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

L. G. IVANOVA, N. P. GOLOVINA

MYCOLOGICAL STUDY OF THE PRODUCERS OF STACHIBOTRIOTOXINS AND FUZARIOTOXINS

(T-2-TOXIN, ZEARALENONE)

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A MYCOLOGICAL STUDY OF THE PRODUCERS OF STACHYBOTRYOTOXINS AND FUSARIOTOXINS (T-2, ZEARALENON)

Ivanova L.G., Golovina N.P.

The Stachybotrys alternans fungus is a cellulolytic saprophite. Under natural conditions it well develops on cellulose-rich substrates: straw, hay, grain, various weeds, and plant remains. It attacks cotton, hemp articles, wood, sackcloth, paper. On straw and grain it forms a black powdery, easily removable coating.

Taken for mycological examination are suspected specimens of straw (weat, rye, oats), threshing residues, more seldom hay (of cereals) and oats.

Straws affected by the fungus are examined with a magnifying glass. The heaviest accumulation of the fungi is observed on the nodules of the straws where one can see a black powdery coating of spores. The dark coating is scraped off and placed into a drop of a 50% aqueous glycerol solution on a slide, covered with a cover glass, and examined microscopically at low magnification. Darkly coloured conidiophores and the fallen spores of the fungus will be seen in the visual field.

For producing a culture of the fungus pieces of infected fodder samples are placed in sterile Petri dishes with filter paper moistened with Van Iterson's fluid medium and kept at 24-26°C. Within 5-10 days the fodder samples will contain a fine powdery black bloom. The bloom is transfered with a loop into test tubes or Petri dishes with Czapek's agar.

The fungus develops well on nutrient media. A colony of St.alternans is black, velvety, surrounded by a white border, excreting a dark brown pigment into the medium. The conidiophores are septate, sympodial-ramified, pale olive-green with a bundle of sterigmata on the tip upon which black elliptical conidia are formed.

An important part in the development of the fungus and toxin formation is played by temperature and humidity. The optimum temperature is 20-25°C and the best humidity is 60-75%.

The fungi of the Fusarium genus are widespread in nature and very frequently cause the spoiling of fodder in store-houses.

Organoleptically, toxic fodders quite frequently cannot be distinguished from high-quality ones. If undersized, rugose, light grey or pinkish-red grains are detected among the samples, the contamination of the grain with fusaria may well be suspected.

For revealing the in-depth contamination of grain with Fusarium fungi, the grain is preliminarily disinfected with a 3% formalin solution or 70% ethyl alcohol. About 50-70 grains wrapped in a gauze napkin are placed into a beaker with a disinfecting solution. After 5-7 minutes they are transfered into a beaker with sterile water and rinsed. The gauze is unfolded and the grains are arranged with sterile forceps on the surface of the nutrient medium so that they do not touch one another.

Small grains (wheat, oats, barley, rye, millet, etc.) are arranged in groups of 20, larger ones (maize, beans, peas) — in groups of 10. The number of Petri dishes for the inoculation

of small grains should not be less than 5 and for the inoculation of large ones, not less than 10.

For detecting surface mycoflora (contamination with spores), the spores are washed out in water. The resulting suspension of spores is diluted with sterile water and placed onto the surface of a nutrient medium. One ml of the suspension is placed on each Petri dish.

In order to isolate fungi of the Fusarium genus from flour, bran, mixed fodder, grist, the pouring method is employed.

Pouring method. 10 g ofmixed fodder are placed in a sterile flask and 100 ml of sterile water are added to obtain the basic dilution of 1:10. The flask is shaken for 10-20 minutes. The 1:100, 1:1000, 1:10000 dilutions are prepared from the basic dilution (suspension) by adding sterile water. Mixed fodder is usually inoculated in a 1:1000 dilution onto a nutrient medium in 5 dishes. The dilution is performed by means of sterile pipettes (takin a separate pipette for each dilution). 1 ml portione of the prepared diluted suspension are transfered to the Petri dishes.

All the inoculations from all the fodders are kept at 22-25°C; the dishes are inspected on the 3rd, 5th, 7th and 10th day.

Around the grains contaminated with Fusarium fluffy or creeping white, yellowish or pinkish colonies develop. The material from the colonies is passaged into test tubes containing nutrient media. Potato agar, wort agar and Czapek's ager are used for cultivating Fusarium.

The species of the Fusarium genus are differentiated on the

basis of the morphology of macroconidia, the presence or absence of microconidia, chlamydospores, the formation of sclerotia, fruit bodies, the coloration of the stroma during development on rice grains.

The following fungi are producers of T-2 toxin: F.sporotrichiella var. tricinctum, F.sporotrichiella var.sporotrichioides, F.sporotrichiella var.poae (syn.F.tricinctum, F.sporotrichioides, F.poae).

The colonies of F.sporotrichiella on potato agar are fluffly or arachnoid, frequently powdery; the colour of the mycelium is white, pinkish, yellowish, with mass formation of pseudopionotes or sporodochia. The nutritive medium assumes crimson shades.

Microscopic study of the aerial mycelium reveals microconidia of two types: spherical, spherical pear-shaped or lemon-shaped and elongated or elliptical-spindle-shaped. The macroconidia have from three to five septa, they are sickle-or spindle-shaped, tapered at the tips. The chlamydosphores are formed in chains.

The fungus developing on rice grains acquires yellowish-brown colour with crimson shades and the grains of rice become brown-olive.

Different variants of the F.sporotrichiella fungus differ from one another by their macroconidia. In F.tricinctum the macroconidia have three septa; in F.sporotrinchioides are macroconidia with five septa prevail; finally, F.poae has no macroconidia at all, or possesses only occasional ones.

Zearalenone or F-2 toxin are oestrogenic metabolites of the F.graminearum, F.culmorum, F.moniliforme, F.roseum, and

F.semitectum fungi.

The colonies of F.graminearum (the main producer of zearalenone) on nutrient media are fluffy, white or golden-yellow; the substrate mycelium is crimson-red, pseudopionnotes are abundant. The macroconidia are spindle-shaped, slightly curved, with a rather narrowed apical cell. There are no microconidia.

The colonies of F.culmorum are fluffy, whitish-pink; the substrate is of crimson colour; sporodochia and pseudopionnotes are formed. The macroconidia are spindle-and sickle-shaped, broad, with five septa. There are no microconidia.

The colonies of F.moniliforme are fluffy-velvety, white with a pinkish or slightly lilac shade. The macroconidia are awl-shaped or slightly spindle-shaped and possess three septa. There are numerous oval microconidia in long chains with one septum or none at all.

The colonies of F.roseum are fluffy, arachnoid, pale-pink, with abundant sporodochia and pionnotes. The macroconidia are awl-shaped, narrow, long, with five septa. There are no microconidia.

The colonies of F.semitectum are fluffy, whitish-yellow or ochre-yellowish, pinkish. The macroconidia are spindle-and sickle-shaped, elliptically curved or nearly straight, narrowing towards both tips and having from 3 to 5 septa. The microconidia are spindle-and-sickle-shaped, smaller than the macroconidia with 1-3 septa.

Zearalenone is usually formed on fodder in storages under conditions when moderate and cool periods alternate (12° and 25°). The nutrient substrate for the development of oestrogen

producers are various cereal cultures and coarse fodder. These Fusarium species usually object maize stored under unfavourable conditions. This is why the most frequent cause of the oestrogenic syndrome is mouldy maize. Zearalenone has also been isolated from rice, rice bran, wheat, forage flour, and other fodders.

The optimum temperature for the development of the mycelium on fodders during storage is 18-24°C with relative air humidity in excess of 70%.

Zearalenone is relatively resistant to high temperatures and ultraviolet irradiation.

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