3-14 and 12-13% respectively; for low-oil sunflower seeds - 10-11%, high-oil seeds 6-9% and flax seeds - 8-9%. Equilibrium humidity is created as a result of sorption by the seeds of water vapour under conditions of increased relative air humidity (80-90% and higher).

As regards temperature, fungi are divided into the following groups:

1. Mesophilic, growing with in the range of 3 to 37-38°C, the optimal temperature being 18-27°C. This group includes the predominant number of fungi species affecting grain and coarse fodders. Some of them, though slowly, may grow even at -3 and 5°C. The optimal temperature for other fungi is 16-18°C and, when the cereals and the straw are harvested late, they are affected in considerable degree.

2. Thermotolerant, capable of growing at temperatures of 40 to 50°C and even higher, but also well growing at temperatures suitable for mesophilic fungi; the optimal temperature for their growth is usually higher than that of the latter, being in the overwhelming majority of cases above 30°C.

3. Thermiphilic fungi, unable to grow at a temperature which is optimal for mesophilic fungi, and growing at temperatures of 40-60 and up to 80°C; the optimal temperature for their growth is about 40-45°C. The group is the least numerous.

Grain and fodder humidity values are closely correlated with temperature. Norms and regulations for lasting and short-term

storage of grain are drawn up, as a rule, with account of humidity and temperature conditions.

In connection with these or other climatic features the problem arises of grain storage and its protection against moulding. In zones with cold and moist harvesting periods the main concern is the drying of the harvested grain, in zones with a warm and dry harvesting period priority goes to the problem of airing and the prevention of self-warming of grain.

The degree of the grain's maturity, of its intactness the composition of the motley grasses in hay, the presence of weeds in straw also affects the vulnerability to fungi, including toxicogenic ones. It has been established, for example, that small and feeble grains withhold more moisture and are more susceptible to fungal attack than large and well-filled grains. The grain with damaged hull is less resistant to fungal penetration and affection.

The humidity range within which spore production takes place is usually much narrower than that which is conducive to mycelium growth.

The most optimal relative air humidity for the majority of fungal species is within 90-100 and usually approaches 100%. However, a number of xerophilic species may grow and produce spores at a much lower air humidity - 80 and even 75%.

It may be assumed that the most dangerous storage periods for grain and coarse fodder with increased humidity are the seasons when ambient air temperature rises above 0°C, i.e., in summer and autumn. During harvesting of bread cereals, the temperatures are so favourable for the development of fungi that at an increased humidity they might succumb to considerable fun

gal contamination within the matter of a few days, which not only would considerably reduce the quality of these products, but could impart toxic properties to them. In the lower temperatures of winter and spring, the processes of fungal invasion of fodders with higher humidity are naturally slowed down as compared to the summer months, but their intensity is still high. One should also point out the competitive capacity of a number of toxinogenic fungi to grow and produce toxic metabolites at lower temperatures. This is true, first of all, of *Fusarium sporotrichiella*, which has demonstrated a higher toxin content when cultivated at the temperature of 12 to 18°C, as compared to that of 26-24°C. On the other hand, in the case of toxinogenic fungi of the genus *Aspergillus* (for example, *A. Flavus*), which preeminently affects fodder grains in the more southern zones, it is advisable to study the processes of toxin production during their cultivation at higher temperatures as well. The geographic distribution of toxinogenic fungal species is probably connected to a certain extent with the distribution of the plants subject to affection, rather than with the climatic features of the geographic zones concerned. The substrate-related properties of toxinogenic fungal species are not narrow, yet one may, nevertheless, indicate that, for example, *Stachibotrys alternans*, *Dendrodochium toxicum* predominantly affect coarse fodders (straw, cereals, hay), *Fusarium sporotrichiella* - the grain of food cereals, particularly when stored under unfavourable conditions, *Aspergillus flavus* - the grain and beam, cultures and in the localities of its cultivation - groundnuts, *Aspergillus fumigatus* - various kinds of combined fodders, particularly at increased temperatures of cultivation, though these and other species

are capable of growing on other substrates as well.

A characteristic property of toxinogenic fungi is the formation during the metabolic process of a complex close in chemical structure to compounds possessing different degrees of toxicity and producing a pathologic effect.

One of the substantial mycological aspects of fungal toxicoses is the need for accurate characterization of the isolates of toxinogenic fungal species not only by the degree of their toxicity (atoxic, mildly toxic, toxic, very toxic), but also by the toxic components, both in natural isolates, and under definite conditions of cultivation in different ecogeographical zones. At present these data are lacking. Meanwhile, they could be used to explain a number of matters connected with processes of toxic race formation within the limits of a species, the epidemiology of mycotoxicoses, the manifestations of their diverse pathologic effects, etc.

When studying toxinogenic processes in natural isolates during the natural invasion of fodders or foodstuffs and during their cultivation, the establishment of the composition of the intraspecific population according to their pathogenic properties may also provide valuable data not only concerning purely mycological, but also epidemiological lines.

Closely linked with the ecology of toxinogenic fungi are problems of toxin transformations in the natural habitats and under competitive experimental conditions. As mentioned above, during the cultivation of many species of toxinogenic fungi, with the advance of time, there is either a considerable reduction in the content of intra- and extracellular toxins or they become undetectable. In this case they undergo metabolic inactivation

by the enzymatic systems of the producer fungus. Revealing these enzymatic systems, and of the mechanism of transforming toxins into atoxic metabolites is not only of considerable theoretical interest, but may prove of practical importance. Also known are some cultivating conditions for toxinogenic fungal species which are not accompanied by the biosynthesis of mycotoxins. This, however, will be the subject of special lectures.

REFERENCES

1. Bilai V.I., Pidoplichko N.M. Toksinoobrazuyushchiye mikro-skopicheskiye griby i vyzyvayemye imi zabolevaniya cheloveka i zhivotnykh (Toxinogenic microscopic fungi and diseases of man and animals caused by them). Naukova dumka Publishers, Kiev, 1970, pp. 290 (in Russian).
2. Bilai V.I. Osnovy mikologii (Principles of mycology). Viahcha Shkola Publishers, Kiev, 1980, pp. 346. Experimental Mycology (in Russian).
3. Moreau C.L. Larousse des champignons. Paris. Librairie Larousse, 1978.
4. Barnett J.H. Fundamentals of Mycology, Ind., Arnold Ind., 1968.