ORIGINAL RESEARCH PAPER
Volume-9 | Issue-2 | February-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

INCIDENCES OF FUNGAL CONTAMINATION BY ASPERGILLUS SECTION FLAVI IN MAJOR SPICES BEING SOLD IN CENTRAL INDIA

Microbiology

Anjali Dongre*
Department of P.G. studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur 482001 (MP) India *Corresponding Author

ABSTRACT

Aspergillus species belonging to section *Flavi*, are potential aflatoxin producing fungi. A total of 100 samples belonging to 11 different spices from Central India were examined for the fungal contamination with special reference to *Aspergillus* species. *Aspergillus* section *Nigri* and *Flavi* were the dominant sections, isolated from 78.5% of the samples, infected with fungi. Two members of *Aspergillus* section *Flavi*; *Aspergillus flavus* and *A. parasiticus* were isolated from 36 samples. Their aflatoxigenic potential was checked via thin layer chromatography, which showed that 59% of the isolates showed mycotoxigenic properties, with aflatoxin B1 as predominant aflatoxin. The results showed that the spices, sold in Central India, are prone to be contaminated with aflatoxins and can present a severe health hazard.

KEYWORDS

Spices, Aspergillus section Flavi, Mycotoxin, Aflatoxin, Aspergillus flavus, Aspergillus parasiticus

INTRODUCTION

In tropical and subtropical environments, the fungal contamination of various foodstuffs and agricultural commodities is a major problem, due to the climatic conditions that favour the fungal growth (Mandeel, 2005). Fungal infestation not only renders the foodstuffs with unpleasant taste, odour and compromised physical appearance, but also can produce toxic chemicals, such as mycotoxins (Garcia *et al*., 2018). Mycotoxins have been reported to be carcinogenic, teratogenic, tremorogenic, haemorrhagic, and dermatitic to a wide range of organisms and to cause hepatic carcinoma in man (Alshannaq and Yu, 2017; Puntscher *et al*., 2018). In India, Food safety and Standards Authority of India has set a maximum limit of 30 µg kg^{-1} aflatoxin in spices, although the spices sold in the market, do not get their products tested. Furthermore, the ground spices usually are prepared with raw material of inferior quality agricultural products, which always have a probability of fungal infestation during their harvest, transportation or storage. The present paper deals with the occurrence of toxin producing fungi, especially belongs to *Aspergillus* group.

MATERIALS AND METHODS

Sample collection

Ground spice samples were procured from the local market. The major spices chosen were red chilli powder, coriander powder, cinnamon powder, turmeric powder, black papper powder and curry powder (garam masala). The samples contained both branded and unbranded products, but the priority was given to the local packers of these spices.

Isolation and Identification of fungi

The samples of purchased spices were subjected for the isolation of fungi associated with them. The dilutions of the samples were prepared in sterile water and plated onto potato dextrose agar (Kalil *et al*., 2017). Once the growth is observed, the fungi were isolated for many generations. The macro and micro-morphological characters were used to identify the fungi using standard identification keys (Alexopoulos *et al*., 1980).

Estimation of aflatoxins from spice samples

In order to check wheather the isolated fungi are capable of producing aflatoxins or not, the isolated fungi were mass cultivated in potato dextrose broth for 7 days. The cell free filtrate of the cultures is obtained by filtration and the filtrate was freezed and kept at -20°C till further use. Various aflatoxins i.e. aflatoxin B1, B2, G1 and G2, were estimated qualitatively by thin layer chromatography (TLC). For extraction, 45 ml of culture filtrate was mixed with 55 ml of methanol, 200 ml of hexane and 4 g NaCl. The suspension was blended for one minute and then centrifuged for 5 min at 2000 rpm. The lower aqueous methanol phase (25 ml) was extracted thrice with 25 ml chloroform and the chloroform layer was passed through anhydrous sodium sulphate into a 250 mL beaker. The extract was evaporated and redissolved in 200 µL benzene–acetonitrile (98+2) and spotted on TLC plate (Silica gel G with binders) along with aflatoxin standards (Sigma, India). The plates were developed in chloroform–acetone (9+1) solvent mixture. The aflatoxins were detected as fluorescent bands under long wave UV lamp (JSGW, India) (Zahra *et al*., 2018).

RESULTS

The spice samples, mainly in powderd form, except bay leaves and cloves, were collected from local market. A total of 100 samples belonging to 11 spices were collected (Table 1). Out of these, 93 samples were found to be infested with atleast one fungus, although many of them showed presence of more than one fungi. 73 samples yielded the presence of various species of the genus *Aspergillus*. Major *Aspergillus* species included *A. flavus*, *A. niger*, *A. fumigatus*, *A. parasiticus* and *A. terreus*. Under the section *Flavi*, *A. flavus* and *A. parasiticus* were the members with predominance of *A. flavus* (31 cases) as compared to *A. parasiticus* (5 cases) (Fig 1).

Table 1: Incidencences of fungal infestations in different Indian spices due to *Aspergillus* genus with emphasis on *Aspergillus* section *Flavi* members from the spice samples collected from Central India during 2016-17.

S. No.	Type of spice	No. of samples collected	Number of samples with fungal infestation	Samples having presence of Aspergillus species	Presence of member of Aspergillus section Flavi	
					A. flavus	A. parasiticus
1.	Bay Leaves	7	7	3	1	0
2.	Black cumin	4	4	4	1	0
3.	Black pepper	4	4	2	0	0
4.	Cinnamon	10	10	8	4	1
5.	Clove	10	9	4	3	0
6.	Coriander	18	18	17	11	4
7.	Cumin seeds	5	5	3	1	0
8.	Fenugreek	4	3	2	0	0
9.	Curry Powder	11	11	9	2	0
10.	Red Chilli	15	12	12	5	0
11.	Turmeric	12	10	9	3	0

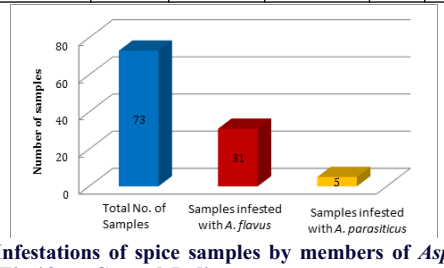

Fig 1: Infestations of spice samples by members of *Aspergillus* section *Flavi* from Central India.

All the isolates of *A. flavus* and *A. parasiticus* were further cultured in the laboratory. Culture filtrates of *A. flavus* and *A. parasiticus* were further tested by thin layer chromatography yielded four bands, representing aflatoxin B1, B2, G1 and G2 which culture filterate of 36 isolates, associated with one aflatoxin in 21 samples (Table 2). Cultute filtrates obtained from rest of the isolates failed to show any aflatoxin band. Aflatoxin B1(AFB1) was the predominat aflatoxin, and was present in *Aspergillus flavus* and *A. parasiticus* isolates. Presence of AFG1 and AFG2 could be attributed to *A. parasiticus* only, with more cases of AFG2 as compared to AFG1.

Table 2: Aflatoxigenic potential of *Aspergillus* isolates, belonging to the section *Flavi* from spice samples of Central India.

S. No.	Spices	Date collected	Isolated fungi	Aflatoxins identified			
				AFB1	AFB2	AFG1	AFG2
1.	Bay leaves	August 2016	*A. flavus*	+	+	-	-
2.	Black Cumin	August 2016	*A. parasiticus*	+	+	-	+
3.	Black Cumin	November 2016	*A. flavus*	+	-	-	-
4.	Black pepper	October 2016	*A. parasiticus*	+	-	-	+
5.	Cinnamon	June 2017	*A. parasiticus*	+	-	-	+
6.	Clove	October 2016	*A. flavus*	+	+	-	-
7.	Coriander	May 2016	*A. flavus*	+	+	-	-
8.	Coriander	June 2016	*A. flavus*	+	-	-	-
9.	Coriander	June 2016	*A. parasiticus*	+	+	-	+
10.	Coriander	July 2016	*A. flavus*	+	-	-	-
11.	Coriander	September 2016	*A. flavus*	+	-	-	-
12.	Coriander	March 2017	*A. flavus*	+	-	-	-
13.	Curry Powder	June 2016	*A. flavus*	+	+	-	-
14.	Curry Powder	July 2016	*A. parasiticus*	+	+	+	-
15.	Curry Powder	May 2017	*A. flavus*	+	+	-	-
16.	Cumin	December 2017	*A. parasiticus*	+	-	+	-
17.	Red Chilli	April 2016	*A. flavus*	+	-	-	-
18.	Red Chilli	July 2017	*A. parasiticus*	+	+	-	+
19.	Red Chilli	December 2017	*A. flavus*	+	-	-	-
20.	Turmeric	April 2016	*A. flavus*	+	-	-	-
21.	Turmeric	January 2017	*A. flavus*	+	+	-	-

DISCUSSION

Spices and condiments are indispensible part of Indian cousine, and hence has a huge domestic market is available. However, since, most of the spices are sold in ground (powdered) form, it is not possible to look for the fungal contaminations and the mycotoxins. Further, there is no available regarding the nature of the toxigenic fungi that may contaminate spices from local region of Central India. The results showed the presence of members of *Aspergillus* section *Nigri* and *Flavi* in almost all types of spices collected during the studies. The results are in complete agreement with Jeswal and Kumar (2015).

Aspergillus section *Flavi* contains some of the most important species in the genus, that cause mycotoxicosis and are important in food safety and human health (Frisvad *et al*., 2019). *Aspergillus flavus* is reported as the leading aflatoxin producer in food articles. For years, three main aflatoxigenic species were commonly considered in the section *Flavi*: *A. flavus*, *A. parasiticus* and *A. nomius* (Cary *et al*., 2006). The present survey did not find any presence of *A. nomius* though.

Among spices, coriander was the spice highest contaminated with members of *Aspergillus* section *Flavi*, while bay leaves were least contaminated. Higher incidence of aflatoxins in coriander as observed during the present investigation have also reported by Dongre and Pandey (2019). Although, overall fungal contamination was seen in almost all the spice samples. This may also be due to the handling of spices by hand, and storage in unhygienic, and damp area during the transportation and processing (Mandeel, 2005). Our results showed that almost 59% of strains were able to show the production of aflatoxins. Makhlouf *et al*. (2019) recently showed that 80% strains of *Aspergillus* section *Flavi*, isolated from spice samples from Lebanon, showed toxigenic properties.

Acknowledgement

I am thankful to the Head, Department of P.G. Studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur for laboratory and library facilities and University Grant Commission, New Delhi for financial assistance by prestigious Rajiv Gandhi National Fellowship. I am also thankful to Late Dr. Jamaluddin and Dr. A. K. Pandey for helping in various ways.

REFERENCES

1. Alexopoulos, C.J., Bold, H.C. and Delevoryas, T., 1980. Morphology of Plants and Fungi. Harper & Row.
2. Alshannaq, A. and Yu, J.H., 2017. Occurrence, toxicity, and analysis of major mycotoxins in food. International Journal of Environmental Research and Public Health, 14(6): 632.
3. Cary, J.W. and Ehrlich, K.C., 2006. Aflatoxigenicity in Aspergillus: molecular genetics, phylogenetic relationships and evolutionary implications. Mycopathologia, 162(3):167.
4. Dongre A. and Pandey A. K., 2019, Biodiversity of Aflatoxin Producing Fungi Associated with Ground Coriander (Indian Spice). Int J Recent Sci Res. 10(10): 35440-35442.
5. Frisvad, J.C., Hubka, V., Ezekiel, C.N., Hong, S.B., Nováková, A., Chen, A.J., Arzanlou, M., Larsen, T.O., Sklenář, F., Mahakarnchanakul, W. and Samson, R.A., 2019. Taxonomy of Aspergillus section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Studies in Mycology, 93:1-63.
6. Garcia, M.V., Parussolo, G., Moro, C.B., Bernardi, A.O. and Copetti, M.V., 2018. Fungi in spices and mycotoxigenic potential of some Aspergilli isolated. Food Microbiology, 73:93-98.
7. Jeswal, P. and Kumar, D., 2015. Mycobiota and natural incidence of aflatoxins, ochratoxin A, and citrinin in Indian spices confirmed by LC-MS/MS. International Journal of Microbiology, 2015: 242486. doi:10.1155/2015/242486
8. Kalil, M.I., Hussein, G.S. and Shareef, A.M., 2017. Aflatoxin production and genes assay in Aspergillus flavus isolated from spices. International Journal of Enhanced Research in Science, Technology and Engineering, 6(9):60-67.
9. Makhlouf, J., Carvajal-Campos, A., Querin, A., Tadrist, S., Puel, O., Lorber, S., Oswald, I.P., Hamze, M., Bailly, J.D. and Bailly, S., 2019. Morphologic, molecular and metabolic characterization of Aspergillus section Flavi in spices marketed in Lebanon. Scientific Reports, 9(1):5263.
10. Mandeel, Q.A., 2005. Fungal contamination of some imported spices. Mycopathologia, 159(2):291-298.
11. Puntscher, H., Kütt, M.L., Skrinjar, P., Mikula, H., Podlech, J., Fröhlich, J., Marko, D. and Warth, B., 2018. Tracking emerging mycotoxins in food: development of an LC-MS/MS method for free and modified Alternaria toxins. Analytical and Bioanalytical Chemistry, 410(18): 4481-4494.
12. Zahra, N., Khan, M., Mehmood, Z., Saeed, M.K., Kalim, I., Ahmad, I. and Malik, K.A., 2018. Determination of Aflatoxins in Spices and Dried Fruits. Journal of Scientific Research, 10(3):315-321.