KAVAKA 54: 89-95 (2020) doi:10.36460/Kavaka/54/2020/89-95

Toxigenic mycoflora and natural co-occurrence of toxins in red chillies from Jammu and Kashmir

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(Submitted on March 23, 2020; Accepted on May 30, 2020)

ABSTRACT

Quality deterioration of dried red chillies during storage poses a major threat as they are hygroscopic. Moreover, due to unscientific methods of collection, drying and post-harvest storage, red chillies are prone to fungal contamination and their toxins. In view of this, samples of dried red chillies were collected from different regions of Jammu and Kashmir and analysed for fungal species and associated toxins. Investigations revealed the presence of several fungal species, which are known producers of aflatoxins (*Aspergillus flavus*), cyclopiazonic acid (*Aspergillus flavus, A. fumigatus, A. tamarii, A. oryzae, A. versicolor, Penicillium griseofulvum* and *P. chrysogenum*) and sterigmatocystin (*Aspergillus nidulans, A. versicolor, A. sydowii, Chaetomium* and *Emericella* spp.). Qualitative analysis by thin layer chromatography (TLC) and then quantification by high performance liquid chromatography (HPLC) showed that 36 % of the samples were positive for aflatoxin B1 (0.24 - 2.14 µg/g), 5 % samples were positive for aflatoxin B2 (2.08 - 2.14 µg/g), 31 % of samples were positive for cyclopiazonic acid (1.65 - 144.78 µg/g) and 23 % samples were positive for sterigmatocystin (5.10 - 24.03 µg/g). In addition, co-occurrence of mycotoxins like aflatoxin and cyclopiazonic acid; aflatoxin and sterigmatocystin; aflatoxin, cyclopiazonic acid and sterigmatocystin were detected in 15, 5 and 5% samples, respectively. Detection of more than one mycotoxin from chilli pericarp samples is a matter of great concern as the co-occurrence of mycotoxins may generate additive or synergistic effect in consumers.

Keywords: Chilli pericarp, mycoflora, aflatoxins, cyclopiazonic acid, sterigmatocystin, co-occurrence.

INTRODUCTION

Chilli (*Capsicum annuum* Linn.) belonging to family *Solanaceae* is one of the most important spice cum vegetable crop grown in India both for domestic consumption as well as for export. It is the second largest consumed spice throughout the world, being next to black pepper, and is often called as hot red pepper, red chilli pepper, pod pepper, cayenne pepper, paprika and capsicum in different parts of the world.

In the Indian sub-continent, chillies are produced throughout the year but they grow best at 20-35°C and can be grown over a wide range of altitude from sea level up to nearly 2100 meters. Today, India is the largest producer, exporter and consumer of chillies in the world. It contributes about 36% of the total world production of chillies (Poulos, 1992; Geetha and Selvarni, 2017). It is grown over an area of 7,75,000 HA with annual production of 14,92,000 tonnes (DAC and FW 2016). However, high temperature and high relative humidity in the production areas results in fungal proliferation and subsequent mycotoxin contamination. Further, unscientific methods of collection, extended periods of drying in the open air, storage practices and transportation system enhances contamination and quality deterioration of red chillies as they are hygroscopic in nature. Absorption of moisture may result in caking, discoloration, hydrolytic rancidity and growth of mycotoxin producing strains of filamentous fungi such as, *Aspergillus, Penicillium* and *Alternaria* species (Seenappa *et al*., 1980; Giridhar and Reddy, 1999).

In India, there is marked fluctuation of temperature and relative humidity in different seasons, which favours the growth of storage fungi and incidence of mycotoxic contaminants on red chillies (Kiran *et al*., 2005; Saha *et al*., 2007; Jeswal and Kumar, 2014). Mycotoxin contamination is a very complex process and sometimes even more than one type of mycotoxin may occur (Miller, 1991; Murphy *et al*., 2006). This co-occurrence of mycotoxins lead to extreme toxic interactions, which provoke multimycotoxic aetiology (Stoev, 2010). In view of the hazards of mycotoxin ingestion, most of the countries have strict regulations on the level of mycotoxins permitted in traded commodities, of which European Union (EU) regulations remain the most stringent.

Red chillies from Jammu and Kashmir are famous worldwide for their deep red colour, fragrance and taste. However, when these red chillies are dried to a satisfactory degree before storage, they may develop local pockets of fungal growth, many of which may consist of toxigenic strains. So far, no attention has been paid to this problem in this region. Therefore, an attempt was made to assess the dried red chilli pericarp from Jammu and Kashmir for the presence of associated mycoflora and mycotoxic contaminants like aflatoxins, cyclopiazonic acid and sterigmatocystin as the pericarp constitutes the major portion of powdered chilli sold in the markets.

MATERIAL AND METHODS

Recovery of mycoflora associated with samples of dried chilli pericarp: During the investigation period, samples of dried red chillies were collected from households and various local markets of Jammu and Kashmir. To avoid external contamination the samples were brought to the laboratory in sealed polyethylene bags. Mycoflora associated with the dried red chilli pericarp was determined by following the method given by Harrigan (1998). For this purpose 5 g of ground chilli pericarp sample was homogenized in 45 mL of sterilized water for 15 minute. After this 1 mL of suitable tenfold serial dilutions prepared was poured in Petriplates by using a sterilized pipette. To recover maximum number of fungal propagules from each sample, three different media, mainly Czapek Dox Agar (CDA), Dichloran 18% Glycerol Agar (DG-18) and Malt Salt Agar (MSA) were used. Five replicates were maintained for each medium. Petriplates thus prepared were incubated at 28 + 2 ºC for 7 days. Fungal species recovered from the samples were purified by streaking. All the purified fungal isolates were maintained on

sterilized Potato Dextrose Agar (PDA) medium slants and stored in the refrigerator at 8-10 ºC. Identification of the recovered fungal species was done by using relevant literature and keys (Subramanian, 1961; Raper and Fennel, 1965; Booth, 1971; Ellis, 1971; Samson, 1974; Pitt, 1979; Sutton, 1980). Identity of some of the fungal species was confirmed from National Fungal Culture Collection of India, Agharkar Research Institute, Pune (India).

Percentage frequency of occurrence (%) was calculated by using formula:

$$\text{Frequency (\%)} = \frac{\text{Number of samples from which an organism was recovered}}{\text{Total number of samples tested}} \times 100$$

Extraction of sample for mycotoxins analysis: Samples of dried red chillies were analyzed for aflatoxins, cyclopiazonic acid and sterigmatocystin by using modified multimycotoxin method given by Roberts and Patterson, (1975). For this purpose 25 g of finely ground sample was extracted for 30 minutes on horizontal shaker in a mixture of 100 mL of acetonitrile and 4% potassium chloride (90:10v/v). The extract was filtered through Whatman no. 41 filter paper. The filtrate was defatted and extracted twice with 50 mL iso-octane in a 250 mL separating funnel. The upper iso-octane layer was later discarded. To the lower acetonitrile layer 12.5 mL of distilled water was added. To extract basic mycotoxins, the acetonitrile layer was extracted three times with 20 mL of chloroform each time. The chloroform acetonitrile layer was drained through Whatman no.41 filter paper containing a bed of anhydrous sodium sulphate. The extract collected in the flask was marked as extract I. To the upper aqueous layer 1 mL of 1.0 N HCL was added, acidic mycotoxins were extracted from this layer three times by using 10 mL of chloroform each time. The collected chloroform layers were passed through anhydrous sodium sulphate bed, and marked as extract II. Both the extracts were combined and evaporated to dryness on a water bath. The residue obtained was dissolved in 1 mL of chloroform and stored in freezer in clean vial till further use. For pigment separation the residue was dissolved in 12.5 mL of acetonitrile. The mixture was transferred to a dialysis sac prepared from dialysis tubing. The dialysis sac was equilibrated against 25 mL of acetone water mixture (30:70 v/v) in a flask for 16 hours by gentle shaking on a wrist action shaker. To improve recovery of mycotoxins, dialysis sac was equilibrated again for 6 hours against 25 mL of acetone water mixture (30:70 v/v). Aqueous acetone dilysates were combined and extracted three times in a separating funnel using 15 mL of chloroform each time. Then 3 mL of methanol was also added to it for the clear separation of layers. Chloroform extracts were combined, passed through anhydrous sodium sulphate bed and dried on a water bath. Dried residue was dissolved in 1 mL of chloroform and stored in a small screw cap vial for qualitative and quantitative analysis of mycotoxins.

Estimation of aflatoxins (AF): For the estimation of aflatoxins (AF), 50 mL of sample extract was spotted on the activated TLC plate along with the aflatoxin standards (B1 and B2) as reference spots. Spots were then developed with solvent system consisting of toluene: isoamyl alcohol: methanol (90:32:2 v/v). After air drying, the developed TLC plates were examined under long wave UV light (365nm). Aflatoxin spots were located after comparing their fluorescence colour and Rf value with that of standard spots. Quantitative estimation of aflatoxins was done by standardizing the method described by Sigma-Aldrich (2000). The analytical equipment for high performance liquid chromatography (CLASS - LC10 SHIMADZU) consisted of a liquid chromatographic pump LC-10AT, having an auto-injection system (SIL-10A) with a 50 µL sample loop. It was having a variable wavelength absorbance UV-VIS detector SPD-10 set at 365 nm. The analytical column was CLC-ODS (4.6 x 250 mm), filled with ODS (M), RP-18 material, 5 µm particle size (Merck). The mobile phase consisted of water: acetonitrile: methanol (60:30:10 v/v) set at a flow rate of 1.5 mL/minute. Estimation was done at room temperature of 25-30 ºC and data was recorded in HP Desk Jet 670 C. Injection volume for extract solution was 20 µL. Estimation was done by comparing the retention time of aflatoxins (AFB1-6.0 min. and AFB2-5.1 minutes) and peak areas of aflatoxin standards with those of samples.

Estimation of sterigmatocystin (STER): Detection of sterigmatocystin (STER) was done by using the method given by Athnasios and Kuhn (1977). For this purpose 50µL of sample extracts were spotted on the activated TLC plates along with the standard and developed in a solvent system consisting of benzene: acetic acid (9: 1 v/v). Air dried plates were then sprayed with 20% aluminium chloride solution and then heated at 80 ºC for 10 minutes. The spots were then located as yellow fluorescent spots under short wave UV light. Quantitative estimation of sterigmatocystin was done by high performance liquid chromatography, by modifying a method given by Engelhart *et al.* (2002). In this method, mobile phase consisted of methanol: water (80: 20 v/v) set at a flow rate of 1.5 mL/minute. Estimation was performed at room temperature (25-30 ºC). Volume of injection used for extract solution was 10 µL. A variable wavelength absorbance UV-VIS detector set at 365 nm was used. Quantification of sterigmatocystin was done by comparison of retention time (4.2 minutes) and peak area observed in the standard with those observed for samples.

Estimation of cyclopiazonic acid (CPA): For detection of cyclopiazonic acid (CPA) contamination, known amount of sample extracts (50 µL) were spotted on activated TLC plates along with the standard and developed in a solvent system consisting of benzene: methanol: acetic acid (90: 16: 8 v/v). After drying the plate, spots of CPA were visualized under short wave UV light as purple spots. Chemical confirmation of CPA was done by spraying freshly prepared Ehrlich's reagent (by dissolving 1.0 g P-dimethyalaminobenzaldehyde in 10 mL distilled water and 20 mL concentrated hydrochloric acid) on the plate. Under visible light, CPA showed purple spots. Quantitative estimation of CPA was done by modifying the HPLC method of Motta and Soares (2001). Mobile phase consisted of 0.3% aqueous ZnSO4.7H2O: methanol (70:30 v/v) set at a flow rate of 0.6 mL/minute. Injection volume for extract solution was 5 µL. Quantification of CPA was done by comparison of the retention time (7.4 minutes) and peak area observed in the CPA standard with those observed in the samples.

RESULTS AND DISCUSSION

The samples of dried chilli pericarp collected by undertaking survey of 39 market places and domestic areas of different parts of Jammu and Kashmir showed an association of 69 fungal species (**Table 1**). Perusal of data generated shows (**Fig. 1**) that among the recovered fungal species, zygomycetous fungi were represented by 3 genera (5 species), ascomycetous fungi by 5 genera (13 species) and mitosporic fungi were represented by 14 genera (51 species). Present study revealed the dominance of *Aspergillus* species on the chilli pericarp (**Table 1**). Among the various recovered aspergilli, *Aspergillus niger* formed the dominant component of fungal mycoflora as it was detected from 62.0 % samples

Table 1: Frequency of fungal species recovered from the pericarp of dried red chillies.

Fungal species recovered	Frequency (%)
ZYGOMYCOTA	
Mucor racemosus	8.0
Rhizopus arrhizus	15.5
R. oryzae	10.0
R. stolonifer	28.5
Syncephalastrum racemosum	10.5
ASCOMYCOTA	
Chaetomium sp.	2.5
C. homopilatum	2.5
Eurotium amstelodami	44.0
E. chevalieri	36.0
E. chevalieri var. *intermedius*	15.5
E. herbariorum	2.5
E. repens	8.0
E. rubrum	20.5
Emericella nidulans var. *latus*	2.5
E. quadrilineata	8.0
E. striata	5.0
Sartorya fumigata	2.5
Thielavia sepedonium	2.5
MITOSPORIC FUNGI	
Acremonium implicatum	5.0
Aspergillus aculeatus	8.0
A. candidus	2.5
A. ficuum	10.0
A. flavipes	5.0
A. flavus	56.5
A. flavus var. *columnaris*	10.5
A. fumigatus	13.0
A. japonicas	2.5
A. nidulans	2.5
A. niger	62.0
A. ochraceus	18.0
A. oryzae	2.5
A. parasiticus	2.5
A. sydowii	13.0
A. tamarii	7.5
A. terreus	8.0
A. terricola var. *americana*	5.5
A. ustus	2.5
A. versicolor	18.0
Alternaria alternata	36.0
Alternaria raphani	10.0
Curvularia pallescens	5.0
Cladosporium cladosporioides	28.0
C. oxysporum	2.5
Colletotrichum capsici	8.0
Drechslera australiensis	2.5
Fusarium concolor	7.5
F. verticillioides	5.0
F. verticillioides var. *Subglutinans*	7.5
F. semitectum	10.5
Nigrospora oryzae	5.0
Penicillium brevicompactum	7.5
Penicillium chrysogenum	15.5
Penicillium citrinum	18.0
P. expansum	2.5
P. fellutanum	10.5
P. griseofulvum	18.0
P. herquei	5.5
P. islandicum	2.5
P. miniolutеum	5.0
P. olsonii	5.0
P. oxalicum	2.5
P. pinophilum	2.5
P. puberlum	18.0
P. verrucosum	2.5
P. waksmanii	7.5
Paecilomyces victoriae	2.5
Scopulariopsis brevicaulis	2.5
Tritirachium roseum	2.5
Wallemia sebi	7.5

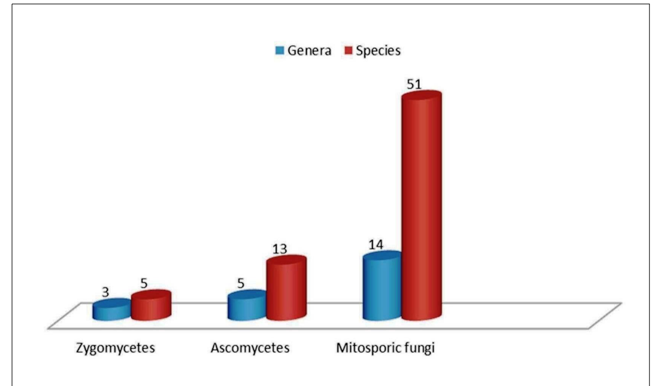

Fig. 1: Total number of fungal genera and species recovered from market and domestic samples of dried red chillies.

(**Table 1**). Earlier, Giridhar and Reddy (1999) also reported prevalence of *A. niger* in stored chillies from Andhra Pradesh. As has been documented presently, workers from other countries have also reported dominance of *A. niger* on *Capsicum annuum* (Atanda *et al*., 1990; Mandeel, 2005; Santos *et al*., 2011; Gambacorta *et al*., 2018). During the present investigation, besides *Aspergillus niger*, potentially toxigenic *A. flavus* was recovered from 56.5 % samples, whereas 18.0 % samples were having *A. ochraceous* (**Table 1**). These results are in conformity with the studies undertaken from elsewhere by number of investigators (Christensen *et al*. 1967; Pal and Kundu, 1972; Flannigan and Hui, 1976) in which *A. flavus* has been documented as the dominant component of the mycoflora of red peppers. While working with the chilli samples from warehouse in Cochin, Seenappa *et al*. (1980) also documented the dominance of *A. flavus* and *A. ochraceus* on the surface of pepper pods. Recently, Jeswal and Kumar (2015) also detected *A. flavus* in 32% of red chilli samples from Bihar. During the present investigations, representatives of versicolor group (*A. sydowii* and *A. versicolor*) were detected from 13.0% and 18.0% of the examined samples, respectively. As compared, the per cent frequency of other aspergilli, *viz. A. flavipes, A. terricola* var. *americana, A. tamarii, A. terreus* and *A. fumigatus* varied between 5.0-13.0% (**Table 1**). Ath-Har *et al*. (1998) also reported frequent association of *A. flavus, A. niger, A. nidulans, A. ochraceus, A. sydowii, Penicillium* and *Rhizopus* species with *Capsicum frutescens* and other Indian spices.

Present investigation showed *Penicillium* as the next most

Table 2: Percentage frequency of major fungal genera contaminating the pericarp of dried red chillies.

Fungal Genera	No. of species	Positive samples (%frequency) (n =39)
Aspergillus	19	32(82.0)
Penicillium	15	29(74.0)
Eurotium	6	25(64.0)
Rhizopus	3	16(41.0)
Alternaria	2	19(49.0)
Fusarium	4	11(28.0)
Cladosporium	2	12(31.0)
Emericella	3	5(13.0)

n = total no of samples assessed

frequent contaminant in decreasing order. It was recovered from 74.0% samples of chilli pericarp (**Table 2**). In all, 15 species of *Penicillium* were recovered from the samples. Among these, *P. citrinum, P. griseofulvum* and *P. puberlum* were recovered from 18.0% samples (**Table 1**). Percentage frequency of other *Penicillium* species, *viz. P. pinophilum, P. oxalicum, P. minioluteum, P. herquei, P. waksmanii, P. fellutanum* and *P. chrysogenum* varied between 2.5% and 15.5% **(Table 1**). Among the recovered *Eurotium* species, *E. amstelodami* was the major contaminant associated with 44.0% samples followed in decreasing order by *E.chevalieri*, which contaminated 36.0% chilli pericarp samples (**Table 1**). Other species of *Eurotium* included *E. chevalieri* var. *intermedius* (15.5%), *E. rubrum* (20.5%) and *E. repens* (8.0%). The other major fungal contaminants documented during the present investigation (**Table 2**) are *Rhizopus* (41.0%), *Alternaria* (49.0%), *Fusarium* (28.0%), *Cladosporium* (31.0%) and *Emericella* (13.0%).

Perusal of data generated during the present investigations (**Tables 1, 3 & 4**) show that 56.5% of the samples were positive for *A. flavus* contamination, whereas 36% were positive for AFB1 and 5% were positive for AFB2 contamination. Jeswal and Kumar (2015) detected approximately 56% isolates of *A. flavus* collected from red chillies to be toxigenic, producing aflatoxins up to 33.6 µg/L. However, Yogendrarajah *et al*. (2014) reported at least 77% chilli samples from Sri Lanka to be contaminated with aflatoxins, which is much more in comparison to the present findings. This proves that amount of aflatoxins produced by aflatoxigenic fungi vary with the climate and storage conditions available. Similar observations have been reported by Cotty and Garcia (2007) who detected variation in the quantity of aflatoxigenic fungi depending upon the climatic conditions under which the crops are grown and stored. The aflatoxigenic fungi are known to compete poorly under cool conditions and therefore the concentration of toxins produced by them in such areas is low in comparison to the warmer regions. However, still the percentage of fungal contamination and amount of toxin detected in chillies is alarming.

Table3: Contamination of aflatoxins (AFB1 and AFB2) cyclopiazonic acid (CPA) and sterigmatocystin (STER) detected from market and domestic samples of dried red chillies from Jammu and Kashmir.

SAMPLE CODE (Kashmir) n=20	Mycotoxins detectedµg/g				SAMPLE CODE (Jammu) (n=19)	Mycotoxins detected (µg/g)			
	AFB1	AFB2	CPA	STER		AFB1	AFB2	CPA	STER
K-1	-	-	-	-	J-1	-	-	-	-
K-2	-	-	-	-	J-2	0.33	-		6.71
K-3	0.32	-	2.14	-	J-3	0.34	-	144.78	5.10
K-4	-	-	-	12.07	J-4	-	-	39.64	14.75
K-5	0.79	-	-	-	J-5	0.77	-	-	-
K-6	-	-	-	-	J-6	2.14	-	-	13.99
K-7	0.91	-	15.44	-	J-7		-	-	-
K-8	0.58	-	-	-	J-8	0.24	-	77.23	5.73
K-9	-	-	-	-	J-9	-	-	-	-
K-10	0.67	-	40.54	-	J-10	-	-	-	-
K-11	-	-	-	7.78	J-11	-	-	38.75	-
K-12	-	-	-	-	J-12	-	-	-	-
K-13	-	-	-	-	J-13	-	-	-	-
K-14	-	-	-	-	J-14	-	-	-	-
K-15	-	-	-	11.2	J-15	1.57	-	37.68	-
K-16	-	-	-	-	J-16	0.34	2.08	1.65	-
K-17	0.47	-	138.21	-	J-17	-	-	29.91	-
K-18	-	-	-	-	J-18	-	-	34.26	-
K-19	-	-	-	24.03	J-19	-	2.14	-	-
K-20	0.25	-	-	--					

-, not detected

Among the various aflatoxins (AF) detected from chilli pericarp samples, AFB1 was found to be most frequent, being detected in 36.0% of the examined samples and concentration varied from 0.24 to 2.14 µg/g (**Tables 3 and 4**). It is reported to be the most potent natural carcinogen (Squire, 1981) and is one of the major aflatoxin produced by toxigenic strains (Bennett, 2003). The maximum permissible level of aflatoxin B1 in dried fruits of chillies is 5µg/Kg, whereas for total aflatoxins (AFB1, AFB2, AFG1 and AFG2) it is 10 µg/Kg (EC, 2010). Earlier AFB1 has been detected as the predominant mycotoxin contaminating at least 77% of the Srilankan red chilli samples (Yogendrarajah *et al*., 2014). In the present investigation only 5.0% samples procured from Jammu region were found to be positive for AFB2 (2.08-2.14 µg/g), whereas it was not detected from the samples procured from Kashmir region (**Table 3**). Jalili and Jinap (2012) reported 14.0% samples of red chillies from supermarkets of Malaysia to be positive for AFB2 and the detected level of contamination was high ranging from 0.1 to 11.45 ng/g of red chillies. Similarly, Ozbey and Kabak (2012) also detected AFB2 contamination (0.04–1.28 µg/kg) in red chilli flakes sampled from Turkey. Yogendrarajah *et al*. (2014) detected AFB2 in 10% samples of chilli flakes and whole chillies (6.1-31.5 µg/kg) from Sri Lanka. Additionally, many more workers have also reported high concentration of aflatoxins in chillies from different parts of the globe (Martins *et al*., 2001; Paterson, 2007; Iqbal *et al*., 2011;Santos *et al*., 2010; Golge *et al*., 2013; Singh and Cotty, 2017).

Sterigmatocystin (STER) is a precursor of aflatoxin biosynthesis, but is reported to be less carcinogenic than aflatoxin B1 (Casteel and Braun, 1992; Versilovskis and De Saeger, 2010). Maximum permissible level for sterigmatocystin has not been set yet. (EFSA, 2013). It is produced by many species of *Aspergillus* including frequently occurring *A. versicolor, A. sydowii, A. nidulans* and some species of *Bipolaris, Chaetomium* and *Emericella*. While assessing the mycoflora of chilli pericarp, *Aspergillus versicolor*, the most significant producer of sterigmatocystin, was recovered from 18% chilli pericarp samples (**Table 1**). In addition, other known producers like *A. sydowii, A. nidulans, Emericella* and some *Chaetomium* species were also recovered from the pericarp samples (**Table 1**).While investigating these samples for sterigmatocystin, 23.0% were found to be positive and the concentration varied from 5.10-24.03 µg/g (**Table 4**). Earlier, El-Kady *et al*. (1995) reported sterigmatocystin contamination (10-23 µg/kg) in red pepper samples from Egypt. Later, Bokhari (2007) also detected sterigmatocystin (11-25 µg/kg) from red peppers of Saudi Arabia. Recently, Yogendrarajah *et al*. (2014) have reported 38% of red chilli samples of Sri Lanka to be contaminated with sterigmatocystin (32 µg/kg of sample). Sterigmatocystin is a carcinogenic compound and classified as 2 B carcinogen by the International Agency for Research on Cancer and there are many reports about its toxicity and mutagenicity (IARC, 1987).

Presently while investigating the samples of chilli pericarp, 31.0% of the samples were found to be positive for cyclopiazonic acid (CPA) contamination (**Table 4**). The level of CPA contamination was detected to be quite high, varying

Table 4: Chilli pericarp samples showing per cent occurrence of various mycotoxins (AFB1, AFB2, CPA and STER).

Mycotoxins detected	Percentage of samples detected positive	Range of contamination (µg/g)
AFB_1	36.0	0.24-2.14
AFB_2	5.0	2.08-2.14
CPA	31.0	1.65-144.78
STER	23.0	5.10-24.03

between 1.65-144.78 µg/g (**Table 3**). Cyclopiazonic acid is a mycotoxin produced by several species of *Aspergillus* and *Penicillium* (Dorner *et al*., 1984). However, discovery of CPA production by *Aspergillus flavus*, a commonly found mould species, has attracted the attention of scientists (Luk *et al*., 1977). This mycotoxin is unique as it is reported to affect muscle tissue in animals (Norred, 1990). A recent research has also reported that it is cytotoxic and immunotoxic to human cells (Hymery *et al*., 2014). Just as sterigmatocystin, CPA is also yet to be evaluated by IARC programme (Ostry *et. al*., 2017).

Perusal of data generated (**Fig. 2**) shows that only 3.0% of the chilli pericarp samples showed co-occurrence of AFB1 and AFB2, whereas 15.0% samples showed co-occurrence of AF and CPA. Similar trend as have been observed presently is also reported in many other fresh and dried commodities (Bamba and Sumbali, 2005; Zorzete *et al*., 2013). The overall effect of aflatoxins and CPA is reported to be additive (Ostry *et al*., 2018). Presently co-occurrence of AFB1 and STER was found in 5.0% samples (**Fig. 2**). In a study from Sri Lanka similar co-occurrence has been reported in 33% samples of chillies (Yogendrarajah *et al*., 2014). During the present investigation, additional 5.0% samples of chilli pericarp were also detected to have co-occurrence of three mycotoxins *viz*. AFB1, CPA and STER (**Fig. 2**). Such co-occurrence of different mycotoxins in food samples is quite common but at the same time it is a risky proposition. Since it is an established fact that the combined intake of different mycotoxins at variable concentration levels may lead to a higher risk than their individual intake (Speijers and Speijers, 2004).

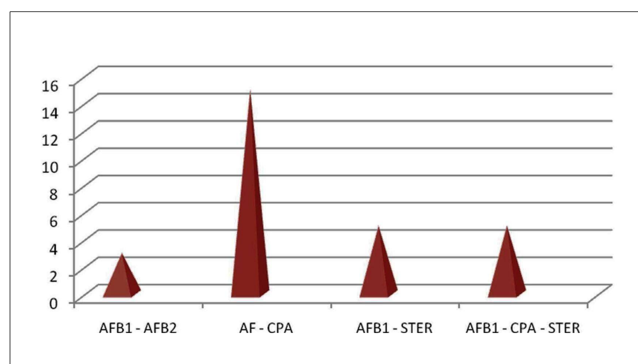

Fig. 2: Percentage of chilli pericarp samples showing co-occurrence of various mycotoxins (AFB1, AFB2, CPA and STER).

CONCLUSION

Detection of high concentration of aflatoxins, cyclopiazonic acid and sterigmatocystin in market and domestic samples of dried red chillies indicates that they form a good substrate for mycotoxin production under poor conditions of storage. Some samples even showed co-occurrence of mycotoxins, which is a matter of great concern as this may result in additive or synergistic effects in humans who consume them frequently. Creating awareness among farmers and retailers to use scientific methods for storage is an important aspect which needs to be emphasized. In addition to this there is an urgent need for developing new methods of drying and storage so as to improve the quality as well as to decrease the probability of infection by toxigenic fungi and formation of such toxic contaminants in stored food items.

ACKNOWLEDGEMENTS

Thanks are due to the Department of Botany, University of Jammu for financial support, to the Department of Environmental studies, University of Jammu for providing HPLC facilities and to UGC, New Delhi for providing assistance under Faculty improvement Programme.

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