INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 6, p. 213-225 2016

RESEARCH PAPER OPEN ACCESS

Natural occurrence of multiple fungi in variable germplasms of red chillies from Kunri, Pakistan

Shaista Akhund[1*], Abida Akram[1], Rahmatullah Qureshi[1], Farah Naz[2], Nafeesa Qudsia Hanif[3], Brian Gagosh Nayyar[1]

[1]*Department of Botany, PMAS-Arid Agriculture University, Rawalpindi 46300, Pakistan*

[2]*Department of Plant Pathology, PMAS-Arid Agriculture University, Rawalpindi 46300, Pakistan*

[3]*Romer labs, Rawalpindi 46300, Pakistan*

Key words: Red chilli, Kunri, Agar plate, Blotter paper, Deep freezing.

http://dx.doi.org/10.12692/ijb/9.6.213-225 Article published on December 17, 2016

Abstract

Red chilli(*Capsicum annuum*L.) is an important crop which is grown all over the world for its variable uses in food and medicines. However, it is vulnerable for bacterial and fungal diseases due to which its production is declining with the passage of time.The present study aimsto evaluate the natural occurrence of multiple fungi in variable germplasms of red chilli.In this regard, a mycological survey was carried out in Asia's biggest chilli production center "Kunri" Pakistan. A total of 69 samples belonging to six local cultivars namely Nagina, Maxi, Kunri, Tall Round, Tall Pointed and Drooping Type were collected and analyzed by Agar plate, Blotter paper, Deep freezing and Dilution method. All samples were positive for fungal occurrence. The most frequently isolated fungal genera were Alternaria(14 species),Aspergillus(8 species), Fusarium(6 species), Curvularia(6 species), Penicillium(4 species). However,Bipolaris, Cercospora, Cladosporium, Drechslera,Helicorhoidion, Rhizomucor, Rhizopus, Syncephalstrum andScolecobasidiumwere less frequent genera represented by single species. Overall 47 species were isolated, among them the leading contaminants were *A.flavus* (61.4%),*F. oxysporum* (44.50%), *A.niger* (39.19%) and *Al.alternata*(38.22%). The data obtained by percent frequency, incidence and contribution revealed that Nagina was highly susceptible cultivar. This is the first ever report of mycofloraisolated from six local cultivars of red chilli. These results indicate possible health hazards for human consumption of such contaminated food by mycotoxigenic fungi. Moreover, this baseline data about the prevalence of mycoflora contamination will certainly help to devise the effective strategies to tackle this significant problem.

* **Corresponding Author:** Shaista Akhund ✉ shaistaakhund@hotmail.com

Introduction

Red chilli (*Capsicum annuum* L) is a member of family Solanaceae and is native of tropical America. It is rich source of vitamin C and is grown throughout the world for its variable uses as it can be used fresh, cooked, pickled, in sauces, soups, stews and as powder for its heat and pungency. This pungency is due to the presence of capsaicin which is digestive and a cure of rheumatic problems. Owing to their vital pharmacological activity, chillies are also used in medicine (Reyes-Escogido *et al.*, 2011).

Red Chilli is amajorcash crop of Pakistan that acquires 6th position in export of this crop all over the world. (Iqbal *et al.* 2010a).These are cultivated on 62.5 thousand hectares with a production of 145.1 thousand tons and average yield of 2.32 tons per hectare for domestic use and export during 2013-2014 (Pakistan Economic Survey 2013-2014). The crop covers 20% of the total area under vegetable cultivation and is mainly cultivated in province Sindh and Punjab followed by Khyber Pakhtunkhwa and Baluchistan. Pakistan possessesa very diverse climatic conditions and this crop is able to grow in different ecological zones in each province. However, in Punjab,the crop is mainly grown in Layyia and in Sindhit grows in many areas like Tharparkar, Kunri and Hyderabad.(Anonymous, 2009-10). Pakistan's 85% chillies are produced in Sindh province where a small town "Kunri" is known as a biggest chilli market of Asia (Hussain and Abid, 2011).

This region has very hot summers upto 46 ºC with recurrent dust storms, and rainfall of about 100 mm. The optimal temperature ranges between 24°C to 32°C (Anonymous, 2010).Nevertheless, such warm and moist conditions are the most important factors that engouragefungal growth, its propagation and production of mycotoxins (Atanda *et al.* 2013). As soon as the crop gets contaminated, the fungi start to multiply and remain in every phase of production like harvesting, transportation and storageas long as theyget conducive environmental conditions.(Bennett and Klich 2003).In this way, they remain in food as normal microflora component and starts spoilage of food and mycotoxin formation at any stage (Aziz *et al.* 1998; Hitokoto *et al*, 1980; Roy and Chourasia, 1990; Chourasia, 1995). Basically fungi are grouped into two broad ecological categories: field and storage fungi. Field fungi attack living plant parts while in field, for example species of Alternaria, Fusarium, Cladosporium, Rhizopus and Mucor. Whereas, storage fungi likeAspergillus andPenicilliumthrives well at relatively low moisture content found in stored products. (Kiran *et al.*, 2005; Mandeel, 2005).

Fungal contamination of chilli has been considered bymany researchers from Pakistan and all over the world: Mushtaq and Hashmi (1997) recovered fungi from red chillies of Mirpur Khas District, Sindh, Pakistan. Hussain *et al* (2013) collected chilli samples from markets of lower Sindh including Hyderabad, TandoAllahyar, Mirpurkhas, Umerkot, Kunri, Samaro, Kot Ghulam Muhammad and Digri and reported several fungi associated with chilli. An Indian study reports that the most frequent fungi from *Capsicum frutescens*were *A. flavus, A. nidulans,A. niger, A. ochraceus,A. sydowii,Penicillium* and *Rhizopus* spp. (Ath-Har *et al.* 1988). Some of them are xerophilicmould species especially *A. flavus,A. fumigatus,A. ochraceus,niger*found in most pepper samples (Seenappa and Kempton, 1980; Mathyastha and Bhat, 1984; Delcourt*et al.*, 1994; El-Kady *et al.*, 1995; Adegoke *et al.*, 1996; Freire *et al.*, 2000; Vrabcheva, 2000). The role of these and some other fungi like *A. terreus, A. candidus, A. sclerotium, Fusariumsporotrichioides,F.moniliforme,Syncephala strumracemosum, Penicilliumcorylophilum* and *Paecilomycesvariotii*in spoilage was observed by Prasad *et al.* (2000). Theywere commonly found on decaying fruits of chilliin storage.

Generally, the food mycoflora has been given less attention as compared to bacterial flora (Kneifel and Berger, 1994).And food safety is a major concern for human health and national economy (Manjula*et al.* 2009).Keeping these things in mind, present study was conducted to determinethe predominant mycoflora and level of fungal contamination of red

chilliobtained from a hub (Kunri) of red chilli production and export. All the previous reports of Pakistani red chillies were focused on market/commercial samples. Therefore in this study the samples of six local cultivars of red chilliwere obtained directly from fields of Kunri, Sindh and were analysedfor the first time to investigate the varietal difference for the presence of multiple fungi.

The results will be valuable to assess and differentiate the quality of variable germplasm and indicate the possible potential for mycotoxin production at pre-harvest stage.

Materials and methods

Collection of Samples

Sixty nine samples of red chillies belonging to 6 different cultivars (cv) namely, Kunri, Nagina, Tall Round (T.R), Tall Pointed (T.P), Drooping Type (D.T) and Maxi were collected from the Kunriresearch station.

The chillies were harvested during August to December 2012 by hand picking at monthly intervals, when the pods change the colour from green to red. These samples were placed in air tight polyethylene bags and brought in laboratory within 48 hours, where they were sundried for 10-15 days. The samples were tested for the presence of different genera of fungi which infect varieties of red chillies in Kunri.

Detection of mycoflora

The samples were analyzed for the presence of mycoflora from all parts (seed & pericarp) of fruit in order to investigate external and internal mycoflora.

For this, a variety of methods were applied including standard protocols of International Seed Testing Association (ISTA, 2001) viz. Agar Plate, Blotter Paper, Deep Freezing Method along with Dilution Plate Method. The seeds and pericarp were surface disinfected with 2 % Sodium hypochlorite (NaOCL) for 2 minutes followed by rinsing three times with distilled water. Surface non-disinfected seeds and pericarp were also used.

Agar Plate method

Surface disinfected and non-disinfected seeds and pericarp were placed at the rate of 25 seeds and 15 pieces per plate respectively on sterilized potato dextrose agar (PDA). Plates were incubated for seven days at 28°C.

Blotter Paper method

Three filter papers were moistened and transferred in sterilized petri plates. Surface disinfected and non-disinfected seeds (25/plate) and pericarp (15/plate) were placed on filter papers and incubated at 28°C for 7 days.

Deep Freezing method

In this method plating of seeds and pericarp were same as blotter paper method. But the plates were incubated for 1 day at 28°C followed by -20°C for 24 hours and then at 28°C for 7 days.

Dilution method

In this method, the sample was ground and ten gm powder was added in 100 ml of distilled water to make stock suspension from which tenfold dilutions were made. One ml of each dilution was poured on PDA and incubated at 28°C. After incubation period, the fungus was isolated, purified and maintained in PDA slants.

Fungal colonies were observed for their typical colonial and conidial characteristics and were counted.Further, the occurrence frequencies and incidence were calculated using the formulae (Marasas, 1988):

$Fr (\%) = (Ns/N) \times 100$

$In (\%) = (ng/Ng) \times 100.$

Where Fr represents the frequency of occurrence (%) of a fungus, Ns is the number of fungi in samples, N is the number of samples, In (%) represents incidence, ng is number of infected seeds or pericarp and Ng is the total number of seeds or pericarp. Colony forming units (cfu) were also calculated in dilution plate method and percent contribution was calculated by using the formula:

Contribution (%) = cfu of particular species/ cfu of all species x 100.

Isolated fungi was examined periodically and identified by cultural and morphological characteristics and followed the taxonomic schemes of Domsch *et al.* (1980); Ellis, (1971, 1976); Booth (1971); Raper and Thom (1949); Raper and Fennel (1965); Simmons (2007) and Leslie and Summerell(2007).

Results

Sixty nine red chillisamples (whole and powdered) were screened for the presence of fungi, all samples were positive for fungal growth. A total of 47 species viz. *Aspergillus candidus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. ochraceus, A. penicilloides, A. tamarii, Alternaria. destruens, Al. tomaticola, Al. alternata, Al. brassicicola, Al. chlamydospora, Al. citri, Al. dianthicola, Al. godetiae, Al. infectoria, Al. longipes, Al. subulata, Al. tangelonis, Al. triticina, Al. vaccariae, Bipolarissorokiniana, Cercospora*sp., *Cladosporiumuridinicola, Curvulariaovoidae, Cu. brachyspora, Cu. lunata, Cu. pallesence, Cu. tuberculata, Cu. trifolii, Drechslera*sp., *Fusarium anthophilum, F. oxysporum, F. semitectum, F. solani, F. sporotricioides, F. tabacinum, Helicorhoidionbotryoideum,Penicilliumcorylophilum , P. expansum, P. rubrum, P. rugulosum, Rhizomucor*sp., *Rhizopusoryzae, Scolecobasidium*sp.,*Syncephalstrumracemosum*belonging to 14 genera were isolated from six local cultivars.The data showing overall frequency of multiple fungi isolated from six local cultivars is presented in Table 1.

Table 1. Percent frequency of multiple fungi isolated from six local cultivars of red chilli.

S.No	Name of Fungi	Sample Type	Method	KUNRI	Maxi	TR	TP	DT	Nagina	Average	S. E
1	*A.candidus*	Powder	D	---	---	---	---	---	6.67	1.11	1.11
2	*A.flavus*	Percicarp, seed & Powder	A,B,C,D	66.67	50.00	75.00	50.00	33.33	93.33	61.39	8.72
3	*A.fumigatus*	Seed	B	---	---	---	---	---	13.33	2.22	2.22
4	*A.nidulans*	Pericarp	A,D	27.27	---	14.29	---	---	53.33	15.82	8.74
5	*A.niger*	Percicarp, seed & Powder	A,B,C,D	45.45	16.67	21.43	68.75	42.86	40.00	39.19	7.64
6	*A.ochraceus*	Pericarp & seed	A	18.18	---	---	---	---	20.00	6.36	4.03
7	*A.penicilloides*	Pericarp	A	---	---	7.14	---	---	---	1.19	1.19
8	*A.tamarii*	Pericarp, Seed	A	---	---	---	6.25	---	---	1.04	1.04
9	*Al. dianthicola*	Percicarp, seed & Powder	A,B,D	---	---	---	12.50	---	---	2.08	2.08
10	*Al.alternata*	Percicarp, seed & Powder	A,B,C,D	27.27	---	64.29	62.50	28.57	46.67	38.22	10.02
11	*Al.brassicicola*	Percicarp, seed & Powder	A,B,C,D	---	8.33	28.57	18.75	14.29	---	11.66	4.57
12	*Al.chlamydospora*	Pericarp	A,B,C	---	---	---	---	14.29	---	2.38	2.38
13	*Al.citri*	Percicarp, seed & Powder	A,B,D	54.55	---	35.71	---	---	---	15.04	9.82
14	*Al.destruens*	Pericarp, Seed	A,B,C	---	---	---	---	---	20.00	3.33	3.33
15	*Al.godetiae*	Seed	B	---	---	---	---	---	13.33	2.22	2.22
16	*Al.infectoria*	Pericarp	A	---	---	---	---	---	6.67	1.11	1.11
17	*Al.longipes*	Powder	D	---	---	---	25.00	---	---	4.17	4.17
18	*Al.subulata*	Percicarp, seed & Powder	A,B,C,D	---	---	---	31.25	57.14	---	14.73	9.90
19	*Al.tangelonis*	Pericarp, Seed	B,C	---	---	---	---	---	33.33	5.56	5.56
20	*Al.tomaticola*	Pericarp, Seed	A	---	---	---	---	---	40.00	6.67	6.67
21	*Al.triticina*	Pericarp, Seed	B,C	---	---	---	37.50	---	---	6.25	6.25
22	*Al.vaccariae*	Pericarp, Seed	A,B,C	---	---	---	---	---	6.67	1.11	1.11
23	*B.sorokiniana*	Pericarp, Seed	A,B	---	16.67	---	6.25	---	6.67	4.93	2.68

24	*Cercospora.sp*	Pericarp, Seed	A,B,C	---	---	---	---	---	60.00	10.00	10.00
25	*Cl.uridinicola*	Pericarp, Seed	A,C	9.09	---	---	---	14.29	20.00	7.23	3.53
26	*Cu.brachyspora*	Pericarp, Seed	A,B,C	---	---	42.86	43.75	---	---	14.43	9.13
27	*Cu.lunata*	Pericarp, Seed	A,B,C	---	---	50.00	50.00	---	26.67	21.11	10.06
28	*Cu.ovoidae*	Pericarp, Seed	A	---	---	---	---	71.43	---	11.90	11.90
29	*Cu.pallesence*	Seed, Powder	C	---	25.00	---	---	---	46.67	11.94	8.06
30	*Cu.trifolii*	Pericarp, Seed	B,C	---	---	---	---	---	6.67	1.11	1.11
31	*Cu.tuberculata*	Pericarp, Seed	A,B,C	---	33.33	---	---	---	---	5.56	5.56
32	*Drechslerasp*	Pericarp, Seed & Powder	A,B,C,D	18.18	---	---	---	71.43	---	14.94	11.68
33	*F.anthophilum*	Pericarp, Seed	B,C	---	41.67	---	---	---	---	6.94	6.94
34	*F.oxysporum*	Pericarp, Seed & Powder	A,B,C,D	45.45	50.00	57.14	12.50	28.57	73.33	44.50	8.76
35	*F.semitectum*	Pericarp, Seed	A,B,C	---	---	---	---	---	46.67	7.78	7.78
36	*F.solani*	Pericarp, Seed & Powder	A,B,C,D	---	---	---	---	---	20.00	3.33	3.33
37	*F.sporotrichioides*	Seed	B,C	---	---	---	---	85.71	---	14.29	14.29
38	*F.tabacinum*	Pericarp, Seed & Powder	A,B,C,D	36.36	25.00	7.14	---	---	6.67	12.53	6.06
39	*H. botryoideum*	Pericarp, Seed & Powder	A,C,D	9.09	---	---	---	---	---	1.52	1.52
40	*P.corylophilum*	Pericarp	A,C	18.18	---	---	---	---	---	3.03	3.03
41	*P.expansum*	Pericarp	A	---	---	---	---	---	26.67	4.44	4.44
42	*P.rubrum*	Seed	A	27.27	---	---	---	---	---	4.55	4.55
43	*P.rugulosum*	Powder	D	---	---	14.29	---	---	---	2.38	2.38
44	*Rhizomucor sp.*	Pericarp	A	---	58.33	---	---	---	---	9.72	9.72
45	*Rhizopusoryzae*	Pericarp	A	---	---	---	---	14.29	6.67	3.49	2.42
46	*S. racemosum*	Pericarp	A	18.18	---	7.14	---	---	---	4.22	3.03
47	*Scolecobasidium sp.*	Pericarp, Seed	A,B	---	8.33	0.00	---	---	---	1.39	1.39

Methods categorized as A= Agar plate, B= Blotter paper, C= Deep Freezing, D= Dilution plate
TR= Tall Round, TP= Tall Pointed, DT= Drooping Type
S.E= Standard Error.

This data revealed that *A.flavus* was the leading contaminant found in frequency of 61 %. It was the only fungus isolated from all the varieties and methods. Other leading contaminants found in six varieties were *F. oxysporum* (44.50%), *A.niger* (39.19%) and *Al. alternata* (38.22%). However *Cl. uridinicola* was encountered occasionally.

More number of fungi was encountered from pericarp (39) followed by seeds (34) and powder (16).

According to the incidence data,25 species were isolated from cvNagina;14 fromKunri, 12 from Tall pointed, 10 from Maxi, 11 from Tall Round and Drooping Type each.In Kunri variety, *F. oxysporum* was the most frequent fungi isolated from all methods followed by *A. alternata*, *A. niger, Drechslera sp.* and *F. tabacinum*. It was interesting to note that*A. flavus* was isolated from non-treated samples only. The less frequent isolates in this variety were *P. rubrum, P. corylophilum* and *S. racemosum*. (Table2).

In cv. Maxi, *Cu. tuberculata* was the most frequenthowever,*A. niger*and *Rhizomucor* sp. were less frequentand isolated only from surface non disinfected samples by Agar plate method (Table 3). Tall Round was rich in fungal growth as it yielded more number of colonies.

The most frequent species was*F. oxysporum*. *O*ther predominant species were *A. flavus, A. niger*and *Al. brassicicola*while*A. nidulans* and *S.racemosum* were less frequent(Table 4). Tall pointed yielded lower no of colonies.

Table 2. Percent incidence of various fungi isolated from cvKunri by different methods.

S. No	Fungi	PERICARP						SEED					
		Agar plate		Blotter paper		Deep freezing		Agar plate		Blotter paper		Deep freezing	
		S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**
1	*A.flavus*	---	11.10	---	2.80	---	---	---	13.50	---	6.10	---	3.10
2	*A.nidulans*	---	0.11	---	---	---	---	---	---	---	---	---	---
3	*A.niger*	1.86	3.71	---	---	---	---	2.22	3.55	0.67	0.45	---	---
4	*A.ochraceous*	---	0.06	---	---	---	---	---	0.22	---	---	---	---
5	*Al.alternata*	1.50	2.00	0.33	---	0.47	---	0.31	2.83	1.00		0.17	2.00
6	*Al.citri*	0.67	---	0.17	0.53	---	---	0.50	1.50	---	---	---	---
7	*Cl.uridinicola*	0.33	---	---	---	---	---	---	---	---	---	---	---
8	*Drechslera*sp.	0.50	0.75	0.38	0.15	---	0.19	0.50	0.33	---	---	---	---
9	*F.oxysporum*	2.50	3.83	1.33	2.33	1.83	1.00	7.83	9.17	2.52	5.50	1.00	1.60
10	*F.tabacinum*	---	0.67	---	1.17	---	1.28	---	4.00	---	2.22	---	3.62
11	*H. botryoideum*	---	1.07	---	---	---	0.27	---	0.07	---	---	---	---
12	*P.corylophilum*	3.10	---	---	---	---	0.10	---	---	---	---	---	---
13	*P. rubrum*	---	---	---	---	---	---	2.11	---	---	---	---	---
14	*S. racemosum*	---	0.50	---	---	---	---	---	---	---	---	---	---

*Surface disinfected

**Surface non disinfected.

Table 3. Percent incidence of various fungi isolated from cvMaxi by different methods.

S. No	Fungi	PERICARP						SEED					
		Agar plate		Blotter paper		Deep freezing		Agar plate		Blotter paper		Deep freezing	
		S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**
1	*A.flavus*	1.00	10.00	---	---	---	---	---	---	---	2.60	---	---
2	*A.niger*	---	2.23	---	---	---	---	---	---	---	---	---	---
3	*Al.brassicicola*	---	---	---	---	---	---	---	---	2.20	3.20	2.67	---
4	*B.sorokiniana*	1.60	4.84	---	---	---	---	1.17	2.50	---	---	---	---
5	*Cu.tuberculata*	3.00	7.50	2.50	---	1.10	3.50	12.50	12.52	11.50	9.70	10.50	12.00
6	*F. anthophilum*	---	---	---	---	---	0.65	---	---	0.34	1.67	---	---
7	*F.oxysporum*	3.50	6.00	---	1.20	---	---	---	3.50	---	---	---	---
8	*F.tabacinum*	1.17	0.34	---	6.00	---	---	---	---	---	---	---	---
9	*Rhizomucor sp.*	---	0.67	---	---	---	---	---	---	---	---	---	---
10	*Scolecobasidium*sp	---	---	---	2.20	---	---	6.34	8.50	---	---	---	---

*Surface disinfected

**Surface non disinfected.

The most frequent species in this cultivar was*F. oxysporum* followed by *Cu. lunata* and *Al. dianthicola*. However Aspergillus species and A. subulata were recovered by Agar plate method only(Table 5). In drooping type 6 genera were isolated. The genus Alternaria was dominant in this variety. Whereas *Drechslera* sp. and *A. flavus* were isolated by Blotter paper and agar plate method respectively (Table 6).

The important finding of this study was about cvNagina as it was found to be highly contaminatecd variety.

Ityielded 9 genera and its predominant fungi were*A.flavus*, *A.niger*, Cercospora sp. *Al. vaccariae*, *Al. destruens* and *Al.alternata*.(Table7).

Table 4. Percent incidence of various fungi isolated from cvTall Round by different methods.

S. No	Fungi	PERICARP						SEED					
		Agar plate		Blotter paper		Deep freezing		Agar plate		Blotter paper		Deep freezing	
		S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**
1	*A.flavus*	13.30	1.70	21.70	26.72	1.70	---	11.00	3.00	7.00	2.00	1.00	---
2	*A. nidulans*	---	0.25	---	---	1.67	---	---	---	---	---	---	---
3	*A.niger*	25.00	1.67	24.60	23.30	---	---	21.0	24.00	---	---	---	25.27
4	*A. penicilloides*	---	0.13	---	---	---	---	---	---	---	---	---	---
5	*Al.alternata*	2.88	3.00	---	---	---	---	3.00	6.31	---	7.00	---	---
6	*Al. brassicicola*	---	---	1.50	1.75	1.50	1.25	---	---	2.00	4.00	2.25	4.25
7	*Cu.brachyspora*	0.38	0.50	0.50	---	---	0.38	0.25	0.42	0.50	---	---	---
8	*Cu. lunata*	---	---	---	---	1.12	---	---	---	---	---	1.38	0.75
9	*F.oxysporum*	3.75	7.00	3.51	3.75	2.15	2.52	7.25	9.50	4.51	7.00	6.00	7.25
10	*F.tabacinum*	---	0.33	---	---	---	---	---	1.13	---	2.38	---	0.88
11	*S. racemosum*	0.13	---	---	---	---	---	---	---	---	---	---	---

*Surface disinfected

**Surface non disinfected.

The results obtained by dilution method showed that Nagina carried the highest fungal load ($5.1x10^5$cfu/g) with 7 species. Lowest fungal load was carried by Drooping type ($1.2x10^4$cfu/g) with 5 species followed by Maxi ($1.5x10^4$cfu/g) with 4 species, Kunri ($2.1x10^4$cfu/g) with 6 species, Tall Round ($2.6x10^4$cfu/g) with 7 species and Tall Pointed ($4.6x10^4$cfu/g) with 5 species.

Table 5. Percent incidence of various fungi isolated from cvTall Pointed by different methods.

S. No	Fungi	PERICARP						SEED					
		Agar plate		Blotter paper		Deep freezing		Agar plate		Blotter paper		Deep freezing	
		S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**
1	*A.flavus*	3.00	3.30	---	---	---	---	---	---	---	---	---	---
2	*A.niger*	6.67	8.90	---	---	---	---	---	3.00	---	---	---	---
3	*A. tamarii*	---	0.56	---	---	---	---	---	0.50	---	---	---	---
4	*Al.alternata*	0.80	---	---	---	---	---	0.75	---	---	---	---	---
5	*Al. brassicicola*	---	---	3.00	---	---	2.50	---	---	4.50	5.00	---	6.00
6	*Al. dianthicola*	---	5.20	---	4.00	4.50	---	4.50	10.00	---	6.00	2.00	---
7	*Al. subulata*	2.00	---	---	---	---	---	---	---	---	---	---	---
8	*Al. triticina*	---	---	---	---	---	0.25	---	---	---	2.00	---	---
9	*B.sorokiniana*	---	---	---	---	---	---	0.32	0.25	0.50	---	---	---
10	*Cu.brachyspora*	---	1.25	---	---	---	---	---	---	---	---	---	---
11	*Cu. lunata*	---	---	1.65	2.25	0.20	0.25	---	0.23	3.25	---	---	1.75
12	*F.oxysporum*	5.50	7.75	---	---	---	0.51	7.75	11.75	---	2.00	1.50	1.34

*Surface disinfected

**Surface non disinfected.

The striking feature to be noted was the detection of *A. flavus* in all varieties by this methidand again Naginawas high carrier of this fungus showed 67.80% contributionwhile Kunri carried minimum contribution (0.26%) hence showed the tolerance against this fungus. The data of percent contribution is presented in Figure 1.

Discussion

In general, 100% red chilli samples analyzed in this study were contaminated with fungi. The most common fungi were identified as belonging to genera Aspergillus, Alternaria and Fusarium. We found that the frequency of appearance and percentage occurrence of fungi differed from variety to variety which may be attributed to the biochemical nature

and resistance level of the varieties. It was observed that pericarp yielded more no of fungi as compared to seeds and powder. However agar plate was the most efficient method followed by blotter paper and deep freezing method. The most of the fruit borne mycoflora of chillies isolated in this study is usually encountered as post-harvest disease agents. While the presence of *A. flavus* in high quantity is serious matter as it produces aflatoxin, a cancer causing agent. Moreover fusarium and alternaria are also notorious for toxin production which are harmful for humans and animals.

Table 6. Percent incidence of various fungi isolated from cvDrooping Type by different methods.

S. No	Fungi	PERICARP						SEED					
		Agar plate		Blotter paper		Deep freezing		Agar plate		Blotter paper		Deep freezing	
		S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**
1	*A.flavus*	---	2.20	---	---	---	---	---	---	---	---	---	---
2	*A.niger*	---	4.44	---	---	---	---	4.00	4.20	---	---	---	---
3	*Al.alternata*	---	---	0.33	2.50	0.67	2.70	---	---	0.33	1.00	1.63	0.33
4	*Al. brassicicola*	2.33	---	---	---	---	---	---	---	---	---	---	---
5	*Al. chlamydospora*	---	1.67	---	2.00	2.75	---	---	---	---	---	---	---
6	*Al. subulata*	3.33	5.00	1.50	1.00	2.00	---	---	2.67	---	2.00	---	0.67
7	*Cl. uridinicola*	---	---	---	---	---	---	0.33	3.00	---	---	---	3.70
8	*Cu. ovoidae*	1.00	0.80	---	---	---	---	2.35	---	---	---	---	---
9	*Drechslera*sp.	---	---	0.36	---	---	---	---	---	---	---	---	---
10	*F.oxysporum*	0.33	1.10	---	---	---	---	2.60	4.33	---	---	---	---
11	*F. sporotrichioides*	---	---	---	---	---	---	---	---	0.67	1.67	0.33	1.33

*Surface disinfected

**Surface non disinfected.

The methods comparison shows that the highest fungal population was obtained by Agar Plate method with 32 species from pericarp and 26 from seeds followed by Blotter paper method with 23 species from pericarp and 24 from seeds, deep freezing yielded 21 from pericarp and 20 from seeds while dilution plate method encountered lowest number (15) of species. Results of blotter paper method are similar to Sharfun-Nahar*et al.* (2004) where seeds yielded more no of fungi than pericarp however they differ in case of Deep freezing as in present study pericarp yielded more fungi. However deep freezing method is suitable for the detection of slow growing parasitic fungi because they draw nutrition from dead embryo of seed, furthermore the growth of fast growing saprophytic fungi is checked due to an interrupting deep-freezing period of twenty four hours.

Such a high fungal diversity in red chilli detected from the present study was also supported by earlier reports. Kobina and Ebenezer (2012) investigated the fruit borne mycoflora of *Capsicum annuum* L. from Accra metropolis. He found eighteen fungal species belonging to eight genera from surface sterilized and non-sterilized fruits.

The highest (2.79 log10 CFU/g) fungal load was recorded with *A. flavus* being the most common species.Parey *et al* (2013) reported three isolates of *Colletotricumcapsici*, and single isolate of *Alternariaalternata*, *Fusarium pallidoroseum*, *F. moniliforme*, *F. oxysporum*, and *Aspergillus flavus* from diseased samples of chilli fruits of India. While in 2004, Sharf-un-Nahar *et al.* has reported 47 fungal species from Indian consignment of red chilli. Among them *A. flavus, A. niger, A. alternata, Chaetomiumbostrychodes, F. moniliforme, Paecillomyces* sp. and *R. stolonifer* were predominant species isolated from seeds and pericarp. *A. flavus* was found in 100 % occurrence from pericarp.

Table 7. Percent incidence of various fungi isolated from cvNagina by different methods.

S. No	Fungi	PERICARP						SEED					
		Agar plate		Blotter paper		Deep freezing		Agar plate		Blotter paper		Deep freezing	
		S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**
1	*A.flavus*	25.50	23.20	25.00	28.60	5.60	20.00	19.70	25.10	11.70	27.50	12.00	20.10
2	*A. fumigatus*	---	---	---	---	---	---	---	---	---	0.16	---	---
3	*A.niger*	19.70	22.81	10.37	6.96	0.89	7.11	15.47	13.14	10.13	8.53	0.53	2.40
4	*A. ochraceus*	---	0.47	---	---	---	---	---	---	---	---	---	---
5	*Al.alternata*	---	0.73	0.87	1.20	0.20	0.80	0.40	1.03	1.43	2.07	0.40	1.33
6	*Al. infectoria*	---	0.53	---	---	---	---	---	---	---	---	---	---
7	*Al. citri*	---	---	---	---	---	---	---	---	---	---	---	0.33
8	*Al.destruens*	0.27	---	0.40	0.54	0.20	0.60	0.80	1.00	0.60	0.07	0.67	0.47
9	*Al. godetiae*	---	---	---	---	---	---	---	---	1.33	---	---	---
10	*Al. tomaticola*	---	0.13	---	---	---	---	---	0.27	---	---	---	---
11	*Al. tangelonis*	---	---	---	0.07	---	---	---	---	---	0.20	---	---
12	*Al.vaccariae*	0.67	1.00	0.43	0.40	0.33	0.47	0.67	1.07	0.67	1.13	0.40	1.30
13	*B.sorokiniana*	0.13	---	---	---	---	---	---	---	---	---	---	---
14	*Cercospora*sp	0.33	0.47	0.20	0.33	0.07	0.40	0.47	1.00	0.33	1.33	0.40	0.67
15	*Cl. uridinicola*	1.07	---	---	---	---	---	1.87	---	---	---	---	---
16	*Cu. lunata*	---	0.07	---	---	---	---	0.10	0.27	---	---	---	---
17	*Cu.pallesence*	---	---	---	---	---	---	---	---	---	---	---	0.22
18	*Cu. trifolii*	---	---	0.20	0.47	---	0.33	---	---	0.36	0.60	0.27	0.67
19	*F.oxysporum*	0.28	1.33		0.27	---	---	2.00	4.80	0.73	1.00	0.08	0.26
20	*F. semitectum*	---	---	0.13	0.20	---	---	0.40	0.80	---	1.25	0.40	0.53
21	*F.solani*	0.53	0.40	---	0.53	---	---	1.73	2.00	1.20	1.13	---	0.58
22	*F.tabacinum*	0.07	0.02	---	0.04	---	---	0.16	0.98	0.62	1.04	0.73	1.20
23	*P. expansum*	0.27	---	---	---	---	---	---	---	---	---	---	---
24	*P. rugulosum*	0.13	---	---	---	---	---	---	---	---	---	---	---
25	*R. oryzae*	0.24	---	---	---	---	---	---	---	---	---	---	---

*Surface disinfected

**Surface non disinfected.

It may also be mentioned that species of *Alternaria*, *Colletotrichum*, *Fusarium* and *Phoma*have been reported by Hashmi (1990) from samples of capsicum imported from India. Wadia *et al.* (1983) reported fruit surface mycoflora of *Capsicum annuum*. *A. niger, P. citrinum,* and *F. semitectum*were frequently linked from fruit surface.

However *S. racemosum, P. theae, A. flevipes, C. herbarum, Phoma*sp. *R. minutus,* and *S. oryzae* were isolated less frequently. Mushtaqand Hashmi (1997) found eleven species like *F. anthophilum, A. alternata, Cephalosporiumacremonium, F. moniliforme, F. solani, F. oxysporum, F. proliferatum, Macrophominaphaseolina, Rhizoctoniasolani* and *Pythium aphanidermatum*were detected predominantly from red chillies in Mirpurkhas Sindh. Another report from Sindh has been provided by Hussain *et al.* (2013). Out of five fungi, *A. flavus, A. niger* and *Colletotricumcapsici* were the pre-dominant ones with mean values of 61.6, 48.5 and 47.2% respectively than *A. solani* and *A. alternata*. Jamiolkowska (2009) isolated *A. alternata, Colletotricumcoccodes, F. oxysporum, F. equiseti, F. solani, Gilmaniellahumicola,, P. janczewskii, P. cyclopium, Gliocladiumroseum, T. hamatum and T. harzianum*from red chilli plants of Poland.

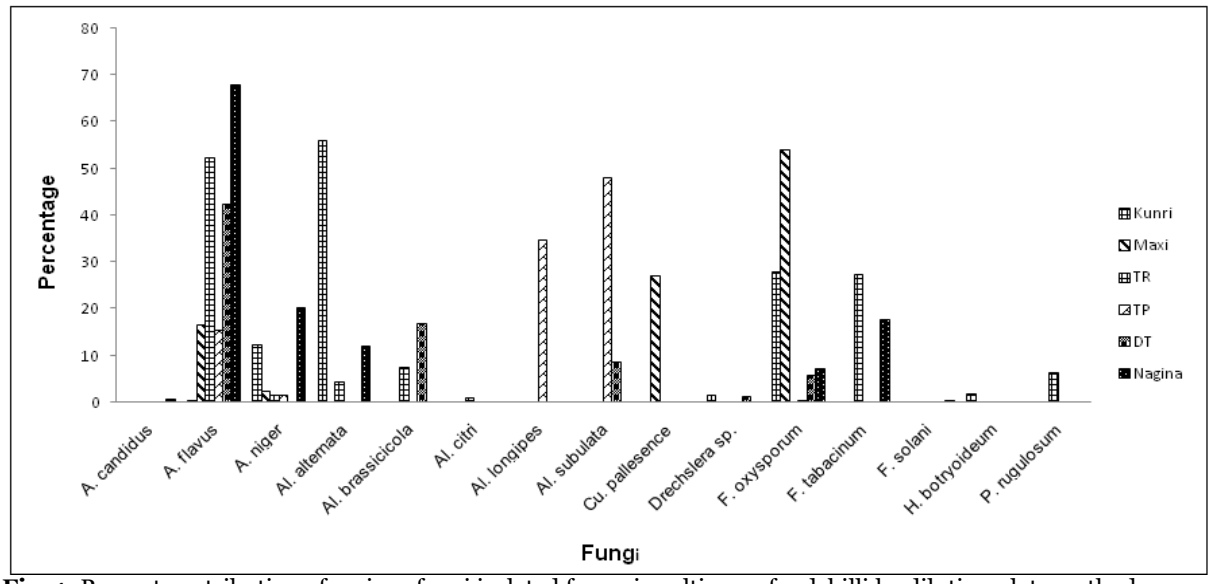

Fig. 1. Percent contribution of various fungi isolated from six cultivars of redchilli by dilution plate method.

The results of present study are in agreement with above mentioned reports which clearly indicate that red chilli is highly threatened commodity for being heavily contaminated with fungal flora at every stage of production. Hence, pre and post-harvest losses of red chillies pose a major challenge to developing countries like Pakistan. Due to this problem quality of both seeds and fruits of this cash crop is being destroyed as these fungi produce mycotoxins which cause health hazards in humans and animals. Number of mycotoxins like deoxynivalenol, zeralenone, fusarubin, bostrycoidin, moniliformin, aflatoxins and ochratoxins has been detected from the fungi isolated from Capsicum.*A. flavus* which is the most predominant fungi of this crop is known to produce Aflatoxins which is carcinogenic in nature. This is the first ever report of mycoflora detection from six local cultivars of Kunri, Sindh. This baseline data about the prevalence of mycoflora contamination will certainly help to devise the effective strategies to tackle this significant problem.

Conclusion

The results of this study have confirmed that fungal species are resident on both the surface and within the tissues of red chilli. The cultivarNagina was found as highly susceptible for the presence of multiple fungi and especially for *A. flavus*. While in drooping type its occurrence was minimal. Although the environmental conditions are suitable for fungal growth but varietal difference for fungal occurrence is considerable.

The high percentincidence, frequency and contributionof mycotoxigenic and plant pathogenic fungi on surface and seeds of chilli fruits suggest an obvious relationship existing between fruit borne mycoflora and fungi responsible for the human and post-harvest diseases. The study recommends that mycotoxin (especially aflatoxin) profile in these cultivars should also be investigated and farmers should be guided to grow tolerant varieties instead of susceptible variety as preventive measures.

Acknowledgement

This work was financially supported by the Higher Education Commission (HEC) of Pakistan under HEC Indigenous 5000 Ph.D. fellowship Program Batch-VII (Pin No-117-7663-Bm7-221). We acknowledge Sindh Horticulture Research Institute (SHRI), Mirpur Khas, Sindh for guidance and help in collection of different chilli varieties used in this research.

References

Adegoke GO, Allamou AE, Akingbala JO, Akanni AO. 1996. Influence of sundrying on the chemical composition, aflatoxin content and fungal counts of two pepper varieties- *Capsicum annum*and

Capsicum frutescens. Plant Foods for Human Nutrition**49(2),** 113-117.

Anonymous. 2009-10. Agriculture Statistic of Pakistan, Government of Pakistan, Ministry of Food, Agriculture and Livestock, Economic Wing, Islamabad.

Anonymous. 2010. Red chilli de-hydration plant-Kunri, Sindh. Sindh Board of Investment, Govt. of Sindh. www.sbi.gos.pk/pdf/Red-Chilli-De-Hydration-Plant.pdf

Atanda O, Makun HA, Ogara IM, Edema M, Idahor KO, Eshiett ME, Oluwabamiwo BF. 2013. Fungal and mycotoxin contamination of Nigerian foods and feeds. In: Makun HA, Ed. Mycotoxin and food safety in developing countries. Croatia: InTech, 3-38. http://dx.doi.org/10.5772/55664

Ath-Har MA, Prakash HS, Shetty HS. 1988. Mycoflora of Indian spices with special reference to aflatoxin producing isolates of *Aspergillus flavus*. Indian Journal of. Microbiology **28,** 125-127.

Aziz NH, Youssef YA, El-Fouly MZ, Moussa LA. 1998. Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. Botanical Bulletin- Academia Sinica Taipei **39,** 279-285.

Bennett JW, Klich M. 2003. Mycotoxins.Clinical Microbiology Reviews **16(3)**, 497-516. http://dx.doi.org/10.1128/CMR.16.3.497-516.2003

Booth C. 1971. The genus Fusarium. Common Wealth Mycological Institute, Kew, Surrey, England. 237.

Chourasia HK. 1995. Mycobiota and mycotoxins in herbal drugs of Indian Pharmaceutical industries. Mycological Research **99(6)**, 697-703. http://dx.doi.org/10.1016/S0953-7562(09)80531-5

Delcourt A, Rousset A, Lemaitre JP. 1994. Microbial and mycotoxic contamination of peppers and food safety.Bollettinochimicofarmaceutico **133 (4),** 235-238.

Domsch KH, Gams W, Anderson T. 1980. Compendium of Soil Fungi. Academic Press, London. 858.

El-Kady IA, El-Maraghy SS, Eman-Mostafa M.1995.Natural occurrence of mycotoxins in different spices in Egypt. Folia Microbiologica **40(3),** 297-300.

Ellis MB. 1971. Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, Kew, Surrey, England. 608.

Ellis MB. 1976. More Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, Kew, Surrey, England. 507.

Freire FC, Kozakiewicz Z, Paterson RR. 2000. Mycoflora and mycotoxins in Brazilian black pepper, white pepper and Brazil nuts. Mycopathologia **149 (1),** 13-19. http://dx.doi.org/10.1023/A:1007241827937

Hashmi MH. 1989. Seed-borne mycoflora of *Capsicum annuum* L. Pakistan Journal of Botany **21,** 302-308.

Hitokoto H, Morozumi S, Wauke T, Sakai S, Kurata H. 1980. Inhibitory effects of spices in growth and toxin production of toxigenic fungi. Appliedand Environmental Microbiology **39,** 818-822.

Hussain F, Abid M. 2011. Pest and diseases of chilli crop in Pakistan: A review. International Journal of Biology and Biotechnology **8,** 325-332.

Hussain F, Shaukat SS, Abid M, Usman F, Akbar M. 2013. Filamentous Fungi Infecting Fruits and Leaves of *Capsicum annuum*L. in Lower

Sindh.International Journal of Biology and Biotechnology **10(1),** 109-116.

Iqbal SZ, Paterson RRM, Bhatti IJ, Asi MR, Sheikh MA, Bhatti HN. 2010a. Aflatoxin B1 in chilies from the Punjab region, Pakistan. Mycotoxin Research **26(3),** 205-209 http://dx.doi.org/10.1007/s12550-010-0055-6

ISTA (International Seed Testing Association). 2001. International Rules for Seed Testing. Rules Amendments. Seed Science and Technology **29,** 1-127.

Jamiołkowska A. 2009. Fungi isolated from underground part of hot pepper (*Capsicum annuum*L.)plants cultivated in the field. Phytopathologia **51,** 37-44.

Kiran DR, Narayana KJP, Vijayalakshmi M. 2005. Aflatoxin B1 production in chillies (*Capsicum annum* L.) kept in cold stores. African Journal of Biotechnology **4(8),** 791-795.

Kneifel W, Berger E. 1994. Microbial criteria of random samples of spices and herbs retailed on the Austrian market. Journal of Food Protection **57,** 893-901. http://dx.doi.org/10.4315/0362-028X-57.10.893

Kobina MJ, Ebenezer O. 2012. Fruit borne mycoflora of *Capsicum annum* L (pepper), *Abelmoschusesculentus* L. Moench (okra), and *Lycopersiconesculentum* Mill. (tomato) from Accra metropolis. African Journal of Food Science **6,** 1-7. http://dx.doi.org/10.5897/AJFS11.189

Leslie JF, Summerell BA. 2006.The Fusarium Laboratory Manual. Blackwell Publishing: Oxford, UK.

Mandeel QA. 2005. Fungal contamination of some imported spices. Mycopathologia **159,** 291-298. http://dx.doi.org/10.1007/s11046-004-5496-z

Manjula K, Hell K, Fandohan P, Abass A, Bandyopadhyay R. 2009. Aflatoxin and fumonisin contamination of cassava products and maize grain from markets in Tanzania and Republic of the Congo. Journal of Toxicology, Toxin Reviews **28,** 63-69. http://dx.doi.org/10.1080/15569540802462214

Marasas WFO, Burgess LW, Anelich RY, Lamprecht SC, Van Schalkwyk DJ. 1988. Survey of Fusarium species associated with plant debris in South African soils. South African Journal of Botany **54,** 63-710.

Mathyastha MS, Bhat RV. 1984. *Aspergillus parasiticus*growth and aflatoxin production on black and white pepperand the inhibitory action of their chemical constituents.Applied and Environmental Microbiology **48(2),** 376-379.

Mushtaq M, Hashmi MH. 1997. Fungi associated with wilt disease of Capsicum in Sindhi, Pakistan. PakistanJournal of Botany **29(2),** 217-222.

Pakistan Economic Survey. 2013-2014. Ministry of Finance.Government of Pakistan. Available at: [accessed 16 July, 2015] http://finance.gov.pk/survey/chapters_14/02_Agriculture.pdf

Parey MA, Razdan VK, Sofi TA. 2013. Comparative study of different fungi associated withfruit rot of chilli and screening of chilli germplasmagainst *Colletotrichumcapsici*. International Journal of Agriculture and Crop Sciences **5,** 723-730.

Prasad BK, Sahoo DR, Mandojkumar Naresh. 2000. Mycotoxins in spices, Voprosyopitaniya **69,** 40-43.

Raper KB, Thom C. 1949. A Manual of the Penicillia. The Williams & Wilkins Company, Baltimore. 875.

Raper KB, Fennel DI. 1965. The genus Aspergillus. Williams & Wilkins Co., Baltimore. 686.

Reyes-Escogido ML, Gonzalez-Mondragon EG, Vazquez-Tzompantzi E. 2011. Chemical and Pharmacological Aspects of Capsaicin. Molecules **16,** 1253-1270. http://dx.doi.org/10.3390/molecules16021253

Roy AK, Chourasia HK. 1990. Mycoflora, mycotoxin producibility and mycotoxins in traditional herbal drugs from India. The Journal of General and Applied Microbiology **36,** 295-302. http://dx.doi.org/10.2323/jgam.36.295

Seenappa M, Kempton AG. 1980. Aspergillus growth and aflatoxin production on black pepper. Mycopathologia **70(3),** 135-137. http://dx.doi.org/10.1007/BF00443023

Sharfun-Nahar, Mushtaq M, Pathan IH. 2004. Seed-borne mycoflora of *Capsicum annum* imported from India. Pakistan Journal of Botany **36,** 191-197.

Simmons EG. 2007. An Identification Manual, CBS Fungal Biodiversity Centre Utrecht, Netherlands. 775.

Vrabcheva TM. 2000. Mycotoxin in spices. Voprosypitaniia **69(6),** 40-43.

Wadia KDR, Mahoharachary C, Janaki CH. 1983. Fruit surface mycoflora of *Vitisvinifera* L. and *Capsicum annuum* L. in relation to their fruit rot disease. Proceedings of the Indian National Science Academy**49,** 371-376.