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Incidence of aflatoxins in market samples of mustard seeds at Bhagalpur

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Mustard (*Brassica juncea* L.) is an important oil seed crop and is extensively grown in Bihar State in India but this state also provide ideal environmental conditions for the natural contamination of aflatoxin in different agricultural crops including mustard. Altogether 50 samples of mustard seeds were collected on random basis from different markets located at Bhagalpur and nearby areas during the year 2000. Species of *Alternaria, Aspergillus, Curvularia, Fusarium, Penicillium* and *Rhizopus* were fund to be associated with some of the seeds of those samples, incidence of which varied from 12-60%. Some of the isolates of *Aspergillus flavus* were toxigenic which produced aflatoxins in culture media. Out of 50 samples analysed, seven samples were found to be naturally contaminated with aflatoxins in the range of 790-1120 µg/kg.

Key words: Aflatoxin contamination, mustard seeds, market samples

INTRODUCTION

Aflatoxins are known as natural contaminants of various types of food and feed. Mustard (*Brassica juncea* L.) or Rai, an important oil seed crop of India, is widely grown in various parts of the country, particularly in Bihar. The oil content of the seeds ranges from 30 to 40% and its oil is the main cooking medium in this part of the country. Besides adding a special flavour and palatibility to food, it also acts as a lubricating agent to body tissue. It is also fed to cattle as a feed supplement in the form of oil cake (known as Khalli). At the same time mustard seeds also harbour a number of saprophytic and parasitic micro-organisms which thrive at the cost of host substrate as externally or internally seed borne inocula. Amongst various miccro-organisms invading the seeds, fungi stand at the forefront. Some of the common detriments that can directly be attributed to fungal association include lowring 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, the seeds are stored for different periods for future use. Prasad *et al.* (1987), Sinha (1995) and Ahmad (1999) recorded the levels of contamination in mustard seeds upto 2230, 1248 and 1240 µg/kg in samples of mustard seeds collected from different stages of crop development like pre-harvest, harvest and storage respectively. An attempt has been made in this investigation to record the association of mycoflora and aflatoxin contamination in mustard seeds collected from local markets at Bhagalpur and nearby areas during the year 2000.

MATERIALS AND METHODS

Mustard seeds were collected on random basis from different markets located at Bhagalpur and nearby areas during the year 2000. Moisture contents of the seeds were determined on dry weight basis.

Isolation of the mycoflora associated with different seed samples was done by various standard techniques as recommended by ISTA 1985.

Aflatoxin producing potentials of *Aspergillus flavus* isolates was tested in SMKY liquid medium (Diener and Davis, 1966).

Natural occurrence of aflatoxins in mustard samles were analysed by the methods of Jones (1972).

RESULTS AND DISCUSSION

Table 1 lists the incidence of various fungi found to be associated with mustard seeds collected from different markets which varied from 12-60%. *Aspergillus flavus* and *A. niger* were having the highest incidence. The incidence of *Alternaria alternata* and *A. brassicae* was 50% while *Penicillium citrinum* in 38% of seeds. The incidence of *Fusarium moniliforme* was 24%.

Table 1. Mycoflora associated with mustard seeds and their percentage incidence

Mycoflora	% incidence
Alternaria alternata (Fr.) Keissler	50
A. brassicae (Berk) Sacc.	50
Aspergillus flavus Link ex Fries	60
A. niger van Tiegh.	60
Chaetomium sp.	14
Cladosporium sp.	20
Curvularia lunata (Wak.) Boed.	26
Fusarium moniliforme Sheld.	24
Helminthosporium sp.	12
Penicillium citrinum Thom.	26
Rhizopus stolonifer Ehrenh. ex Fr.	38

At the time of seed collection, atmospheric temperature and relative humidity ranged from 14° to 28°C and 28 to 87% respectively.

As is evident from Table 2 altogether 33 isolates of *A. flavus* obtained from mustard seed samples were screened, out of which 8 (21.12%) produced aflatoxins in the range of .02-7 µg/1 and B_2 were produced by 4 and 2 isolates whereas aflatoxin G_1 was elaborated along with B_1 and B_2 by 2 isolates of *A. flavus,* respectively. Table 3 shows that out 50 samples of mustard seeds analysed, 7 samples were naturally contaminated with aflatoxin B_1 in the range of 790-1120 µg/kg.

Table 2 : Aflatoxin producing potentials of *Aspergillus flavus* isolates associated with mustard seeds

Fungi	No. of isolates screened	No. of toxigenic isolates	% Toxigenicity	Aflatoxins produced	Range of aflatoxin production (µg/ml)
A. flavus Group	33	8	21.21	Afls.	0.2-7
		4	12.21	Afl. B_1	
		2	6.06	Afl. B_1 & B_2	
		2	6.06	Afl. B_1 B_2 & B_2	

Table 3 : Natural occurrence of aflatoxins in mustard seeds

Total No. of sample screened	Range of moisture content (%)	No. of contaminated samples	Amount of Aflatoxin B_1(µg/kg) in contaminated samples
50	10-14%	MS-2	1120
		MS-6	1070
		MS-16	1090
		MS-27	970
		MS-35	880
		MS-39	790
		MS-47	1030

The moisture contents of the mustard seed samples ranged from 10-14%.

Incidence of various fungi found to be associated with mustard seeds during collection period was variable. Species of *Alternaria, Curvularia, Fusarium, Penicillium* and *Rhizopus* were also found to be associated with seeds samples. Associations of fungal organisms as well as their incidences are actually governed by the nature of seed substrates, methods of harvesting, storage and prevailing environmental conditions. Earlier reports also indicated varied pattern of fungal incidences with different samples of maize (Sinha, 1990), wheat (Sinha, 1991) and mustard (Sahay, 1988).

It has earlier been indicated that aflatoxin producing potentials of *A. flavus* group depend on the variations of genome (Detroy *et al.,* 1971). Toxigenic and non-toxigenic isolates have identical morphology and growth rate. These isolates differ in the pattern of their metabolism (Tulpule, 1969; Rambo and Bean, 1974).

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. Aflatoxin contamination in mustard samples mainly appears to be due to faulty and inadequate agriculture and storage practices used by cultivators. In the field conditions, the seeds may be damaged by the biotic agents like insects, birds, etc. which become more receptive towards the attack by toxigenic fungi. The farmers also leave the

harvested crop in the field for sundrying and then store them in traditional storage structures such as gunny bags, kothi, bukhari, underground structures or metal bins. Freshly harvested seeds placed into store houses as generally contaminated by field and storage fungi. In Bihar, the atmospheric temperature remains very high in most parts of the year and during summer and rainy season. This ranges between 30-42°C. High temperature is also coupled with high rainfall leading towards humid condition conducive to the growth of toxigenic fungi.

Occurrence of aflatoxin B_1 levels in the present investigation is supported by the results of some earlier surveys made in this region by Prasad *et al.* (1987) who detected aflatoxin B_1 upto 2230 µg/kg in mustard sample obtained from kothi. Variation in toxin level may also be due to variation in length of storage, varietal resistance, nature of substrates etc. It is important to note that all the contaminated samples contained aflatoxin above 30 ppb, the tolerance level fixed by W.H.O. for human consumption. Therefore extra caution in consumption of mustard seeds and their products should be taken.

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