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

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**GENERAL METHODS
OF MYCOLOGICAL STUDIES**

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GENERAL METHODS OF MYCOLOGICAL STUDIES

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In mycological studies usually the same instruments and devices are used as in bacteriological and chemical laboratories with only few modifications in the mycological technology.

Instruments. Needles, the loose ends of which are bent at right or obtuse angles (mycological hook) and flattened in the form of a spatula, are used for inoculation. The needles are inserted into a needle holder. Straight (preparation) needles, forceps, and scalpels are utilized.

Glassware. Various flasks, Petri dishes, slide and cover glasses, test tubes with diameters of 14 and 20 mm, measuring pipettes are used.

Equipment. Microscopes, magnifying glass, pH-meter.

Reinoculation techniques. Reinoculation is performed in the following order: opening of a fungus-containing dish; taking an inoculum (particles of mycelium, conidia, etc.), transfer of the inoculum (into a new vessel, test tube); inoculum fixing on a new nutritive medium.

1. Inoculum transfer (parts of a colony; fragments of mycelium, spores) is performed with a needle.
2. Spore suspension reinoculation is done with a pipette.

Mycological study of fodder

The mycological study includes: isolation of fungi from fodder, quantitative determination and differentiation of fungi, isolation of pure cultures from primary inocula.

Nutritive media. For primary fungi isolation Czapek's agar medium, wort agar, and Sabouraud's agar are used. For species

differentiation, special media are applied in every individual case.

Techniques of fungi isolation

Fungi isolation from grain (seeds)

1. Identification of surface microflora. Spores washing off in water or nutritive medium is performed and the technique of deep pouring in a medium is used.

2. Identification of deep layer microflora. Preliminary disinfection of grain with 3% formalin, 70° ethyl alcohol, or other antiseptics is performed. Treated grain is placed on top of nutritive medium.

Fungi isolation from coarse fodder (hay, straw)

Humid chambers are used. A layer of filter paper in a Petri dish is impregnated with van Iterson medium.

Fungi isolation from flour, mixed fodder, grist, etc.

1. Technique of pouring.
2. Technique of direct inoculation.

The quantitative registration of fungi and the differentiation of species are performed on the 2nd-3rd day after fodder sample inoculation. Total volume of colonies of a given fungus species is determined on the basis of 1 g of the studied fodder.

In grain fodder the contamination degree is assessed on the basis of percentage ratio of grown colonies of every fungus species to the total volume of inoculated grains.

Methods of identification of smut fungi in fodder

Smut affects mainly cereals. The affected parts of plants often look like covered with soot or charred due to a large num-

ber of spores with dark stained membranes.

In grain fodder smut can be detected both in the form of whole smut grains in ears (when the grain is completely damaged and its coat is filled with fungus spores having a rounded form and grey-brown colour) or the form of diffused spores.

The percentage of damaged grain in a weighed portion is determined through the isolation of spores by washing them off grain with benzene, ether, or water.

Technique of ergot identification in fodder

Ergot of rye, barley, cultivated and wild growing cereals is characterized by the formation of violet-black sclerotia (instead of seeds) in the form of horns 4 cm long and clearly visible in grain. The ergot content in grain fodder should not exceed 0.1-0.5%.

In mixed fodder, flour, or bran ergot identification is performed through washing off dark ergot particles with chloroform or alcohol.

Fungi isolation from other substrates

Fungi are isolated from the air by capture of spores settling from the air on a horizontal surface. Recommended exposure of open Petri dishes with agar medium in streets and in town is from 30 sec to 6 min; in laboratories - 30 min; in the field - 1-4 hours, in the forest - 10-24 hours.

Isolation of fungi from water and other liquids is performed by a technique of pouring.

Isolation of fungi from meat and meat produce is done through inoculation with meat particles or by means of a smear.

Isolation of fungi from milk and dairy produce and eggs

is performed by the technique of pouring if the outward appearance of a product is unchanged or by a direct inoculation technique in case of changed colouring or apparent presence of fungi membranes on the product.

Isolation of fungi from succulent fruits and vegetables in the presence of the fructification or spore-bearing of fungi on the surface of fruits, roots, bulbs, tubers is performed by direct transfer of fungi fragments onto nutritive medium. If a fungus grows within tissues, surface disinfection of the product is recommended.

Isolation of fungi from soil is possible through fungus isolation into pure culture with a technique of direct inoculation and that of pouring and also with the help of a technique of overgrowing plates (proposed by N.G.Kholodny).

The determination of mold fungus contamination of walls is done by means of the inoculation of the scrapes of the whitewash surface layer.

The air of refrigerating chambers is analysed by a technique of the capture of spores settling from the air on a horizontal agar surface of a Petri dish.

Techniques of separation, Isolation of fungi into pure culture and the applied media

Selective media, bacteria-suppressing acidification, various development conditions (temperature, etc.), physical elimination, difference of growth rates, etc. are utilized for separation.

Among biological methods of study of fungi there are no methods which by their precision, effect, and availability could have greater significance than that of pure cultures. To obtain

pure fungi cultures, the technique of consecutive dilutions broadly used.

A universal growing medium for the majority of fungi is wort agar. For the cultivation of mould fungi Csapek's and potato agars are also used. To isolate cellulose destroying fungi moist filter paper is utilised.

Fodder toxicity identification techniques

Toxicobiological method of fodder analysis (rabbit skin test through the application of ether extract, mice test, ingestion and subcutaneous injection, eye test, test on cats, immunofluorescent techniques, chromatographic tests).

Fodder sampling

An average straw or hay sample is taken from various places in lots subject to testing, in the amount not less than 5 kg for every 25 tons of unpressed and 50 tons of pressed straw or hay. Out of the average sample a specimen with the mass not less than 100 g is taken; the average grain or mixed fodder samples weighing not less than 1 kg are collected from various places suspicious in their quality.

Organoleptic analysis

Determination of smell, colour, friability.

Assessment of fodder by the results of study.

Mould-smelling and mould-affected (more than by 10%) hay and straw, grain fodder with mould-rotten smell, animal origin fodder with stuffy, mould, and rotten smell, are to be destroyed.

Straw and chaff the samples of which turned out to be toxic and affected by toxic variants of the Stachybotrys alternans fungi, are strictly prohibited for consumption without decontamina-

tion.

Hay affected by Dendrodochium toxicum is destroyed.

Coarse fodder, affected by toxic fungi variants from the Fusarium genus and also from the Aspergillus, Penicillium, Mucor, Rhizopus genera (with the exception of the Sporotrichiella section) which are low toxic in skin tests and which produced negative results at feeding, are permitted as admixtures to other fodder in a volume not exceeding 25% of the total meal.

Coarse fodder, mixed fodder, grain fodder characterized by high toxicity and which have been proved toxic on the basis of a biotest, are not subject to consumption.

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