

Evaluation of Mycotoxigenic Fungi and Aflatoxins, Ochratoxin A & Citrinin Contamination in some Dried Fruits and Nuts from Bihar (India)

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Abstract—In this study natural occurrence of three important mycotoxins such as aflatoxin, ochratoxin & citrinin were examined in five different dried fruits and nuts (almond, pistachio, cashew nut, raisins and figs) of Bihar. 154 samples of dried fruits and nuts were collected from different district of Bihar. Association of mycotoxigenic fungi were examined by using Potato dextrose agar (PDA) and Standard blotter paper method from different samples of dried fruits and nuts. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *Penicillium verrucosum* and *P. citrinum* were the dominant mycotoxigenic fungi isolated from dried fruits and nut samples. Natural incidence of aflatoxins, ochratoxin A & citrinin in dried fruits and nuts samples were assessed by Enzyme-linked immunosorbent assay (ELISA) method. Aflatoxins, ochratoxin A and citrinin were commonly detected mycotoxins from almond, pistachio, cashew nut and raisins samples whereas figs were contaminated with only aflatoxins. Highest amount of aflatoxins were detected from pistachio (442.8 ng/g) whereas the Fig. samples have lowest aflatoxin contamination (35.4 ng/g). The results of present investigation suggest that the almond, pistachio, cashew nut and raisins are susceptible substrate for fungal growth and further mycotoxin production. The amount of aflatoxin detected was sufficiently high to induce carcinogenesis.

Keywords: Mycotoxins, ELISA, Dried fruit & nuts, mycotoxigenic fungi

1. INTRODUCTION

Mycotoxins are toxic secondary metabolites mainly produced by the three fungal genera *Aspergillus*, *Penicillium* and *Fusarium*. More than 400 mycotoxins are known so far the most important mycotoxins are aflatoxin, ochratoxin and citrinin. Aflatoxins are generally produced by *A. flavus* and *A. parasiticus*, which produce four different aflatoxins i.e AFB₁, AFB₂, AFG₁ and AFG₂ in agricultural commodity. Among all aflatoxins, AFB₁ is most toxic and potent hepatocarcinogen [1,2] and has been placed as grade-I carcinogen by International Agency of Cancer Research, Lyon, France. It is the most common contaminant of edible substance including dried fruits [3,4]. Ochratoxin A is an important mycotoxin mainly produced by *Aspergillus ochraceus* and *Penicillium verrucosum* and considered as hepato-renal toxin. Citrinin is a

well known nephrotoxin having adverse effect on renal system and generally found as co-contaminant with ochratoxin A [5,6].

Dried fruits and nuts (almond, pistachio, cashew nut, raisins and figs) are packed are an abundant source of proteins, carbohydrates, vitamins, minerals, dietary fibre and have antioxidants properties. They keep heart diseases at bay, prevent anaemia, maintain cholesterol, improve haemoglobin levels and beneficial for proper functioning of crucial body organ. Pistachios contain vitamin B₆ that prevents heart problems. Almonds also help in the formation of red blood cells. Raisins are essential to fight or prevent anaemia and beneficial for bone formation and preventing weakening of vision. A daily consumption of almonds, cashew nut and pistachios will go a long in maintaining cholesterol levels [7,8].

A large number of literature are available regarding mycotoxins contamination in cereals, oil seeds, pulses and spices from India and different parts of world but very fragmentary reports are available regarding association of mycotoxigenic fungi and mycotoxins contamination in dried fruit and nuts from India and these are mainly concern with aflatoxins contamination. [9-11].

The present study was conducted to assess and examined the fungal association and natural occurrence of aflatoxin, ochratoxin A & citrinin mycotoxins in pistachio, almonds, cashew nut, raisins and figs from Bihar. During the investigation, it has been observed that aflatoxins, ochratoxin A and citrinin were detected from all types of dry fruits and nuts samples except figs and the detectable amount were sufficiently high to induce mycotoxicosis. These dried fruit and nuts are commonly used in Bihar and presence of mycotoxins in these dried fruits and nuts can directly affect the health of consumers.

2. MATERIAL AND METHODS

2.1 Sampling

A total of 154 dried fruits and nut samples (32 each of *almond* and *pistachio* & 30 samples of each *cashew nut*, *raisins* and *figs*) were collected from local market of different districts of Bihar. Each sample was put into the sterile cellophane bag and then put into the sterile brown envelope and stored at 4°C to arrest any mycotoxin formation before analysis.

2.2 Isolation and identification of fungi

All the samples of dried fruits and nuts were randomly plated on the freshly prepared Potato dextrose agar (PDA) and Standard blotter paper and incubated at $28 \pm 2^{\circ}\text{C}$ for 7 days and examined daily. Fungal colony count was recorded after 5 to 7 days. Identification was carried out by morphological characteristics and followed and the taxonomic schemes of Maren [12] for genus *Aspergillus*, Pitt [13] for *Penicillium*, Nelson *et. al.* [14] for *Fusarium* and Funder [15] for other genera.

2.3 Potentiality of mycotoxin producing isolated fungi

Mycotoxin producing potentiality of *A. flavus*, *A. ochraceus*, *A. paraciticus*, *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.* [16] for aflatoxins producing potentiality of *Aspergillus* spp.; Schwenk *et.al.* [17] and Davis *et.al.* [18] for testing ochratoxin A and citrinin producing potentiality of *A. ochraceus*, *P. citrinum* and *P. verrucosum*.

2.4 Detection of natural contamination of mycotoxins by ELISA

The qualitative and quantitative detection for natural occurrence of mycotoxins in dried fruits and nuts samples were analysed by enzyme linked immunosorbent assay (ELISA). Aflatoxins were detected in the sample by using Total Aflatoxin (AF) assay kit (TO-E0006) and Ochratoxin A assay kit (To-E0001) was used for detection of ochratoxin A and RIDASCREEN FAST citrinin Assay (R6302) for Citrinin detection by the methods of Turner *et. al.* [19].

3. RESULT AND DISCUSSION

3.1 Percent incidence 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 5 fungal genera belong to 15 species were isolated (Table 1). Fungi were identified on the basis of their culture and morphological characteristics, these were identified as *Aspergillus parasiticus*, *A. niger*, *A. flavus*, *A. ochraceus*, *A. versicolor*, *A. fumigatus*, *A. terreus*, *Penicillium citrinum*, *P. islandicum*, *P. verrucosum*, *Fusarium oxysporum*, *F.*

moliniiforme, *Rhizopus nigricans* R. *Oryzae* & *Mucor hiemalis*. *Aspergillus* was the most dominant genera followed by *Penicillium* and *Fusarium* (Fig.1). Alghalibi *et. al.* [20] was also isolated *A. flavus*, *A. niger*, *A. terreus*, *A. ochraceus* from raisins and figs samples of Yemen. Zohri [21] reported association of *A. fumigatus*, *A. flavus* and *A. versicolor* in figs and raisins samples of Egypt and also detected the level of ochratoxin A was up to 120mg/kg in Fig. samples.

Table 1: Percent incidence of isolated fungi from dried fruits and nuts of Bihar

Name of Fungi	Dry fruit & nuts				
	Almond	Pistachio	Cashew nut	Raisin	Fig
<i>Aspergillus parasiticus</i>	5.4	5.1	3.4	4.5	1.2
<i>Aspergillus niger</i>	2.1	4.2	1.2	3.2	0
<i>Aspergillus flavus</i>	15.6	24.8	13.4	12.4	4.8
<i>Aspergillus ochraceus</i>	14.2	12.1	11.6	15.5	6.5
<i>Aspergillus versicolor</i>	1.4	3.8	4.2	2.7	0
<i>Aspergillus fumigatus</i>	0	0	1.1	2.6	0
<i>Aspergillus terreus</i>	1.2	0	2.1	0	0
<i>Penicillium citrinum</i>	3.6	2.1	2.4	1.3	1.2
<i>Penicillium islandicum</i>	0	0	1.2	0	0
<i>Penicillium verrucosum</i>	12.4	11.6	13.4	13.8	0
<i>Fusarium oxysporum</i>	2.8	1.2	5.1	1.2	0
<i>Fusarium moniliforme</i>	3.1	0	1.2	0	1.8
<i>Rhizopus nigricans</i>	2.8	1.1	0	0	1.4
<i>Rhizopus oryzae</i>	0	2.5	1.8	0	0
<i>Mucor hiemalis</i>	2.1	1.1	3.1	2.1	1.2



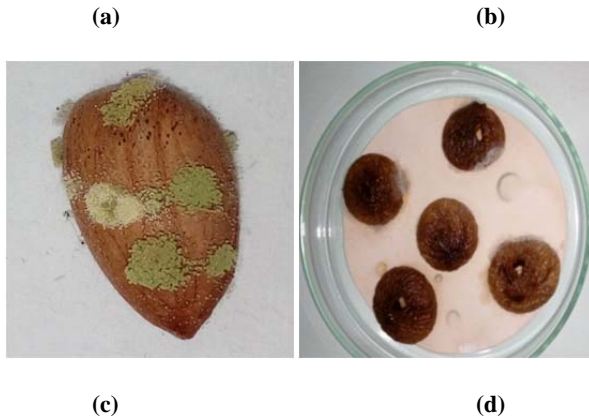


Figure 1: Showing A – Vigorous contamination of *Aspergillus flavus* and *A.niger* on pistachio, B – Raisin contaminated with *A. flavus* and *A. niger*, C – Almond seed having toxigenic strain of *A. flavus* and *A. versicolor*, D – Figs samples on petriplate having less contamination of toxigenic fungi.

3.2 Mycotoxin producing potentiality of isolated fungi

Aflatoxins, ochratoxin A and citrinin producing potentiality of *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, *Penicillium citrinum* and *P. verrucosum* were presented in Table 2. 44% of *A. flavus* was found to be toxigenic and produced aflatoxins which range between 15.7µg/l – 22.8 µg/l whereas *A. parasiticus* also produced aflatoxins but the level of potentiality was less than *A. flavus*. *A. ochraceus* and *P. verrucosum* produced ochratoxin A with potentiality upto 16.5 µg/l and 18.7µg/l respectively. In present finding ochratoxin A was produced by *P. verrucosum* and *A. ochraceus* both. Niessen *et. al.* [22] carried out the molecular diagnostic of similar fungi for ochratoxin A production.

Table 2: Mycotoxins producing potentiality of fungi isolated from dried fruit and nuts

Fungi examined	Positive/N.I.A ¹	% toxicity	Mycotoxin detected	Potential range (µg/l)
<i>Aspergillus flavus</i>	11/25	44.0	Aflatoxins	15.7 – 22.8
<i>Aspergillus ochraceus</i>	9/25	36.0	Ochratoxin A	6.5 – 16.5
<i>Aspergillus parasiticus</i>	5/18	27.7	Aflatoxins	5.4 – 9.2
<i>Penicillium citrinum</i>	7/18	38.8	Citrinin	3.5 – 9.3
<i>Penicillium verrucosum</i>	3/10	30.0	Ochratoxin A	8.4 – 18.7

¹Number of isolates analyzed

3.3 Natural occurrence of mycotoxins in dried fruits and nuts

In present investigation, aflatoxins, ochratoxin A and citrinin were detected in the samples of dried fruits and nuts collected from different region of Bihar. The results of natural incidence

of aflatoxins, ochratoxins A and citrinin in 5 different types of dried fruits and nuts has been shown in Table 3. 68.7% of pistachio samples were contaminated with aflatoxins followed by 59.3% of almond and 52.5% raisin samples and figs samples had only 12.5% contamination. Highest amount of aflatoxins was recorded in pistachio samples (442.8 ng/g) whereas in almond and raisins samples, it was 245.8 ng/g and 184.1 ng/g respectively. The lowest amount of aflatoxin was present in figs samples (Fig. 2). Masood *et. al.* [23] have also reported aflatoxins contamination in dried fruits of Pakistan. The amount detected was ranges from 3.28 µg/kg to 7.89 µg/kg. They observed lowest

Table 3: Natural occurrence of amount of mycotoxins contamination in dried fruits and nuts.

Sample s	Number of sample analyzed	% Contamination	Amount (ng/g)		
			Aflatoxins	Ochratoxin A	Citrinin
Almond	32	59.3	245.8 ± 34.2	154.2 ± 18.8	184.1 ± 28.5
Pistachio	32	68.7	442.8 ± 35.1	193.8 ± 35.7	158.0 ± 45.7
Cashew nut	30	43.3	214.5 ± 37.2	179.5 ± 28.5	98.8 ± 47.3
Raisin	30	52.5	184.1 ± 20.2	215.1 ± 30.2	174.1 ± 28.5
Fig.	30	12.5	35.4 ± 24.8	ND	ND

amount of aflatoxins in dried figs (3.28 µg/kg) and highest in Pistachios without shell (7.89 µg/kg). In this study, Ochratoxin A and citrinin was also detected from all types of dried fruits and nuts except figs. The highest amount of Ochratoxin A was detected in raisin samples (215.1 ng/g) and lowest in almonds (154.2 ng/g). Citrinin contamination was maximum in almonds (184.1 ng/g) and minimum in cashew nut (98.8 ng/g). All 5 types of dried fruits and nuts were contaminated with aflatoxins whereas ochratoxin A and citrinin was not detected from Fig. samples.

4. CONCLUSION

On the basis of the present study, it may be concluded that the dried fruits and nuts are rich substrate for fungal growth and further mycotoxin productions. All 5 types of dried fruits and nuts are contaminated with aflatoxins and the detection level was extremely higher than the permissible limit of EU. Ochratoxin A and citrinin was also detected from pistachio, raisins, almond and cashew nut samples and the detected amount was sufficiently high to induced toxicity. Fig. samples were resistant to ochratoxin A and citrinin production and none of the samples of figs were found contaminated with ochratoxin A and citrinin. The amount of aflatoxins detected from figs samples were less than other dried fruits and nuts samples. Dried fruits and nuts are used regularly for health

benefits but presence of aflatoxins, ochratoxin A and citrinin is hazardous for the health of humans. It is very important to care in processing, handling and transportation to reduce the contamination of these hazardous mycotoxins in dried fruits and nuts.

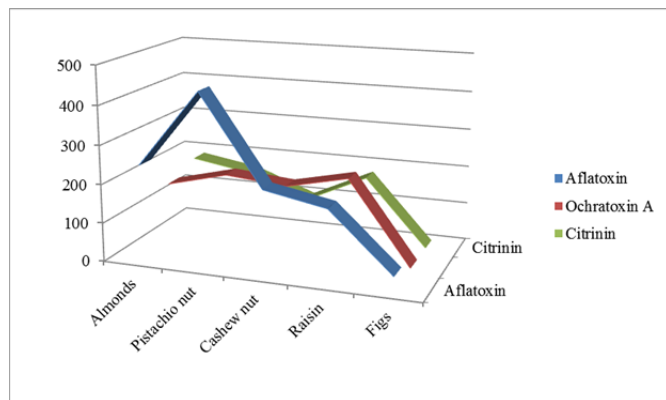


Fig. 2: Showing pistachio had maximum aflatoxin contamination

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