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SURVEY OF FOOD SAMPLES FOR THE PRESENCE OF MYCOTOXINS

By

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Systemetic investigation of toxin producing molds in food samples of rice, wheat, oilcakes revealed that *Aspergillus* and *Penicillium* species are predominant in those samples. Frequency of occurrence of *Aspergillus flavus* is maximum in rice whereas *Aspergillus* and *Penicillium* species both occur in wheat. Most of the mycotoxins are characterised by typical fluorescence, but no correlation can be made between the intensity of fluorescence & toxicity. Ochratoxin is produced by two new strains of *Aspergillus wentii*.

INTRODUCTION

A significant proportion of world production is destroyed by insects and micro-organisms. In the tropical and subtropical countries particularly in India the losses due to mold spoilage are of greater magnitude. A great deal of work has been done to show the occurrence of variety of fungi in stored food grains (Kirksey and Cole, 1974). Mycotoxin problem has got a worldwide fluvus. Since

1960, a number of mycotoxins have been discovered. The occurrence of mycotoxins in stored food grains depends on the type of fungi invading the grains. It seems to be necessary to isolate different fungal species and to determine the toxicity of each mold metabolites.

A large number of reports indicate that the ability to produce mycotoxins was mostly restricted among species of *Penicillium* and *Aspergillus* (Raper and Thom 1949). Very limited studies have, however, been made in India in this regard. The present paper describes a study on the distribution of toxin producing fungi in different stored food samples.

MATERIALS AND METHODS

Isolation & Identification of species of fungi: Most of the food samples specially, rice & wheat (both long stored and damaged) and few oil cakes such as, Niger Oil Cake, Ground Nut-oil Cake were collected from Food Corporation of India, Calcutta, Zonal Godown. These samples were then kept separately at 28°C and relative humidity at 85 to 90% for about one month to allow sufficient growth of different molds.

In a typical experiment, 10 g of moldy rice were mixed with 100 ml of sterile water in a 250 ml Erlenmeyer flask and shaken in a rotary shaker (120 R. P. M.) for 10 minutes. 0.5 ml of the suspension was then added to 15 ml of molten Czapek-Dox agar and then plated. The plates were incubated at 28°C for seven days. Streptomycin solution was added at a concentration of 30 µg per ml. prior to plating.

After seven days, fungal isolates were picked and examined under microscope. The identification of different species was done mainly on the basis of morphological characters (Raper and Funnell, 1965; Wells, John and Payne, 1975). For the measurement of fruiting structure due to great variability, at least five measurements were taken and the figure was taken from their average.

RESULTS

Distribution of toxic molds in different food grains : As a result of systematic investigation, 200 strains of fungi were isolated from 35 different food samples having their storage period ranging from 6 months to 3 years. It has been found that different species of the two genera *Aspergillus* and *Penicillium* are the predominant types. Out of 200 strains, 70% belong to different species of *Aspergillus* and the rest to *Penicillium* species. The results are shown in Table 1.

Table 1. Fungal isolates from different food samples

Food Samples	Storage Period	Fungal isolates
Rice	1 to 3 years --	<i>Aspergillus flavus</i> , <i>A. ochraceus</i> , <i>A. wentii</i> , <i>A. parasiticus</i> , <i>A. oryzae</i> , <i>A. tamarii</i> , <i>A. clavatoflavus</i> , <i>A. cremeus</i> , <i>A. versicolor</i> , <i>A. foetidus</i> , <i>A. niger</i> , <i>A. fumigatus</i> , <i>A. carbonaceous</i> , <i>Penicillium</i> <i>verruculosum</i> , <i>P. nigricans</i> , <i>P. islandicum</i> , <i>P. luteum</i> .
	2 to 3 years --	<i>A. fumigatus</i> , <i>A. pulvinus</i> , <i>A. thomii</i> , <i>A. spinulosus</i> , <i>A. ochraceus</i> , <i>A. terreus</i> , <i>A. zonatus</i> , <i>A. candidus</i> , <i>A. flavus</i> , <i>A. ochraceus</i> , <i>A. melleus</i> , <i>A. sulphureus</i> , <i>A. wentii</i> , <i>P. urticae</i> , <i>P. citrinum</i> , <i>P. chermesinum</i> , <i>P. meleagrinum</i> .
Wheat	2 to 3 years --	<i>A. fumigatus</i> , <i>A. pulvinus</i> , <i>A. thomii</i> , <i>A. spinulosus</i> , <i>A. ochraceus</i> , <i>A. terreus</i> , <i>A. zonatus</i> , <i>A. candidus</i> , <i>A. flavus</i> , <i>A. ochraceus</i> , <i>A. melleus</i> , <i>A. sulphureus</i> , <i>A. wentii</i> , <i>P. urticae</i> , <i>P. citrinum</i> , <i>P. chermesinum</i> , <i>P. meleagrinum</i> .
Oil-cakes	6 months to --	<i>A. flavus</i> , <i>A. oryzae</i> , <i>A. parasiticus</i> ,
	1 year	<i>A. niger</i> , <i>A. tamarii</i> , <i>A. ochraceus</i> , <i>A. cervinus</i> , <i>A. wentii</i> , <i>A. terricola</i> , <i>P. citrinum</i> , <i>P. steckii</i> , <i>P. viridaenutants</i> , <i>P. frequentans</i> , <i>P. nigricans</i> .

The frequency of occurrence of *A. flavus* group is maximum in rice and oil-cakes; whereas in wheat samples both *Aspergillus* and *Penicillium* species are predominant.

Preparation of crude toxins : At first the inoculum was prepared by subculturing the fungal isolates in potato-dextrose agar (PDA) medium and incubating at a temperature of 30°C for 7 days. A heavy spore suspension was prepared in sterile water.

Fermentation was carried out in 500 ml. Erlenmeyer flasks each containing 50 g of rice. The sample was moistened by tap water and kept for 2 hours with frequent shaking and then sterilized. After cooling each flask was inoculated with 20 ml of spore suspension and incubated at 28°C for 10 days. The flask content was stirred thoroughly with sterile glass rod every twenty four hours to break the rice clumps.

Table 2. Toxicity of crude culture-extract

LD 50 range (mg/g)	Organisms
.01-.02	<i>A. flavus</i> , <i>A. tamarii</i> , <i>P. cyclopium</i> , <i>A. wentii</i> , <i>A. clavato.flavus</i> , <i>A. parvathecius</i> , <i>A. fumigatus</i> , <i>P. citrinum</i> .
0.021-0.03	<i>A. clavato-flavus</i> , <i>A. zonatus</i> , <i>P. nigricans</i> , <i>A. parasiticus</i> , <i>A. oryzae</i> , <i>P. steckii</i> , <i>A. terricola</i> , <i>P. puberulum</i> , <i>A. flavus</i> .
0.031-0.04	<i>A. foetidus</i> , <i>P. janthinellum</i> , <i>A. niger</i> , <i>A. cervinus</i> , <i>P. chermesinum</i> , <i>A. multicolor</i> , <i>A. candidus</i> , <i>A. terreus</i> , <i>A. versicolor</i> , <i>A. sulphureus</i> , <i>P. terrestre</i> .
0.041-0.045	<i>A. ochraceus</i> , <i>P. funiculosum</i> , <i>A. flavus</i> , <i>A. nidulans</i> , <i>P. luteum</i> , <i>p. digitatum</i> , <i>A. paradoxus</i> , <i>A. cremeus</i> , <i>A. pulvinus</i> , <i>A. glaucus</i> , <i>P. variable</i> , <i>A. giganteus</i> , <i>P. urticae</i> , <i>A. janus</i> , <i>P. frequentans</i> , <i>A. melleus</i> , <i>P. verruculosum</i> , <i>A. cervinus</i> .
0.046-0.05	<i>P. roqueforti</i> , <i>A. viridi-nutans</i> , <i>P. citrinum</i> (variant) ¹ , <i>A. thomii</i> , <i>A. spinulosum</i> , <i>A. duricaulis</i> , <i>P. meleagrimum</i> , <i>A. chevalieri</i> .

After fermentation, 10-15 ml of Chloroform was added to each flask to destroy the fungus. After one hour moldy rice was taken in a mortar and finely ground. The pigmented toxic product was isolated by adding the mixture of chloroform and methanol (97 : 3) and kept overnight. Next this mixture was shaken thoroughly in 500 ml separating funnel. The pigmented toxic factor went in to the chloroform-methanol layer and collected. Then chloroform extract was concentrated in a rotary vacuum evaporator to dryness and pigmented solids were collected.

Toxicity studies with crude extracts : 40-50 mg of crude solid was taken in 10 ml propylene glycol and a clear solution was obtained. Then a definite amount of it was injected intraperitoneally to mice on body weight basis (15-25 g) and effect of toxic factor was noted after 24 hours, and the LD₅₀ range of toxin was then calculated as mg per g mice (Table 2).

DISCUSSION

As a result of systematic investigation, 200 strains of fungi were isolated from 35 different food samples having their storage period ranging from 6 months to 3 years. It has been found that different species of the two genera *Aspergillus* and *Penicillium* are predominant in the samples studied. They were identified upto the species level. Out of 200 strains 70% belong to different species of *Aspergillus* and the rest to *Penicillium* species. The frequency of occurrence of *Aspergillus flavus* group is maximum in rice and oil-cakes; whereas in wheat samples both *Aspergillus* and *Penicillium* species are common. Out of five strains of *Aspergillus flavus* tested for toxicity only two of them are highly toxic; Out of two *Aspergillus parasiticus* only one is toxic; none of the *Aspergillus ochraceus* is highly toxic; out of three *Aspergillus wentii* two are highly toxic. One *Penicillium citrinum* is highly toxic. Most of the mycotoxins are characterised by typical fluorescence on TLC plate when placed under ultraviolet light. The chloroform extract of the fungus shows bright to moderate green fluorescence under U. V. light. No direct correlation could be made between the intensity of fluorescence and toxicity. The most interesting observation, not reported

earlier is the production of ochratoxin by two strains of *Aspergillus wentii*. *P. citrinum* produces toxic citrinin. The production of citrinin by *Penicillium steckii* also deserves mention.

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