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

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BIOLOGICAL TECHNIQUES OF MYCOTOXIN DETECTION IN FOOD AND FEEDS

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BIOLOGICAL TECHNIQUES OF MYCOTOXIN DETECTION IN FOOD AND FEEDS

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A considerable number of toxin-forming fungi species affecting feed and food products is known nowadays. To fungi toxins are metabolites of differing chemical origin with a more or less specific pathological action on the organism of man, animals, plants and microorganisms.

The pathological action upon a living organism has been demonstrated in relation to the following fungi: Claviceps purpurea, Cl. paspeli, Sthachybotris alternans, St. atra. St. lobulata, Dendrochium toxicum, D. caucasicum, Fusarium graminearum, F. sporotrichiella, F. tricinctum, F. sambucinum u gp., Aspergillus flavus, A. parasiticus, A. fumigatus, A. ochraceus, A. clavatus u gp., Penicillium islandicum, P. viridicatum, P. urticae u gp., Pithomyces chartarum and many others.

The ethiological importance of the listed species of toxin-forming fungi in correspending diseases has been related to the ingestion (with food or fodder) of substrates affected by them; it has been reproduced in experiment. Besides the mentioned toxin-forming fungi, more than 140 other species are known which belong to different taxonomic groups (Sarkisov, 1954; Bilai, 1954, 1970, 1977; Spesivtseva, 1964; Moreau, 1974; Kellerman, et al. 1976; Krogh ,1968).

Owing to the broad spread of microscopic fungi in nature and the ability of many of them to form toxin, toxicity and carcinogenicity of some mycotoxins, considerable efforts have been made to achieve effective detection and control

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of microscopic fungi metabolites in food products and feeds (Spesivtseva, 1964; Kvrasova, Kostin, Malinovskaya, 1971, Bilai, 1982).

Different techniques of investigation are applied to prevent human and animal diseases, to elucidate the cause of mycotoxicosis, to identify the pathogen and to ensure the safety of food or feed. However, the investigation techniques have some common principles: sampling, mycological analysis, isolation of pathogenic fungi in a pure culture, extraction of toxic substances from feeds or food, detection and identification of mycotoxins, study of their biological action using different test objects and establishment of the maximum allowable concentration (MAC) for mycotoxins in the studied sample.

Biological techniques may be applied both for the determination of the sanitary standard of foodstuffs and for the determination of the toxigenic activity of fungi and toxin preparations. Considering varying susceptibility of animals and birds to mycotoxins, the more frequently used animals in biological tests are chicken, ducklings, hens, pigeons, mice, rabbits, some fishes, plants, microorganisms, tissue culture, etc.

Biological techniques

Alimentary tests on animals are used to determine the general toxic action of feeds and food products.

Mice, guinea-pigs, rabbits, cats, chicken, pigeons and ducklings are found to be susceptible to toxic substances of fungi belonging to the series of Fusarium, Aspergillus, Den-

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drochium, Penicillium, etc. The toxicity of feeds and food products is determined by feeding grain rations to chicken (5-15 day-old), pigeons, mice, ducklings (1-10 day-old) coarse vegetative feeds - to guinea-pigs and rabbits, products of animal origin - to cats, dogs, mice.

Feeding

The regular daily feed ration is replaced by the studied feed (50-100%) which is fed to test animals for 10 days. The animals are first left, for 5-6 hours, without feed to induce a rapid onset of the toxicosis. Watering is not limited. The amount of ingested feed is recorded daily. Depending on toxicity level and amount of feed eaten by test animals, the onset of the disease or death of the animal may differ in time. Noteworthy clinical symptoms of poisoning of chicken with toxic grain contaminated with fusaria are: cyanosis of the comb and the wattle, sleepiness, not infrequently diarrhea; if the disease takes a protracted course it may be accompanied by anemia, sharp leukopenia, and loss of appetite, drop in weight, and vomiting in pigeons.

Ingestion of grain infected with toxigenic aspergilli, penicilli causes the disease and death of the animals within the range of one to three days with clinical manifestations of damage to the central nervous system(trembling, convulsions, paralyses).

Positive indications of feed toxicity in a bioassay on animals is loss of weight, disturbance of the gastro-intestinal tract and the central nervous system; in birds - cyanosis of the comb and wattle, anemia. Sharply toxic samples

of feed are liable to induce death without clinical manifestations of intoxication.

Intragastric administration

To determine the toxicity of flour, groats or products of animal origin a water mash (1:2 or 1:3) of the studied sample is administered directly into the stomach.

Mice (3-5) are administered a water extract at a dose of 0.5 ml or an extract of the studied sample (mg) through a probe directly into the empty stomach for three days. A blunt and slightly bent needle put on a syringe may be used for the proble. Toxicity of feeds or food products is determined by the time of death of mice and the amount of the extract of the studied sample or the water extract administered to them (Kurasov V.V., Kostin V.V. Malinovskaya L.S., 1971).

To study food products damaged by the A. flavus fungus Allcroft R and Carnaghan R.B.A. (1963) suggested the technique of administering to one-day old ducklings the extract of the studied sample directly to the forestomach.

To carry out the bioassay on ducklings the aflatoxin of the studied sample is extracted by methanol, ester or chloroform. Water suspension of the extract is administered directly into the ducklings stomach at a dose of 0.5 ml on the first day, 0.75 ml on the second day, 1.25 ml on the third day and 1.5 ml on the fourth day. The ducklings are kept under observation for seven days, are sacrificed and their liver is studied.

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Positive indications of toxicity and contamination with aflatoxins of the studied products are: fatty dystrophy of the liver in mice and ducklings, proliferative processes in the biliary ducts, multiple haemorrhages, edemas.

Rabbit skin test

This technique is used to determine the toxicity of grain, food products, friable products, products of animal origin.

To do this, 50 g of a minced product is placed in packets of filter paper and extracted in Soxhlet's apparatus for six hours using ester (methanol, chloroform). The extract is then placed into an evaporation dish or a volumetric flask and is evaporated in a fume cabinet at room temperature until the smell of the solvent disappears.

In the absence of Soxhlet's apparatus 50 g of minced feed are placed into glass jars (0.5 l.) with a ground glass stopper and ether or alkohol ether (1:3), benzol or methanol is poured in so that the liquid would cover the sample for 2-3 cm. Extraction continues for 24 hours at room temperature, and the jar is regularly shaken. Then the liquid is poured out through a paper filter to an evaporating dish and left in the fume cabinet until all solvent is evaporated. The extract from grain products looks like vegetable oil while the extract of plant products are crumbly in consistency therefore two drops of vegetable or cod oil are added prior to the skin test.

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The extract is placed on well-shaven but undamaged skin of the rabbit (4-5 cm) and is slightly rubbed into the skin using a glass rod or palette. The extract is administered twice at a 24 hour interval. To prevent the rabbit from licking the applied extract a carboard plywood collar are put on the animals neck. The reaction is registered daily. The inflammatory process on the skin develops on the first and the second day after the application of the extract, it is intensified later on and by the fourth-fifth day (at times by the seventh) reaches its peak.

The rabbit skin test as a technique of determining the toxicity of feed or fungi cultures has been studied by many investigators. It was noted that different parts of the skin of different rabbits have differing susceptibility to toxic substances, the most susceptible skin is on the rabbit's back or the sides.

It has been also found that the sensitivity of man and animal skin is not the same. The skin of guinea pigs has medium sensitivity while the skin of cattle, horses, sheep, hen, geese, dogs and cats is just as susceptible as rabbit skin. The skin of white mice is the least susceptible.

The skin test following the application of the extract, allows one to consider the extent of toxicity of the studied product by the nature and depth of the inflammatory process (1, 2, 3, 4). The third and fourth degrees are brought about by toxic or highly toxic feeds and food products. Control is by extracts of wholesome grain. Some species of toxin-forming fungi (patulin, for instance) cause no dermonecro-

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tic reaction.

Determination of food toxicity by subcutaneous introduction of extracts to mice, ducklings and chicken

A sample of the studied product (200-250 g) is ground and placed in an acidified ester-alkohol mixture (200 ml ester + 100 ml alkohol + 1 ml of strong hydrochloric acid). The mixture is extracted for 2-3 days in a refrigerator, the solvent is filtered. The filtrate is evaporated in a fume cabinet up to the disappearance of the solvent's smell. 4.5-9 ml of sterile neutral oil is added to 0.5-1 ml of the fat extract and is thoroughly mixed.

Mice are administered subcutaneously 0.5 ml of the studied extract, ducklings and chick - 0.5-1 ml; control is 0.5 ml of neutral oil. Animals are kept under observation for 3-5 days. Highly toxic products kill the animals in 6-12 hours, or in two days. Extracts from weakly toxic feeds cause only necroses which develop on the site of application. Control animals do not show any changes.

Intracutaneous administration of the extract into hen's wattle

Ester or alkohol-ester extracts (1:2 or 1:3) are prepared; ground grain or some food product is immersed in the solvent, extracted for 24 hours, and then filtered. The solvent is evaporated. The oily extract, at a dose of 0.1-0.2 ml, is injected into one of the hen's wattles; into the other - the

extract of a high-quality product. The reaction is assessed by the presence of an inflammatory process, an edema, heamorrhage and necroses at the site of administration. The wattle thickness is measured by a cutimeter (Birbin S.S., 1967).

Determination of the maximum allowable concentration (MAC) of the mycotoxin in the studied sample

To determine the extent of toxicity of studied samples of feed, food products, fungi culture appropriate extracts with the known weight or volimetric content of mycotoxins are prepared from the sample and administered to animals in increasing amounts intraperitoneally or intravenously.

In case of intraperitoneal administration, mice are injected with 0.5-1 ml of the studied substance, guinea-pigs - 2-3 ml, rabbits - 5 ml.

Intravenous administration requires that the injection substance should be absolutely sterile. It is injected at a dose ranging from 0.5 to 10 ml depending on the animal species.

To assess tolerance and toxicity of the studied substance it is neçessary to determine the maximum endurable dose (MED), the absolutely lethal dose (LD 100) and the dose which kills 50% of test animals (LD 50). The study is conventionally started with the determination of these doses for albino mice. To do this the preparation is administered at different doses: ranging from those which do not produce any toxin phenomena in the animals up to a dose which kills

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all the test mice. Every dose is administered to 5 or 10 mice. The preparations are usually administered intraperitoneally. The number of animals which die throughout the observation time is recorded (Pershin, 1959).

All the animals which have been used in biotests to detect mycotoxins in food products and to study their biological action were dissected and morphological pathologies in their organs and tissues were studied.

Besides the biological test techniques used for determining the content of mycotoxins in feeds and food products, studies are conducted with the protozoa Paramaecium caudatum, Tetrahymena pyriformis, with eggs of the mollusk Bankia setaceae, with eggs of a marine borer, larvae of the Zebra fish Brachydanio rerio, rainbow trout, sturgeon fishes, the guppy, larvae of Artemia salina, rat erythrocytes, chick embryos, tissue cultures, microorganisms, chlorella, and okpa plant (Abelmoschus esculentus).

The protozoa

This technique is used mainly to determine fungi culture toxicity in both known and unknown species, extracts (aqueous) from the fungal film, cultural liquid and also extracts from feeds.

The most frequently used test objects are cultures of Paramaecium caudatum and Tetrahymena pyriformis. It is only necessary to use pure cultures of protozoa which are obtained from natural reservoirs by passing with a thin pipette under a magnifying glass. The protozoa are cultivated on a hay brew and milk media. Pure cultures of Paramaecia should

be continually maintained and renovated.

The criterion for the determination of sensitivity is the time lag from the onset of the tested extract action to the death of Paramaecia.

Paramaecia die under the action of the extract from fungal cultures in 8 minutes - when the fungi are highly toxic in 10 to 20 minutes - toxic; 2 hours - weakly toxic.

Non-toxic strains of fungi did not kill Paramaecia or cause any morphological changes even after 24 hours (Spesivtseva N.A., 1964).

Paramaecia are highly sensitive to aflatoxins, sporodesmiotoxin, stachibotriotoxin, dendrotoxin, fusariotoxin, fumigatin and some other toxins.

Mollusk eggs

To determine aflatoxin toxicity fertilized eggs of the Bankia setaceae mollusk are used. The eggs and sperm of adult mollusks are obtained by opening the gonodial tissue, the eggs and sperm are suspended in marine or tab water containing different concentrations of aflatoxin, and incubated at 10-20°C. The bicellular stage of the mollusk develops in the control (without the aflatoxin) after two hours, after 3-4 hours - the multicellular and the trichofores, the freely swimming forms develop in approximately 18 hours. The division of fertilized eggs and formation of cellular envelope is inhibited in the presence of aflatoxin at a concentration of 0.05-40 µg/ml so that cells containing but several nuclei are found but after 3-5 hours.

Presence or absence of freely swimming larvae is the

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criterion of the aflatoxin action (Townsley P.M., Lee E.G. H., 1967).

Fishes

The toxicity of cereals is determined on aquarium fishes of the guppy (Lebistes reticulatus) species. The studied sample (50 g) is thoroughly crushed in a laboratory mill and placed in a flat bottom flask with a ground glass stopper. 100 ml of acetone is poured in and extracted in the shaker for two hours. The extract is paper-filtered into a porcelain dish and dry steamed in a water bath at 60°C. The dry residue is weighed and dissolved in 5 ml of acetone, and is placed in a glass jar with 500 ml of water from the aquarium (17-20°C). Five guppy fish regardless of sex or age are placed in the solution. Their behaviour is observed for 24 hours and the time of death is recorded. Subject to the extent of toxicity of the studied samples guppy died within one to 24 hours.

The control is a 1% acetone water solution in which guppy should stay alive for at least 3 days (Kurmanov, Talanov, 1975).

Aberi L.H. and McKinley W.P. (1968) suggested a technique for aflatoxin determination in feeds using of eggs and larvae of the Zebra fish (Brachydanio rerio). Aflatoxin B_1 at a concentration of 1 μg/ml causes abnormal motion of the larva in 30 minutes, and kills all larva within 5-6 hours. The inclusion of aflatoxin B_1 at an amount of 0.1 μg/kg into the ration of rainbow trout induces the formation

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of hepatomas in 10% of all fish, an increase in the aflatoxin concentration to C.5 μg/kg, and results in the development of hepatocellular carcinomas in 40%. The carcinogenic effect in rainbow trout is less pronounced in case of aflatoxin C_1, B_2 and M_1.

The sensitivity of aflatoxins compared to that in rainbow trout is 20 times less pronounced in sturgeon fishes and 30 times less pronounced in the catfish.

The formation of hepatomas in sturgeon fishes was observed when the ration contained 12 μg/kg of aflatoxin B_1.

Bird embryos

Chick embryos are used to detect aflatoxin B_1, B_2, G_1, G_2 in feeds and food products and also to detect fusariotoxins, fumitoxin A, and some other toxins. Five - day-old embryos are used to introduce mycotoxins into egg yolk; one-day-old embryos—to introduce it into the air cavity; and 9-day-old embryos - into the chorionallantoise envelope (Platt B.S., 1962, Verrett M.G., 1964; Ivanov A.T., 1967; Debeaupuis J.-P. 1978, Kulikova, Minasyan, Razbitskaya, 1980).

Mycotoxins introduction technique

a) into the yolk

An aperture is made in the shell over the air cavity and a long thin needle is used to introduce 0.5 ml of the studied extract with different content of mycotoxins into the yolk sack (under the ovoscope). Then the opening is closed and the tray with embryos is thermostated.

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b) into the air cavity

Prior to aflatoxin injection the eggs are examined and the line of the air sack is marked by a penscil. Then an aperture made in the shell over the air sack and the tested solution at a volume up to 0.04 ml or less is introduced onto the egg membrane; the aperture is then sealed with adhesive tape.

The embryos are placed in a thermostate for incubation at 37°C and a 52% relative air humidity.

c) into the chorionallantoise envelope

This technique is used to determine toxicity of different mycotoxins and extracts from the studied food products.

A thin needle is used to introduce 0.2 ml. of the tested mycotoxin extract into the chorionallantoise envelope through an aperture in the shell over the air sack; proper introduction is assured by keeping the egg over an ovoscope.

The infected embryos are examined twice a day for 21 days. The biological action of mycotoxins is determined by the time of death of embryos and by anatomic examination.

Toxic extracts from the studied products and feeds infected by Fusarium fungi kill the embryos in 18-25 hours.

Weakly toxic extracts kill the embryos in 30-90 hours. The blood vessels on the chorionallantoise envelope are flat. The embryo is hyperemic, specifically, the head and wings. The liver is marble-like, the kidneys are blood filled and there are haemorrhages on the cardiac sack.

Chick embryos are sensitive to the toxins of Aspergillus genus. LD_{50} is 325-780 μg/embryon. The chorionallantoise

envelope of dead embryos shows multiple punctate haemorrhages, hyperemia, filling with blood and flacidity of the internal organs.

Administration of aflatoxins into the yolk and the air space of the egg inhibits embryos growth and usually kills it on the fifth-tenth day; if it survives for a longer time it never hatches. The dissection has shown an edema, haemmorhages and underdevelopment of the brain, spottiness and degenerative changes in the liver. Underdevelopment or absence of the beak, shorter legs; poor feathering are also observed.

LD_{50} for aflatoxin B_1 when it is administered into the yolk is 0.048 μg, and when it is introduced into the air space of the egg - 0.025 μg per egg.

Fumitoxin A kills embryos at 0.2 μg per egg.

Tissue culture

This technique is mainly used to study the cytotoxic action of mycotoxins upon different types of cell cultures. Sensitivity to mycotoxins has been demonstrated for the cultures of chick embryos liver, liver, lungs and kidneys of baby rats; calf and monkey kidneys, liver of human embryo; fibroblasts of human skin, Hela, Hep-2, Vero and other cells.

Embryo tissue cultures of birds and mammals are obtained from embryos of chicken, ducklings, mice, rats and rabbits.

Monolayer tripsinized cell cultures are obtained by dispersing bits of tissue using enzymes to obtain suspensions of individual cells. To obtain a complete deaggrega-

tion of tissue cells a 0.25% trypsin salt solution (generally the Difco solution) is made or its mixture with pancreatin. The trypsinization of embryo tissues is performed at 37°C for 2-3 hours. The liquid with suspended cells is collected and the cells are precipitated by centrifuging (1500 pm for 7-10 minutes), then they are resuspended in a nutritive medium.

Unpurified aflatoxin at different concentrations (100, 50, 10, 1, 05 μg/ml) is introduced into the tissue culture and tests are performed after the passage of 1,3,6,12 and 24 hours. Aflatoxin-free culture is used as the control.

In parenchyma cells, at aflatoxin's final concentration of 0.5 μg/ml, degeneration of nuclei takes place due to the inhibition of RNA and protein synthesis.

The toxic action of aflatoxins on cell cultures of different types is shown by characteristic pathomorphological changes in cells due to disruption of cell organoids in the monolayer, including the lysosomes, changes in the nuclear-plasmic ratio, slower mytotic division rate and disturbances in functional activity of cells.

Aflatoxin B_1 at a concentration of 0.05 μg/ml completely inhibits the growth of lung cells in a human embryo. LD_{50} for the Hela cells is 5-7 μg/ml, for the liver cells of adult persons it is 14.3 μg/ml. Alongside with aflatoxin B_1, toxic action on liver cells of a human embryo is caused by aflatoxin G_1,G_2,B_2 (Stich H.F., 1975, Benumovich M.S., 1973).

A 10^{-4} concentration of mycotoxin PR (Penicillium roguforti producent) makes structure the liver cell nuclei structure less and causes their death.

Cultures of calf kidney epithelium cells and transplanted kidneys cells of a pigling exhibit high sensitivity to toxins F. sporotrichiella. A dilution of toxins and extracts from infected grain ranging from 1:1000 to 10^{-5} alters nuclei size decreases mytotic division and causes lysis cell (Nazynov M.N., Titov V.V., 1977). Kidneys cells of a pigling embryo are used to detect the presence of fusariotoxins and stachiobotriotoxins in meat of animals which were slaughtered due to mycotoxicoses. The cytotoxic action of extracts from the studied meat manifests itself in the cells rounding appearance of granules, pigment and cell necrosis (Boikov, Yu.I. Khasanova E.M., 1980).

Hela cells, Hep-2 cells, fibroblasts of a human embryo are sensitive to toxic metabolites of A. fumigatus, A. flavus and A. niger. Penicillic acid at a concentration of 10 µg/ml stimulates the mytotic activity of Hela cells (Wilson D.M., 1976).

The study of cytotoxic action of mycotoxins of different chemical origin upon Hep-2 cells showed that mycotoxins exert varying effects on the mytotic regimen of transplanted cells of a human larynx carcinoma. Thus, dendrotoxin and gelvolic acid at a concentration of 0.25 µg/ml sharply inhibit cell mytotic activity; fusaric acid and patulin at a dose of 1 and 0.5 µg/ml increase the number of pathological mytoses due to the chromosomes damage. Fusarin-treated cells results in an increase in the number of K-mytoses. A delay of mytosis in the metaphase stage has been influenced by fumigatine. Vero cells proved to be more resistant to expo-

sure to the studied mycotoxins (Nagornaya N.I., Barshtein Yu.A., Kurbatskaya Z.A., 1979, 1981).

In rat erythrocytes

The biological method of determining mycotoxin effect on the erythrocytes destruction rate in growing animals is used to determine toxicity of extracts from food products and feeds. A hemolytic effect has been established upon exposure to extracts of feeds affected by toxic fungi of the aspergillus genus (Kovarsky V.A. Bushanskaya T.Ya., Berezhinsky M.Ya., 1980). The authors concluded that the application of the technique based on erythrocyte hemolysis rate is effective for a rapid detection of feed toxicity. They have likewise studied effects of pure preparations of aflatoxin B_1, T-2, ochratoxin A, and zearalenone on hemolysis at LD_{50} doses. An aflatoxin B_1 dose of 2 μg/kg of the feed is taken as a unit of toxicity. It has been experimentally found that the toxicity of aflatoxin B_1 is 1.0 μg/kg; that of T-2 - 0.5 μg/kg, ochratoxin A - 1.5 μg/kg and zearalenone - 3.0 μg/kg.

Larvae

Durashkova Z., Betina V. (1976) developed and proposed a bio-autographic technique of detecting mycotoxins in feeds and food products using Artemia salina. After extracting mycotoxins from the examined lot of feeds, the extracts are applied to glass plates which are covered with a fixed layer of silicagel dissipate and in a solvent system. After drying the chromatograms, spots of mycotoxins are removed from them and incubated with a standard larvae suspension. Mycotoxin

toxicity level is assessed by the time of larvae death. Fumitoxin A, at a concentration of 25 µg/ml kills 100% of larvae. This technique can be applied for the detection and titring of aflatoxin B_1, kojic acid, sterigmatocystin in different samples of feeds, food products and farm raw materials.

Microorganisms

The studies by Bilai V.I. (1960, 1961), Barmeister H.R. (1966), Giegler A. (1966), Dakhnovsky V.I. (1972) and others contributed greatly to research into antibiotic and fungicidal properties of mycotoxins. It has been found that many mycotoxins, alongside with high toxicity, have a pronounced antibacterial action as well.

The microbiological technique of detecting mycotoxins in food products and feeds is based on the idea of a direct action of examined extracts on live cells of bacterial and yeast test cultures sensitive to mycotoxins. In a specific case, the most sensitive microorganism is applied to every mycotoxin. The presence of mycotoxins in the examined samples is detected by the delay zone pertaining to the growth rate of the indicator test culture. The microbiological technique makes it possible to detect negligible amounts of mycotoxins. The sensitivity of the technique is within the range up to 0.03 µg/ml.

This technique is applied both for qualitative and quantitative determination of mycotoxins in the studied samples.

As a rule, the more frequently applied technique for the qualitative determination of mycotoxins is that of paper

disks cylinders, holes and streaks. The series dilutions technique is used for quantitative determination of mycotoxins. This technique makes it possible to determine the content of mycotoxin not only in conventional but in weight units, too.

The bioautographic technique of mycotoxin identification in extracts of studied products is also promising. This technique may be useful while detecting (in the presence of a witness-substance) mycotoxins even if they are contained in negligible amounts and, moreover, in a mixture with other substances.

In plants and algae

It is known that prevailing majority of mycotoxins which have been thus far studied - aflatoxin B_1, patulin, penicillic acid, rubratoxin B, citrinin, trichothecenes alongside with general toxicity for warm-blooded animals possess well-pronounced phytotoxic activity.

Kang M.S., Chohan V.S. (1978) suggested for the determination of aflatoxin B_1 a sensitive species of the ochra-plant (Abelmoschus esculentus), the cotyledonous leaves of which demonstrate different degree of chlorosis subject to the concentration of the effective mycotoxin.

A high degree of sensitivity to dendrotoxin, fusariotoxin, stachibotriotoxin and to other toxins has been reported for the sprout of maize (Zea mays), beans (Phaseolus vulgaris), cross lettuce (Lepidium sativum), wheat (Triticum vulgare), oats (Avena sativa) and other plants (Bilai V. I., 1982).

Some physiological peculiarities common to monocellular algae and higher plants made it possible for Ikava et al. (1960), Borovkov, Zolnikova (1977, 1979) to work out a technique of detecting fungi-producents of mycotoxins using Chlorella vulgaris for a bioassay. This technique has been widely tested in isolating patulin, roridin A, verrukarin A, penicillic acid, citrinin, etc.

Chlorella as a test-object is known for its wide sensitivity which makes it possible to use it for the detection of fungi capable of producing both mycotoxins and phytotoxins.

The biological techniques of detecting mycotoxins in feeds and food products which have been suggested here correspond, in terms of sensitivity (and some by far surpass it to the MAC levels for aflatoxins and mycotoxins in food products).

It should be stressed that biological techniques of detecting mycotoxins in food products compared to physicochemical techniques are more labour and time consuming , but they are indispensable in proving the presence of mycotoxins in the studied samples since they determine their toxicity level in relation to different biological objects and indicate the complexity and diversity of pathological and clinical manifestations of mycotoxicosis.

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