

Some more additions to the mycotoxin profile of dried red chillies from Union Territory of Jammu and Kashmir

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ABSTRACT

A study was undertaken to isolate and enumerate species of *Aspergillus* and *Penicillium* and their toxic secondary metabolites associated with the pericarp of dried red chillies sampled from Jammu and Kashmir (UT). To recover maximum species, three different media, viz., Czapek Dox Agar (CDA), Dichloran 18% Glycerol Agar (DG-18), and Malt Salt Agar (MSA) were used. Mycoflora assessment indicates that chilli pericarp is capable of harbouring a number of species belonging to these two genera and their perfect states. Overall, *Aspergillus* and *Penicillium* were equally represented by 15 species each, followed in decreasing order by their perfect states, *Eurotium* and *Emmericella*, which were represented by three and two species, respectively. In view of the detection of fungal species, which are known producers of toxic metabolites, the samples were investigated for some more toxins like ochratoxin A, patulin and citrinin by using multimycotoxin method and HPLC analyser. Results revealed the presence of all the three toxins in 15-70 per cent samples. Detection of these mycotoxins from the dried red chillies marketed in Jammu and Kashmir is of great concern as it is an important spice of commerce, which is used daily in almost every household for cooking and seasoning.

KEY WORDS: Chilli pericarp, *Aspergillus*, *Penicillium*, ochratoxin A, patulin, citrinin.

INTRODUCTION

Red chillies (*Capsicum annuum* Linn.) are one of the favourite spices consumed as the second largest spice throughout the world because of their colour, aroma, pungency and taste. In India, they are grown over an area of 8,30,800 HA with an annual production of 18,72,000 MT, whereas in UT of Jammu and Kashmir, 600 HA of land is under its cultivation with an annual production of 400 MT (DACFW, 2018). In India, the harvested red chillies are usually sun-dried by spreading on the ground, road sides or roofs of mud houses. The hot and humid climatic conditions, processing methods, long drying periods under non-sanitary conditions may result in the deterioration of the quality of red chillies as these factors are very conducive for the growth of toxic fungal species and subsequently production of mycotoxins. The fungal contamination of chilli pericarp may get initiated from different sources, such as, surface mycoflora of the plants, fungal air-spores, human contact, contamination from dust particles and water (Wirtanen *et al.*, 1993). Many mycotoxin producing fungal species growing in stored agricultural commodities belong to *Aspergillus* and *Penicillium* genera (Alshannaq and Yu, 2017). In the past, some studies have reported *Aspergillus* and *Penicillium* species and their toxic secondary metabolites as the predominant contaminants from dried red chilli pods (Samyal and Sumbali, 2013; 2020; Yogendrarajah *et al.*, 2014; Jeswal and Kumar, 2015; Frimpong *et al.*, 2019). Environmental parameters like water activity, temperature, pH, and substrate composition are some of the important factors that determine colonisation by fungal species and biosynthesis of mycotoxins (Schmidt-Heydt *et al.*, 2008). All the mycotoxins are heat stable and can initiate a number of syndromes, which may sometimes result in fatal consequences (Kamala *et al.*, 2018). At times co-occurrence of two or more mycotoxins may also occur, which also leads to extreme toxic interaction that provokes multimycotoxic aetiology (Stoev, 2010).

Earlier, the authors assessed market and domestic samples of

dried red chillies and their seeds from the Union territory of Jammu and Kashmir for the mycoflora and mycotoxigenic contaminants like aflatoxins, cyclopiazonic acid and sterigmatocystin (Samyal and Sumbali, 2020 a; 2020 b). In view of the detection of high concentration of these three mycotoxins and their co-occurrence in a number of samples, an attempt was made to assess the samples for some other mycotoxigenic contaminants. This communication reports assessment results of dried chilli pericarp samples for the spore load of species of *Aspergillus*, *Penicillium* and their perfect states and some mycotoxigenic contaminants like ochratoxins A, patulin and citrinin, which are produced by them and are of concern because of the associated human health risks.

MATERIALS AND METHODS

During the investigation period (2009-2010), samples of dried red chilli pods were randomly collected from various households and local markets of Jammu and Kashmir (UT). Seeds were separated aseptically and the pericarps were kept in pre-sterilized polyethylene bags at 5-7°C for conducting studies.

Recovery of aspergilli and penicilli associated with the samples of dried red chilli pericarp: For this purpose 5g of the sample was homogenised in 45 mL of sterilized distilled water for 15 minutes (Harrigan, 1998). After this 1 mL of tenfold serial dilution prepared was poured in sterilized Petri plates. To recover maximum species of *Aspergillus* and *Penicillium*, three media – Czapek Dox Agar medium (CDA), Dichloran 18% Glycerol Agar medium (DG-18) and Malt Salt Agar medium (MSA) were used. Five replicates were prepared for each medium and incubation was done at 28°C for 7 days. Species recovered were streaked for purification and then maintained on slants having potato dextrose agar medium (PDA). The purified cultures were maintained at 8-10°C and identified using relevant literature and keys. (Raper and Fennel 1965; Pitt, 1979). Identity of some of the recovered species was

confirmed from National Fungal Culture Collection of India, (NFCCI), Pune.

Average colony forming units (cfu/g) of the recovered species were calculated by following the formula given by Parikh and Shah (2006).

$$\text{Cfu/g} = \frac{\text{Average number of fungal colonies observed on the Petri plate} \times \text{Dilution factor}}{\text{Dry weight of sample}}$$

Frequency (%) was determined by the following formula:

$$\text{Frequency (\%)} = \frac{\text{Number of chilli samples from which the fungal species was encountered} \times 100}{\text{Total number of chilli samples tested}}$$

Extraction of samples for mycotoxin analysis: Multi-mycotoxin analysis method given by Roberts and Patterson (1975) was followed to extract the mycotoxins from dried red chillies. For this purpose 25 g of dried chilli sample was finely ground and put in 100 mL mixture of acetonitrile and 4% potassium chloride (90:10v/v), extracted for 30 mins. and filtered through Whatman 41. The filtrate thus obtained was extracted twice with 50 mL of iso-octane each time. The upper iso-octane layer was discarded and distilled water (12.5 mL) was added to the lower acetonitrile layer. The acetonitrile layer was extracted three times with 20 mL of chloroform each time and passed through the anhydrous sodium sulphate bed. This was marked as extract I as it contained the basic mycotoxins. To the upper aqueous layer, 1 mL of 1.0 N HCL was added, extracted thrice by using 10 mL of chloroform each time and passed through anhydrous sodium sulphate bed. This was marked as extract II as it contained the acidic mycotoxins. Both the extracts were mixed, dried on a water bath and the residue was dissolved in 1.25 mL of acetonitrile and transferred into a dialysis sac made from dialysis tubing for separation of pigments. The dialysis sac was equilibrated against 25 mL of acetone water mixture (30:70 v/v) in a stoppered conical flask for 16 hours by gentle shaking on a wrist action shaker. Dialysis sac was again equilibrated for 6 hours to improve recovery of mycotoxins. Aqueous acetone dilysates were combined and extracted with 15 mL of chloroform three times in a separating funnel. Methanol (3 mL) was 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 of chloroform (1mL) for detection and quantitative estimation of ochratoxin A, Patulin and citrinin.

Analysis of ochratoxin A (OTA): It was detected by loading sample extracts (50 µL) and OTA standard on the activated TLC plates. The plates were then developed in toluene: ethyl acetate: 90% formic acid in the ratio of 50: 40: 10 v/v. OTA was detected under UV light (long wave) as fluorescent spots and confirmation was done by comparing Rf value with the standard spots. Quantitatively OTA was estimated by following HPLC method given by Vrabcheva *et al.* (2000).

Analysis of patulin (PAT): It was detected from the samples by applying (50 µL) of the sample extract on the activated TLC plates with a micropipette. Standard of patulin was also spotted on the TLC plates as reference spot. The loaded plates

were then developed in toluene: ethyl acetate: chloroform (80: 70: 50 v/v) and 1mL of 90% formic acid. Developed plates were air-dried, sprayed with freshly prepared phenylhydrazine hydrochloride (by dissolving 2g in 100 mL of water) and thereafter heated at 110°C for 5 minutes (Subramanian, 1982). Patulin was detected under visible light as yellow coloured spots. Confirmation was done by comparing their Rf value and colour with that of the standard. Quantification of patulin was done by following HPLC method of Beretta *et al.* (2000).

Analysis of citrinin (CIT): For detection of citrinin contamination, loaded TLC plates were developed in toluene: ethyl acetate: chloroform (80: 70: 50 v/v) and 1 mL of 90% formic acid. Developed plates were observed directly under long UV light. Presence of citrinin showed yellow fluorescent spots. Quantification of citrinin was done by following HPLC method of Marti *et al.* (1978).

RESULTS AND DISCUSSION

1. *Aspergillus* and *Penicillium* species associated with the pericarp of dried red chillies: During the present investigation, samples of dried chilli pericarp showed an association of several xerophilic species belonging to genera *Aspergillus* and *Penicillium*. Both these genera are versatile in their water activity requirement and are most numerous in low water activity habitats (Hocking, 1991). In order to recover maximum number of toxin producing species of *Aspergillus* and *Penicillium*, three culture media, viz. CDA, DG-18 and MSA were used. It was observed that the recovery of various species on different media was not uniform (**Table 1**). Among the media used, CDA is a routine laboratory medium and most useful for counting the mould colonies. Out of the recovered species, *Aspergillus parasiticus*, *A. ustus*, *A. oryzae*, *Emericella quadrilineata*, *Penicillium expansum* and *P. verrucosum* were recorded exclusively on CDA. Similarly, DG-18 supported the growth of 2 exclusive species, namely *Aspergillus japonicus* and *Penicillium oxalicum*, whereas MSA exclusively supported the growth of *Aspergillus nidulans*. Third medium DG-18 used is well known for enumerating the xerophilic fungi from low moisture foods and was developed by Hocking and Pitt (1980). Few other investigators have also used DG-18 for enumeration of moulds from dried chillies and their products (Martin *et al.*, 2005; Hell *et al.*, 2009; Samyal and Sumbali, 2013, 2020).

1 a Sampling from Jammu division: Present study revealed dominance of *Aspergillus* species (95%) from the dehydrated chilli samples obtained from Jammu division (**Table 2**). Among the 11 species of *Aspergillus* recovered from the samples, *A. niger* formed the dominant component of fungal mycoflora as it was detected from 89.0 per cent samples with colony count up to 2.7×10^3 cfu/g (**Table 1**). Earlier, Giridhar and Reddy (1999) also reported maximum prevalence of *A. niger* in stored chillies from Andhra Pradesh. From other countries also few investigators have reported dominance of *A. niger* on *Capsicum annum* (Atanda *et al.*, 1990; Santos *et al.*, 2011; Mandeel, 2005). In fact, *A. niger* is reported to show highest per cent incidence on other Indian spices also (Hedawoo and Chakranarayan, 2011). In the present

Table 1: Frequency percentage and total colony count (cfu/g) of *Aspergillus* species, *Penicillium* species and their perfect states recovered from dried pericarp of chilli pods on different growth media.

Species recovered	Jammu Division				Kashmir Division			
	Freq. %	cfu/g			% Freq.	cfu/g		
		CDA	DG-18	MSA		CDA	DG-18	MSA
<i>Aspergillus niger</i>	89.0	2.7×10^3	2.4×10^3	2.1×10^3	35.0	8.4×10^1	1.1×10^3	8.1×10^2
<i>A. flavus</i>	68.0	2.5×10^3	1.6×10^3	1.9×10^3	45.0	1.3×10^3	1.1×10^3	1.0×10^3
<i>A. flavipes</i>	5.0	8.4×10^1	-	3.1×10^1	5.0	7.0×10^1	-	-
<i>A. flavus</i>	68.0	2.5×10^3	1.6×10^3	1.9×10^3	45.0	1.3×10^3	1.1×10^3	1.0×10^3
<i>A. fumigatus</i>	16.0	2.9×10^2	-	1.5×10^2	10.0	8.0×10^1	1.5×10^2	-
<i>A. japonicas</i>	-	-	-	-	5.0	-	6.0×10^1	-
<i>A. nidulans</i>	-	-	-	-	5.0	-	-	6.0×10^1
<i>A. ochraceus</i>	26.0	3.8×10^2	2.7×10^2	-	10.0	1.0×10^2	9.0×10^1	5.0×10^2
<i>A. oryzae</i>	-	-	-	-	5.0	1.1×10^2	-	-
<i>A. parasiticus</i>	5.0	5.2×10^1	-	-	-	-	-	-
<i>A. versicolor</i>	16.0	2.6×10^2	2.4×10^2	6.4×10^2	20.0	2.5×10^2	9.0×10^1	1.5×10^2
<i>A. sydowii</i>	11.0	-	1.3×10^2	5.2×10^2	15.0	-	2.1×10^2	2.2×10^2
<i>A. tamaritii</i>	5.0	-	4.2×10^1	-	10.0	5.0×10^1	8.0×10^1	4.0×10^1
<i>A. ustus</i>	-	-	-	-	5.0	5.0×10^1	-	-
<i>A. terreus</i>	11.0	4.2×10^1	-	4.2×10^1	5.0	-	-	3.0×10^1
<i>Eurotium amstelodami</i>	58.0	1.5×10^3	1.5×10^3	2.2×10^3	30.0	2.1×10^2	9.4×10^2	1.2×10^3
<i>E. chevalieri</i>	42.0	3.8×10^2	8.1×10^2	1.0×10^3	30.0	4.6×10^2	9.0×10^2	9.9×10^2
<i>E. repens</i>	11.0	4.2×10^1	6.2×10^2	4.7×10^2	5.0	-	1.4×10^2	1.5×10^2
<i>Emericella nidulans</i>	5.0	7.3×10^1	-	-	-	-	-	-
<i>E. quadrilineata</i>	11.0	7.3×10^1	-	-	5.0	1.0×10^1	-	-
<i>Penicillium brevicompactum</i>	-	-	-	-	15.0	3.8×10^2	1.8×10^2	2.5×10^2
<i>P. chrysogenum</i>	11.0	3.4×10^2	2.1×10^2	3.6×10^2	20.0	2.1×10^2	2.2×10^2	2.0×10^2
<i>P. citrinum</i>	21.0	3.6×10^2	6.3×10^1	3.5×10^2	15.0	3.4×10^2	5.0×10^1	1.9×10^2
<i>P. expansum</i>	-	-	-	-	5.0	7.0×10^1	-	-
<i>P. fellutanum</i>	16.0	1.0×10^2	1.0×10^2	6.3×10^1	5.0	1.7×10^1	-	-
<i>P. griseofulvum</i>	21.0	2.7×10^2	2.2×10^2	1.3×10^2	15.0	5.5×10^2	1.8×10^2	2.0×10^2
<i>P. herquei</i>	11.0	8.4×10^1	9.4×10^1	-	-	-	-	-
<i>P. islandicum</i>	-	-	-	-	5.0	-	7.0×10^1	1.0×10^2
<i>P. minioluteum</i>	5.0	5.2×10^1	-	-	5.0	-	7.0×10^1	-
<i>P. olsonii</i>	-	-	-	-	10.0	2.9×10^2	1.9×10^2	1.2×10^2
<i>P. oxalicum</i>	5.0	-	5.2×10^1	-	-	-	-	-
<i>P. pinophilum</i>	5.0	7.3×10^1	-	1.4×10^2	-	-	-	-
<i>P. puberulum</i>	21.0	2.0×10^2	2.3×10^2	1.4×10^2	15.0	2.3×10^2	1.8×10^2	2.2×10^2
<i>P. verrucosum</i>	-	-	-	-	5.0	4.0×10^1	-	-
<i>P. waksmanii</i>	5.0	2.2×10^2	6.3×10^1	-	10.0	1.4×10^2	-	8.0×10^1
-, not detected								

investigation, potentially toxigenic *A. flavus* (2.5×10^3 cfu/g) and *A. ochraceus* (3.8×10^2 cfu/g) were recovered from 68.0 and 26.0 per cent samples, respectively (Table 1). Seenappa *et al.*, (1980) also detected *A. flavus* and *A. ochraceus* on the surface of pepper pods obtained from a warehouse in Cochin and both these organisms are reported to have dominated the pepper pods at 95% RH. From Jammu samples, representatives of versicolor group, that is, *A. versicolor* and *A. sydowii* were detected from 16.0 and 11.0 per cent samples, respectively (Table 1). Ath-Har *et al.* (1988) also reported frequent association of *Aspergillus niger*, *A. flavus*, *A. ochraceus*, *A. nidulans*, *A. sydowii* and some species of *Penicillium* with *Capsicum frutescens* and other Indian spices. Dominance of *Aspergillus* species and their perfect states have also been reported from pericarp of chilli pod during harvesting, sun drying, transportation and marketing (Samyal and Sumbali, 2020b). In the present study, the spore load of perfect states like *Eurotium amstelodami*, *E. chevalieri*, *E. repens*, *Emericella nidulans* and *E. quadrilineata* were found to vary from 1.0×10^1 to 2.2×10^3 (Table 1).

As presented in table 2, *Penicillium* species were recovered from 74 per cent samples of dried chilli pericarp collected from Jammu division. In all, ten species of *Penicillium* were recovered from these samples and their colony count varied from 5.2×10^1 – 3.6×10^2 cfu/g (Table 1), thus collectively occupying a high share in total fungal load of chilli samples. The high counts of *Penicillium* species could be due to the unhygienic methods of handling, storage and marketing, which pose a health risk for both the sellers and the consumers.

1 b. Sampling from Kashmir Division: Mycological analysis of household and market samples of dried red chillies collected from Kashmir division showed an association of 14 species of *Aspergillus* (Table 1). Out of these, *A. flavus* was detected in maximum number of (45%) samples with a colony count of up to 1.3×10^3 cfu/g, whereas *A. niger* was associated with 35% samples with a colony count of up to 1.1×10^3 cfu/g. However, the colony count of *Aspergillus* species in the evaluated Kashmir samples was

less in comparison to Jammu samples. It is probably due to the temperate variation and climatic conditions of Kashmir division that the storage fungi multiply and deteriorate the dried chillies at a slow rate in comparison to chillies stored at Jammu where conditions are very warm and humid from April to October. Most of these species have also been isolated from other spices by several researchers (El-Kady *et al.*, 1995; Abdulkadir *et al.*, 2002; Bokhari 2007). Some perfect states of *Aspergillus* species belonging to *Eurotium* (55%) and *Emericella* (10%) were also detected (**Table 2**). In most of the chilli producing countries of the world, the climatic conditions contribute largely to the high fungal loads recorded on the red chilli pods (Almela *et al.*, 2007; Yongendrarajah *et al.*, 2014; Ham *et al.*, 2016; Singh and Cotty 2017).

Samples from Kashmir division were also found to be contaminated with 12 species of *Penicillium* (75.0 per cent frequency). The frequency of occurrence of *P. chrysogenum*, *P. citrinum*, *P. griseofulvum*, *P. puberulum* and *P. brevicompactum* varied between 15-20 per cent, whereas that of *P. expansum*, *P. fellutanum*, *P. islandicum*, *P. minioluteum*, *P. verrucosum*, *P. olsonii* and *P. waksmanii* varied between 5-10 per cent with their colony forming units varying from 1.7×10^1 to 5.5×10^2 cfu/g.

2. Detection of mycotoxins from dried pericarp of chilli pods: While investigating the chilli pods for mycotoxin contamination, all the three mycotoxins, viz. ochratoxin A, patulin and citrinin for which assessment was conducted were found to be positive. Earlier, the authors had detected aflatoxins, sterigmatocystin and cyclopiazonic acid from the samples (Samyial and Sumbali, 2020b).

2a Ochratoxin A (OTA): It is a nephrotoxic and hepatotoxic secondary mould metabolite produced by some *Aspergillus* and *Penicillium* species. The most important species in this regard being *Aspergillus ochraceus*, *A. niger*, *Penicillium chrysogenum* and *P. verrucosum* (Abrunhosa *et al.*, 2001; O'Callaghan *et al.*, 2003). Incidentally all these producer species were recovered during the present investigation (**Table 1**). Presently OTA was detected as a moderate contaminant of chilli pericarp. From Jammu division, 53.0 per cent samples of dried red chillies were found contaminated with OTA and the level of contamination was found to range between 0.006 to 0.71 µg/g (**Table 3**). In comparison, from Kashmir division only 45.0 per cent samples were detected with this toxic contaminant with their amount varying from 0.007 to 0.10 µg/g. Earlier, some investigators from other countries have also reported OTA contamination (up to 474.7 µg/kg) in about 75% capsicum samples (Abrar, 2009; Ahn *et al.*, 2010; Santos *et al.*, 2010; Ozbey and Kabak, 2012; Jalili and Jinap, 2012). It is an established fact that OTA is carcinogenic for human consumption (IARC, 1993) and has a remarkably long resistance time in the animal body (Moss, 2002). The permissible maximum level of OTA in chillies is reported to be 20 µg/Kg (CCCF, 2017). However, in the chilli pod samples procured from Jammu and Kashmir divisions investigated presently, it is on much higher side than the permissible level.

Table 2: Frequency percentage of *Aspergillus*, *Penicillium*, *Eurotium* and *Emericella* species contaminating the sampled dried red chillies.

Major fungal genera	Jammu Division (n=19)		Kashmir Division (n=20)	
	Number of species detected	Number of Positive samples (frequency%)	Number of species detected	Number of Positive samples (frequency%)
<i>Aspergillus</i>	11	18 (95.0)	14	14 (70.0)
<i>Penicillium</i>	10	14 (74.0)	12	15 (75.0)
<i>Eurotium</i>	3	14 (74.0)	3	11 (55.0)
<i>Emericella</i>	2	3 (16.0)	1	2 (10.0)

2 b Patulin (PAT): It is a heat stable toxic fungal metabolite produced by some species of *Penicillium* and *Aspergillus* especially *P. expansum*, *P. griseofulvum*, *P. chrysogenum*, *P. melinii*, *Aspergillus amstelodonii*, *A. repens*, *A. echinulatus*, *A. terreus* and *A. fumigatus* (Frisvad, 1985; Jimnez *et al.*, 1991; Kadakal and Nas, 2003). During the present investigations, most of these species were commonly encountered as fungal contaminants of dried red chilli pods (**Table 1**). All these species are known to have strains that are active producers of patulin (Frisvad, 1985). Among the samples of chilli pericarp collected from Jammu division, 58.0% were found to be positive for patulin contamination and the level of toxin varied from 0.05-0.99 µg/g.

Red chilli samples from Kashmir division were found to have patulin (70.0%) level up to 1.47 µg/g, which is much more in comparison. Its exposure is considered to be teratogenic, gastrointestinal, neurological and immunological in nature (Llewellyn *et al.*, 1998; Drusch and Ragab, 2003; Boussabbeh *et al.*, 2015). Several countries have established permissible limit of patulin in food at 50 µg/kg or 50 µg/L (Welbe *et al.*, 2009). In view of this patulin contamination found in dried red chillies is quite high then the permissible limit. Therefore, such chilli pod may result in many health issues as chilli is used whole or in powdered form without processing or with minimal processing.

2 c Citrinin (CIT): It is another toxic metabolite produced by *Penicillium* and *Aspergillus* species like *P. expansum*, *P. viridicatum*, *P. fellutanum*, *P. notatum*, *Aspergillus terreus* and *A. niveus* (Scott *et al.*, 1972; Mintzloff *et al.*, 1972) and is reported to possess both nephrotoxic and genotoxic potential (Follmann *et al.*, 1998; Ammar *et al.*, 2000). During the present investigation, three citrinin producer fungal species, namely *Penicillium expansum*, *P. fellutanum* and *Aspergillus terreus* were recovered from chilli pericarp samples. From Jammu division, 26.0 per cent samples were found to be positive for this toxin and the range of toxin contamination varied from 5.04-8.99 µg/g (**Table 3**). As compared, from Kashmir division, only 15 per cent samples were found to be contaminated with citrinin and the amount of this toxin varied

Table 3: Contamination of ochratoxin A, patulin and citrinin detected in the sampled dried red chillies.

Mycotoxin detected	Jammu Division		Kashmir Division	
	% samples positive	Contamination range (µg/g)	% samples positive	Contamination range (µg/g)
Ochratoxin A	53	0.006 - 0.71	45	0.007 - 0.10
Patulin	58	0.05-0.99	70	0.05-1.47
Citrinin	26	5.04-8.99	15	4.16-5.76

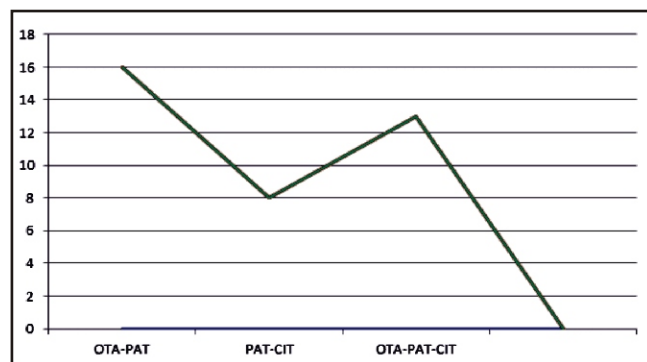


Fig.1: Percentage of chilli pericarp samples showing co-occurrence of various mycotoxins

from 4.16-5.76 µg/g. Earlier, Jeswal and Kumar (2015) reported 47.2 per cent samples of red chillies to be contaminated with citrinin. So far, no specific regulations exist for this mycotoxin but it is fixed to 2000 µg/kg for fermented rice foods (EC, 2014).

2d. Co-occurrence of OTA, PAT and CIT in the chilli pod samples: Data generated through this investigation showed that 16% samples had co-occurrence of OTA and PAT, 8.0% samples showed co-occurrence of PAT and CIT, whereas 13% samples showed the presence of all the three mycotoxins (**Fig.1**) Many studies have shown co-occurrence of several other mycotoxins in chillies like aflatoxins, ochratoxins, sterigmatocystin, cyclopiazonic acid and citrinin (Ozbey and Kabak, 2012; Santos *et al.*, 2010 Yongendrarajah *et al.*, 2014; Samyal and Sumbali, 2020b). OTA is hepatotoxic and CIT is nephrotoxic and these two mycotoxins together cause hepatorenal carcinogenesis, which shows that this may have additive or synergistic effect in humans consuming them. (Santos *et al.*, 2011) Earlier, Speijers and Speijers (2004) have also stated that combined intake of different mycotoxins at variable concentration levels may lead to higher risk than their individual intake.

CONCLUSION

Detection of ochratoxin A, patulin and citrinin from the investigated dried chilli pod samples suggests that they are favourable substrates for the production of toxic fungal metabolites. Although dried red chillies and their products are used in small amounts for cooking and seasoning, yet their daily intake is deleterious for the health of consumers. Since most of the developed countries have adopted stringent laws with respect to mycotoxin contamination in spices because of which export rejected consignment may find way to the domestic markets, which is again a matter of concern therefore, good post harvest practices need to be implemented in our country so as to decrease the probability of mycoflora and their toxic metabolites and improve the quality of dried red chillies and their products.

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