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Mycotoxin contamination of mustard (*Brassica juncea* L.) seeds at the time of harvest

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Fifty samples each of mustard pods and seeds were collected at the time of harvest from different farms located near Rajendra Agiculture College, Sabour during 1995 and 1996 crop years. Species of *Alternaria, Aspergillus, Curvularia, Fusarium, Penicillium* and *Rhizopus* were found to be associated with some of pods and seeds of those samples. The percentage incidence of these fungi varied from 8-12%. Isolates of *Asperigillus flavus*, *Fusarium moniliforme* and *Penicillium citrinum* were also toxigenic which produced aflatoxins, zearalenone and citrinim, respectively, in culture media. Out of 50 harvested samples analysed, 11 in pod and 14 in seed samples were found to be naturally contaminated with aflatoxins, zearalenone and citrinin in the range of 960-1240 and 860-1140 µg/kg, 120-260 and 120 to 240 µg/kg and 40-120 and 60-80 µg/kg in pods and seeds samples, respectively.

Key words : Mycotoxin contamination, mustard seeds, harvest

INTRODUCTION

The worldwide occurrence of mycotoxin contamination in food and feed stuffs continues to have an extensive impact on the welfare of human and animal populations. Mustard (*Brassica juncea* L.) is one of the important oil-seed crops in India and is largely grown in Northen, Central and Eastern part of the country. Mustard seeds also harbour a number of saprophytic and parasitic microorganisms which thrive at the cost of host substrate as externally or internally seed borne inocula. Amongst various microorganisms invading the seeds, fungi stand at the front (Christensen and Kaufmann, 1960). Some of the common detriments that can directly be attributed to fungal association include lowering of the seed quality, deterioration of nutritional components, failure of seed germinability and elaboration of toxic metabolites.

Mustard is cultivated as *rabi* crop in the country. After harvesting, a wide range of fungi attack the pods/seeds. Sahay (1988) as well as Sinha (1995) reported the dominance of *Aspergillus flavus, A. niger, A. ochraceus, Alternaria alternata, Fusarium moniliforme, Cladosporium* sp., *Curvularia lunata, Penicillium citrinum, Rhizopus stolonifer* etc. on mustard seeds. Mustard seeds have also have been shown to be contaminated with aflatoxins and other mycotoxins (Sahay, 1988; Kumar, 1995).

Since mustard crop is extensively grown in this region of Bihar, an attempt has been made in this investigation to record the incidence of various mycoflora and mycotoxins associated with mustard pods/seeds at the time of harvesting.

MATERIALS AND METHODS

Mustard pods/seeds were obtained at the time of havesting from the farms located near Rajenda Agriculture College, Sabour, Bhagalpur during 1995 an 1996 crop years. Fifty samples each of mustard pods/seeds as well as atmospheric tempe-rature and relative humidity were also recorded at the time of sample analysis. Isolation of the mycoflora associated with different samples was done by the techniques recommended by ISTA (1985).

The aflatoxin producing potentials of *Aspergillus*

flavus isolates were tested in SMKY liquid medium (Diener and Davis, 1966). Methods of Schwenk *et al.* (1958) were followed for the determination of citrinin production by *Penicillium citrinum* isolates whereas moist-rice medium was used for testing zearalenone producing ability of *Fusarium* sp. (Scott *et al.*, 1970). Natural occurrence of mycotoxins in these samples was estimated by the methods suggested by Jones (1972), as well as by Roberts and Patterson (1975). Qualitative estimation of these mycotoxins was done on TLC (toluene : methanol : acetic acid 24:2:1, v/v/v). For aflatoxins, solvent system (toluene : iso-amyl alcohol : methanol, 90:32:2, v/v/v) as suggested by Reddy *et al.* (1970) was also employed. Chemical confirmations were done by treatment with suitable chemical reagents for different mycotoxins viz. aflatoxin (Trifluoric acid and sulpuhric acid), zearalenone and citrinin (acidic anisaldehyde solution) as suggested by Stack and Pohland (1975) and Scott *et al.* (1970), respectively.

Quantitative estimation of mycotoxins was done with the help of spectrophotometer and by "dilution to extinction technique" (Nabney and Nesbitt, 1965; Jones, 1972).

RESULTS AND DISCUSSION

Table 1 : Mycoflora associated with mustard pods/seeds at the time of harvest and their percentage incidence in two crop years (i.e. 1995-1996).

Mycoflora	1995		1996	
	Pods	Seeds	Pods	Seeds
Alternaria alternata (Fr.) Keissler	6	8	6	8
A. brassicae (Berk) Sacc.	12	8	10	12
Aspergillus flavus Link ex Fries	8	6	6	6
A. niger van Tiegh	2	8	8	8
Chaetomium sp.	–	2	8	6
Cladosporium sp.	6	2	8	4
Curvularia lunata (Wak.) Boed.	8	8	6	8
Fusarium moniliforme Sheld.	4	6	8	4
Helminthosporium sp.	8	8	4	–
Penicillium citrinum Thom.	8	6	4	8
Penicillium sp.	2	2	–	2
Rhizopus stolonifer (Ehrenh. ex Fr)	10	12	10	8

The data in Table 1 showed percent incidence of various fungi isolated from mustard pods/seeds after harvesting. The percentage incidence of these fungi varied from 8-12%. *A. brassicae* was having the highest incidence in pods/seeds throughout the year whereas percentage incidence of *A. flavus* was 6-8% on those samples. *P. citrinum* was having the incidence in the range of 2-8% whereas *F. moniliforme* had incidence of 4-8%. At the time of harvesting period the temperature and relative humidity of the surrounding were 14° to 37° C and 28 to 87%, respectively.

Table 2 : Mycotoxin producing potentials of the fungi isolated from mustard pods/seeds at the time of harvest.

Fungi	No. of isolates screened	No. of toxigenic isolates	% Toxigenicity	Mycotoxins produced	Range of mycotoxin production (µg/ml)
Isolated from pod samples at the time of harvest					
A. flavus group	34	11	32.35	Afl.	0.2-16
		7	20.58	Afl.B_1	
		2	5.88	Afl. B_1 & B_2	
		2	5.88	Afl. B_1, B_2 & G_1	
F. moniliforme	18	4	22.22	Zea.	0.1-2.0
P. citrinum	6	2	33.33	Cit.	1.5-5.0
Isolated from seed samples at the time of harvest					
A. flavus group	54	26	48.14	Afl.	0.4-18
		12	22.22	Afl.B_1	
		6	11.11	Afl. B_1 & B_2	
		8	14.81	Afl. B_1, B_2 & G_1	
F. moniliforme	45	11	24.44	Zea.	0.3-5.0
P. citrinum	6	3	30.00	Cit.	0.1-2.0

As is evident from Table 2, out of 34 and 54 isolates of *A. flavus,* obtained from mustard pod and seed samples at the time of harvest were screened out of which 11 (32.35%) and 26 (40.14%) produced aflatoxins in the range of 0.2-16, 0.4-18 µg/ml, respectively. Of which 4 and 10 isolates were high toxin producers whereas the remaining 7 and 16 isolates were low toxin producers. About 4 and 11 (out of 18 and 34) isolates of *F. moniliforme* and 2 and 3 (out of 6 and 10) isolates of *P. citrinum* produced zearalenone and citrinin in the range of 0.1-2.0 and 1.5-5.0 µg/ml, and 1.5-5.0 and 1.0-3.0 µg/ml, respectively.

In the above cases, all the toxigenic isolates of *A. flavus* invariably produced aflatoxin B_1 in the liquid medium (Table 2). Another 2 and 6 toxigenic isolates obtained from harvested pod and seed

samples, elaborated B_2 in addition to aflatoxin B_1 and 2 and 8 isolates of *A. flavus* also produced aflatoxin B_1, B_2, and G_1 respectively.

As is evident from Table 3 aflatoxin was the most common mycotoxin encountered as natural contaminant in mustard samples. Out of 50 samples each of pods and seeds screened, 11 and 14 samples were found to be contaminated with different mycotoxins, respectively.

Aflatoxin B_1 was analysed in 7 and 9 samples and the levels of aflatoxins B_1 were in the range of 960-1240, 860-1140 µg/kg, respectively in those cases. Zearelonone was present in 4 and 6 samples, respectively, and the range was 120-260 µg/kg. Citrinin was detected in the range of 40-120 µg/kg in 3 samples.

Table 3 : Natural occurrence of mycotoxins in mustard pod/seeds at the time of harvest.

Total no. of samples screened	Range of moisture content	No. of contaminated samples	Amount of mycotoxins (µg/kg) in contaminated samples		
			Afl. B_1	Zea.	Cit.
Mustard Pods					
50	11-13%	MSS-1	1240	--	--
		MSS-5	--	260	120
		MSS-10	1080	--	--
		MSS-17	1040	--	--
		MSP-24	1220	--	--
		MSP-31	960	--	--
		MSP-37	--	120	--
		MSP-39	--	180	--
		MSP-41	--	160	60
		MSP-47	1120	--	--
		MSP-49	1040	--	--
Mustard Seeds					
50	10-12%	MSS-2	1080	--	--
		MSS-4	1140	--	--
		MSS-8	--	240	80
		MSS-12	--	180	--
		MSS-15	860	--	--
		MSS-18	860	--	--
		MSS-22	920	--	--
		MSS-23	--	160	60
		MSS-36	--	140	--
		MSS-39	--	180	60
		MSS-42	1020	120	--
		MSS-45	960	--	--
		MSS-48	1080	--	--
		MSS-50	940	--	--

The moisture contents of mustard pod and seed samples ranged from 11-13 and 10-12%, respectively, (Table 3).

Association of fungal organisms as well as their incidences are actually governed by the nature of pod/seed substrates, methods of harvesting, and prevailing environmental conditions. Earlier reports also indicated varied patterns of fungal incidences with different samples of maize (Kumari, 1988), wheat (Sinha, 1991), gram (Kumar, 1995) mungbean (Kumari, 1988) and mustard (Kumar and Sinha, 1992).

Among the isolated fungi there were several species which are known to elaborate mycotoxins. These fungi exhibited mycotoxin producing capacities. However, high potentials for aflatoxin elaboration were shown by the isolates of *A. flavus* group. Toxigenic islates of *A. flavus* elaborated different components of aflatoxin viz., B_1, B_2 and G_1 in varying concentrations.

It has earlier been indicated that aflatoxin producing potentials of *A. flavus* group depended on the variations of genome (Detroy *et al.*, 1971). Toxigenic and non-toxigenic isolates have identical morphology and same growth rate. These isolates differed in the pattern of their metabolism (Rambo and Bean, 1974). Some reports were also available regarding the zearalenone and citrinin producing abilities of *Fusarium moniliforme* and *Penicillium citrinum,* respectively (Singh, 1984; Scott, 1990).

A toxigenic fungal strain, a suitable food base and congenial climatic conditions are the three major factors which contribute to mycotoxin contamination under natural conditions. Earlier reports also indicated the incidence of several mycotoxins as natural contaminants in food and feed items (Bilgrami and Sinha, 1984). Higher percentage of aflatoxin contamination in mustard samples mainly appears to be due to faulty and inadequate agricultural practices used by cultivators. In the field conditions, the pods and seeds might have been damaged by the biotics agents like insects, birds etc. which become more respective towards the attack by toxigenic fungi and mycotoxin elaboration in due course of time.

Occurrence of aflatoxin B_1 in high levels in the present investigation is supported by the results of some earlier surveys made in this region by Prasad *et al.* (1987) who had detected aflatoxin B_1 up to 2230 µg/kg in a mustard sample obtained from

Kothi. Occurrence of other toxins in comparatively low levels might be due to toxigenic potentials of the related fungi, length of storage, varietal resistance, nature of substrates etc. It is important to note that all the contaminated samples contained aflatoxin above 20 ppb, the tolerance level fixed by W.H.O. for human consumption (Anonymous, 1976). This is an alarming situation and we should be very cautious in consuming mustard seeds and their products.

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