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Aflatoxin production potentiality of *Aspergillus flavus* strains associated with maize rhizosphere

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Altogether 61 *Aspergillus flavus* strains isolated from maize rhizosphere soil of Jharkhand state were screened for their aflatoxigenicity. Of which 32 isolates were found positive. Out of these, 14 strains of *A. flavus* produced only aflatoxin B_1, 17 strains both B_1 and B_2 and only one strain was found to produce G_1 aflatoxin but none of the isolate was noticed to produce G_2. The quantity of aflatoxin B_1 produced by all the toxigenic strains varied from trace to 830 µg/l.

Key words: *A. flavus,* Aflatoxin production, maize rhizosphere

INTRODUCTION

Aflatoxins, highly toxic and carcinogenic chemical compounds are produced as secondary metabolites by certain toxigenic strains of *A. flavus* isolates (Sargeant *et al.,* 1961). Natural occurrence of aflatoxins in agricultural commodities viz. maize grains, pulses, oil seeds, other cereals, dry fruits and crude herbals is a world wide problem but greater in tropic than temperate zone (Roy *et al.,* 1988; Roy, 1989). Bilgrami *et al.* (1982) have reported the natural contamination of aflatoxin in maize and their food products beyond the tolerance level as fixed by WHO i.e. 30 ppb in Indian context. Sinha (1980) has also reported that maize is one of the richest substrates for growth of *A. flavus* and aflatoxin production. Bilgrami *et al.* (1981) after an exhaustive survey of almost all maize growing areas of old Bihar (including Jharkhand) state have recorded high % incidence of aflatoxin in standing maize crops but he could not evaluate the role of rhizospheric isolates of *A. flavus* on aflatoxin contamination in standing maize. The population of toxigenic *A. flavus* groups has been isolated from the rhizosphere zone of cotton, peanut, maize and other cereals by several earlier workers (Cotty, 1997; Horn and Dorner, 1998; Wicklow, 1998). Preharvest contamination in relation to standing maize crops is due to the landing of toxigenic inoculums of *A. flavus* originating either from aerosphere or from rhizosphere. Keeping this in view the present study is targeted to examine aflatoxin production potentials of toxigenic *A. flavus* strains in maize rhizosphere.

MATERIALS AND METHODS

Survey and collection of sample

Nine districts of Jharkhand State viz., Sahibganj, Pakur, Dumka, Deoghar, Giridih, Hazaribagh, Ranchi, Chaibasa and Daltonganj were surveyed for the collection of rhizosphere soil of maize plant having the age group up to 45 days. Ten samples from each district were randomly collected in separate polybag and properly maintained. *Aspergillus flavus* strains were isolated from all the samples collected from each district by serial dilution technique on PDA medium.

Evaluation of aflatoxigenicity of A. flavus

A. flavus isolates were screened in SMKY liquid medium for their aflatoxigenicity (Sucrose-200, $MgSO_4$, $7H_2O$-0.5, KNO_3-3 and Yeast extract-7 g./l) by following the methods of Diener and Davis (1966). *A. flavus* isolates were grown on 25 ml of sterilized SMKY medium for 9 days at 28±2°C and thereafter culture filtrates were extracted with chloroform.

Qualitative and quantitative assay of aflatoxin

For qualitative assay of aflatoxin thin layer chromatography method by using Toluene: Isoamyl alcohol and Methanol (90 : 32 : 2) as running solvent system (Reddy *et al.*, 1970) was followed. The developed plate was studied under UV light in order to determine congers of aflatoxins on the basis of their Rf value and colour. Quantitative assay of aflatoxin B_1 spot developed on TLC plate was assayed by Spectrophotometric method of Nabney and Nesbitt (1965). Chemical confirmation of aflatoxin was made by Trifluoroacetic acid as suggested by Stack and Pohland (1975).

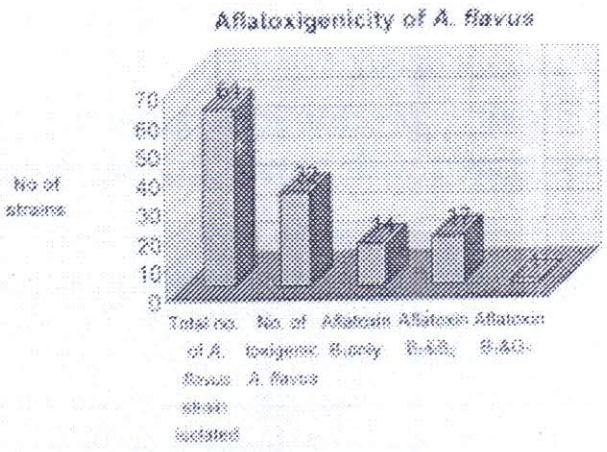

Fig. 1 : Aflatoxigenicity of *A. flavus* isolates

RESULTS AND DISCUSSION

A total of 61 strains of *A. flavus* were isolated from maize rhizosphere soil samples of which the maximum no. (9) of strains were isolated from the Sahibganj and Ranchi samples whereas minimum i.e. 4 from the samples of Pakur district. Several earlier workers (Agnihothrudu, 1955; Edward, 1962; Nesci and Etcheverry, 2002) isolated fungi dominated with species of *Aspergillus, Fusarium and Penicillium* and bacteria from rhizosphere soil of various crops. During the screening of aflatoxigenic isolates of *A. flavus*, only 32 were found to be positive for aflatoxin production, however, their production potential varied from trace to 830 µg/l. Mehan and Chouhan (1973) screened 21 isolates of *A. flavus* obtained from seed surface of cotton, maize and wheat and recorded only 16 as toxigenic. Richard and Cysewski (1971) screened 15 strains of *A. flavus* isolates from mould corn sample and found only 7 having aflatoxin production potentials. Results also indicated that there was qualitative variation in production of aflatoxin congers, as some strains produced both B and G series of aflatoxins and some others only B series. Of all 32 toxigenic isolates 14 strains were found to produce aflatoxin B_1 only, 17 strains both B_1 and B_2 and only one isolate produced both B_1 and G_1 but none of the strain was noticed to produce G_2 (Fig. 1). Hesseltine *et al.* (1968) observed similar phenomenon in a number of toxigenic isolates. This behaviour of different isolates for producing different types of aflatoxin might also assigned to their genetical differentiation. None of the isolates was able to produce G_2 series of aflatoxin which supports the findings of Bennett (1987). Hesseltine *et al.* (1966) also reported that *A. flavus* produced both aflatoxin

Table 1: Isolation and screening of *A. flavus* isolates for their aflatoxin production potentials.

Source of *A. flavus* isolates	No. of *A. flavus* isolates	No. of Toxigenic isolates	toxigenic strain (%)	No. of Aflatoxigenic isolate			Range of afl. B_1 (µg/l)
				B_1	B_1B_2	B_1G_1	
Sahibganj	9	5	55	2	3	–	102-636
Pakur	4	3	75	2	1	–	65-830
Dumka	8	4	50	2	2	–	350-636
Deoghar	6	4	66	1	2	1	T-355
Giridih	5	3	60	1	2	–	T-630
Hazaribagh	8	3	37	1	2	–	75-460
Ranchi	9	4	44	2	2	–	92-830
Chaibasa	6	3	50	2	1	–	72-630
Daltonganj	6	3	50	1	2	–	120-680

'T' denotes Trace

B_1 and B_2 but little or no G_1 and G_2. Quantitative variation of aflatoxin in different strains might be assigned to the difference in the nature of strain. Maggon *et al.* (1969) proposed the genetical hypothesis to explain differences in the quantitative elaboration of toxin by the various isolates.

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