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

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THE ECOLOGY OF FUNGI — PRODUCERS OF MYCOTOXINS

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Fungi are omnipresent organisms. There is no media where they are not present. Fungi have broad adaptive properties and therefore occupy most varied ecological niches which are most frequently inaccessible for other microorganisms.

They are present in the environment in the form of diaspores, i.e. germs. The function of the latter can be performed by special spores, bits of a vegetative body -- mycelium or pieces of the fruit body which are cells specialized for multiplication. Fungi can be isolated from air, soil, water, plants, from different industrial materials and commodities, from any objects in nature or in a premise. In one case fungi find conditions and develop on these substrates. In other cases they make use of the substrate as a support and develop only after being carried by the air, by water or animals to objects which are favourable for their life.

By their mode of existence fungi are either parasitic or saprotrophic. The former feed on live organisms. The latter feed on dead organic material. There are also transitional forms. Both of them may be obligatory and in this case cannot develop in one or another type of nutrition or they may be facultative, capable, under definite favourable conditions, to pass also to another type of nutrition.

Parasites, usually are more or less narrowly specialized. As for saprotrophs, they are characteristic of a relatively broad specialization. The saprotrophs include a good number of polyphages which developed on most differing substrates and cosmopolites which occur in all or many continents. Some polyphages are concurrently cosmopolites (Aspergillus niger, A. flavus, A. fumigatus, Alternaria tenuis and others).

Parasitic fungi are rather poorly studied as regards their toxicogenicity. This is associated with the fact that parasites, specifically obligatory ones, can hardly be grown on artificial medium outside of the organism which feeds the fungus. Meanwhile, we are aware that for toxicological analysis we need a definite biomass of the fungus. It should be also noted that a number of parasitic phytotrophic (those which develop on plants) species of fungi have already shown their phytotoxicity.

All fungi are microscopic organisms. However, a small part of them, in the cycle of development, forms macroscopic fruit bodies which range from several mm to several tens of cm. For instance, fruit bodies of tinder fungi (Polyporales), hymenomycets -Agaricales. Such wellknown toxic fungi as the pathogens of argot form, in the cycle of development, large, reaching up to 15-20 cm horn-like sclerocia which contain a number of toxins.

The most wide spread in nature are soprotrophic fungi which comprise 3/4 of all known fungi. The first place among them is held by the fungi which cause the molding of the substrate. They are called mold fungi, while still more correct is to call them mold-forming fungi. Asexual multiplication predominates in the majority of them in their development cycle. Many species occur but in an asexual gaploid state and therefore they are classed with Deuteromycetes (the division of the Deuteromycotina).

Toxicity has been identified for the majority of these fungi while the rest may be viewed as potential producers of mycotoxins. This conclusion is based on the fact that the toxigenic property of fungi is still poorly studied and every new investigation adds to the list of toxic fungi. Moreover, as it has been shown by our and other studies the toxicogenic property is variable and depends on a variety of ecological factors and their combinations.

Conditions of development of toxigenic fungi

The growth and physiological activity of fungi is decisively influenced by many environmental factors. Particularly important among them are humidity, temperature and the concentration of oxygen and carbonic acid. Every species of a fungus is a unique system and therefore the response of different species to one and the same factors of the environment differs.

As regards the attitude to temperature fungi are subdivided into: a) psychrophilic, which grow in the range of 3 to 10°C (and psychrotolerant -- which grow even with lower temperatures); b) mesophilic -- which grow in the range of a comparatively moderate temperature -- 10-38°C; c) thermophilic -- growing in the range of 10-50° and even higher. Thermophilic fungi (T.I. Bilai, 1979) may be subdivided into: obligatory (true) thermophils which grow well under 45-55°C and at a maximum temperature reaching 65° but which do not grow at a temperature below 20-25°C;
4064 facultative thermophils are characteristic of an optimum growth at a temperature of 40-45°C, maximum 65°C, but they also can grow at a temperature under 20°C.

The minimum, maximum and optimum temperatures differ for growth, sporulation and physiological activity of fungi. For instance, the pathogen of "snow mold" - Fusarium nivale grows better at $0^{\circ}C$, while for the growth of another species of the same fungus, F. sporotrichiella, the temperature should be $18-24^{\circ}C$. However, optimum conditions for toxinformation are developed when they are cultivated alternatwely in one or another temperature regimen.

Psych hilic fungi include a number of species belonging to the genera of Penicillium and Fusarium. These are the species and isolates which are capable of developing on food products during their storage in refrigerators. Besides this psychrophils include some species of Cladosporium and mucoral fungi.

The majority of mold-forming fungi belong to mesophilic representatives. They include many species, isolates from the genera of Penicillium, Fusarium, Oospora, Cladosporium, Trichothecium, Botrytis and mucoral fungi. All Aspergillus genera are relatively more thermophilic. They include a good number of thermophilic fungi, specifically those which are facultative.

The majority of food products, during their production or further processing, are subjected to high temperatures and at that time fungi which contaminate raw materials and products, perish. However, some of them retain viability and shortly afterwards induce the formation of a mold on a product. Those are thermophilic species, isolates.

Thermophilic fungi occur also in soil, specifically so in its surface layer, on vegetative substrates, particularly so in self-heating, like hay, straw, grain. Therefrom being transported by air they can reach food products.

Apsergillus fumigatus is the most typical facultative thermophil. As far back as in 1889 Cohn demonstrated that self-warming of moist grain of barley is caused by the development of A. fumigatus in it. The temperature of the grain mass reaches 60-70°C in the bulk of grain. Subsequently it was found that a number of other fungi is engaged in the process. The composition of these fungi varies with temperature. Thus the prevailing species at a temperature of 25-30° are Penicillium, Rhizopus, Fusarium, and at a temperature of 35-40°C and higher -- A. fumigatus, A. candidus and other species of this genus. According to Pidoplichko (1953) the temperature maximum for A. fumigatus is 57°C. The facultative thermophilic fungi which inhabit soil include also A. niger, A. terreus, A. flavus, A. niveus, A. carneus, A. nidulans for which the minimum temperature is 11°C, the maximum 52°C and the optimum is higher than 40°, whereas for Penicillium these indicators are 17, 47, 38-40°C, respectively. A considerable part of true and facultative thermophilic species and isolates are isolated from soils of southern regions.

To isolate thermophilic fungi from food products one has to cultivate them at higher temperatures, approximately reaching 40-50°C.

In our studies thermophilic fungi were isolated from canned products including juices, jams, tomatos. A. versicolor and A. nidulans have been repeatedly isolated from butter and later on
4064 from the products of its rendering and pot butter. This process, in keeping with the technology of the production, occurs at a temperature of 80°C.

The humidity of products and the relative humidity of air in the field or in a storage place is decisive for affecting food products with fungi. In case of increased humidity, conditions develop for the growth of microorganisms, fungi primarily, in products. The critical limit of humidity for the grain of wheat, rice and barley is 14.5-15.5%, and it is 3-14 and 12-13% for the grain of maize and millet, respectively; it is 10-11 for sunflower seed of low oil percentage and 6-9% for sunflower seed of high oil percentage, it is 8-9% for flax (Bilai, 1970).

A combination of high humidity and high temperature facilitates the damage of food products by mold forming fungi. Under these conditions we see the development of Aspergillus, dark coloured hyphomycets -- Alternaria, Stemphylium. The growth of fungi under the same humidity but lower temperatures is considerably inhibited. The prevailing contaminants in this case are the species of Penicillium, Fusarium and Mucor.

The species composition of contaminants of a product from one and the same area can vary subject to the season and weather conditions during harvesting or during industrial processing. Therefore, prior to placing vegetative food products for storage, grain specifically that was harvested during humid weather the grain should be dried.

Fungi are known as aerobic organisms. However, among them, specifically among mold forming fungi are many such which are capable of growing and sporulation in conditions of low oxygen content. For instance, this is the case in natural conditions when a fungus penetrating stoma of plants or through mechanical damages develops lavish sporulation in tissues or in internal cavities of stalks, tubers, root crops and fruit. Species of Alternaria frequently damage the pulp of oranges; species of Cladosporium and Penicillium are found in fruits of apples, in cavities where seeds are formed. P. resticulosum, which is of low toxicity, is a typical contaminant of fruits of pomegranates within which it produces abundant sporulation. When socculant raspberry, dewberry and other socculant fruit like seabuckthorn are kept in a deep container (a bucket, a casserole) for two-three days under room temperature sporulation of Penicillium species begins in the deep layers. During this process fruit which are in the surface layers remain without mold for some time. The development of mold forming fungi is observed in the bulk of such products as butter, cheese etc.

The facts of mold development in preserves also attest to the ability of fungi-contaminants to develop in relative unaerobic media.

When fungi grow in conditions of oxygen insufficiency there may be morphological and physiological variability. In such cases mycelium disintegrates into individual cells and becomes yeast like. Sporulation is either inhibited or, to the contrary, is intensified. Increased content of carbonic acid stimulates sporulation.

An important ecological factor influencing the choice of a fungal substrate is pH of the medium. Its level is decisive
4064 for the growth and the development of a fungus, the activity of enzymes, the development of pigments, vitamins, antibiotics, toxins and the organs of reproduction. The most favourable media

for the majority of fungi is weakly acid with a pH of 5.0-6.0. Some fungi can grow on substrates with a wide range of pH, for instance, Penicillium variable (pH = 1.6-11.1), Fusarium bullatum (pH = 2.2-11.2). The species of Penicillium which affect citrus fruit are capable of growing at very acid pH reaching 3.0 and even less than that (Bekker, 1963). There are fungi which prefer an alkali medium, for instance, Aspergillus clavatus grows and sporulates at pH = 13.0 (Bilai, 1980).

Fungi are capable of altering the acidity of the medium during their growth. For instance, developing in a sugary medium Aspergillus niger accumulates citric acid in it, Penicillium vitale -- gluconic acid.

Fungi react differently to osmotic pressure. There are osmophilic fungi which are capable of growing on media with a high osmotic pressure. Such fungi grow, for instance, in jams, syrups, sweet dry fruit which contain high concentrations of sugar (Torula saccharina, Trichothecium roseum, Catenularia pidoplichzkoi). Osmotolerant species do not grow on media with a high osmotic pressure but do not lose their viability in that medium.

There are fungi which live in media with a high concentration of common salt or other salts. These are fungi of salty soils, salted vegetables (Aspergillus niger, some species of Penicillium, Oospora lactis).

Habitat of toxigenic fungi

Soil is the main reservation of the majority of microscopic and macroscopic fungi. Fungi inhabit soil in the form of sporulation, structures at rest and active mycelium. Their role in soil is considerable and varied.

There are from several thousands to several hundreds of thousands fungi germs in one gram of soil. However, the number of toxin forming microscopic species in soil is much lower. While in soil, they, most likely, do not represent any immediate danger for food products and foods. However, it is soil that becomes the main source of the spread of toxigenic fungi which contaminate raw materials, food products, feeds and which induce diseases in man. Practically all species of toxic contaminants of food products are initially soil fungi.

The content of saprotrophic microscopic fungi of soils depends upon the type of soil and considerably upon vegetation and the organic residues in soil. It undergoes seasonal changes during which there takes place the change of fungal associations.

Typical soil fungi are the majority of species of *Penicillium*, *Aspergillus*, *Trichoderma*, *Fusarium*, *Cladosporium*, *Mucor*, *Rhizopus* etc. Many of them are known as toxic fungi.

Species of *Penicillium* prevail in soils of all ecologo-geographical regions but they favour soils of temperate or cold latitudes. The species of *Aspergillus* favour warm soils of southern regions.

The microflora of cultivated soils and soils with varied vegetative cover is particularly rich. Soils devoid of vegetation contain a meagre and uniform composition of fungi species.

For some of the fungi soil performs the function of natural habitat where they are active -- where they grow, multiply, participate in the transformation of organic substances. Others reach the soil with vegetative or animal remnants, with water, with dust particles. They stay in soil in an inactive state and continue their development in the presence of favourable medium -- in the presence of vegetative, animal or other organisms.

The second habitat for fungi, just as for those which produce mycotoxins, are plants. Parasitic species prefer vegetating plants. Their mycotoxicological properties, with rare exceptions are not studied since many parasites, obligatory ones specifically, are not cultivated in artificial conditions or the isolation of their pure cultures is extremely difficult. The better known among them is the fungus Claviceps purpurea -- the pathogen of grain argot. Its sclerotia produce ergotoxin and a number of other toxins.

Plants are an excellent substrate for the epiphytic and saprophytic mycoflora. Epiphytic fungi only settle on the surface of plants during vegetation without damaging them. However, during harvesting and storage of seeds, fruit and other food and feed parts of plants the spores of these fungi can grow and if there are any mechanical damages and sufficient humidity they can induce the spoilage of products. Saprotrophic fungi develop on vegetative remnants: twigs, wood, fallen leaves, dry remnants of grassy plants, and during storage -- on fruit, rootcrop, tubers.

The conditions for the development of fungi are particularly favourable during the storage of forage. Molded forage or feeds are the most important link in the alimentary chain which afford the possibility for the penetration of mycotoxins into food products of man.

Dozens of different species of fungi may develop on vegetative feeds and food product. Some of them are already known as producers of toxins while others, the unknown part, should be regarded as potential producers of mycotoxins.

This conclusion may be drawn on the basis of the findings of studies conducted by us at the Erevan University. We have examined large numbers of fungi-contaminants of numerous food products, including those of vegetative origin in fresh and processed condition, of home and industrial production. 300 types of contaminants have been identified. 74% of those tested for toxigenicity have proved positive. Table 1, 2 (data for Armenia).

This information confirms that vegetative medium is most favourable for the development of mold forming fungi in general and for toxin forming fungi, in particular. An acid medium of vegetative organisms is not favourable for the development of bacteria.

At the same time bacteria are known to develop well in an animal organism increasing its alkaline reaction. Under these conditions fungi are poor rivals of bacteria and therefore they are found in smaller numbers on animal substrates compared to vegetative substrates. This can be confirmed by the results of our studies.

Approximately 250 species of fungi have been isolated from different groups of fresh vegetative food products. At the same time only 36 species have been isolated from meat and meat products.

The current view is that food products of animal origin are also poorly contaminated with fungi. Our studies of many years on a large number of varied processed food products of animal origin have indicated that many of them -- butter, pot butter, cheeses, sour milk, dehydrated milk, cottage cheese provide a favourable medium for molding which in most cases is caused by toxigenic fungi. The risk of molding increases with an increase in the fat content of a product. As a rule, in this medium several species develop while the genera of Penicillium and Aspergillus predominate.

A comparative assessment of the content of fungi which contaminate food products of vegetative and animal origin reveals that there is a community of saprotrophic genera of fungi in both groups of products. However, on the level of species this community decreases which indicates a certain confinement of species of fungi to specific products.

The species composition of fungi which develop on fresh vegetables and fruit during storage is particularly varied. This is due to the fact that besides saprotrophic fungi of "storage" the species which are characteristic of the vegetation period -- "field" fungi which belong to parasites continue to develop on them.

Several species are specialized for vegetative products and do not occur in meat and dairy products like, for instance, Botrytis and Trichothecium.

Most harmful and widespread contaminants of food products are the numerous species of Penicillium and Aspergillus.

There are not many species of fungi which affect processed vegetative food products.

Processed animal products with an admixture of plants like cheese or cured meat are particularly affected by molding.

The edible pileate (hymenomycetes) mushrooms are regarded as a favoured food by many nations. Their fresh fruit bodies, even while in the field, are affected by microscopic moldforming fungi which are known as mycotrophs or mycophyls. Thus, the fruit bodies of fungi should also be viewed as a habitat of fungi-contaminants of food products while the toxigenic property of the latter should be an object of specific studies.

The least studied habitat of fungi are water basins and water streams. In recent years fungi have attracted the attention of mycologists. This is a varied and at the same time specific ecological group of organisms with a broad range of adaptation to growth and development in different types of water bodies. They inhabit fresh and seawater, water surface and, to the contrary, the bottom layers. The water mold varies by the mode of nutrition. They include obligatory parasites, saprotrophs and facultative species. They parasitise on algae, invertibrates and submerged vegetative and plant remnants. The composition of water mold includes representatives of all classes of fungi but particularly oomycetes and gyphomycetes. The water mold include typical water mold like Saprolegnia, Achlia, Aphanomyces and others and the so-called air-water fungi, i.e. those which enter water arriving from air. The latter are more numerous on vegetative remains in the coastal zone of marine and freshwater bodies. These are Alternaria, Cladosporium, Fusarium, Trichoderma, Aspergillus, Penicillium.

The toxicogenic property of water mold is unknown. The reason is possibly the difficulty of isolating pure cultures. The need in this study, however, is pressing since the network of man-made ponds is growing. It is known that some fish, like the rainbow trout, are highly sensitive to mycotoxins and are well used as test organisms when identifying the toxicity of strains. Mycotoxicoses of fish may occur also when ingesting molded feed and contracting fungal diseases. In both cases, a decision has to be taken on the permissibility of eating fish affected by fungi or products of their metabolism.

Air is not a habitat for microorganisms including fungi. However, they spread by means of the air. Wherever air can reach, fungal diaspores can be carried. Microorganisms are everpresent in the solid particles of aerosols. Over land surface--these are mainly fungi, over the seas--primarily bacteria.

Fungi enter air during the active spread of spores or are carried by air currents when the spores are passively spread. In the air, fungi are present in the form of fragments of a mycellium.

The number of diasposes in air varies depending on natural and geographical conditions, on the season of the year and even the time of the day. The number of spores descreases with distance from the ground. The maximum number of spores is found in the light time of the day, and the minimum--at night. Variation relate not merely to the number of spores but their qualitative composition too. For instance, Conidia of the Cladosporium species predominate in the light time of the day, while basidospores--Sporobolomyces predominate at night in the air of the temperate and subtropical climatic belts.

Plants which are affected by fungi are believed to be the main suppliers of fungi diaspores to the atmosphere. Viable spores are practically all fungi which have surface sporulation may occur in air.

Studies conducted in Erevan (the Armenian SSR) have shown that the prevailing (numerically) genus is Cladosporium. In winter, the number of its spores in 1 m^3 drops practically 28 fold compared the autumn months. During the same seasons the numerical change in the spores of Penicillium and Aspergillus genera is insignificant.

The arrival of spores from soil and plants into air is inhibited in some seasons, they are washed from the atmosphere by rainfall, when the temperature decreases their survivability also drops. Many Penicillia and Aspergilli are polyphages and therefore the possibility of their entering air increases.

The conditions of existence of microorganisms in air are not favourable for them. The amount of nutritives there is limited ~~and in~~ it is a form which is not very suitable for assimilation. The action of sunrays and specifically of ultraviolet ones is also unfavourable just as the abrupt changes of temperature and relative humidity. To a certain extent the diaspores of mold forming fungi are protected against these influences in the area of clouds where humidity is sufficient, temperature is stable and sunrays are screened.

The increased content of fungi spores in industrial premises can be the cause of allergic diseases or profound mycoses, mycotoxicoses in workers.

Vegetative products compared to products of animal origin during their ripening and up to harvesting have a greater contact with environmental factors. The variety of the latter definitely influences the species of fungi-contaminants and might be even decisive. It is known that some vegetable plants form edible organs (tubers, rootcrops, roots, bulbs)in soil. Being in direct contact with soil and being exposed to specific conditions which include microflora they, following harvesting, become the carriers of diaspores of numerous fungi.

Food products, in the main, develop on the surface parts of plants and contact the air. They are exposed to desiccating wind, aeration, fluctuations of temperature and humidity, insolation. Many of these factors inhibit the development of microorganisms. Thus the potential of fungal contamination is definitely greater in the subsoil parts of plants which are used for food.

When food products are stored in special premises, in depots, there are conditions when all environmental factors have a cumulative action. Their combination is reflected on the qualitative composition of fungal flora and its development. Thus, in the storages of vegetables, fruits, the factors which determine the qualitative and quantitative composition of fungi which cause their molding include the following: the remnants of soil on products, the product proper which serves as a substrate for fungi, its nature, air temperature and humidity, its composition and the content of diaspores, i.e. the sanitary-hygienic state.

The decisive factors of building up the species composition of fungi-pathogens of molding in industrial shops where food products are processed (preserves of vegetative and animal products, butter, cheese, salamis etc) are the large numbers of spores on raw materials, equipment, packing materials, tare, the technology as such. The latter does not always provide for such a temperature regimen and such pressure which would have fungicidal action on diaspores of fungi which contaminate raw materials and equipment.

In closed premises where no protection has been provided to prevent the development of spores and where sanitation and hygienic conditions are not adequately observed there is always a reciprocal contamination: raw materials, equipment, contaminate air; air, in its turn, contaminates raw materials, equipment and finished products.

A monopoly development of fungi on products is not frequent. It occurs on fresh fruit and vegetables and also on processed vegetative and animal products. More frequently, with the advent of ageing, fungi groupings are formed on products. Their components enter into different relations where aggressive species predominate. They inhibit the development of other species. Thus the spread of fungi in food products depends upon the product, its physiological state, the composition of fungi-contaminants and their interaction.

It follows from the aforesaid that the characteristics of a species cannot be extrapolated on the entire genus as it happens, not infrequently, in literature. This relates primarily to mold forming fungi which by nature are saprotrophs and possess high adaptive properties. Besides this, the species composition of fungi-contaminants, their biological activity and specifically the ability of forming mycotoxins are influenced by geographical, natural-climatic conditions and even by weather.

Table 1

Taxonomic composition of fungi which contaminate food products (Armenian SSR)

Class, order	Genus	Vegetative products		Animal products		
		Number of species				
		fresh	pro-cessed	meat	butter	cheese
1	2	3	4	5	6	7
Oomycetes						
Peronosporales	Phytophthora	1				
Zygomycetes						
Mucorales	Circinella	1				
	Mucor	5	6	3	2	3
	Rhizopus	5	2			
	Mortierella	3				
Ascomycetes	Pleospora	1				
	Sclerotinia	1				
	Capnodium	1				
	Dimerina	1				
	Sphaerotheca	2				
Deuteromycetes	Hyalodendron		1			
	Monilia	4	2			1
	Oospora	4	2		2	1
	Trichoderma	2			2	
	Geotrichum	2	1		1	
	Oidiodendron	1				

Table 1 (continuation)

1	2	3	4	5	6	7
Deuteromycetes	Aspergillus	12	13	6	15	6
	Acremonium	1				
	Sterigmatocystis	1				
	Scopulariopsis	1				
	Penicillium	42	21	18	38	27
	Botrytis	16	1			
	Verticillium	2			1	
	Trichothecium	1	1		1	
	Ramularia	1				
	Torula	3				
	Papularia	1				
	Catenularia	1	1			
	Cladosporium	7	2	3	2	2
	Fusicladium	3				
	Pleiochaeta	1				
	Triposporium	1				
	Heterosporium	1				
	Helminthosporium	1			1	
	Drechslera	1			2	
	Cercospora	2				
	Alternaria	14	9	1	3	1
	Sporodesmium	1				
	Fumago	1				
	Stemphylium	5		1	2	
	Cylindrosporium	1				

Table 1 (continuation)

1	2	3	4	5	6	7
Deuteromycetes	Colletotrichum	3				
	Sphaceloma	1				
	Gloeosporium	1				
	Dendrodochium	1				
	Fusarium	30	3	4	2	
	Vermicularia	1				
	Phoma	1				
	Gloeodes	1				
	Zythia	1				
	Sphaeropsis	1				
	Leptothyrium	1				
	Mycelia sterilia	1			1	
	Rhizoctonia	1				
		199	65	36	75	44

Table 2

Results of testing fungi isolated from different groups of food products for toxicity (Armenia)

Product	Number of isolated species of fungi	Number of strains	
		Tested for toxicity	Proved to be toxic
Vegetables	136	21	15(71.4%)
Fruit	138	23	18(78.2%)
Processed vegetative products	58	12	9(75.0%)
Meat	36	18	11(61.1%)
Food fats	47	26	21(80.8%)
Cheese	39	27	20(74.1%)
Dehydrated milk	7	4	3(75.0%)
Total	300*	131	97(74.0%)

* The total figure has been belittled owing to the repetition of species

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