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Isolation of toxigenic fungi from stored cereal grains

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Study was conducted to determine toxigenice fungi associated with the stored grains is Rice, Wheat and maize.The collected grains were surface sterilized and plated on Czapek's Dox Agar media. Seven different storage fungi were isolated all together namely Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger, Penicillium sp., Rhizopus sp., Mucor sp. and Fusarium sp. In these Aspergillus flavus and Penicillium sp. were more prominent. Selected grains were subjected to thin layer chromatigraphic technique. However no toxins were identified.

Key words: *Storage fungi, Czapek's dox agar, Thin layer chromatography.*

Introduction : Growth of commonly occurring filamentous fungi in foods may result in production of toxins known as mycotoins, which may cause a variety of ill effect in human, from allergic responses to immunosupression and cancer. The term mycotoxin are usually reserved for toxic chemical products produced by fungi that readily colonize crops (Turner 2009). The production of toxins depends on the intrinsic and extrinsic environments and toxins vary greatly in their severity depending on the organism infected and its susceptibility, metabolism and mechanism (Hussein and Brassel 2001). The storage fungi especially *Aspergillus, Penicillium, Rhizopus* and *Mucor* species infect grains after harvest and grow on them during storage (Adams 1977) others toxigenic fungi frequently found on grains are *Alterneria, Paecilomyces, Chaetomium* and *Acremonium* (Dooley 2001). Toxicgenic fungi producing mycotoxins are potential problem for health and economic perspective. Generally, mycotoxin have been implicated as causative agent for different human and animal health disorder (Ciegler and Bennet 1980). In 1974, an outbreak of hepatitis that affect 400 Indian people, of whom 100 died, almost certainly resulted from aflatoxin (Krishnamachari et al 1974), as aflatoxin is most toxin and widespread mycotoxins, derives its name from fact that it was originally found to be produced by Aspergillus flavus (Agrios 1978). So, the main objective of the study was to isolate toxigenic fungi associated with wheat, rice and maize kept in different storage conditions. These findings will alert the consumers from consuming poorly stored grains.

Materials and Methods :

(a) Isolation of fungi associated to grains : 10 samples of wheat, rice and maize were collected from three different storage conditions. Then the selected grains were surface sterilized with 2% HgCl2 for 2 minutes and then rinsed with sterile water. Now, these grains were aseptically plated on Czapeks dox agar media composition: sodium nitrate – 2.0g, dipotassium hydrogen phosphate – 1.0g, magnesium sulphate – 0.5g, potassium chloride – 0.5g, ferrous sulphate – 0.01g, sucrose – 30.0g, agar – 15.0g for 1000.0ml distilled water (K.R .Aneja). The plates were incubated for 24 to 48 hours at 26 - 27°c. then the fungal growth of media were subjected to microscopy for identification.

(b) Detection of mycotoxin : mycotoxins are well suited for analysis by TLC since most of the compounds fluoresce strongly under long wave U.V light. Firstly the grains extracts was prepared in chloroform and stored in amber coloured bottle. Then the TLC plates were prepared with the help of silica gel G and 10µl grains extract was loded on it and kept in a solvent system containing toluene – ethyle acetate – formic acid (6:3:1). After 50 minutes the plates were observed under ultra violate chamber (Practical microbiology, Dubey and Maheshwari).

Result :

(a) Isolation of fungi from stored grains : 10 samples from each rice, wheat and maize were surface

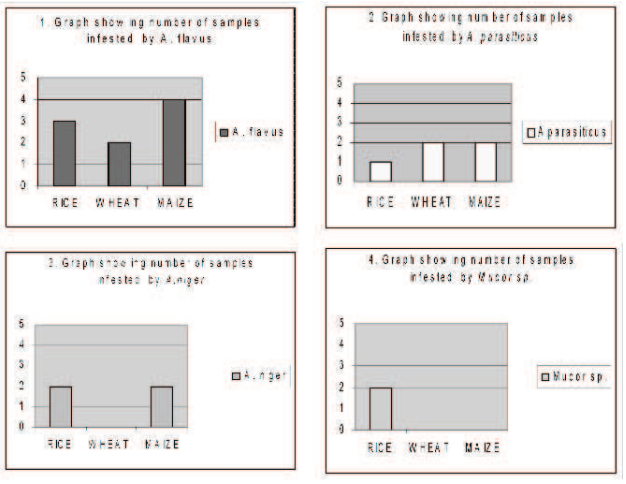
sterilized and were placed on Czapek's dox agar media. the plates were incubated at 27^{0}c for 48 hours. The colonies which emerged were identified, counted and number of plates with different fungal species, with respect to different samples were noted as shown in the table :-

Sl. N.	samples	Total no. of sample plated	No. of plated samples infested by fungal species						
			A. flavus	*A. parasiticus*	*A. niger*	*Mucor sp.*	*Rhizopus sp.*	*Penicillium sp.*	*Fusarium sp.*
1.	Rice	10	3	1	2	2	–	2	2
2.	Wheat	10	2	2	–	–	4	1	1
3.	Maize	10	4	2	2	–	–	3	–

1) Isolation fungi from rice : In rice out of total samples plated on czapek's dox agar media 3 samples had shown growth of *Aspergilus flavus*, 1 of *Aspergiillus parasiticus*, 2 of *Aspergillus niger;* 2 of *Penicillium sp*. and 2 of *Fusarium sp*.

2) Isolation fungi from wheat : In wheat out of total samples plated 2 samples had shown growth of *Aspergilus flavus,* 2 of *Aspergiillus parasiticus,* 4 of *Rhizopus sp*., 1 of *Penicillium sp.* and 1 of *Fusarium sp.*

3) Isolation fungi from rice : In maize out of total samples plated 4 samples had shown growth of *Aspergilus flavus*, 2 of *Aspergiillus parasiticus*, 2 of *Aspergillus niger* and 3 of *Penicillium sp*.



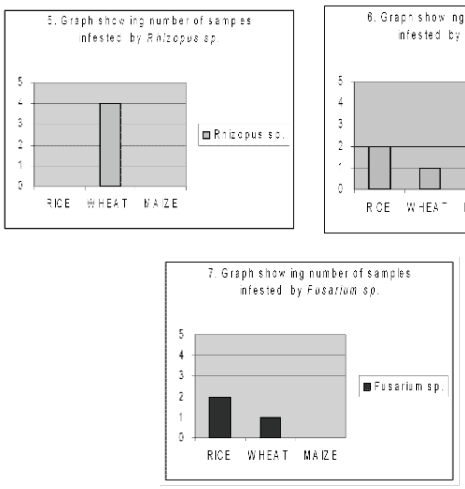


Figure (1- 7):-Showing number of plates of different grain samples infested by storage fungal species

The above figure shows that all the grains were infested to various degrees with storage fungi. And in all the infestation of grain by species of *Aspergillus* is higest while fungi such as *Rhizopus sp.* and *Penicillium sp*. Also infest the grains but at lower frequency than *Aspergillus sp.* Other fungi such as *Mucor sp*. and *Fusarium sp.* were also isolated in present study.

(b) Detection of mycotoxin ; For this the cereal grains extracted were prepared and loaded on TLC plates, these plates were kept into system of solvent and then kept in U.V. chamber for observation, no fluorescent spots were visible. Hence no result on TLC of grain extract.

Discussion and conclusion:-

7 fungi were isolated from stored maize, rice and wheat samples in this study.

The Aspergillus species isolated *Aspergilus flavus*, *Aspergiillus parasiticus* were recovred in high count in all most all the cereals while *Aspergillus niger* were recovered, comparativly lesser. According to Agrios (1978), the most common storage fungi are *Aspergillus* and *Penicillium species*, and also stated that some major species of Aspergillus produce toxin called aflatoxin for example *Aspergilus flavus. Penicillium sp.* were also recovered from cereal samples in moderate or low frequency of occurance. *Pencillium sp*. are reported to produce ochratoxin A (Vanwalheek , 1969). *Mucor sp.,*

Rhizopus sp. Fusarium sp. and other genera and species isolated in this study were found to occur either in low frequencies or rare frequencies on the Czapek's dox agar media plates. There are several mycotoxin produced by these fungi for example rhizonin A secreted by Mucor damage kidney and liver of rat (Visconti & Sibilia, 1994), zerelenone and chlamydosporol secreted by Fusarium sp. (Marasus, 2001). These toxins cause serious health problems in humans and animals hence , there is need of more epidemiological studies in present era.

Detection of mycotoxin on fungal contaminated cereals; the cereals with positive growth of mycoflora were selected and extract were prepared and loaded on TLC plates for mycotoxin detection in U.V. light, but no florescent spot were observed this may be because of low concentration of mycotoxin production in cereals. Prolonged improper storage can cause production of mycotoxin by the isolated strains of fungi.

From this study we can conclude by saying, however no mycotoxins were observed in this study yet the fungus obtained for example *A. flavus, A. parasiticus, Penicillium sp., Fusarium sp*. etc. can potentially produce mycotoxin (as per literature review). Hence, further studies on mycotoxin production by such fungal would be of great interest.

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