J. Indian bot. Soc Vol. 86 (3 & 4) 2007 : 60-63

BIO-SCREENING OF MAIZE RHIZOSPHERE MYCOFLORA TO CONTROL AFLATOXIN PROBLEMS

N. L. MANDAL, A. N. SINGH, S. ROY AND A. K. ROY

University Dept. of Botany
T. M. Bhagalpur University, Bhagalpur-812007.

Aflatoxigenic strains of *A. flavus* inhabiting in rhizosphere soil are the bioagents of maize seed contamination and aflatoxin production in standing crops. To prevent this biological event in developing maize, nine co-existing fungi were individually tested *in vitro* against highly aflatoxigenic strain of *A. flavus* (860µg/lt).Though interaction behaviours were varied type, moreover percent aflatoxin production was reduced to 34%-75% in SMKY liquid medium. A significant loss in dry mycelial weight was also recorded.

Key words: Maize rhizosphere fungi, *A. flavus*, aflatoxin control.

Among all known mycotoxins, aflatoxins are very common and natural contaminants of foods and feeds. These are produced as secondary metabolites by toxigenic *A. flavus* and are reported to be highly carcinogenic, mutagenic, and teratogenic compounds posing serious health hazards to consumers. In nature, *A. flavus* is frequently found associated with numerous other microorganisms. Its co-existence may influence the production of aflatoxin in stored seeds and grains (Reddy & Reddy, 1983). In order to prevent the growth of aflatoxigenic *A. flavus* and subsequently aflatoxin production some earlier workers (Singh *et al.*, 1989; Roy and Chourasia, 1990; Chourasia and Sinha, 1993; Chourasia and Roy, 1993; Roy and Kumar, 1996, 1997; Janisiewic and Korsten, 2002 and Hua *et al.*, 2003) have screened different fungi isolated either from air or from agricultural commodities or crude herbals but none of them could study interaction between co-exiting fungi of maize rhizosphere and *A .flavus*. Maize is an important crop of India and in new Bihar state it is cultivated on large scale and consumed directly as food. Starchy nature of maize seeds followed with warm and humid condition of India in general and this region in particular make it as a susceptible crop for *Aspergillus* contamination and aflatoxin production. In addition, maize plants secrete more root exudates than any other crop resulting more microbial diversity. Therefore, an attempt has been made to isolate different fungi along with *A. flavus* from maize rhizosphere to achieve workable data of co-existing fungi and their interaction against toxigenic *A. flavus* strains which may be one of the important sources of aflatoxin contamination in standing maize crop.

MATERIALS AND METHODS

Survey and collection of samples: For this entire maize growing area of North Bihar state was divided into seven sites viz- Bagha, Bettiah, Vaishali, Samastipur Saharsa, Khagaria , Purnia and samples in triplicate were collected from each site randomly and tagged as S_1,S_2,S_3,S_4,S_5,S_6,and S_7 respectively.

Isolation of mycoflora: *A. flavus* and co-existing fungi were isolated from maize rhizosphere by serial dilution technique on PDA medium.

Evaluation of toxigenic potential of *A. flavus*: *A. flavus* isolates obtained from rhizosphere soil were screened for their aflatoxin production potentials in SMKY liquid medium (Sucrose-200 gm, $MgSO_4.7H_2O$- 5gm, KNO_3-3gm & Yeast extract-7gm/lit) by following the methods of Diener & Davis (1966). *A. flavus* isolates were grown on 25 ml of sterilized SMKY medium for 9 days at 28± 2^0C and thereafter culture filtrates were extracted with chloroform to screen the presence of aflatoxin.

Table 1: Diversity and % occurrence of fungi in maize rhizosphere soil samples.

S.N	Fungal Isolates	S1	S2	S3	S4	S5	S6	S7
1	*Aspergillus flavus*	60	40	50	20	40	30	80
2	*A. niger*	60	60	30	40	60	40	50
3	*A. candidus*	20	10	-	30	40	20	10
4	*A. terreus*	40	-	40	60	20	30	50
5	*A.nidulans*	20	10	-	-	20	10	-
6	*A.fumigatus*	-	10	20	20	40	10	10
7	*A. ochraceus*	-	20	10	-	30	-	10
8	*A. sydowi*	20	10	-	10	10	20	30
9	*Alternaria tenuis*	40	20	30	10	10	20	-
10	*Alternaria alternata*	60	30	10	-	-	20	20
11	*Cephalosporium roseum*	50	-	-	20	10	30	-
12	*Cladosporium herbarum*	30	-	20	-	-	-	10
13	*Curvularia lunata*	20	10	20	20	10	10	40
14	*C.clavatum*	10	-	-	-	10	20	10
15	*Fusarium oxysporum*	60	30	-	20	20	10	-
16	*F.solani*	-	-	20	30	10	-	10
17	*F.semitectum*	30	20	-	10	10	20	20
18	*F.chlamydosporum*	20	-	-	10	30	-	10
19	*Trichothecium roseum*	-	-	10	-	20	-	-
20	*Mucor mucedo*	30	60	60	20	30	10	40
21	*Monilia brunnea*	20	-	-	-	-	10	-
22	*Rhizoctonia solani*	-	-	10	20	-	20	20
23	*Helminthosporium g ramineum*	20	20	-	40	30	20	20
24	*Penicillium chrysogenum*	30	10	10	10	20	-	10
25	*P.rubrum*	-	-	-	10	30	20	-
26	*P.citrinum*	10	-	20	30	-	20	20
27	*Verticillium sp.*	-	30	20	-	-	20	30
28	*Botrytis sp.*	-	10	-	10	-	10	20
29	*Periconia sp.*	40	10	-	30	20	-	-
30	*Stachybotrys atra*	30	20	10	-	-	40	30
31	*Rhizopus stolonifer*	30	10	-	10	30	10	10
Fungal diversity		23	20	17	22	23	24	23

Soil sample representing collection sites S1-Bagha, S2- Bettiah, S3-Vaishali, S4- Samastipur, S5- Saharsa, S6- Khagaria , S7- Purnia

and G_2 were separated. Quantitative assay of each aflatoxin spot on TLC was assayed by Spectrophotometric method of Nabney & Nesbitt (1965). Chemical confirmation of aflatoxin was done by Trifluoroacetic acid as suggested by Stack and Pohland (1975).

Screening of antagonistic fungi: Interaction between toxigenic *A.flavus* and co-existing fungi was observed by dual culturing technique on PDA medium at 28± 2^0C for 5-8 days. Interaction were recorded and antagonists were categorized as A, B, C, D and E type on the basis of criteria laid down by Johnson & Curl (1972).

Aflatoxin assay in co-culture medium: Effect of co-existing fungi on aflatoxin production by *A. flavus* was studied under laboratory condition in SMKY medium. For this, 25 ml of sterilized SMKY was taken into 150 ml of conical flask and antagonists were allowed to grow. One day after, 1 ml spore suspension of *A. flavus* was poured aseptically in each flask where no. of spores maintained almost the same. Control sets were kept with *A. flavus* only. The inoculated flasks were incubated at 28± 2^0C for 9 days and thereafter aflatoxin was extracted (Diener and Davis, 1966). Percentage inhibition of aflatoxin production in

Table 2: Screening of *A. flavus* isolates for Aflatoxin production potentials.

Source of *A. flavus* isolates	No. of *A. flavus* isolates	No. of toxigenic strains	% toxigenic strain	Qualitative assay of aflatoxin			Range of afl. B_1 (µg/lt)
				B_1	B_1B_2	B_1G_1	
S1	5	3	60	1	2	-	320-785
S2	4	2	50	-	1	1	535-760
S3	2	1	50	-	1	-	690
S4	3	1	33	1	-	-	320
S5	4	1	25	1	-	-	658
S6	6	2	33	1	1	-	86-520
S7	5	2	40	1	1	-	380-720

Qualitative and quantitative assay of aflatoxin: For qualitative assay of aflatoxin Thin Layer Chromatography method of Reddy *et al.*, (1970) was followed by using Toluene: Isoamyl alcohol and Methanol (90:32:2) as running solvent system and congeners of aflatoxin i.e. B_1, B_2, G_1 relation to loss in mycelial biomass was also determined against control.

RESULTS AND DISCUSSION

Altogether 31 fungi were isolated from maize

Table 3: Interaction behaviors of co-existing fungi against aflatoxigenic strains of *A. flavus*

Name of the fungi	Mycelial dry wt. of *A.flavus*./25 ml. (in mg).	Type of interaction	% inhibition of mycelial dry wt.	Afla. B_1 production (in µg/lit.)	% inhibition of afla B_1 production
Aspergillus flavus (control)	750	-	-	860	-
A. terreus	225	B	70	215	75
A. candidus	405	C	46	516	40
A. nidulans	367	A	41	431	49
Fusarium chlamydosporum	240	B	68	240	72
Penicillium chrysogenum	210	E	72	249	71
P. rubrum	277	D	63	301	65
Helminthosporium gramineum	450	B	46	455	47
Rhizoctonia solani	330	A	56	412	52
Trichothecium roseum	540	A	29	567	34

Note: Type A- mutual intermingling of the two organisms; B- Mutual inhibition on contact; C- mutual inhibition at a distance; D- inhibition on contact, the antagonist continues to grow, unchanged or reduced rate, through the colony of the inhibited organism; E- inhibition at a distance, the antagonist continues to grow through the resulting clear zone at an unchanged or reduced rate.

rhizosphere soil samples and their diversity and percentage occurrence were determined (Table-1). The results clearly illustrate that diversity and percentage occurrence of fungi were found to vary with the sites. Soil sample Nos S_1, S_5 and S_7 had 23 fungi whereas highest diversity of fungi was recorded in soil sample S_6.Out of fungi isolated *A. flavus A. niger*, *Curvularia lunata* and *Mucor mucedo* were isolated from all samples. Species of *Aspergillus*, *Fusarium* and *Penicillum* constituted dominant flora .The percentage occurrence of target fungus i.e. *A. flavus* was recorded above 20% in all samples, however, its highest occurrence (80%) was recorded in S_7 sample. Out of 29 isolates of *A. flavus* isolated only twelve strains were found to have aflatoxin production potentiality with a range of 86-860µg/lt (Table-2).

On preliminary screening only nine co existing fungi were found to inhibit the growth of toxigenic *A. flavus* as well as aflatoxin production (Table-3). In the dual culture of *A. flavus* and *Rhizoctonia solani*, *Trichothecium roseum*, and *Aspergillus nidulans* separately showed A type interaction whereas *Helminthosporium gramineum*, *Aspergillus terreus* and *Fusarium chlamydosporum* B type; *Aspergillus candidus* C type; *Penicillium rubrum* D type and *Penicillium chrysogenum* E type.

The maximum percentage inhibition of aflatoxin production was recorded by *A. terreus* (75%) which was followed by *F. chlamydosporum* (72%), *P. chrysogenum* (71%), *P. rubrum* (65%), *R. solani.* (52%), *A. nidulans* (49%), *H. gramineum* (47%), *A. candidus* (40%) and minimum i.e.34% was recorded in case of *T. roseum.* Percent increase/decrease in mycelia dry biomass of antagonists was also recorded to compare with the production of aflatoxin.

Earlier workers including Roy and Chaurasia (1990), Choudhary and Sinha (1993), and Roy and Kumar (1997) have also noticed similar result of inhibition of aflatoxigenic strains associated with different crops and crude herbal drugs. In general they have suggested that such inhibitory response is due to antibiosis which might be accomplished by the secretion of test fungal diffusates.

Authors are thankful to Head, University Department of Botany, T.M. Bhagalpur University, Bhagalpur for providing laboratory facilities and also to DST, Government of India for financial assistance.

REFERENCES

Chaudhary A K & Sinha K K 1993 Competition between a toxigenic *Aspergillus flavus* strain and other fungi on stored maize kernels. *J Product Res* **29**(1) 75-80.

Chourasia H K & Roy A K 1993 Growth and aflatoxin production by *Aspergillus parasiticus* with co-occurring fungi and bacteria. *J Indian Bot Soc* **72** 131-134.

Diener U L & Davis ND 1966 Aflatoxin production by isolates of *Aspergillus flavus*. *Phytopath* **55 (12)** 1390 -1393.

Dorner J W Cole R J & Wicklow DT 1999 Aflatoxin reduction in corn through field application of competitive fungi. *J Food Protection* **62** 650-656.

Janisiewic W & Korsten L 2002 Biological control of post harvest diseases of fruits. *Ann Rev Phytopathol* **40** 411- 441.

Hua S-ST 2003 Reduction of aflatoxin in pistachiothrough biological control of *Aspergillus flavus*. In *California pistachio commission 2002 Production Research Report Fresno* CA pp 197-202.

Johnson L F & Curl E A 1972 *Methods for research on the ecology of soil borne plant pathogens.* Burgess Publishing Co Minnesota, pp 305.

Nabney J & Nesbitt B E 1965 A spectrophotometric method for determining the aflatoxin. *Analyst, Lond* **90** 155–160.

Reddy T V, Viswanathan L & Venkitasubramanian TA 1970 Thin layer chromatography of aflatoxin. *Anal Biochem.* **38** 568–571.

Reddy A S & Reddy S M 1983 Interaction of different seed borne fungi on Til (*Sesamum indicum* L.) and its effect on aflatoxin production by *Aspergillus flavus.* Proc Symp In Food & Feed pp 293.

Roy AK & Chourasia H K 1990 Inhibition of aflatoxin production by microbial interaction. *J Gen Appl Microbial* **36** 59-62.

Roy A K & Kumar S 1996 Effect of some medicinal plants extracts on *Aspergillus flavus* growth & aflatoxin production. *J Indian Bot Soc* **75** 225-227.

Roy A K & Kumar S 1997 Microbes for the prevention of aflatoxin contamination in herbal medicines. In *Phytodiversification and Human Welfare* eds. AK Roy, JVV Dogra and SK Verma. M D Publications, New Delhi pp 27-34.

Singh P, Ahmad S K & Bhagat S 1989 Microbial interaction and aflatoxin production. *J Indian Bot Soc* **68** 169-171.

Stack M E & Pohland A E 1975 Collaborative study of a method for chemical confirmation of identity of aflatoxin. *J Assoc Anal Chem* **58** 110-113.