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

A. I. KOTIK

BIOAUTOGRAPHIC METHOD FOR DETERMINATION OF TRICHOTHECENE MYCOTOXINS IN GRAIN AND CEREAL PRODUCTS

Centre of International Projects, GKNT

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Kotik A.N.

Trichothecene mycotoxins (TM) are closely related metabolites produced by several species of Fusarium, Myrothecium, Stachybotrys, Trichoderma and some other genera of imperfect fungi. Being widespread on goods and feedstuffs these fungi cause serious toxicoses in man and farm animals (fusariotoxicosis, stachybotryotoxicosis, dendrodochiotoxicosis) and are, therefore, referred to the most dangerous contaminants of grain and cereal products. Nowadays detection of TM in grain and cereal products is carried out mainly by gas chromatography and mass-spectrometry. Research on the trichothecenes content is sporadic due to its complexity, and, therefore, information on the cases of grain contamination with TM is scarce. Nevertheless to prevent toxicoses induced by TM, large scale research is needed to control the level of TM contamination of foods and feedstuffs. Simple and reliable methods are required for this purpose.

We have worked out a method for TM detection in grain and cereal products based on extraction with organic solvents of mycotoxins from the test sample and their further identification and quantification, using thin layer chromatography of the extract in the presence of witness-agents with further bioautographic development of chromatograms with the help of a strain of microorganisms sensitive to TM.

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Apparatus, Materials, Reagents

Drying cabinet, thermostat for 28°C, table with controlled surface level, shaker, distiller for organic solvents, labora-

tory balance, shallow pans 30 x 40 cm for arranging a moist chamber, 50 ml separating funnel, 500 ml flask with ground stopper, 200 ml flask, conical funnel, 10-12 cm dia., TCL chamber, 1 ml pipette, 5 ml pipette, 0.1 ml micropipette, glass plates 9 x 12 cm, paper filters, hectographic pencil, silica gel LCL_{254} 5/40 with 13 per cent of gypsum, acetone, diethyl ether, hexane, toluene, methanol, ethyl acetate, distilled water, wort agar in 100 ml bottles, 24 hr culture of Candida pseudotropicalis, strain 44 pk, T-2 toxin solution in chloroform, 20 μg/ml, solution of HT-2 toxin in chloroform, 20 μg/ml solution of stachybotryotoxin D.

Preparation of Thin-Layer Silica Gel Plates

Weigh 10 g of silica gel into 200 ml flask, add 50 ml of distilled water and shake the content vigorously. Apply 7 ml of suspension to each plate, and using pipette, spread it evenly over the surface; let the plates rest strictly horizontally on the table, dry coated plates for 12-14 hours at the room temperature. Before using activate the plates for 1 hr at 120°C.

Preparation of 24 hr Culture of Candida Pseudotropicalis Strain 44 pK.

Transfer a colony of Candida pseudotropicalis, strain 44 pK, grown on slanted agar into a test tumbe with liquid nutrient medium prepared from beer-wort diluted with distilled water 1:1 and sterilized for 1 hr at 121°C. Incubate the tube at 28°C for 24 hr. Candida pseudotropicalis, strain 44 pK can be obtained at the Ukrainian Scientific Research Institute of poultry breeding. Wort agar is used for culturing the strain. The strain is reseeded once in 6 months and is stored at +4°C .

Analysis Procedure

Extract 20 g of flour with 50 ml of acetone for 15 min using a shaker, repeat the operation three times. Combine the extracts and filter them through a filter paper, evaporate on a water bath to complete removal of acetone and dissolve the extract in 10 ml of methanol or ethanol. Dispose the oily residue, evaporate the solution on a water bath; determine the volume of the obtained extract with the help of a micropipette. Apply 10-15 µl of the 50-obtained extract to chromatoplates; apply TM solutions (for example solutions of T-2 toxin, HT-2 toxin, stachybotryotoxin D) as witness agents. Develop the plates in the system of diethyl ether-hexane (I:I), then repeat development in the system of ethyl acetate-toluene (3:1) and dry the plates. Apply 8 ml of melted wort agar to these plates and let them rest on the table strictly horizontally. Apply to and spread evenly 0.5 ml of 24 hr Candida pseudotropicalis, 44 pk strain culture over the set surface of the agar. Place the plates seeded with the culture to a humid chamber and incubate at 28°C for 16 hr. After incubation of the plates in a thermostat examine them for growth inhibition and morphological aberrations, determine R_f of yeast growth inhibition zones. The presence of the yeast growth inhibition zones with the centres in the points whose R_f is equal to R_f of the witness-agents is indicative of the presence of respective mycotoxins in the test sample. It is possible to determine the quantity of mycotoxins according to the size of the yeast growth inhibition zone (for example, it has been established in respect of T-2 toxin that a zone 10 mm in diameter corresponds to 0.12 µg of this mycotoxin, 11 mm -

- to 0.2 μg, 13 mm - to 0.26 μg, 14 mm - to 0.33 μg, 15 mm - to 0.43 μg).

The described method is simple, specific and highly sensitive (the lower limit of detection of T-2 toxin in the samples is 300-350 μg per kg). At present this method is applied in a number of laboratories (in the Ukrainian Scientific Research Institute of Experimental Veterinary Science, in the Ryazan Agricultural Institute) for detecting TM in grains and cereal products, for determining the toxin-formation capacity of TM-producing fungal strains, as well as in elaborating methods for detoxication of foods and feeds contaminated with TM.

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