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QUALITATIVE ANALYSIS OF AFLATOXIN FROM *ASPERGILLUS FLAVUS* ISOLATES

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Abstract

Different isolates of *Aspergillus flavus* were isolated from five varieties of groundnut. These isolates were grown on liquid SMKY medium. By using TLC the cultural filtrate produced by these isolates were screened for Aflatoxin production. About 80% of *A. flavus* isolates were found to produce aflatoxin, out which 100% isolates produces aflatoxin (B_1), 30% isolates produces B1 and B2, 10% isolates produces B1 and G1 aflatoxin.

Key Words: Isolates; *Aspergillus flavus*; TLC; Aflatoxin.

Introduction

The species of *Aspergillus* are found commonly occurring as post harvest molds in storage conditions. Most of the species of *Aspergillus* are dominant and play vital role in the seed biodeterioration. Nearly 250-300 fungal species are reported to be associated with seeds in varieties of ways. Fungal organisms plays significant role in infection, altering quality and longevity of seeds during the storage (Christensen and Kanfman, 1969).

Sargeant *et. al* (1961) first reported toxin production by *Aspergillus flavus* grown on sterile peanuts and in Czapek's solution. Austwick and Ayerst reported that 11 of 59 isolates of *A. flavus* produces toxin. In western parts of India 100% contamination of maize samples and oil seeds with aflatoxin was reported by Krishnamachri *et a*l. Consumption of aflatoxin contaminated food has also been correlated with the occurrence of Indian childhood cirrhosis (*Amla et al.* 1974) and hepatomegaly (Sreenivasamurthy, 1975).

Though many studies on the incidence of aflatoxigenic fungi and natural occurrence of aflatoxin in oil seeds, oil cakes and chewing products have been made earlier by Verma *et al.* (1991, 1995). The present investigation is an attempt to screen aflatoxin from *Aspergillus flavus* isolates, isolated from abnormal groundnut seeds.

Material and Methods

Collection of Oilseed samples and Isolation of Aspergillus flavus from oil seeds

Four different varieties of groundnut seeds (i.e. Pasari, Tag-11, Tag-45, Ghungru) were collected from market places, store houses, fields from different parts of Marathwada region of Maharashtra state. These seeds were then packed in pre-sterilized polythene bags. Mycoflora associated with groundnut seeds were detected using agar plate method (ISTA, 1996). Ten seeds per pre-sterilized petriplates were equispaced aseptically on autoclaved PDA and RBA media. Plates were then allowed to incubate at room temperature for seven days. On seventh day of incubation the seeds were examined under stereoscopic microscope for the preliminary determination of fungal growth on them. Detail observation of fungal characters was done under the binocular microscope and their identification was confirmed with standard literature (Ellies1971; Mukadam, 1997). Fungal colonies formed were identified and percent incident of each fungus was calculated.

Screening of Aspergillus flavus isolates for Aflatoxin producing ability

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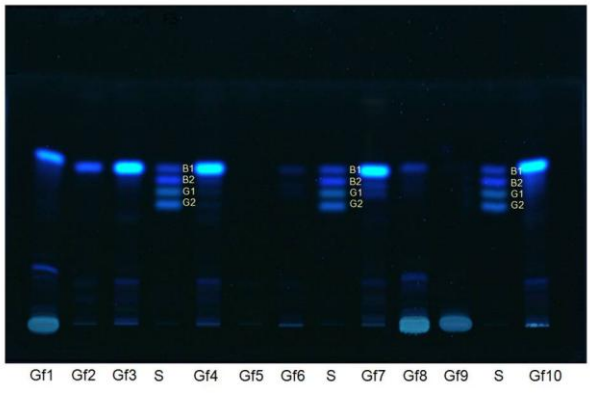
Isolates of *Aspergillus flavus* obtained were screened for their Aflatoxin-producing potentials in SMKY liquid media (Sucrose- 200gm, Magnesium sulphate- 0.5gm, Potassium nitrate- 3gm, yeast extract- 7dm and distilled water 1000ml) (Diener and Davis, 1966). 25ml of SMKY liquid medium was taken in 250 ml flasks and culture of *Aspergillus flavus* was inoculated with 25 ml medium aseptically. Triplicates were maintained for each isolates of *A. flavus.* These were kept at 26 to 30°C for 8 days. On 9th day mycelial mat was separated from the medium through whatmen filter paper number 1. 5ml of culture filtrate was extracted twice with 10 ml of chloroform in a separating funnel. The polled chloroform extract was passed through a bed of anhydrous sodium sulphate, which was evaporated, to dryness on water bath (60°C). The residue was dissolved in 1 ml of chloroform and kept in a small vial.

C) Qualitative assay of Aflatoxin By TLC

i) Preparation of TLC plate: Uniform glass plates (20 X 20) was thoroughly cleaned with distilled water. 30 gm of silica gel was mixed with 60 ml of distilled water in a stopper flask (250 ml). And was shaken vigorously for one min. the resulting slurry was poured to the plate spreader which was adjusted to 25 mm with a firm and smooth action the spreader was drawn across the plates to coat them. The chromatography plates were left to semi dry in a dust free atmosphere for about 16 minutes and then transferred to an oven at 110 °C for 1hrs. Activated plates were then used on the same day.

HPTLC showing the Screening of B1, B2, G1, G2 aflatoxin



ii) Spotting on TLC plate: A line at 15 cm from the bottom edge of the activated but cooled TLC plate was ascribed as solvent spot. The final sample extract was dissolved in 5 ml chloroform in a small vials. 50 µl of chloroform extract was spotted on TLC plate along with the standard aflatoxin at 4 cm from bottom edge. Spotting was done with micro pipette and TLC guide was used for making all the spots in straight line, paralleled to bottom edge.

iii) Development of Chromatogram: **S**potted chromatography plates were developed in TLC tank containing running solvent of Toluene : Isoamyl alcohol : Methanol in ratio of 90:32: as suggest by Reddy *et al.,* (1970) 100 ml of solvent system was poured in TLC tank well before the development of chromatoplates for homogenous saturation. The developed plates were air dried and then observed under ultraviolet light (360 nm.).

Results and Discussion

Amongst 10 fungi isolated from the different groundnut varieties *A. flavus, A. niger, A. ustus* and *A. terrus* species were more frequent. The incidence of *A. flavus* was highest in Pasari, Tag-11, Tag-45 as compare to Ghungru (Table 1).

Table 1. Percent incidence on different varieties of groundnut

Isolates	Varieties of groundnut			
	Pasari	Tag-11	Tag-45	Ghungru
A. flavus	30	30	30	10
A.fumigatus	10	-	-	10
A. glaucus	-	10	10	-
A. nidulance	-	-	10	-
A. niger	30	20	20	20
A. parasitus	-	-	-	10
A. terrus	10	20	-	-
A. ustus	20	10	20	30
A.versicolor	-	-	10	-
A. oryzae	-	10	-	10

Table 2. Aflatoxin production by *Aspergillus flavus* isolates from different varieties of groundnut

Name of Isolates with groundnut Seed	B_1	B_2	G_1	G_2
Variety (Pasari)				
G.f1	++	-	-	-
G.f2	++	-	-	-
G.f3	+++	+	-	-
Variety (Tag-11)				
G.f4	+++	+	-	-
G.f5	-	-	-	-
G.f6	+	-	-	-
Variety (Tag-45)				
G.f7	+++	++	+	-
G.f8	+	-	-	-
G.f9	-	-	-	-
Variety (Ghungru)				
G.f10	+++	+	-	-

+++ - Maximum; ++ - Moderate; + - Minimum

Out of ten isolates of *Aspergillus flavus,* eight isolates produce aflatoxins. Isolate Gf3 of var. Gf4 of var. Tag-11, Gf7 of var. Tag-45, Gf10 of var. Ghungru are able to produce the aflatoxin B1 at maximum level. On the other hand Gf2 and Gf1 of var. Pasri are moderately produce the aflatoxin B1. Aflatoxin B2 is produced at

minimum level by isolate Gf3 of var. Pasri, Gf4 of Tag-11, Gf10 of var. Ghungru, except isolate Gf7 of var. Tag-45 which produce B2 moderately. It is interesting to note that only Gf7 of var. Tag-45 show production of aflatoxin G1 at minimum level. Aflatoxin G2 is absent in all the isolates (Table 2).

References

1. Austwick, P. K. and Ayesrst C. 1963. Toxic products in groundnuts. Groundnut microflora and toxicity, Chem. And Ind. (London) 1963:55-61.
2. Sargeant J., A. Sheridan, J. O'Kelly and R. B. A. Carnaghan. 1961. Toxicity associated with certain samples of groundnuts. Part II. Nature 192:1096-1097.
3. Amla, I., Sreenivasamurti, V., Jayaraj, A.P. and parpia, H.A.B. (1974). Afltoxin in India childhood cirrhosis-A review *J. Environment child health* 20: 28-33.
4. Krishnamachri, K.A.V.R., Bhat, R.V., Nagarajan, V., Tilak, T.B.G. (1975). Hepatitis due to aflatoxicosis: An outbreak in western India. *Lancet,* 1: 1061-1063.
5. Sreenivasamurthy, V. (1975). Mycotoxin in foods. *Proc. Nutr. Soc., India* 19: 1-6.
6. Verma, R.J., Raval, P.J. and Dube, H.C. (1991). Effect of aflatoxin on liver and blood cells of rats. *Indian J. Microbiology* 31:87-89
7. Christensen, C.M. and Kaufman, H.H. 1969. *Grain storage*. The role of fungi in quality losses. Univ. Minnesota, Press Minneapolis.ISTA (1996) *Seed Sci. Tech*. 24 (suppl.): 335-337.
8. Ellies, M.B. (1971). Dematiaceous hypomycetes,Cab International,Wallingford.
9. Mukadam, D.S. 1997. The Illustrations of Fungi. Akshar Ganga Prakashan, Aurangabad
10. Diener, U. L. and Davis, N. D. (1966). Aflatoxin production by isolates *Aspergillus flavus. Phytopathology* 56:1390-1393.
11. Reddy, T. V., Viswanathan, L. and Venkitasubramanian, T.A. (1970). Thin-layer chromatography of aflatoxin. *Anal. Biochem.* 38: 568-571.