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## FUNGAL AND AFLATOXIN CONTAMINATION IN SOME CULTIVATED MEDICINAL PLANTS

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### ABSTRACT

Drug plants especially roots of *Withania somnifera* (Dunal.), *Asparagus racemosus* (Willd.) and *Rauwolfia serpentina* (Benth.), rhizomes of *Glycyrrhiza glabra* (Linn.) and fruits of *Embllica officinalis* (Gaerth.) showed fungal contamination in Ayurvedic reparations, out of which *Aspergillus* was found to be dominant. Aflatoxins B<sub>1</sub> and B<sub>2</sub> were also found to be associated with *A. flavus*

**Key words:** Medicinal plants, *Aspergillus*, aflatoxin, contamination

### Introduction:

The medicinal plants get affected by number of fungi during cultivation as well as storage, affecting their medicinal value. It has been observed that the roots of *Withania somnifera* (Dunal.), *Asparagus racemosus* (Willd.) and *Rauwolfia serpentina* (Benth.), rhizomes of *Glycyrrhiza glabra* (Linn.) and fruits of *Embllica officinalis* (Gaerth.) often get contaminated with various pathogenic fungi, which produce Aflatoxins. The fungi *Aspergillus flavus* and *A.parasiticus*. produce Aflatoxins B<sub>1</sub> and B<sub>2</sub>. *A.parasiticus*. produce Aflatoxins B<sub>1</sub> and B<sub>2</sub>. Roy et al.(1988) reported that among 50 isolates of *A.flavus*, obtained from different medicinal plants, 21 isolates were toxigenic, 12 could produce aflatoxin B<sub>1</sub>, while 9 isolates were capable of producing aflatoxin B<sub>1</sub> and B<sub>2</sub>. Present study was undertaken on the fungal contamination and aflatoxin production by *A. flavus* isolates, and resulting aflatoxin contamination in crude herbal drugs.

### Material and Methods:

The strains of *A.flavus* were isolated from seeds, fruits, rhizomes, roots and stem samples of different medicinal plants and

maintained on Czapek Dox agar medium. These isolates were screened for their aflatoxigenic nature by organic yellow or reverse pigmentation method (Bothast and Fennel 1974).

The aflatoxin producing potential of *A. flavus* isolates was measured on semisynthetic SMKY liquid medium (Diener and Davis, 1966) For this purpose, the isolates were grown on SMKY medium in 100 ml Erlenmeyer flasks and incubated for 8 days at 25° 1°C. After incubation period, contents of each flasks were filtered through Whatman No.1 filter paper. The filtrates of each isolates were collected in pre-sterilized bottles and termed as crude toxin preparations.

The crude toxin preparation thus obtained was mixed with equal volume of chloroform in a separating flask and shaken well. After the separation of two phases, the chloroform phase was taken into a flask containing 10 g sodium sulphate to absorb remaining water. The clear chloroform solution was concentrated to a known volume by evaporating chloroform and stored in an amber coloured vial under refrigeration.

About 30g silica gel 'G' (with CaSO<sub>4</sub> as binder) was taken in a stopper flask and mixed with 60 ml distilled water. The slurry was uniformly spread using applicator on clean plate keeping the thickness of layer 0.25 mm.

The dry plate was activated at 110°C for 30 min. in a hot air oven. The gel on plate was divided into a number of portions by drawing lines with sharp needle.

The chloroform extracts of toxins were then spotted in known volume in various lines carefully with micro-capillary tube on an imaginary line 2.5 cm away from one end of the plate. The plate was developed in solvent system of toluene : ethyl acetate: formic acid (6:3:1) in a chromatographic tank for 50 min till solvent reached upto 20 mm below the top end of the plate.

The plate was dried at room temperature and the flourescing spots of toxins were visualized under UV lamp in an UV cabinet (360 nm). The blue flourescent spots were identified as aflatoxin B<sub>1</sub> and B<sub>2</sub> on the basis of the intensity of the flourescent spots.

**Results and Discussion**

**Table 1. Production Aflatoxins (B<sub>1</sub> & B<sub>2</sub>) in different isolates of *Aspergillus flavus* on SMKY medium.**

Isolate with source	B <sub>1</sub>	B <sub>2</sub>
<i>Withania somnifera</i>		
WIAF-1	++	++
WIAF-2	+++	+++
WIAF-3	++	+
<i>Asparagus racemosus</i>		
ASAF-1	++	+++
ASAF-2		
<i>Glycyrrhiza glabra</i>		
GLAF-1	+++	+++
GLAF-2	+++	+++
GLAF-3	++	++
<i>Rauwolfia serpentina</i>		
RAAF-1	+	+
RAAF-2		
<i>Emblica officinalis</i>		
EMAF-1	+	+
EMAF-2	-	-

+ = very poor, ++ = poor, +++ = moderate, ++++ = high, and +++++ = luxuriant

WIAF *A. flavus* isolated from root of *W. somnifera*,

ASAF *A. flavus* isolated from root of *A. racemosus*,

GLAF *A. flavus* isolated from rhizome of *G. glabra*,

RAAF *A. flavus* isolated from root of *R. serpentina*,

EMAF *A. flavus* isolated from fruit of *E. officinalis*

**Table 2. Effect of temperature on aflatoxin production.**

Plant Materials	10	20	30	40
<i>W. somnifera</i> (Root)	-	+	++	+
<i>A. racemosus</i> (Root)	-	++	++	++
<i>G. glabra</i> (Rhizome)	-	+	++	++
<i>R. serpentina</i> (Root)	-	+	++	+
<i>E. officinalis</i> (fruit)	-	++	++	++

**Table 3. Effect of incubation period (5 to 60 days) on aflatoxin production.**

Plant Materials	5	10	20	30	60
<i>W. somnifera</i> (Root)	-	-	+	++	++
<i>A. racemosus</i> (Root)	-	-	+	++	++
<i>G. glabra</i> (Rhizome)	-	-	+	++	++
<i>R. serpentina</i> (Root)	-	-	+	++	++
<i>E. officinalis</i> (fruit)	-	+	++	++	++

Twelve strains of *Aspergillus flavus* isolated from five different medicinal plants were screened for their ability to produce aflatoxins on SMKY semisynthetic liquid

medium. The results are summarized in Table 1.

It was observed that three isolates of *A. flavus* from *Withania* roots produced both aflatoxin B<sub>1</sub> and B<sub>2</sub>. Among the isolates WIAF-2 proved to be moderate producer of aflatoxin, while WIAF-1 and WIAF-3 produced lesser amount.

Among two isolates of *A. flavus* from *Asparagus* roots, ASAF-1 proved to be highly aflatoxigenic which was followed by isolate ASAF-2.

Among the three isolates from *Glycyrrhiza* rhizome, isolates GLAF-2 proved to be moderate producer of aflatoxin, while GLAF-1 and GLAF-3 were found to be very poor in aflatoxin production.

In case of the two isolates from *Rauwolfia* roots, isolates RAAF-1 were found to be moderate producer of B<sub>2</sub> aflatoxin, RAAF-2 produced very poorly. From *Emblica* fruits, isolate EMAF-1 proved to be very poor in production of aflatoxin, while in EMAF-2, there was no production at all.

It is clear from the results given in Table 3, that none of the isolate showed aflatoxin production up to 10 days of incubation

period, while in the samples incubated for 30 days to 60 days the production of aflatoxin was slightly increased.

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