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AFLATOXIGENICITY OF *A.flavus* ISOLATED FROM PHYLLOSPHERE OF BANANA PLANT

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ABSTRACT

Aflatoxins are toxic metabolities produced by certain fungi in/on foods and feeds.They are the best known and most intensively researched mycotoxins. The occurrence of Aflatoxins is influenced by certain environmental factors; hence the extent of contamination varies with geographic location, agricultural and agronomic practices, and the susceptibility of commodities of fungal invasion during pre-harvest, storage and/or processing periods. Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans.

KEYWORDS: *Aspergillus flavus*, Aflatoxin, Phyllosphere, Banana

Aflatoxin is a mycotoxin produced by the members of *Aspergillus flavus* group (Raper and Fennell, 1965) of fungi (Kulik and Holaday, 1966). Among the A.flavus group, *A.flavus* link and A.parasiticus species are the most active aflatoxin producers (Hesseltine *et al,* 1966). Their presence in the phylloplane of papaya (Prasad, 1976), Banana (Jha, 1977), leaves as well as in the aeromycosporal complex of grain godowns of Bairginia (Prasad, 1992) and aerospora of Samastipur (Roy, 1993) and Bhagalpur (Ghani, 1994) in Bihar and Gulberga (Murlimanohar and Reddi, 1995) of Bhilai (M.P), (Shahu, 1996) have been extensively studied and their results further confirm the present observation that *A.flavus* isolates were more potent among the 51 other isolates of *Aspergillus* species encounterd in the phyllosphere of Banana (Var., Malbhog) plant during the present investigation. Therefore, the Aflatoxin producing potency was evaluated to highlight the gravity of situation of high incidence of *A. flavus* isolates in phylloplane of banana plant and their Aflatoxigenic potency.

MATERIALS AND METHODS

For the purpose of screening of Aflatoxigenic isolates, individual isolates were inoculated in a culture flask containing liquid rice flour medium and were allowed to incubate for 10 days at room temperature (28°±2°c). The inoculated flasks were daily shaken for ½ hour manually or on a wrist action electric shaker. At the end of incubation period the culture filtrates were collected after harvesting the mycelia mats over whatman filter paper no. 1. 5.0ml of the culture filtrates were taken in separating funnel (250ml) and were added with 25ml of chloroform. The mixtures were thoroughly shaken for 20 minutes after which the clear lower aliquots were allowed to pass through a bed of anhydrous sodium sulphate kept on the filter paper. The sodium sulphate was rewashed with 10ml. chloroform.

For the estimation of Aflatoxins, T.L.C. technique was applied. The solvents were allowed to run upto 15cm in vertical direction after which the plates were taken out of the chromatography tank and air dried in a dust free condition. The plates were later observed under long wave UV- lamp (365nm) in complete darkness for the spot of Aflatoxin. The confirmation of Aflatoxins were done by the derivative formation with TFA (Tri fluro acitic acid) as suggested by Stack and Pohland (1975).

RESULTS AND DISCUSSION

The *Aspergilli* Constitute a major component of the aerobiota especially around the Banana field, of which some eleven species were found associated with the Phylloplane mycoflora of this plant. The secondary metabolite of *A. flavus* is well known for its toxigenic ,teratogenic and carcinogenic potency. In total 51 *A.flavus* isolates were collected of which 12 were found to be Aflatoxigenic in nature (i.e 23.4 % of the total). Among *Aspergilli, A. niger, A. flavus, A. fumigatus* and *A. terreus* are well known toxigenic as well as allergenic forms and if such forms dominate in the aerospora then there may be greater possibility of Aflatoxicosis of lungs to the human beings living around the orchard.

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On the basis of present findings it can be said that the incidence of toxigenic isolates are more frequent on the nature of agricultural commodities including fruit and vegetables. However, the frequency of appearance may vary in different geographic regions during varying climatic conditions. Table 1 and Table 2 have shown the screening of several isolates collected from leaf surface of banana and quantitative production of Aflatoxin from A.flavus respectively.

Table 1: Showing screening of *Aspergillus flavus* isolates collected from leaf surface of banana during different months for their toxigenic potentialities on RFM media in 11 days of incubation at room temperature (24° ± 2°C)

Sr. No.	Isolates of the months	Presence or absence of toxin on RFM medium
1	A.f. Jan - (1-3) 3 isolates	-
2	A.f Feb - (4-7) 4 isolates	-
3	A.f March - (8-9) 2 isolates	++
4	A.f April - (10 – 15) 6 isolates	++++
5	A.f May - (16 – 19) 4 isolates	++++
6	A.f June - (20 – 24) 5 isolates	++++
7	A.f July - (25 – 31) 7 isolates	++
8	A.f Nov. - (32 – 42) 11 isolates	++
9	A.f Dec. - (43 – 51) 9 isolates	++

Total isolates: 51, RFM: Rice flour medium (Misra & Sinha, 1979)

Table 2: Showing quantitative production of aflatoxin B1 and qualitative elaboration of other aflatoxins by the 12 toxigenic isolates of *Aspergillus flavus* collected from phyllosphere complex of Banana (malbhog variety) plant on RFM medium in 11 days of incubation at room temperature. (24° ± 2° C)

Sr. No.	Isolate No. (Month & Isolate no.)	Quantity of aflatoxin B1 μ*g*/25 ml.	B_2	G_1	G_2
1	A.f. Ma. - 9	110.50 μ*g*	+	t	-
2	A.f. Ap. - 11	186. 64 μg	++	+	t
3	A.f. Ap - 12	180. 88 μg	++	-	-
4	A.f. Ap. - 15	206.30 μg	++	+	-
5	A.f. My. - 17	247.30 μg	++	++	+
6	A.f. My. - 18	218.84 μg	++	+	t
7	A.f. Jn. - 20	198.62 μg	++	t	-
8	A.f. Jn. - 22	210.50 μg	++	++	+
9	A.f. Jn. - 23	194.46 μg	-	+	-
10	A.f. Jl. - 25	102.60 μg	-	+	-
11	A.f. Nov. - 41	93.30 μg	++	+	t
12	A.f. Dec. - 44	82.85 μg	++	+	-

Mean Average --- 169.482 μg

STASTISTICAL ANALYSIS OF TABLE

SE = 0.1670

CD = ± 0.6399 at 1 % level of P

Summary of statistical analysis:

Replicates - Non - Significant

Isolates - Highly Significant

Isolate No. (Month & Isolate no.)	Quantity of aflatoxin B1 μ*g*/25 ml.
A.f. Ma. - 9	110.50 μg
A.f. Ap. - 11	186. 64 μg
A.f. Ap - 12	180. 88 μg
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A.f. Jn. - 20	198.62 μg
A.f. Jn. - 22	210.50 μg
A.f. Jn. - 23	194.46 μg
A.f. Jl. - 25	102.60 μg
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