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Mycotoxins and their Producing Fungi from Spices of Bihar (India)

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Abstract—*Mycotoxins (Aflatoxins, ochratoxin A & citrinin) and there producing fungi were examined in 6 different commonly used spices of Bihar. Red chilli, turmeric, coriander, cumin, ginger and garlic were selected for examination of mycotoxins contamination and their producing fungi. Aspergillus flavus was the most dominant species present in all types of spice except in garlic. Red chilli has the highest incidence of fungal contamination followed by coriander and turmeric. 45.4 % of A. flavus was toxigenic and produces aflatoxins up to 16.4 µ/l. 26.8 % of A. niger was toxigenic and produce OTA whereas 24 % of Penicillium citrinum were produced CTN. Qualitative and quantitative detection of mycotoxins in spices were analyzed by Enzyme-linked immunosorbent assay (ELISA). 52.4 % of red chilli and 48.6% of coriander samples were contaminated with aflatoxins up to 198.4 ppb and 116.5 ppb respectively whereas 40% and 34% were contaminated with ochratoxin A. Citrinin was also detected from few of these spices but less in amount as compression to aflatoxins and ochratoxin A. The results of this study suggests that the red chilli and coriander are susceptible substrate for fungal growth and mycotoxin production and the detectable amount of mycotoxins are sufficiently high to induce carcinogenesis.*

1. INTRODUCTION

Worldwide, food trends are changing with a marked health orientation. Safety of the food is the basic needs of human in this era and it is under major focus of international and national organization or bodies over the few years. Mycotoxins contaminated food crops are cultivated in the warmer climatic areas of the world [1] and this is not the problem of producing nation but it is worldwide problem, where it is stored and consumed. Fungus can grow on any type of foods and during deterioration and decomposition of these foods they produce various secondary metabolites.

Mycotoxins are secondary metabolites of fungi which are toxic in nature and generally produced on foods and feeds. Generally they are stable compounds and not destroyed during food processing or home cooking. They have ability to induce verity of toxicosis in animals as well as in human beings when ingested in unrestricted manner with foods and feeds [2]. About 20 different types of aflatoxin are produced in nature in which AFB_1 is considered as the most toxic mycotoxin. The most significant mycotoxigenic species are *Aspergillus flavus, Aspergillus parasiticus* and *Asprgillus nomius* which produce different aflatoxins.

Toxigenic fungal species are very common in nature and can be grown on different agriculture substrate like cereals, oilseeds, vegetables and spices. Spices are widely used in whole world as ingredient in food preparation which provides distinctive colour, flavour and aromas compounds and have also used as medicine in the Ayurveda [3]. Red chilli, turmeric, coriander, cumin, ginger and garlic are commonly used spices in cooking food in Bihar state.

Fragmentary reports are available regarding mycotoxins contamination in spices from different part of world [4-6]. However, very few reports are available regarding mycotoxins contamination in different spices from Bihar [7].

The present study was conducted to ascertain the predominant mycoflora associated with red chilli, turmeric, coriander, cumin, ginger and garlic and the natural occurrence of aflatoxins, ochratoxin A and citrinin in spices of Bihar. This is the first report of mycotoxins in garlic from Bihar.

2. MATERIAL AND METHODS

2.1 Survey and sample collection

A survey of different area of Bihar was under taken for the evaluation of natural occurrence of mycotoxigenic fungi and mycotoxins contamination in spices. 25 samples of each spices (red chilli, turmeric, coriander, cumin, ginger and garlic), total 150 samples were collected from different market of Bihar. Each sample was put into the sterile cellophane bag and then put into the sterile brown envelop and stored at 4°C to arrest any mycotoxin formation before analysis.

2.1 Isolation and Identification of fungi

All the samples had randomly placed on the freshly prepared Potato dextrose agar (PDA) and on Blotter paper and incubated at 28 ± 2°C for 7 days and examined daily. The counts were recorded after 5 to 7 days. Identification was carried out by morphological characteristics and followed the taxonomic schemes of Maren [8] for genus *Aspergillus*, Pitt [9] for *Penicillium,* Nelson et. al. [10] for *Fusarium* and Funder [11] for other genera.

Table1: Percent incidence of isolated fungi from spices of Bihar						
Name of the fungi	**Spices**					
	Red chilli	**Coriander**	**Turmeric**	**Cumin**	**Ginger**	**Garlic**
Alternaria alternata	1.8	-	-	-	-	-
Aspergillus parasiticus	7.2	4.2	3.1	5.2	1.6	-
Aspergillus niger	13.9	12.4	-	11.4	7.8	-
Aspergillus flavus	25.2	9.3	14.9	10.2	20.2	5.8
Aspergillus ochraceus	4.8	1.2	6.2	2.1	4.5	1.5
Aspergillus versicolour	7.4	-	-	-	-	-
Aspergillus fumigatus	-	2.4	-	-	2.8	12.4
Aspergillus terrus	-	-	1.1	2.1	3.0	-
Aspergillus sydowi	-	3.5	-	-	-	-
Penicillium citrinum	5.9	5.8	14.6	2.4	5.2	-
Penicillium islandicum	-	-	-	-	-	-
Penicillium verrucosum	8.1	6.9	7.1	-	6.8	-
Fusarium oxysporum	-	-	5.6	2.8	2.9	1.4
Fusarium moliniforme	6.4	-	6.4	-	3.8	-
Rhizopus nigricans	-	6.2	-	2.6	-	1.2
Rhizopus oryzae	3.1	-	4.2	3.0	2.8	-
Mucor hiemalis	5.2	7.1	3.5	-	-	-
Neurospora crassa	1.8	-	-	-	-	-

2.3 Analysis for Potentiality of mycotoxin producing isolated fungi

Mycotoxin producing potentiality of *A. flavus*, *A. ochraceus*, *A. paraciticus, A. terreus, P. citrinum* and *P. verrucosum* were examined. The qualitative and quantitative estimation of mycotoxins producing potentiality of fungi were done by the method of Diener et. al. [12] for aflatoxins producing potentiality of *Aspergillus* spp.; Schwenk et. al. [13] and Davis et. al. [14] for testing ochratoxin A and citrinin producing potentiality of *A. ochraceus, A. terrus, P. citrinum* and *P. verrucosum*.

2.4 Natural occurrence of mycotoxins

The Qualitative and quantitative detection for natural occurrence of aflatoxins, ochratoxin A and citrinin in spice samples were analyzed by enzyme linked immunosorbent assay (ELISA) and TLC. Samples were analyzed by AgraQuant Total Aflatoxin (COKAQ1000) for AFT and AgraQuant Ochratoxin (COKAQ2000) for ochratoxin A from ROMER LAB (ASTRIA).

Whereas qualitative and quantitative estimation of citrinin was done by the method of Wilson, [15] and Robert & Patterson, [16].

3. RESULT AND DISCUSSION

3.1 Natural occurrence of toxigenic fungi

In our present study, numerous of fungi were isolated, in which some of them are well known for their mycotoxin production. A total of 7 different fungal genera belong to 18 species were isolated (Table 1). Fungi were identified on the basis of their culture and morphological characteristics, these were identified as *Alternaria alternata, Aspergillus paraciticus, A. niger, A. flavus, A. ochraceus, A. versicolour, A. fumigatus, A. terreus, A. sydowi, Penicillium citrinum, P. islandicum, P. verrucosum, Fusarium oxysporum, F. moliniforme, Rhizopus*

Fig. 1: Fungal association with turmeric, red chilli and coriander samples

nigricans, R. oryzae, Mucor hiemalis and Neurospora crassa. *Aspergillus* was the most dominant genera followed by *Penicillium and Fusarium* (Fig 1). Elshafie et. al. [17] also reported some of these fungi from cumin, ginger and coriander samples. Red chilli samples were highly contaminated with *A. flavus* (25.5) followed by turmeric (14.9) and the lowest incidence was recorded in garlic samples (5.8). The present study revealed the wide range of fungal contamination in spices in which *A. flavus* and *A. niger* were the most dominant and present in all 6 different spices, the present finding supports the report of Bokari [18]. Our finding says that red chill and coriander are susceptible substrate for growth of *Aspergillus niger, A. flavus, A. ochraceus, Penicillium citrinum, P. verrucosum, F. moliniforme* and *Rhizopus oryzae.*

3.2 Mycotoxin producing potentiality of fungi

Aflatoxins, ochratoxin and citrinin producing potentiality of *Aspergillus flavus, A. paraciticus, A. ochraceus, A. terreus, Penicillium citrinum* and *P. verrucosum* were analyzed (Table 2). 45.4% *A. flavus* were positive and produces aflatoxins with the potential level ranges from 2.1 – 16.4 µg/l. 33.3% of *A. paraciticus* also produces aflatoxins but less potential than *A. flavus. A. ochraceus* shows 31.2% of toxicity and produces ochratoxin A. None of the isolates of *A. terreus* were found toxigenic. *Penicillium citrinum* shows 36.1% of toxicity and produces citrinin with the level ranges from 2.6 – 5.9 µg/l, whereas 43.7% of *Penicillium verrucosum* were toxigenic and produces two different mycotoxins i.e ochratoxin A and citrinin with the potentiality ranges from 3.8 – 8.6 µg/l. Our finding is well agreement with some other researchers [19, 20].

Table2: Mycotoxin producing potentiality of isolated fungi

Detected fungi	N.I.E.	N.I.P. (% toxicity)	Potential range (µg/l)
Aspergillus flavus	55	25(45.4)	2.1 – 16.4
A. niger	41	11(26.8)	3.5 – 5.9
A. paraciticus,	42	14(33.3)	3.4 – 9.2
A. ochraceus	32	10(31.2)	1.5 – 11.4
A. terreus	24	0	
Penicillium citrinum	36	13(36.1)	2.6 – 5.9
P. verrucosum	32	14(43.7)	3.8 – 8.6

N.I.E- Number of isolate examined, N.I.P. – Number of isolates positive

3.3 Natural occurrence of mycotoxins in spices

In present investigation, we were only concern to aflatoxins, ochratoxin A and citrinin contamination in spices. The result of natural occurrence of aflatoxins, ochratoxin A and citrinin in different concentration in 6 different types of spices has been shown in Table 3. During the analyses, 52.7% of red chill samples and 48.6 % of coriander samples were positive to aflatoxins followed by cumin (41.5%) and garlic (8.4%) has the lowest contamination. Highest amount of aflatoxins was recorded in red chilli samples (198.4ppb) where as in cumin and ginger samples, it was 151 ppb and 125 ppb respectively. The lowest concentration of AFT was recorded in garlic sample (25 ppb). All 6 different types of spices are contaminated with aflatoxins whereas ochratoxin A was not detected in cumin & garlic and citrinin was not identified from turmeric and garlic samples. Ochratoxin A contamination was maximum recorded in turmeric (40.5%) where as in red chill, coriander and ginger samples it was only 40%, 34% and 20.2% respectively. Our finding is well agreement with Jalili & Jinap [21]. In our result, 32.5% of red chilli, 30.2% of coriander, and 18.6% of ginger samples were contaminated with citrinin and the detected amount were 80ppb, 55 ppb and 65 ppb respectively. None of the samples of turmeric and garlic were positive to citrinin. So, it may be possible that the growth of *P. citrinum* or *P. verrucosum* were inhibited by these spices or may be that the basic nutrients for the growth of *P. citrinum* and *P. verrucosum* were not present in these spices.

Table 3: Natural occurrence of aflatoxins, ochratoxin A and citrinin in spices

Spices	Aflatoxins (ppb)/ (% Contamination)	Ochratoxin A (ppb) / (% Contamination)	Citrinin (ppb) / (% Contamination)
Red chilli	198.4/ (52.7)	94/ (40)	80/ (32.5)
Coriander	116.5/ (48.6)	118/(34)	55/ (30.2)
Turmeric	65/ (36.4)	124/ (40.5)	-
Cumin	151/ (41.5)	-	75/ (10.5)
Ginger	125/ (35.8)	86/ (20.2)	65/ (18.6)
Garlic	25/ (8.4)	-	-

4. CONCLUSION

On the basis of the present study, it may be concluded that the spices are rich substrate for fungal growth and mycotoxin productions. All 6 types of spices were contaminated with aflatoxins whereas ochratoxin A was not present in cumin and garlic samples and none of the samples of turmeric and garlic was contaminated with citrinin. It may be that turmeric and garlic are resistant to citrinin producing fungi. In our result aflatoxin and ochratoxin A were present in higher concentration and can cause carcinogenesis. Citrinin was also present in the spice samples and the amount was sufficient to induce toxicity in humans and animals and this is the first report of mycotoxins in garlic from India. The present investigation will also help in the reduction of mycotoxin contamination in spices. India is the largest producer of the spices, about 75 % of the spices of the world produced here but only 40 % were exported because the contamination level is extremely higher than the permissible level of mycotoxin contamination in many countries. So, it is very important to care in processing, handling, transportation and storage system to reduce the production of hazardous mycotoxins.

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