Science Research Reporter, **5**(1):24-29, (April - 2015)

Online available at http://jsrr.net
ISSN: 2249-2321 (Print); ISSN: 2249-7846 (Online)
Research Article

Culture characteristics and aflatoxin producing strains of *Aspergillus flavus* from maize grains

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Article Info

Received: 01-02-2015,

Revised: 16-03-2015,

Accepted: 21-03-2015

Keywords:

Aspergillus flauvs, aflatoxin, ammonia vapors, test maize, HPTLC.

Abstract

The known mycotoxin the most important which have been studied mostly because of possible hazards to human health are the afltoxins.Aflatoxins produced very commonly by two species of Aspergillus viz. *Aspergillus flavus* and *Aspergillus parasitic.* In this study growth pattern and culture characteristics of *Aspergillus flavus* isolates were studied. Twelve strains of A. flavus were isolated from three varieties of maize seed. To study the growth pattern of different isolates of *A.flavus*, CZA and GNA media were used. The different strains of *A.flavus* showed great variation in diameter of colony and growth pattern. There was also variation in the colour of colony from both sides. *Aspergillus flavus* is the most important aflatoxin producing mould. This species in maize produces carcinogenic, mutagenic and teratogenic secondary metabolites. Estimation of aflatoxin is generally monitored by ELISA & HPTLC.. Twelve isolates of *Aspergillus flavus* isolated from three maize varieties were tested by cultural method for aflatoxin detection, which is inexpensive and rapid. Two isolates viz. AF1 and AF12 were found highly toxic which showed dark pink colour development ,while AF2 were found moderately toxigenic ,because these isolates turned into moderate pink color, while eight isolates viz. AF3, AF4, AF5, AF7, AF8, AF9, AF10, AF11 exhibited light pink colour after treatment with ammonium vapor ,were low toxigenic. Among all the isolates tested only one viz. AF6 isolate were found to be nontoxigenic. The results of ammonia vapors test were up to 90% with the result of toxin detection by high pressure thin layer chromatography (HPTLC).

INTRODUCTION

Aflatoxins, metabolites of the fungus *A. flavus*, are potent liver toxins and carcinogens in animals and may also be human carcinogens (Mohamed Zain, 2011). The fungus *A. flavus* produces the mycotoxin known as aflatoxin on a number of crops including corn, peanut, and cotton. Typically, the fungus has a yellow green appearance when it is growing on corn kernels. The fungus is quite common in nature, but its population increases during hot dry weather. Aflatoxin contamination is greater in corn that has been produced under stress conditions (Mogle, 2014). Some analytical issues such as sampling, methods for their analysis and emerging mycotoxin detection techniques Mahedra Rai *et al.*, (2012). Aflatoxin is an important carcinogenic toxin produced by *Aspergillus flavus, and Aspergillus paracitus.*

In the present research work production of aflatoxin at the level of G1, G2, B1, B2 of different isolates of *Aspergillus flavus* were studied. Total 12 isolates of *Aspergillus flavus*, from three varieties of maize were screen for the ability of aflatoxin production by using HPTLC technique. Conventional method used to differentiate aflatoxin production and non-producing strains of *Aspergillus flavus* group are based on culture on natural and or on artificial media which permits the release of aflatoxin, (Bennet and Papa, 1988). There are many highly specific and sensitive method for determining aflatoxin concentration in commodities or in cultures, such as high performance liquid chromatography (HPTLC), enzyme linked immunosorbent assay (ELISA) and thin layer chromatography (HPTLC) etc. (Margos and Thompson, 1999 and Dorner, 2002). Usually these methods are expensive and consuming. In this study twelve isolates of *Aspergillus flavus* isolated from maize grains were tested for their toxigenicity by Ammonia vapors test and compare with thin layer chromatography for percent accuracy Saito and Machida, 1999). Ammonia vapour treatment method is inexpensive and less time consuming, and would be ideal for pre-screening of large number of *Aspergillus flavus* isolates to identify non-aflatoxigenic isolates that may be evaluated as potential bio control agent or use in limiting mould infection and aflatoxin production in agricultural crops.

MATERIALS AND METHODS

In this method, pre-sterilized corning glass petriplates of 10cm diameter were poured with 15ml of autoclaved potato dextrose agar (PDA) medium. On cooling the medium, ten seeds per petriplates of the test sample were placed at equal distance aseptically. Incubation conditions and other details were maintained.

Isolation of *Aspergillus flavus* strains:

To study the growth pattern of different isolates of *A. flavus*, CZA and GNA media were used. Three varieties of maize namely Kaweri, Supper-900 and All-rounder were used as a source of *Aspergillus flavus* isolated. The seeds of maize varieties were incubated on PDA medium for 4 days at 28 ± 2°C and thereafter *Aspergillus flavus* strains were isolate. Culture maintained on PDA medium for further experiments.

The dishes were inverted and 2 ml of concentrated ammonia solution (SRL extra pure AR Grade) were poured onto the inside of the lid. Within ten minutes the undersides of aflatoxin producing isolates turned into pink/red, from creamy or yellow colour. Addition of few drops of glacial acetic acid into lid of ammonias vapour treated plates converted the colour of culture back to normal as before the ammonia vapour treatment, Saito & Machida (1999).

Evaluation of toxigenic Potential of *A. flavus*:

A. flavus isolates obtained from maize seeds were screened for their aflatoxin production potential in SMKY liquid medium (Sucrose 200gm, Mg 30g. $7H_2O$- 5 gm, KNO_3-3gm and Yeast extract 7gm / lit) by following the methods of Diener and Davis (1966). *A. flavus* isolated were grown on 25ml of sterilized SMKY medium for 9 days at 28 ± 2°C and thereafter culture filtrates were extracted with chloroform to screen the presence of aflatoxin.

Estimation of aflatoxin:

The qualitative estimation of aflatoxins was carried out from a 10 µ of samples applied with the help of instrument CAMAG automatic HPTLC samples 4 (ATS 4) on activated HPTCL plates. The aflatoxin standard (B_1, B_2, G_1 and G_2) were also applied on HPTLC plated 10 the form of bands and then developed with a solvent system consisting of chloroform: acetone: water developed HPTLC plates were examined under long UV light (366) estimation was done by comparison of retention their and peak height & areas observed in the aflatoxin standards with those observed for samples.

RESULTS AND DISCUSSION

1) Growth pattern and culture characteristics of *Aspergillus flavus* isolates:

To study the growth pattern of different isolates of *A. flavus*, CZA and GNA media were used (Table no. 01 and photo plate no. 1). It is clear from the table and photographs, that, the different strains of a*pergillus flavus* showed great variation in diameter of colony and growth pattern. Af1, Af4, Af7, Af9 and Af11 showed constricted colony pattern on CZA medium whereas Af2, Af8 and Af10 shows lawn smooth colony and Af5, Af6, Af12 shown zonate colony pattern. Diameter of colony were more in Af6, Af8 and Af12, on the other hand it was smaller in Af2, Af4, Af9. On glucose nitrate agar medium, colony pattern were concentric in Af1, Af2, Af3, Af7, Af9, Af11 and Af12 whereas lawn in Af3, Af5, Af6 and Af10 were with diameter in between 4.5 to 8.5 mm.

Table 1: Growth Pattern of different isolates of *Aspergillus flavus*.

Source f *A.*olate	No. of *A. flavus* isolte	On CZA Medium				On GNA Medium			
		Colony Diameter (cm)	Colony Pattern	Colour of Colony		Colony Diameter(cm)	Colony Pattern	Colour of Colony	
				Front	Reverse			Front	Reverse
Maize seeds variety Kaweri	Af1	8.5	Constricted	Yellowish Green	Pale Yellowish Orange	7.0	Concentric rings	Durty Green	White
	Af2	8.5	Lawen, in hieat morgine	Yellowish green & dark green	Yellowish	4.5	Concentric rings	Greenish	White
	Af3	8.0	Lawn constricted margine	Yellowish green	Gray	6.5	Lawn gron	Durty green	White
	Af4	5.5	Constricted	Yellowish green	Brown orange	5.5	Concentric rings	Green	White
Supper 900.	Af5	8.0	Zonate	Yellowish green	White	4.5	Lawn	Durty green	White
	Af6	9.0	Zonate	Green	Durty white	3.5	Lawn	Green	White
	Af7	7.5	Constricted at center & smooth at margine	Yellow at center green at margine	Orange	4.5	Concentric	Green	white
	Af8	9.0	Lawn	Green	Durty white	6.5	Lawn at center and Concentric ring	Durty Green	White
All Rounder	Af9	6.5	Constricted smooth at margine	Durty green	Plae orange	7.0	Concentric rings	Green	White
	Af10	7.5	Lawn, smooth at margine	Yellow at center green at maize	Durty white	8.5	Lawn	Green	White
	Af11	4.0	Constricted intiremargire	Green yellow green at margien	Orange	4.5	Concentric rings	Green	White
	Af12	8.8	Zonate	Green	Orange	5.0	Concentric rings	Green	White

There was also variation in the colour of colony from both sides i.e. front and reverse. Mostly yellowish green colony showed on CZA medium whereas on glucose agar medium it was green colour.

Screening of *A. flavus* isolates for Aflatoxin Production Potentials:

For qualitative assay of aflatoxin, Thin Layer Chromatography method was followed. Twelve isolates of *A. flavus* from three maize varieties were screened for Aflatoxin production potential and results are summarized in table no. 2 and photo plate no 2.

It is clear form Table that, form twelve isolates of *Aspergillus flavus*, nine isolates showed the production of Aflatoxin, two isolates viz. Af5 & Af6 which isolated from maize verity Supper-900 and one isolate Af10 from verity All rounder did not produce aflatoxin. All the nine aflatoxin producing strains were showed the presence of B_1 and B_2 in variable quantities. At very high concentration isolates Af2 and Af3 were produce

aflatoxin G_1, G_2. Concentration of aflatoxin B1 was very high in all nine isolates. The concentration aflatoxin B_2 was comparatively low. On the basis of aflatoxin production potentiality *Aspergillus flavus* strains which isolated from verity Kaweri were 100% toxigenic, verity Supper 900 were 50% and verity All rounder were 75% toxigenic

Rapid detection of aflatoxin and non toxigenic isolates of *Aspergillus flavus* by Ammonium vapour test:

It is clear from the Table no.2 and photo plate no 2 that among twelve isolates of *A. flavus* two isolates viz. Af1 and Af12 were found highly in nature, which showed dark pink colour development, while Af2 were found moderately toxigenic, because these isolates turned into moderate/pink colour, while eight isolates viz. Af3, Af4, Af5, Af7, Af8, Af9, Af10 and Af11 exhibited light pink colour after treatment with ammonium vapour, were low toxigenic. Among all the isolates tested, only one viz. Af6 isolate were found to be non-toxigenic. Aflatoxin:

Seed health emerged as an important component of food security. Though seed health is not a mandatory quality attributed its relevance to yield and quality loss of food grains cannot be ignored. In fact, it has emerged as a major concern in trans-boundary spread and in International seed trade. The seed borne pathogen can reduce the plant stand and contribute for disease epidemics. The seed health information is also required to evolve management strategy (Prakash and Udaya Shankar, 2009; Mogle, 2013).

It is clear from the literature that all the isolates of *Aspergillus flavus* not found to be potential to produce aflatoxins. This has also been observed in the present investigation. All the twelve isolates of *Aspergillus flavus* were screened for aflatoxin production only two of these were not found to be aflatoxigenic in nature. It was also observed by rapid detection method for toxigenic and nontoxigenic isolates of *Aspergillus flavus* (table no.1 and 2 and photo plate no.3). Similar types work done by Jaimez *et al.* (2001) and Sangit kumar *et al.* (2007).

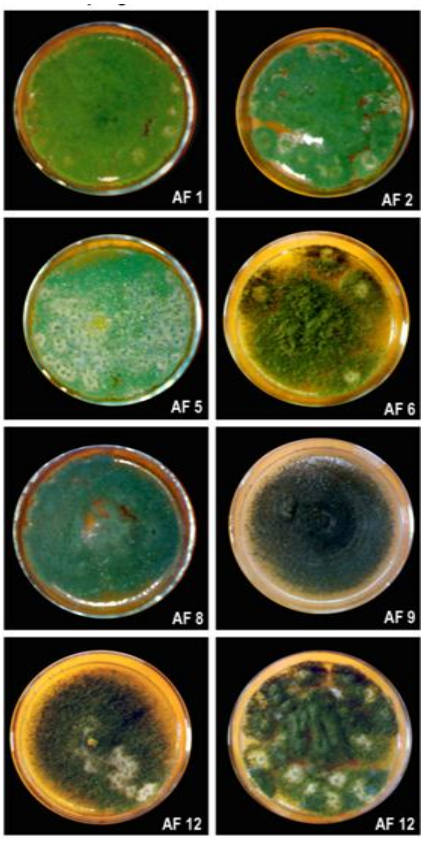

Plate no. 1. Growth pattern of different isolates of *Aspergillus flavus* on CZA medium

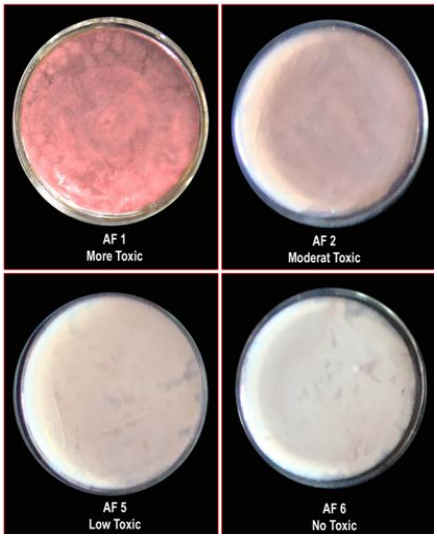

Plate no. 2. Rapid technique for identification of toxigenic and Non-toxigenic isolates of *Aspergillus flavus*

Table No. 2. Rapid Identification of toxinogenic and nontoxinogenic strains of *Aspergillus flavus* by ammonium vapour method.

***Aspergillus flavus* Isolates.**	**Intensity of Pink Colouration**	**Toxicity**
Af1	Dark Pink Colour	More Toxic
Af2	Pink Colour	Moderat Toxic
Af3	Light Pink Colour	Low Toxic
Af4	Light Pink Colour	Low Toxic
Af5	Light Pink Colour	Low Toxic
Af6	No Pink Colour	No Toxic
Af7	Light Pink Colour	Low Toxic
Af8	Light Pink Colour	Low Toxic
Af9	No Pink Colour	No Toxic
Af10	Light Pink Colour	Low Toxic
Af11	Light Pink Colour	Low Toxic
Af12	Dark Pink Colour	More Toxic

Table No. 3: Production of Aflatoxin in different isolates of *Aspergillus flavus*.

Name of Isolates with Source	**B_1**	**B_2**	**G_1**	**G_2**
Maize Seed Variety Kaweri (M_1)				
Af1	++	+	+	-
Af2	+++	+	-	-
Af3	++	-	-	-
Af4	++	+	+	-
Maize Seed Variety Supper 900 (M_2)				
Af5	-	-	-	-
Af6	-	-	-	-
Af7	++	-	-	-
Af8	++	-	-	-
Maize Seed Variety All Rounder (M_3)				
Af9	++	-	-	-
Af10	+	-	-	-
Af11	++	-	-	-
A12	+++	+	+	-

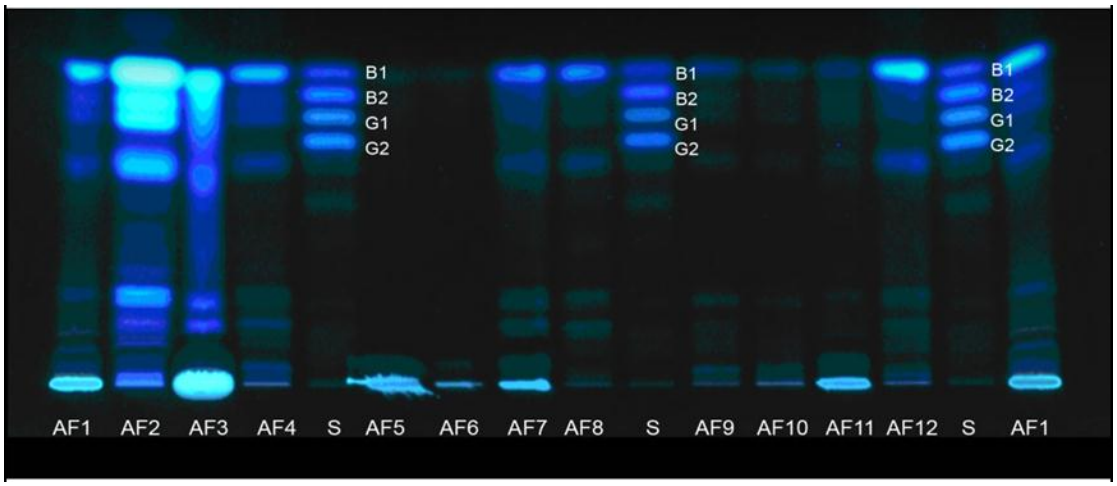

Plate no. 3. HPTLC showing the screening of Aflatoxin B1, B2, G1 and G2

ACKNOWLEDGEMENT
Authors are thankful to UGC for their financial assistance of this work and Principal, Lal Bahadur Shastri Senior College Partur (M.S.) for providing research facilities.

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How to Cite this Article:
Anil U. Kulkarni and Ashok M. Chavan, 2015. Culture characteristics and aflatoxin producing strains of *Aspergillus flavus* from maize grains. *Science Research Reporter*, **5**(1):24-29.