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RESEARCH ARTICLE

QUALITATIVE ANALYSIS OF AFLATOXIN FROM *ASPERGILLUS FLAVUS* ISOLATES FROM *CITRUS* SPP.

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ABSTRACT

Most of the species of Aspergillus are dominant and play vital role in the biodeterioration of fruits. Fungal organisms plays significant role in infection, altering quality and longevity of fruits during the storage. The present investigation is an attempt to screen aflatoxin from. Aspergillus flavus isolates, isolated from different citrus spp. Different isolates of *Aspergillus flavus* were isolated from Orange (*Citrus aurantium*), Lemon (*Citrus aurantifolia*) and Mosambi (*Citrus sinensis*). These isolates were grown on liquid SMKY medium. By using TLC the cultural filtrate produced by these isolates were screened for Aflatoxin production. About 75% of *Aspergillus flavus* isolates were found to produce aflatoxin, out which 95% isolates produces aflatoxin (B_1), 30% isolates produces B1 and B2, 10% isolates produces B1 and G1 aflatoxin.

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INTRODUCTION

Fruits are important food items of our diet. The high concentration of various sugars, minerals, vitamins and amino acids provide a good platform for the successful growth and survival of various parasitic and saprophytic forms of fungi (Fatima *et al*., 2010). Post harvest deterioration of fruits may take place in any stages viz. storage, transit or trans- shipment, during handling processes required to move the crop from the grower to the whole sale dealer and to retailer and finally to consumer. These are responsible for enormous qualitative and quantitative losses of fruits in the market. The fruit infection phase of the disease can result in serious economic loss. 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 fruit losses. Nearly 250-300 fungal species are reported to be associated with fruits in varieties of ways. Fungal organisms plays significant role in infection, altering quality and longevity of fruits during the storage (Christensen and Kanfman, 1969).

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Austwick and Ayerst reported that 11 of 59 isolates of *A. flavus* produces toxin. Though many studies on the incidence of aflatoxigenic fungi and natural occurrence of aflatoxin in fruits. The present investigation is an attempt to screen aflatoxin from *Aspergillus flavus* isolates, isolated from Citrus fruits.

MATERIALS AND METHODS

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

Three different fruits of Citrus (Orange (*Citrus aurantium*), Lemon (*Citrus aurantifolia*) and Mosambi (*Citrus sinensis*) 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 citrus fruits were detected using agar plate method (ISTA, 1996). Fruit peels 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

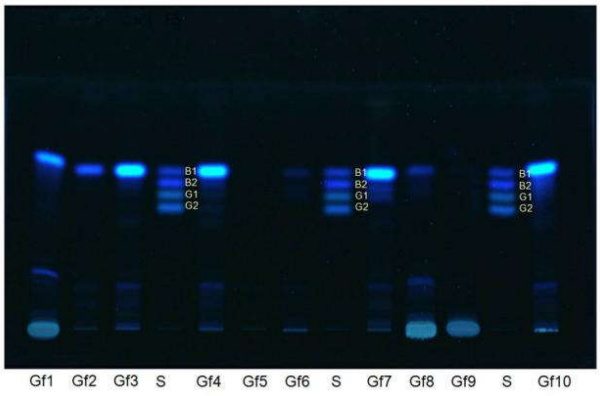
standard literature (Ellies, 1971; Mukadam, 1997). Fungal colonies formed were identified and percent incident of each fungus was calculated.

Screening of Aspergillus flavus isolates for Aflatoxin producing ability

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^{o}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^{o}C). The residue was dissolved in 1 ml of chloroform and kept in a small vial.

Qualitative assay of Aflatoxin By TLC

Preparation of TLC plate: a uniform glass plate (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 oC for 1hrs. Activated plates were then used on the same day.



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 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.

Development of Chromatogram: Spotted 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 Citrus fruits *A. flavus, A. fumigatus, A. niger, A. ustus* and *A. terrus* species were more frequent. The incidence of *A. flavus* was highest in *C. sinensis*, *C. reticulate* as compare to *C. linom.* Whereas *C. linom* is absent in *C. reticulate* (Table 1).

Table 1. Percent Incidence of *Aspergillus* species on citrus fruits

Aspergillus species	*C. linom*	*C.Sinensis*	*C. reticulate*
A. flavus	17.4	27.4	27.4
A. fumigatus	2.3	2.3	2.3
A. glaucus	0.5	0.2	0.4
A. nidulance	0.2	0.5	0.5
A. niger	25.2	31.3	37.4
A. oryzae	0.1	2.1	1.2
A. parasiticus	0.5	1.0	0.6
A. terreus	2.2	2.7	1.5
A. ustus	2.1	1.4	0.7
C. linom	0.2	0.7	-

Among all isolates of *Aspergillus flavus,* all isolates produce aflatoxins. Isolate *A. flavus* 4 of *C. linom* produce B2. In *C.Sinensis* isolates of *A. flavus* produce B2 as well as G1. In *C. reticulate* some isolates of *A. flavus* produce B2 and one isolate i.e. *A. flavus 6* produce G1. It is interesting to note that *C. linom* show production of aflatoxin B2 at minimum level. Aflatoxin G1 and G2 is absent in all the isolates of *C. linom.* G2 is absolutely absend in all isolates of all fruits (Table 2).

Table 2. Qualitative detection of a Aflatoxin by *Aspergillus flavus* isolates from different Citrus fruits

Isolates	B_1	B_2	G_1	G_2
C. linom				
A. flavus 1	+	-	-	-
A. flavus 2	+	-	-	-
A. flavus 3	+	-	-	-
A. flavus 4	+	+	-	-
A. flavus 5	+	-	-	-
C.Sinensis				
A. flavus 1	+	+		
A. flavus 2	+	-	-	-
A. flavus 3	+	+	+	-
A. flavus 4	+	-	-	-
A. flavus 5	+	+	+	-
A. flavus 6	+	-	-	-
A. flavus 7	+	+	+	-
A. flavus 8	+	+	-	-
A. flavus 9	+	+	-	-
C. reticulate				
A. flavus 1	+	-	-	-
A. flavus 2	+	-	-	-
A. flavus 3	+	+	-	-
A. flavus 4	+	+	-	-
A. flavus 5	+	-	-	-
A. flavus 6	+	+	+	-

+ Present - Absent

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