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EFFECT OF SOME CO-EXISTING MOULDS ON AFLATOXIN PRODUCTION IN WHEAT GRAINS UNDER COMPETITIVE ENVIRONMENT

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ABSTRACT

The effect of co-existing mycoflora with *Aspergillus flavus* was observed on wheat grains for its influence on aflatoxin B_1 and G_1 production. All 17 types of associations of different fungal species inhibited aflatoxin B_1 production at different levels (48.21-90.23%). Among the screened co-existing moulds, the minimum inhibition in aflatoxin B_1 production was caused by *A. japonicus* and the maximum inhibition was caused by *Penicillium citrinum* (90.23% and *Trichoderma viride* (90.15%). However, aflatoxin G_1 production was completely inhibited by *Aspergillus fumigatus, A. niger, Candida albicans, Chaetomium globosum, Penicillium citrinum, Trichoderma viride* and a toxigenic strain of *Aspergillus flavus.* These results suggest the potential of co-existing moulds in biological control against pre and post harvest aflatoxin contamination of agricultural commodities.

KEY WORDS: Biocontrol, Aflatoxin, *Aspergillus flavus,* wheat

Aflatoxins, the toxic metabolites of *Aspergillus flavus* and *Aspergillus* parasiticus pose serious health hazards as potent carcinogens to human beings and domestic animals, because of their frequent occurrence in agricultural commodities particularly cereals and oil seeds (Jones, 1972 and Mixon et al. 1984). In nature, *Aspergillus flavus* and a number of other fungal species are known to co-exist and share a common habitat. Mishra and Daradhiyar (1991) noted presence of 62 fungal species with 178 samples of wheat collected from different places. The possibility of the interaction of fungal co-inhabaitants with *Aspergillus flavus* at different stages of growth and metabolism cannot be ruled out. The potential of the fungi to produce mycotoxins depends upon the prevailing environmental conditions, nature of substrate and genetic set up of fungal strain. However, in nature co-existing moulds may greatly affect the production of mycotoxin by competing for space and nutrients.

A few studies have demonstrated the potential significance of other fungi in influencing aflatoxin production by *Aspergillus flavus* on maize and groundnut. However, this field of investigation needs to be substantiated and explored because of its importance in biological control of aflatoxin contamination. With this objective, different co-existing moulds isolated from wheat grains were screened for their ability to reduce aflatoxin production by toxigenic strain of *Aspergillus flavus* on wheat grains.

MATERIALS AND METHODS

In all 62 fungal species including 15 Aspergilli were isolated from 178 samples of wheat grains. One strain of *Aspergillus flavus* (TA-30) was found to produce maximum aflatoxin B_1 and G_1 is SMKY liquid medium. This strain was used in the present study. Sixteen co-existing moulds including one atoxigenic strain of *Aspergillus flavus* were selected on the basis of their frequency of occurrence in association with *A. flavus.* Spore suspension (10^6 spores per ml) of each species was prepared from 8 days old cultures grown on PDA. In order to study the influence of co-existing moulds on aflatoxin production, 50g approximately healthy and aflatoxin free wheat grains were soaked in distilled water for 2 hours and autoclaved for 10 min. Then 0.5 ml spore suspension of toxigenic strain (TA-30) of *A. flavus* was inoculated on the seed lots with each of the possible combinations of dominant co-existing moulds. The flasks were incubated at 28±2ºC for 10 days. All the treatments were run in triplicate. Flasks inoculated with only toxigenic strain (TA-30) of *A. flavus* served as control.

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After incubation of period, the contents of flasks were assayed for qualitative and quantitative estimation of aflatoxins by TLC (Thin layer chromatography) following the methods of Thomas et al., (1975). Qualitative assay of aflatoxins was carried out using toluene /isoamyl/ alcohol/methanol (90:32:2v) solvent system. Quantity of aflatoxin B_1 and G_1 was determined by spectrophotometer following Nabney and Nesbitt (1965).

RESULTS AND DISCUSSION

It is evident from Table-1 that practically all dominant co-existing moulds inhibited aflatoxin B_1 and G_1 production to variable extent. None of the moulds screened could completely inhibit aflatoxin B_1 production. However, aflatoxin G_1 production was completely inhibited by *Aspergillus fumigatus, A. nidulens, A. niger, A. tamari, Candida albicans, Chaetomium globosum, Penicillium citrinum, Trichoderma viride* and atoxigenic strain of *Aspergillus flavus*. Further, the maximum inhibition in aflatoxin B_1 production was caused by *Penicillum citrinum* (90.23%) followed by *Trichoderma viride* which caused inhibition in aflatoxin B_1 production to the extent of 80.70%. It was followed by *Aspergillus niger* and *Chaetomium globosum* which inhibited aflatoxin B_1 production to the extent of 79.30% and 79.20% respectively. Other forms which caused more than 70%

Table 1 : Effect of some promising antagonistic moulds on aflatoxin production on wheat grains under competitive environment

S.No.	Name of antagonistic fungi	Aflatoxin production (in ppb)		% of aflatoxin inhibition	
		B_1	G_1	B_1	G_1
1	*Aspergillus flavus* (TA -30) control	3680	810	-	-
2	AF+*Aspergillus fumigatus*	1220	-	66.84	100
3	AF+*A. japonicus*	1840	120	48.21	85.18
4	AF+ *A. nidulans*	1090	-	70.38	100
5	AF+ *A. niger*	760	-	79.38	100
6	AF + *A tamarii*	1090	-	70.38	100
7	AF+ *Candida albicans*	1000	-	72.8	100
8	AF+ *Chaetomium globosum*	750	-	79.2	100
9	AF+ *Chaetomium spirale*	900	120	75.5	85.18
10	AF+ *Cladosporum cladosporiodes*	1450	120	60.86	85.18
11	AF+ *Fusarium oxysporum*	1420	180	61.41	77.77
12	AF+ *Memnoniella echinata*	1440	210	60.86	74.07
13	AF+ *Mucor haemalis*	1250	180	67.01	71.77
14	AF+ *Penicillium citrinum*	280	10	90.23	100
15	AF+ *P. funiculosum*	1470	210	60.05	74.07
16	AF+ *Torula convulata*	1880	260	48.91	67.9
17	AF+ *Trichoderma viride*	320	-	90.15	100
18	AF+ Atoxigenic strain of *Aspergillus flavus*	710	-	80.7	100

inhibition in aflatoxin B_1 production include *Aspergillus nidulans, A. tamarii* (70.38% each) *Candida albicans* (72.8%) and *Chaetomium spirale* (75.50%). Among the screened moulds, the minimum inhibition in aflatoxin B_1 production to the extent of 48.21% was caused by *Aspergillus japonicus*.

Inhibition of aflatoxin synthesis by *A. niger* and its detoxification through other microbes have been initially reported by Ashworth et al., (1965). In another study Malini et al., (1983) pointed out that percentage inhibition in radial growth of A. flavus by *Trichoderma viride* and *Aspergillus niger* is related to the percentage reduction in aflatoxin synthesis by *A. flavus.*

Reddy and Reddy (1983) reported that *Penicillium citrinum, Aspergillus japonicus, A. niger and A. terreus* caused almost complete inhibition of Aflatoxin synthesis in liquid culture. Mixon et al. (1984) found that *Trichoderma* can reduce aflatoxin content in peanuts, when supplemented in soil or used as post harvest liquid recorded that all the 13 co-existing moulds inhibited aflatoxin B_1 and G_1 production in the range of 34.3 to 100% on maize kernels. This study revealed that inhibition in aflatoxin production by *Fusarium moniliforme, Penicillium citrinum, Aspergillus niger* and *Trichoderma viride* was noted as 100, 94.9, 86.6 and 80.9% respectively. Studied potential of the biological control of aflatoxin contamination in developing peanuts by atoxigenic strain of A*spergillus flavus.* Later, Bandyopadhyay et al., (2005) reported biological control of aflatoxin contamination in maize in Africa using competitive exclusion mechanism by employing atoxigenic strains of *A. flavus*. Similarly Waliyar et al., (2007) suggested that atoxigenic strains of *A. flavus, Trichoderma viride, Penicillium citrinum, Pseudomonas* sp. and some actinomycetes can be used as biocontrol agents for reducing aflatoxin contamination of agricultural commodities.

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