RESEARCH ARTICLE

MYCOTOXIN RESEARCH AND MYCOFLORA IN SOME DRIED EDIBLE MORELS MARKETED IN JAMMU AND KASHMIR, INDIA

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Abstract: *Morchella* species are major wild edible mushrooms of Jammu and Kashmir, which is both exported as well as largely consumed domestically. The aim of the present study was to characterize the toxigenic moulds and to screen different mycotoxins in dried morels. The most commonly isolated fungi were species of *Aspergillus*, *Fusarium* and *Penicillium* and the important mycotoxins detected were aflatoxins, citrinin, ochratoxin and zearalenol. The mean level of aflatoxin B_1 (125.44± 78.14) was found to be highest among all other mycotoxins. This is the first report on mycoflora and mycotoxin contamination in dried morels from Jammu and Kashmir.

Keywords: *Aspergillus*, *Morchella*, Mycotoxin, Mycoflora

INTRODUCTION

Of the world's 5.1 million estimated species within kingdom Fungi (Blackwell, 2011), true morels (*Morchella*, phylum Ascomycota) are arguably the most charismatic and widely recognized wild edible fungi intensively collected by mycophilic people (Kuo, 2005). Morels are highly prized edible mushrooms and have attracted human attention since time immemorial. Worldwide morels (*Morchella* spp.) are highly cherished and easily recogonizable forest resources of edible fungi (Sher and Shah, 2014). These mushrooms have wide distribution in India and is very common in the temperate forests in Uttarakhand, Himachal Pradesh and Jammu and Kashmir. Total world production of morels is estimated to be about 150 tonnes dry weight, equivalent to 1.5 million tonnes of fresh morels. India and Pakistan are the major morel producing countries, each producing about 50 tonnes of dry morels, almost all of which are exported (FAO, 2002) The state of Jammu and Kashmir ranks second in the morel trade after Himachal Pradesh with Uttarakhand as the third largest producer of morels in India (Shad and Lakhanpal, 1991).

The local people cook ascocarps (the fruiting body) mixed with rice and vegetables, and consider it as nutritious as meat or fish. The local inhabitants in remote areas depend upon folk medicines and household remedies to a great extent. The prevalent practices of indigenous herbal medicines including fungi have descended from generation to generation and include cure of both simple and complicated diseases. Of all the edible mushrooms of the North-West Himalayas, morels have been traditionally used for the cure of ailments such as pneumonia, fever, cough and cold, dehydration, stomach pains and for pregnant and lactating mothers. It is also known to cure all the respiratory ailments and generally they prefer to use black coloured fruiting bodies (Nautiyal *et al*., 2001; Lakhanpal *et al*., 2010). Moreover, the morels have shown noticeably significant pharmacological activities *viz*. antimicrobial, anti-inflammatory, immunostimulatory and anti-tumour properties (Kumar *et al*., 2000; Nitha *et al*., 2006; Halliwell, 2011; Badshal *et al*., 2012; Carocho and Ferreira, 2013).

In Jammu and Kashmir, *Morchella* species are collected systematically during the growing seasons (spring and sometimes after rainy season) and sold to established markets both as fresh and as dried mushrooms. A variety of morels such as *Morchella conica*, *M. esculenta* and *M. deliciosa* grow in the wild. One of the major limitation, *Morchella* species are seasonal and due to high moisture (90%) content they have short post-harvest shelf life making them prone to microbial spoilage and exhibit enzymatic browning which is the major cause of quality loss that accounts for reduction in their marketing as fresh produce (Mohapatra *et al*., 2008). In order to make their availability through the year around, drying has been considered as the best method to minimize biochemical and microbial activities (Kumari *et al*., 2011).

After dehydration, dried morels are packed in various storage containers, such as nylon bags, jute bags, polythene bags, glass jars and tins and are transported to various city markets and main marketing centers of northern India. Like any other agricultural product, morels are also exposed to a wide range of fungal and mycotoxin contamination during pre- and post-harvest period, especially during processing, storage, distribution, sale or use. These mycotoxins are considered to be among the most significant food contaminants in view of their negative impact on public health, food security and the national economy of many countries, particularly the developing ones (FAO, 2001). The mycotoxigenic potential depends on species and strains of fungus, composition of substrate and environmental factors (temperature and moisture) (Fernandez -Cruz *et al*., 2010). Among the thousands

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of fungal secondary metabolites currently known, only a few groups of mycotoxins are important from the safety and economic points of view; namely aflatoxins (AFs), ochratoxin A (OTA), citrinin (CIT) and zearalenol (ZOL) (Streit *et al*., 2013). The majority of mycotoxins are produced by the genera *Aspergillus*, *Penicillium*, and *Fusarium*, the so-called field fungi, that frequently infects various food commodities (Reddy *et al*., 2010).

Among the various mycotoxins, aflatoxins have attracted the attention of many scientists, probably because of their toxicological characteristics and ubiquitous presence as unavoidable contaminants in a variety of foods and feeds (Wood, 1992). These are produced mainly by *Aspergillus flavus* and *A. parasiticus* besides their known production by several other species such as *A. nomius*, *A. pseudotamarii*, *A. ochraceoroseus*, *A. bombycis* etc. The four major aflatoxins, B_1, B_2, G_1 and G_2 are the major toxic contaminants in dried commodities because of their high prevalence in nature and toxicity (Ayar *et al*., 2007). Ochratoxin A (OTA) is mainly produced by *Penicillium verrucosum*, *Aspergillus ochraceus*, and *A. carbonarius* while citrinin (CIT) is produced by *Penicillium* and *Aspergillus* species and is supposed to possess both nephrotoxic and genotoxic potential (Ammar *et al*., 2000).

Numerous reports are available on incidence of mycoflora and mycotoxins in dried commodities across the world (Mandeel, 2005; Lutfullah and Hussain, 2011; Rodrigues *et al*., 2012; Ibrahim *et al*., 2013; Gupta *et al*., 2013, 2017, Sharma *et al*., 2013, 2014; Bala *et al*., 2014, 2016). Few records are available on the spoilage of cultivated edible mushrooms (Kamal *et al*., 2009; Jonathan and Esho, 2010; Ezekiel *et al*., 2013) but no attention has been paid towards the deterioration of dried edible morels. Therefore the present study was designed to investigate the incidence of mycoflora and determination of natural occurrence of mycotoxins (aflatoxins, citrinin, ochratoxin and zearalenol).

MATERIAL AND METHOD

Sample collection

Seventy eight dried samples of morels were randomly selected from different wholesale and retailers of dry fruits, households from various regions of Jammu and Kashmir during March, 2015-February, 2016. The samples were collected in sterilized polythene bags to avoid further contamination and stored in refrigerator at 5ºC till further studies.

Moisture content

Prior to mycological analysis, the moisture content of dried samples was determined following Singh *et al*. (2008).

Mycological analysis

Agar plate method as recommended by the International Seed Testing Association (ISTA, 1966), as well as serial dilution method following Harrigan (1998) was employed to record the mycoflora associated with dried *Morchella* species. Experiment was performed in replicates for both the methods.

Table 1. Frequency percent and total colony count of fungal species recovered from dried morels.

S.No.	Fungal species recovered	% Frequency	Jammu Division Cfu/g×10^3			% Frequency	Kashmir Division Cfu/g×10^3		
			CDA	DRBC	MEA		CDA	DRBC	MEA
	Zygomycota								
1	*Mucor mucedo*	45.8	8.0	3.9	9.3	8.0	-	1.6	3.6
2	*Rhizopus oryzae*	14.5	3.2	-	2.1	16.0	-	1.8	-
3	*Syncephalastrum racemosum*	-	-	-	-	12.0	-	2.0	-
	Ascomycota								
4	*Epicocum nigrum*	27.0	1.2	-	-	-	-	-	-
5	*Eurotium amstelodami*	20.8	2.7	-	8.0	-	-	-	-
6	*Eurotium echinulatum*	18.1	5.0	1.4	6.0	-	-	-	-
	Mitosporic fungi								
7	*Acremonium roseolum*	20.8	2.0	-	5.2	-	-	-	-
8	*Alternaria alternata*	25.0	3.3	1.0	7.9	6.0	6.0	-	1.0
9	*Aspergillus flavus*	75.0	1.1	2.0	-	74.0	1.0	-	1.2
10	*A. niger*	54.1	1.8	-	-	12.0	-	8.0	-
11	*A. oryzae*	-	-	-		8.0	1.4	-	-
12	*A. parasiticus*	70.8	9.1	-	1.0	66.0	7.0	-	8.2
13	*A. sydowii*	-	-	-	-	12.0	0.8	-	-
14	*A. terreus*	-	-	-	-	14.0	-	4.0	-
15	*A. viridinutans*	4.1	1.2	-	1.4	-	-	-	-
16	*A. wentii*	10.4	1.2	-	-	-	-	-	-
17	*Chaetomium globosum*	20.8	1.6	-	-	-	-	-	-
18	*Cladosporium cladosporoides*	14.5	-	1.2	8.0	26.0	-	1.2	1.8
19	*C. oxysporum*	-	-	-	-	12.0	-	8.0	1.4
20	*C. sphaerospermum*	22.9	1.2	-	1.2	4.0	4.0	-	-
21	*C.tenuissimum*	18.7	-	1.6	-	-	-	-	-
22	*Curvularia lunata*	-	-	-	-	18.0	1.6	-	-

23	*Dreschlera australiensis*	37.5	-	0.5	-	-	-	-	-
24	*Fusarium oxysporum*	12.5	-	2.3	-	6.0	-	-	2.8
25	*F. graminearium*	4.1	1.4	-	1.0	4.0	-	8.5	-
26	*F. verticillioides*	-	-	-	-	12.0	3.8	-	-
27	*F. sporotrichioides*	32.1	-	8.0	-	-	-	-	-
28	*Paecilomyces variotii*	-	-	-	-	30.0	4.2	-	3.2
29	*Penicillium citrinum*	22.9	1.2	-	-	-	-	-	-
30	*P. expansum*	27.0	1.4	2.7	-	26.0	2.2	-	-
31	*P. islandicum*	10.4	-	1.1	-	-	-	-	-
32	*P. puberulum*	-	-	-	-	10.0	-	3.0	-
33	*Phoma eupyrena*	40.9	1.8	-	3.7	-	-	-	-
34	*Scopulariopsis brumptii*	8.3	1.2	1.2	-	-	-	-	-
35	*S. brevicaulis*	6.2	-	2.7	-	6.0	1.6	-	-
36	*Trichoderma harzianum*	4.1	-	2.2	1.0	-	-	-	-
37	*T. pseudokoningii*	8.3	0.5	-	-	-	-	-	-
Total number of fungal species		28	20	14	13	21	11	9	8

Not detected

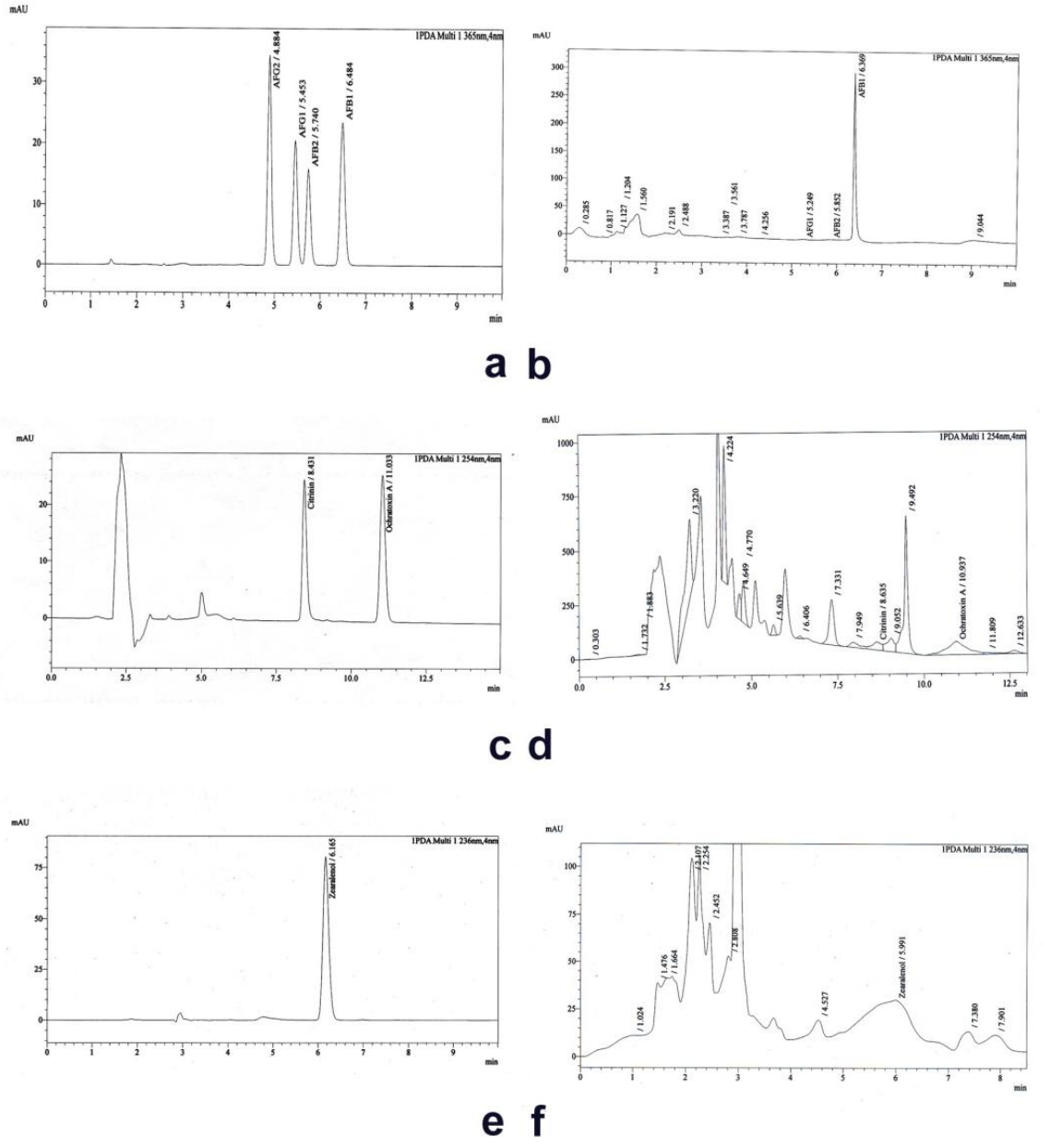

Fig. 1a-f. HPLC chromatograms of standards (AFB1, AFB2, AFG1, AFG2, OTA, CIT AND ZOL) and positive samples

Table 2. Mycotoxin contamination in dried morels.

Mycotoxins	Jammu Division			Kashmir Division		
	Number of samples screened	Number of positive samples	Range of toxin	Number of samples screened	Number of positive samples	Range of toxin
Aflatoxin B_1	42	22(52.38%)	95.33±156.08	36	16 (44.4%)	125.44±78.14*
Aflatoxin B_2	42	17(40.4%)	42.93±48.33	36	10(27.7%)	12.57±16.64
Aflatoxin G_1	42	5(11.9%)	33.62±36.66	36	9(25.0%)	10.36±13.84
Aflatoxin G_2	42	9(21.4%)	35.71±39.83	36	3(8.3%)	10.92±13.51
Citrinin	42	17(40.4%)	53.87±9.71	36	8(22.2%)	22.59±17.87
Ochratoxin A	42	8(19.0%)	49.03±55.81	36	13(36.1%)	26.08±4.52
Zearalenol	42	7(16.6%)	3.31±0.87	36	7(19.4%)	1.47±1.53

*Mean±SD

Agar plate method

Dried *Morchella* samples were surface sterilized with 1% sodium hypochlorite and rinsed three to five times with sterilized distilled water. Ascocarps of *Morchella* were placed equidistantly in Petri Plates containing PDA medium and incubated for 7 days (28±2ºC).

Serial Dilution Method

For determining the mycobiota of dried *Morchella* species, 1g of the powdered sample was added to 9ml of sterlised distilled water in 100ml flask and homogenized thoroughly on an horizontal shaker for 15 minutes. Ten fold serial dilutions were prepared and 1ml portion of suitable dilution was poured in Petri Plates by using a sterilised pipette. For the recovery of maximum number of fungal propagules from each sample, three different media-Czapek dox agar medium (CDA), Dichloran glycerol agar medium (DRBC) and Malt extract agar medium (MEA) were used and for each medium five replicates were maintained. The medium was poured by making rotational movement of Petri Plates so as to ensure uniform spreading of the samples. Petri Plates thus prepared were incubated at 28±2ºC for 7 days. The developing fungal colonies by both the above mentioned techniques were identified with the help of relevant literature and recommended keys.

Percentage frequency of each fungal species was calculated by using the formula given

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

Average colony forming units per gram of sample (cfu/g)

$$N = \Sigma C / Vxnxd$$

N = Number of colony forming units per gram of sample (cfu/g)

$\sum C$ = Sum of all colonies of the count

V = Volume of the dilution pipette in the count plates in ml

n = Number of count plates that can be evaluated

d = Dilution factor

Quantitative estimation of mycotoxins by high performance liquid chromatography (HPLC)

Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2)

Quantitative estimation of aflatoxins was done by modifying the method of Rohman and Triwahyudi (2008). Isocratic elution was done with water: acetonitrile: methanol (54:34:12v/v/v) at a flow rate of 1.0ml/min. Injection volume of extract solution was 30µl. A variable wavelength UV-VIS detector set at 365nm was used. The retention times of AFB_1, AFB_2, AFG_1 and AFG_2 was 6.484 min, 5.740 min, 5.453 min and 4.884 min respectively (Fig. 1a-b).

Ochratoxin A (OTA) and Citrinin (CIT)

For OTA and CIT analysis, the mobile phase comprised acetonitrile and water, acidified with phosphoric acid until pH 3.0 (50:50v/v) and flow rate of 1.0ml/min with 15 min of run time. Injection volume of extract solution was 40µl and analysis was performed at room temperature (25°C-30°C). The UV/VIS light detector wavelength was 220nm and peaks of OTA and CIT were observed at retention times of 8.431 min and 11.033 min respectively (Hackbart *et al*., 2012) (Fig. 1c-d).

Zearalenol

The UV/VIS detector set at 236nm was used for the analysis. An isocratic mobile phase of methanol: water (75:24v/v) was used with a flow rate of 1.0 ml/min and an injection volume of 30µl. Retention time of ZOL was 6.165 min (James *et al*., 1982) (Fig. 1e-f).

Statistical Analysis

All the experiments were replicated thrice and data sets were statistically analyzed using IBM SPSS 20 software. The statistical level of significance was fixed at $P<0.05$.

RESULT AND DISCUSSION

Mycoflora spectrum

The data presented in table 1 showed the prevalence of population of altogether 37 fungal species representing 18 genera. Among the recovered fungal species, mitosporic fungi contributed highest count of 31 fungal species with varying magnitude of incidence whereas Zygomycota and Ascomycota comprised each of three species of *Mucor mucedo*, *Rhizopus oryzae*, *Syncephalastrum racemosum* and *Epicoccum nigrum*, *Eurotium amstelodami*, *E. echinulatum* respectively.

The report of the present study revealed that the highest frequency was of *Aspergillus flavus* (75.0%) followed by *A. parasiticus* (70.8%) and *Aspergillus niger* (54.1%). Aspergilli grow on a large number of substrates and their ability to thrive at high temperatures (30-40ºC) and relatively low available

water (xerophillic nature) make them well suited to colonize a number of dried commodities. Mycoflora spectrum of the samples procured from the markets of Jammu division revealed the presence of 28 fungal species belonging to 15 genera. The results revealed that *Aspergillus flavus* dominated over other fungal species with the heavy mycobial load of 9.1x 10^3 cfu/g and lowest in case of *Dreschlera australiensis and Trichoderma pseudokoningii* (0.5x 10^3 cfu/g). Likewise, 36 samples procured from the markets of Kashmir division revealed the lesser incidence of mycoflora as compared to those from Jammu Division. Data in table 1 show that 21 fungal species representing 11 genera were recovered from dried *Morchella* species. Mitosporic fungi inflicted the maximum contamination (18) followed by Zygomycota (3) while no sample was found contaminated with any member of Ascomycota. The highest colony forming unit was found in *Fusarium graminearum* with 8.5x10^3. Our results are in agreement with Jonathan and Esho (2010) who evaluated the presence of fungi and aflatoxin in stored dried oyster mushrooms from Nigeria.

The percent moisture content, one of the main abiotic factor had a positive correlation with the extent of contamination and species diversity in dried *Morchella* samples. High moisture content of most of the samples may be one of the factors for their biodeterioration. In the present study, the average moisture level of 12.6% was recorded from Jammu division where as in Kashmir division, the moisture level was found to be 10.2%.

The traditional methods of drying in various environments and on exposed surfaces provide a chance for opportunistic saprophytic moulds to invade these dried *Morchella* species. Moreover, the warm and tropical conditions prevailing in the region are also expected to enhance the colonization and proliferation of various storage fungi. In addition to this, rich nutritional and biochemical composition of *Morchella* species could be another important aspect favouring the prolific growth of various microfungi on it. Both, the fruiting bodies and mycelia of *Morchella* species contain an uncommon aminoacid cis-3-amino-L-proline that has been proved to be a good source of fungal nutrition (Thind and Randhawa, 1957; Hatanaka, 1969). *M. esculenta* has been reported to produce high range of calcium (Dursun *et al*., 2006) and therefore, the occurrence of *Chaetomium globosum* on this edible mushroom could be supported by the fact that Ca has been found to stimulate perithecial production in *Chaetomium globosum* (Basu, 1952). *Aspergillus flavus*, one of the predominant species isolated in the highest frequency from dried *Morchella* species, possesses the capacity to produce numerous extracellular hydrolases including serine, proteases, pectinase, amylase and cellulases (Cleveland and Cotty, 1991; Mellon *et al*., 2007) due to which it has greater capacity for growth on complex protein substrates (St. Leger *et al*., 1997).

Literature is sparse on the diversity of microfungi contaminating macrofungi in India (Kotwal, 2010). Moreover, the presence of a wide range of fungal secondary metabolites contaminating mushrooms in India is also not known. Hence, there is a need to assess the mycotoxicological safety of *Morchella* species presented for sale in local markets and potential risk they may pose to consumers.

Mycotoxin analysis

During the present investigation, *Aspergillus flavus*, the potent producer of aflatoxins, was detected as prime fungal species from almost all the investigated samples. However, the magnitude of different aflatoxin contamination varied with the time and type of the place of sampling.

Out of the 78 samples, 42 samples were found contaminated with total aflatoxins. The range of total aflatoxins in the present study was found to be 10.36±13.84 – 125.44±78.14µg/kg. Among the four types of aflatoxins detected in present investigation AFB_1 appeared as a major contaminant with a mean range of 95.33±156.08 – 125.44±78.14 µg/kg. From Jammu division, 85.71% samples were found to be positive for total aflatoxins. The mean level of aflatoxin B_1 and B_2 ranged from 95.33±156.08µg/kg and 42.93±48.33 µg/kg respectively and that of G_1 and G_2 ranged from 33.62±36.66 µg/kg and 35.71±39.83 µg/kg respectively. Ochratoxin was found in 27.78% samples where as citrinin was found in 20.37% with the level of contamination 49.03±55.81 and 53.87±9.71µg/kg respectively. The level of Zearelenol was found to be low as compared to other toxins (3.13±0.87µg/kg). On the other hand, from Kashmir division, 59.18% of the samples were found to be contaminated with aflatoxins. The concentration range of aflatoxin B_1 and B_2 was found to be 125.44±78.14 and 12.57±16.64 µg/kg respectively where as G_1 and G_2 ranged from 10.36 ±13.84 and 10.92 ±13.51 µg/kg respectively. Ochratoxin A was found 25.0% samples with the mean value of 26.08±4.52 µg/kg. In addition to this, 20.45% of samples were found contaminated with citrinin and zearalenol having values i.e. 22.59±17.87 and 1.47±1.53µg/kg (Table 2). These results are in accordance with the results of Jonathan *et al*. (2011a) who recently detected low level of aflatoxins B_1 and B_2 i.e. 0.005 and 0.002µg/kg in *Lentinus squarrosulus* Berk, from Nigeria. Several maximum tolerable limits of aflatoxins have been established by authorities like FAO and EC but no legal limits have been stated for edible mushrooms probably with the fact that mushrooms have not been part of several reports given on contamination of agricultural products by moulds and mycotoxins but the results of the present study showed that samples contained aflatoxins beyond the maximum tolerable limit (MTL) of 4µg/kg set by European Union Commission for

ready to eat dry fruits for human consumption. (EC, 2010). At present there are no specific regulations in the European Union concerning OTA, CIT, ZOL in any kind of mushrooms. Review of literature reveals that this is the first inclusive report on occurrence of mycoflora and mycotoxins from Indian state of Jammu and Kashmir where in several locally edible dried *Morchella* species are consumed.

CONCLUSION

Fungal and mycotoxin contamination is currently regarded as a public health concern and there is a global trend to reduce the resulting health problems. Moreover, considering the fact that dried *Morchella* species consumption is high in Jammu and Kashmir and elsewhere, a strict hygiene mycological measured should be promulgated during harvest, storage and drying to minimize mycobial contamination.

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REFERENCES

Ammar, H., Michailis, G. and Lisovsky, T. (2000). A screen of yeast respiratory mutants for sensitivity against the mycotoxin citrinin identifies the vascular ATPase as an essential factor for the toxicity mechanism. *Current Genetics* **37**: 277-284.

Ayar, A., Sert, D. and Con, A.H. (2007). A study on the occurrence of aflatoxin in raw milk due to feeds. *Journal of Food Safety* **27**: 199-207.

Badshah, H., Qureshi, R.A., Khan, J., Ullah, F., Fahad, S., Ullah, F., Khan, A.M., Hussain, I. and Khan, N. (2012). Pharmacological screening of *Morchella esculenta* (L.) Pers., *Calvatia gigantea* (Batsch ex Pers.) Lloyd and *Astraeus hygrometricus* Pers., mushroom collected from South Waziristan (FATA). *Journal of Medicinal Plants Research* **6**: 1853-1859.

Bala, P., Gupta, D. and Sharma, Y.P. (2014). Mycobiota and aflatoxin (B_1 and B_2) contamination of apricot kernels *Prunus armeniaca* L. from Jammu and Kashmir, India. *Environment Conservation Journal* **15**: 69-76.

Bala, P., Gupta, D. and Sharma, Y.P. (2016). Mycoflora and natural aflatoxin contamination in dried quince seeds from Jammu, India. *Journal of Environment Biology* **37**: 101-106.

Basu, S.N. (1952). *Chaetomium brasiliensis* Batista 81 Pontual; Nutritional requirements for growth and fruiting. *Journal of General Microbiology* **6**: 199-204.

Blackwell, M. (2011). The fungi: 1, 2, 3……5.1 million species. *American Journal of Botany* **98**: 426-438.

Carocho M and Ferreira ICFR. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology* **51**: 15-25.

Cleveland, T.E. and Cotty, P.J. (1991**).** Invasiveness oof *Aspergillus flavus* in wounded cotton bolls is associated with production of a specific fungal polygalactouronase. *Phytopathology* **81**: 155-158.

Dursun, N., Ozcan, M.M., Giyasettin, K. and Celaleddin, O. (2006). Mineral contents of 34 species of edible mushrooms growing wild in Turkey. *Journal of the Science of Food and Agriculture* **86**: 1087-1094.

EC., European Commission (2010**).** Commission regulation N. 165/2010 amending Regulation (EC) N. 1881/2006. 2010. Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. ***Official Journal of European Union* 53**: 8-12.

Ezekiel, C.N., Sulyok, M., Frisvad, J.C., Somorin, Y.M., Warth, B., Houbraken, J.H., Samson, R.A., Krska, R. and Odebode, A.C. (2013). Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. *International Journal of Food Microbiology* **162**: 231-236.

FAO, Food and Agriculture Organization of the United Nations (2001). Manual on the application of the HACCP system in mycotoxin prevention and control. FAO/IAEA training and reference centre for food and pesticide control, Rome, pp. 113.

FAO. Food and Agriculture Organization of the United Nations (2002). Non-wood forest products from temperate broad-leaved trees FAO NWFP Series No. 15, pp Rome 125.

Fernandez-Cruz, M.L., Mansilla, M.L. and Tadeo, J.L. (2010). Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications. *Journal of Advanced Research* **1**: 113-122.

Gupta, D. Sharma, S. and Sharma YP (2013). Mycoflora and natural aflatoxin contamination in some traditional medicinal plants from Jammu, India. *Journal of Mycology and Plant Pathology* **43**: 360-368.

Gupta, D., Bala, P. and Sharma Y.P. (2017). Evaluation of fungal flora and mycotoxin contamination in whole dried apricots (*Prunus armeniaca* L.) from J&K, India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **87**: 81-87.

Hackbart, H.C.S., Prietto, L., Primel, E.G., Garda-Buffon, J. and Badiale-Furlong E. (2012). Simultaneous extraction and detection of ochratoxin

A and citrinin in rice. *Journal of the Brazilian Chemical Society* **23**: 103-109.

Halliwell, B. (2011). Free radicals and antioxidants – quo vadis?. *Trends in Pharmacology Science* **32**: 125- 130.

Harrigan, W. (1998). Laboratory methods in food microbiology. Academic Press, San Diego, pp. 359-375.

Hatanaka, S.I. (1969**).** A new aminoacid isolated from *Morchella esculenta* and related species. *Photochemistry* **8**: 1305-1308.

Ibrahim, M.I.M., Ali, H.S., Sahab, A.F. and Al-Khafia, A.R.S. (2013). Co-occurrence of fungi, aflatoxins, ochratoxin A and fumonisins in date palm fruits of Saudi Arabia. *Journal of Applied Science Research* **9**: 1449-1456.

ISTA., International Seed Testing Association (1966). International Rules of Seed Testing. *Proceedings of International Seed Testing Association* **32**: 565-589.

James, L.J., Mc Girr, L.G. and Smith, T.K. (1982). High pressure liquid chromatography of zearalenone and zearalenols in rat urine and liver. *Journal of the Association of Official Analytical Chemists* **65**: 8-13.

Jonathan, S.G. and Esho, E.O. (2010). Fungi and aflatoxin detection in two stored Oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus pulmonarius*) from Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **9**: 1722-1730.

Jonathan, S.G., Amos-Tautua, B.M.W. and Olawuyi O.J. (2011). Food values, heavy metal accumulation, aflatoxin contamination and detection of exopolysaccharides in *Lentinus squarrosulus* Berk, a Nigerian mushroom. *African Journal of sAgricultural Research* **6**: 3007-3012.

Kamal, A.S.M., Khair, A., Dawltana, M., Hassan, M.T., Begum, F. and Rahim, M. (2009). Evaluation of aflatoxins and pesticide residues in fresh and different processed mushrooms. Bangladesh. *Journal of Scientific and Industrial Research* **44**: 193-198.

Kumar, S.K., Zeireis, W., Wiegrebe, and Mullar, K. (2000). Medicinal plants from Nepal: evaluation as inhibitors of leukotriene biosynthesis. *Journal of Ethnopharmacology* 70(3): 191 - 195.

Kumari, D., Reddy, M.S. and Upadhayay, R.C. (2011). Nutritional composition and antioxidant activities of 18 different wild *Cantharelus* mushrooms of north-west Himalayas. *Food Science and Technology International* **17**: 557-567.

Kuo, M. (2005). Morels. University of Michigan Press, Ann Arbor.

Lakhanpal, T.N., Shad, O. and Rana, M. (2010). Biology of Indian morels. I. K. International Publishing House, New Delhi.

Luttfullah, G. and Hussain, A. (2011). Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. *Food Control* **22**: 426-429.

Mandeel, Q.A. (2005). Fungal contamination of some imported spices. *Mycopatholgia* **159**: 291-298.

Mellon, J.E., Cotty, P.J. and Dowd, M.K. (2007). *Aspergillus flavus* hydrolases : their role in pathogenesis and substrate utilization. Applied Microbiology and Biotechnology **77**: 497-504.

Mohapatra, D., Frias, J.M., Oliveria, F.A.R., Bira, Z.M. and Kerry, J. (2008). Development and validation of a model to predict enzymatic activity during storage of cultivated mushrooms (*Agaricus bisporus*). *Journal of Food Engineering* **86**: 39-48.

Nautiyal, S., Maikhuri R.K., Rao KS., and Saxena K.G. (2001). Medicinal Plant Resources in Nanda Devi Biosphere Reserve in the Central Himalayas. *Journal of Herb, Spices and Medicinal Plants* **8**: 47-64.

Nitha, B., Meera, C.R. and Janardhanan, K.K. (2006). Anti-inflammatory and antitumour activities of cultured mycelium of morel mushroom, *Morchella esculenta*. Department of Microbiology, Amala. Cancer Research Centre Thrissur, India.

Reddy, K.R.N., Raghavender, C.R., Reddy, B.N. and Salleh, B. (2010). Biological control of *Aspergillus flavus* growth and subsequent aflatoxin B_1 production in sorghum grains. *African Journal of Biotechnology* **9**: 4247-4250.

Rodrigues, P., Venancio, A. and Lima, N. (2012). Mycobiota and mycotoxins of almonds and chestnuts with special reference to aflatoxins. *Food Research International* **48**: 76-90.

Rohman A and Triwahyudi (2008). Simultaneous determinations of aflatoxins B_1, B_2, G_1, and G_2 using HPLC with photodiode-array (PDA) detector in some foods obtained from Yogyakarta, Indonesia. *Agritech* **28**: 109-112**.**

Shad, O.S. and Lakhanpal, T.N. (1991). Sociobiology of morels in N.W. Himalayas. Indian Mushroom. Proceedings of the National Symposium on Mushrooms. (Ed) Nair, M.C. Thiruvananthapuram, Kerala. pp 3.

Sharma, S., Gupta, D. and Sharma, Y.P. (2013). Aflatoxin contamination in chilgoza pine nuts (*Pinus gerardiana* Wall.) commercially available in retail markets of Jammu, India. *International Journal of Pharma and Bio Sciences* **4**: 751-759.

Sharma, S., Gupta, D. and Sharma, Y.P. (2014). Natural incidence of aflatoxins, ochratoxin A, patulin and their co-occurrence in chilgoza pine nuts marketed in Jammu, India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **85**: 45-50.

Sher, H. and Shah, A.H. (2014). Traditional role of morels *(Morchella* spp.) as food, medicine and income in Palas Valley, Pakistan. *Biology and Medicine (Aligarh)* **7**: 116.

Singh, P., Srivastava, B., Kumar, A. and Dubey, N.K. (2008). Fungal contamination of raw materials of some herbal drugs and recommendation of *Cinnamomum camphora* oil as herbal fungitoxicant. *Microbial Ecology* **56**: 555-560.

St. Leger, R.J., Joshi, L. and Roberts, D.W. (1997). Adaptation of proteases and carbohydrates of saprophytic, phytopathogenic and entomopathogenic fungi to the requirements of their ecological niches. *Microbiology* **143**: 1983-1992.

Streit, E., Naehrer, K., Rodrigues, I. and Schatzmayr, G. (2013). Mycotoxin occurrence in feed and feed raw materials worldwide: long-term analysis with special focus on Europe and Asia. *Journal of the Science of Food and Agriculture* 93: 2892-2899.

Thind, K.S. and Randhawa, H.S. (1957). Studies on the nutrition of fungi 11. The influence of different source of nitrogen on the growth and sporulation of *Colletotrichum capsici* (Syd.) Butler and Bisby. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **27**: 39-46.

Wood, G. (1992). Mycotoxins in foods and feeds in the United States. *Journal of Animal Sciences* **70**: 3941-3949.