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MYCOFLORA ASSOCIATED WITH THE SEED SAMPLES OF *CUCURBITA PEPO* L. COLLECTED FROM PAKISTAN

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Abstract

Seventeen seed samples collected from Peshawar (2), Swabi (1), Tordher (1), Fatu chack (1), Mardan (2), Karachi (4), Islamabad (1), Murree (1), Abbottabad (1), Sukkur (1), Ghotki (1) and Mandibahuddin (1) areas of Pakistan were analyzed for the seed-borne mycoflora using standard blotter, agar plate and deep-freezing methods as suggested by ISTA. At least 100 fungal species belonging to 49 genera were isolated mutually from all the seed samples analyzed. Seed samples from Peshawar followed by Sukkur & Ghotki were highly infected with fungi. Agar plate method was found best for the isolation of fungi both qualitatively and quantitatively followed by standard blotter method. By using agar plate method, 79 species of 40 genera were isolated while 57 species of 29 fungal genera were isolated by the blotter method. Being frost sensitive, rot and decay of pumpkin seeds was observed in deep-freezing method. Species of *Fusarium*, *Phoma* and *Macrophomina phaseolina* were isolated by all three methods. However, the most dominant fungi were the species of *Aspergillus* followed by *Rhizopus* and *Chaetomium*. Good germination of seeds was observed in surface sterilized seeds treated with 1% Ca $(OCl)_2$, although surface sterilization was found less effective against fungal mycoflora. Atleast 95 species of 47 genera are newly reported from Pakistan.

Introduction

Cucurbita pepo L., of the family Cucurbitaceae is commonly known as zucchini, courgette or summer squash when immature and pumpkin or winter squash when mature. It is native of America but cultivated worldwide with an annual production of 17.7 million tonnes from 1.4 million hectares (Anon., 2002). It is highly susceptible to frost and cultivated mainly during may/June and harvested around October. It is cultivated throughout Pakistan, as a Kharif crop with an annual production of 45217 tonnes from an area of 4027 hectares (Anon., 2009). Pumpkins vary in size and colors. The nutrient profile of pumpkin seeds showed that they are low in calories, however the seeds are rich source of Vitamin A, vitamin B1, B2, B3, B6, B12, vitamin C, vitamin D, vitamin E, vitamin K, pantothenic acid; minerals like calcium, iron, manganese, magnesium, phosphorous, potassium, selenium, sodium, zinc etc., number of amino acids and many other nutrients are present in trace amount. They also contain wide variety of antioxidants phytonutrient. Seeds are found to have some benefits against diabetes, anti microbial activities and cancer etc. (Mateljan, 2006). Seeds of pumpkin are flat and oval with slightly pointed tip; colour may vary from species to species. They are commonly known as Pepita. Pepitas raw or roasted is a rich source of nutrition. Oil is also extracted from Pepitas which is used in folk medicines.

A survey of literature showed that very little work has been done on the seed-borne mycoflora of Pumpkin. The fungi reported on Pumpkin seeds include the species of *Alternaria, Aspergillus, Fusarium, Penicillium*, *Sclerotium* and *Macrophomina phaseolina* (Ahmed *et al*., 1993). Fungi damaging the pumpkin fruit and seeds are mostly soil borne and attack either before or after harvest. Pumpkins are temperature sensitive, storing under direct sunlight or in frost, both cause decay and rot of the fruit. Jamiolkowska *et al*., (2011) isolated the species of *Fusarium* from the roots of zucchini, responsible for damping-off, stunting and stem and root- rot of the plants. The fungi attacking pumpkin includes the species of *Fusarium* causing *Fusarium* rot, *Macrophomina phaseolina* causing char coal rot, *Sclerotinia, Collectotrichum lagenarium* causing Anthracnose, species of *Erysiphales* and *Sphaerotheca* (causing powdery mildew, *Septoria* spp. (*septoria* leaf spot), *Phytophthora* spp. causing *Phytophthora* rot, *Didymella bryoniae* causing black rot, *Cladosporium cucumerinum* (responsible for scab) and *Plectosporium tabacinum* causing *Plectosporium blight* (Zitter *et al*., 1996; Mc Grath, 2011). Seed-borne fungi reported from Pakistan on cucurbits include *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Myrothecium roridum*, *Penicillium* spp, and *Rhizopus* spp. (Sultana & Ghaffar, 2009).

Due to their nutritional values and medicinal properties, pumpkin is gaining interest of researchers and agriculturists. Very little work has been reported previously from Pakistan on pumpkin. Therefore keeping in view their emerging economical importance, a relatively new research work has been done to find out the mycoflora associated with pumpkin seeds from Pakistan.

Materials and Methods

For the detection of seed-borne mycoflora, ISTA (Anon., 1993) techniques i.e. Standard blotter method, Agar plate method and Deep-freezing methods were used. About 400 seeds of each sample were tested.

Collection of seeds: Pumpkin seed samples (17 samples) were collected from the local markets of various areas of Pakistan viz., Peshawar (2), Swabi (1), Tordher (1), Fatu chack (1), Mardan (2), Karachi (4), Islamabad (1), Murree (1), Abbottabad (1), Sukkur (1), Ghotki (1) and Mandibahuddin (1).

Standard blotter method: Untreated and seeds after treatment with 1% Ca $(OCl)_2$ for 2 minutes were placed on three layers of moistened blotter paper, 10 seeds per Petri dish. The dishes were incubated for 5-7 days at 28±2°C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Agar plate method: Untreated and seeds after treatment with 1% Ca $(OCl)_2$ for 2 minutes were placed on Potato dextrose agar (PDA), 10 seeds per Petri dish. The dishes were incubated for 5-7 days at 28±2°C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Deep-freezing method: Untreated and seeds after treatment with 1% Ca $(OCl)_2$ for 2 minutes were placed on three layers of moistened blotter paper, 10 seeds per Petri dish were incubated for 24h, each at 28±2°C and -2°C followed by 5 days incubation at 28±2°C under 12h, alternating cycle of artificial day light (ADL) and darkness (Anon., 1993).

Identification of fungi: Mycoflora observed on seeds were identified after reference to Barnett & Hunter (1998), Domsch *et al*., (1980), Ellis (1971), Gilman (1950), Hanlin (1989), Nelson *et al*., (1983), Raper & Fennell (1965).

Analysis of data: Data was subjected to analysis of variance (ANOVA) following the procedures as suggested by Gomez & Gomez (1984).

Results

Atleast 100 species belonging to 49 genera viz., *Absidia corymbifera* (Cohn) Sacc. & Trotter, *A. cylindrospora* Hagem, *A. glauca* Hagem, *Acremonium cerealis* (Karst).W.Gams. , *A. furcatum* F. & V. Moreau ex W.Gams, *A. kiliense* Grutz, *A. murorum* (Corda) W.Gams. *Acremonium* species Link ex Fr., *Alternaria* (Fr.) Keissler, *A. cucumerina* (Ellis & Everh.) Elliott. *A. dianthicola* Neergaard, *A. longipes* (Ellis & Everh.) Mason., *A.raphani* Groves & Skolko., *A.tenuissima* (Kunze ex pers.) Wiltshire., *Alternaria* species Nees ex Fr. Nees., *Aspergillus flavus* Link ex Gray., *A.fumigatus* Fres., *A. glaucus*. Mich ex Fr., *A.niger* Van Tieghem., *A. oryzae* (Ahlburg) Cohn., *A. parasiticus* Speare, *A. sulphureus* Thom & Church, *A. terreus* Thom, *A. ustus* (Bain.) Thom & Church, *A.versicolor* (Vuill.)Tiraboschi, *Aspergillus* spp. Mich. ex Fr., *Bahusakala olivaceonigra* (Berk. & Br.) Subram., *Botrytis cinerea* Pers. ex Nocca & Balb., *Brachysporium obovatum* (Berk.) Sacc., *Cephaliophora irregularis* Thaxter., *Chaetomium bostrychodes* Zopf., *C.cochliodes* Pall., *C. crispatum* (Fuckel)Fuckel, *C.elatum* Kunze ex Steud., *C.globosum* Kunze ex steud., *C. indicum* Corda, *C. murorum* Corda, *C. spirale* Zopf, *Chaetomium* species Kunze ex Fr., *Chuppia sarcinifera* Deighton, *Cladosporium cladosporioides* (Fres.) de Vries., *C. cucumerinum* Ellis & Arth., *C. spaerospermum* Penz., *Cochliobolus nodulosus* Luttrell, *Coremiella cubispora* Berk. & Curt., *Curvularia lunata* (wakker) Boedijn, *C. pallescens* Boedijn, *C. penniseti* (Mitra) Boedijn, *C. robusta* Kilpatrick & Luttrell, *Drechslera australiensis* (Bugnicourt) Subram. & Jain ex M.B. Ellis, *D. bicolor* Paul & Parbery, *D.hawaiiensis* (Bugnicourt) Subram. & Jain, *Emericella nidulans* (Eidam) Vuill., *E.rugulosa* (Thom & Raper) C.R. Benjamin, *Emericellopsis terricola* van Beyma, *Epicoccum purpurascens* Ehrenb. Ex Schlecht., *Fusarium oxysporum* Schlecht. emend. Sny. & Hans., *F. semitectum* Berk. & Rav., *Fusarium* species Link ex Fr., *Glomerella cingulata* Spauld. & v. Schrenk., *Gonitrichum macrocladium* (Sacc.) Hughes., *Helminthosphaerica clavariarum* (Tul.) Fuckel, *Humicola fuscoatra* Traaen, *Macrophomina phaseolina* (Tassi) Goid, *Melanospora* sp. Corda, *Memnoniella echinata* (Riv.) Galloway, *M. subsimplex* (Cooke) Deighton, *Monilia* sp. Pers. ex Fr., *Monodictys levis* (Wiltshire) Hughes, *Mucor hiemalis* Wehmer, *M. mucedo* Mich. Ex St. Am., *Myrothecium cinctum* (Corda) Sacc., *M. roridum* Tode ex Steudel, *Nectria inventa* Pethybr, *N. ventricosa* C. Booth, *Neosartorya fischeri* (Wehmer) Malloch & Cain, *Nigrospora oryzae* Hudson, *N. sphaerica* (Sacc.) Mason, *Nigrospora* species Zimmermann, *Paecilomyces* species Bain., *Papulaspora irregularis* Hotson , *Penicillium* Link ex Fr., *Phoma eupyrena* Sacc., *P. exigua* Desm., *P. medicaginis* Malbr. & Roum., *Rhizopus arrhizus* Fischer, *R. oryzae* Went & Prinsen Geerligs, *R.stolonifer* (Ehrenb. Ex Link) Lind, *Scytilidium lignicola* Pisante, *Septotrullula bacilligera* Höhnel, *Stachybotrys cylindrospora* C.W. Jensen, *Staphylotrichum coccosporum* J. Meyer & Nicot , *Taeniolella exillis* (Karst.) Hughes, *Trichocladium opacum* (Corda) Hughes, *Trichoderma hamatum* (Bonord.) Bain, *T. harzianum* Rifai, *T. viride* Pers. ex Gray were isolated and identified from the seed samples collected from various localities of Pakistan by ISTA techniques. Out of 100 species isolated, except for *Alternaria*, *Aspergillus*, *Fusarium*, *M. phaseolina* and *Penicillium* (Ahmed *et al*., 1993), all other fungi are newly reported from Pakistan. Agar plate method was found to be more suitable for the isolation of fungi followed by blotter method. Agar plate method yielded 79 fungal species belonging to 40 genera where as blotter method yielded 57 species belonging to 29 genera. Pumpkins as well as its seeds are highly frost sensitive, deep-freezing method yielded around 26 species belonging to 15 genera (Table 1). Pathogenic fungi like *M. phaseolina*, species of *Fusarium* and *Phoma* were observed on seeds causing char-coal rot, damping off, rot and decay of seeds and seedlings. Very heavy infection of seeds was observed by the species of *Aspergillus flavus* ($p<0.001$) and *A. niger* ($p<0.001$), *Rhizopus* and *Chaetomium*. These fungi were responsible for the complete rottening of seeds and seedlings. Mites were also observed on the seed samples infested with *Chaetomium* species. Seeds surface sterilized with 1% Ca $(OCl)_2$ has not produced any significant effect on mycoflora of seeds however good germination was observed during incubation of surface sterilized seeds. Being frost sensitive, rot and decay of seeds subjected to deep-freezing method was observed. Most of the fungi isolated from seed samples (both pathogenic and storage) are known to produce mycotoxins. Seed samples collected from Peshawar, Ghotki and Sukkur were found to be highly infected with fungi.

Table 1. Seed-borne mycoflora associated with *Cucurbita pepo* L.

Name of fungi	Non- surface sterilized seeds						Surface sterilized seeds					
	Blotter method		Agar plate		Deep-freezing		Blotter method		Agar plate		Deep-freezing	
	N.SI	I%±SD	N. SI	I%±SD	N.SI	I%±SD	N. SI	I%±SD	N.SI	I%±SD	N.SI	I%±SD
*Absidia corymbifera**	1	0.06 ±0.0	2	0.24 ±1.41	-	-	-	-	-	-	-	-
*A.cylindrospora**	-	-	3	0.65 ±3.79	-	-	-	-	2	1.00 ±9.19	-	-
*A glauca**	-	-	3	0.29 ± 1.15	-	-	-	-	-	-	-	-
*Acremonium cerealis**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*A furcatum**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*A kiliense**	-	-	-	-	-	-	-	-	2	0.18± 0.71	-	-
*A.murorum**	-	-	1	0.29±0.0	1	0.35±0.0	-	-	1	0.06±0.0	-	-
Acremonuim sp.*	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Alternaria alternate**	1	0.12± 0.0	-	-	1	0.12±0.0	1	0.06±0.0	-	-	-	-
*A.cucumerina**	1	0.12±0.0	1	0.06±0.0	-	-	-	-	-	-	-	-
*A.dianthicola**	1	0.06±0.0	1	0.06±0.0	-	-	1	0.06±0.0	-	-	-	-
*A.longipes**	1	0.12±0.0	-	-	-	-	1	0.06±0.0	-	-	-	-
*A.raphani**	-	-	-	-	-	-	-	-	2	0.12±0.0	-	-
*A.tenuissima**	-	-	-	-	1	0.12±0.0	1	0.12±0.0	-	-	-	-
Alternaria spp.	1	0.06±0.0	2	0.18±0.0	3	0.65±2.65	-	-	3	0.53±0.0	-	-
*Aspergillus flavus**	10	4.65±6.97	14	8.12±10.62	2	0.24±1.41	2	3.47±8.00	13	10.89±18.79	3	0.42±1.53
*A.fumigatus**	1	0.06±0.0	4	1.18±1.41	-	-	2	0.24±1.41	2	0.12±0.0	-	-
*A.glaucus**	-	-	1	0.06±0.0	-	-	-	-	-	-	-	-
*A.niger**	6	1.47±1.94	14	34.75±30.17	1	0.18±0.0	6	3.00±5.05	14	29.47±32.49	1	0.06±0.0
*A.oryzae**	1	0.06±0.0	1	0.06±0.0	-	-	-	-	-	-	-	-
*A.parasiticus**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*A.sulphureus**	-	-	1	0.12±0.0	-	-	-	-	1	0.06±0.0	-	-
*A.terreus**	1	0.06±0.0	-	-	-	-	-	-	-	-	-	-
*A.ustus**	-	-	1	0.06±0.0	-	-	-	-	-	-	-	-
*A.versicolor**	1	0.06±0.0	-	-	-	-	-	-	-	-	-	-
*A.wentii**	3	0.24±0.58	1	0.06±0.0	1	0.06±0.0	-	-	1	0.18±0.0	-	-
Aspergillus spp.	-	-	-	-	-	-	-	-	1	0.177±0.71	-	-
*Bahusakala olivaceonigra**	-	-	1	0.06±0.0	-	-	-	-	-	-	-	-
*Botrytis cinerea**	-	-	1	0.06±0.0	-	-	-	-	-	-	-	-
*Brachysporium obovatum**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Cephaliophora irregularis**	1	0.06±0.0	1	0.12±0.0	-	-	-	-	-	-	-	-
*Chaetomium bostrychodes**	-	-	-	-	-	-	1	0.06±0.0	-	-	-	-
*C.cochliodes**	-	-	1	0.06±0.0	-	-	3	0.47±1.52	1	0.06±0.0	-	-
*C.crispatum**	2	0.29±2.12	-	-	-	-	3	1.71±10.69	-	-	-	-

Table 1. (Cont'd.).

Name of fungi	Non- surface sterilized seeds						Surface sterilized seeds					
	Blotter method		Agar plate		Deep-freezing		Blotter method		Agar plate		Deep-freezing	
	N.SI	I%±SD	N. SI	I%±SD	N.SI	I%±SD	N. SI	I%±SD	N.SI	I%±SD	N.SI	I%±SD
*C.elatum**	-	-	1	0.06±0.0	-	-	2	0.82±1.41	1	0.06±0.0	-	-
*C.funicola**	-	-	1	0.06±0.0	-	-	-	-	-	-	-	-
*C.globosum**	4	1.29±6.14	3	0.18±0.0	1	0.35±0.0	5	2.00±5.26	4	1.65±6.22	2	0.71±1.41
*C.indicum**	1	0.06±0.0	1	1.29±0.0	-	-	2	1.00±0.0	2	1.12±12.02	-	-
*C.murorum**	-	-	-	-	-	-	-	-	-	-	1	0.35±0.0
*C.spirale**	1	0.53±0.0	-	-	-	-	-	-	-	-	-	-
Chaetomium spp.*	7	0.71±1.12	-	-	1	0.53±0.0	5	1.59±4.24	1	0.06±0.0	7	2.41±3.72
*Chuppia sarcinifera**	-	-	-	-	-	-	-	-	1	2.94±0.0	-	-
*Cladosporium cladosporioides**	-	-	1	0.06±0.0	-	-	1	0.06±0.0	3	0.18±0.0	-	-
*C.cucumerinum**	-	-	-	-	-	-	1	0.06±0.0	-	-	-	-
*C.sphaerospermum**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Cochliobolus nodulosus**	1	0.06±0.0	-	-	-	-	-	-	-	-	-	-
*Coremiella cubispora**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Curvularia lunata**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*C.pallescens**	-	-	-	-	-	-	1	0.06±0.0	-	-	-	-
*C.penniseti**	1	0.06±0.0	-	-	-	-	-	-	-	-	-	-
*C.robusta**	1	0.06±0.0	-	-	-	-	-	-	-	-	-	-
*Drechslera australiensis**	-	-	1	0.06±0.0	-	-	-	-	1	0.06±0.0	-	-
*D.bicolor**	1	0.06±0.0	-	-	-	-	-	-	-	-	-	-
*D.cactivora**	-	-	1	0.06±0.0	-	-	-	-	-	-	-	-
*D.hawaiiensis**	-	-	1	0.12±0.0	-	-	1	0.06±0.0	1	0.06±0.0	-	-
*D.revenelii**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Emericella nidulans**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*E.rugulosa**	1	0.06±0.0	-	-	-	-	-	-	-	-	-	-
*Emericellopsis terricola**	-	-	-	-	-	-	-	-	1	0.77±0.0	-	-
*Epicoccum purpurascens**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Fusarium oxysporum**	1	0.12±0.0	-	-	-	-	-	-	1	0.06±0.0	-	-
F.semitectum	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Glomerella cingulata**	-	-	-	-	-	-	-	-	-	-	-	-
*Gonitrichum macrocladium**	-	-	-	-	-	-	1	0.06±0.0	-	-	-	-
*Helminthosphaeriaclavariarum**	-	-	-	-	1	0.06±0.0	-	-	-	-	-	-
*Humicola fuscoatra**	1	0.06±0.0	-		-	-	-	-	1	0.06±0.0	-	-
Macrophomina phaseolina	1	0.06±0.0	3	1.06±7.00	-	-	1	0.06±0.0	2	1.60±10.61	-	-
Melanospora spp.*	1	0.06±0.0	-	-	-	-	-	-	1	0.06±0.0	-	-

Table 1. (Cont'd.).

Name of fungi	Non- surface sterilized seeds						Surface sterilized seeds					
	Blotter method		Agar plate		Deep-freezing		Blotter method		Agar plate		Deep-freezing	
	N.SI	I%±SD	N. SI	I%±SD	N.SI	I%±SD	N. SI	I%±SD	N.SI	I%±SD	N.SI	I%±SD
*Memnoniella echinata**	1	0.41±0.0	-	-	-	-	-	-	-	-	-	-
*M.subsimplex**	1	0.35±0.0	-	-	-	-	-	-	1	0.06±0.0	-	-
Monilia sp.*	1	0.12±0.0	-	-	-	-	-	-	-	-	-	-
*Monodictys levis**	-	-	-	-	1	0.06±0.0	-	-	-	-	-	-
*Mucor hiemalis**	2	0.65±6.36	1	0.59±0.0	1	0.06±0.0	-	-	5	2.53±12.58	1	0.29±0.0
*M.mucedo**	1	0.06±0.0	-	-	-	-	-	-	2	5.9±70.00	-	-
*Myrothecium cinctum**	1	0.06±0.0	-	-	-	-	-	-	1	0.06±0.0	-	-
*M.roridum**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Nectria inventa**	-	-	1	0.06±0.0	-	-	-	-	-	-	-	-
*N.ventricosa**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Neosartorya fischeri**	-	-	-	-	-	-	-	-	1	0.24±0.0	-	-
*Nigrospora oryzae**	-	-	-	-	-	-	1	0.12±0.0	3	1.71±12.29	-	-
*N.sphaerica**	-	-	1	0.06±0.0	-	-	1	0.12±0.0	1	0.12±0.0	-	-
Nigrospora sp.*	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
Paecilomyces sp.*	-	-	-	-	1	0.06±0.0	-	-	-	-	-	-
*Papulaspora irregularis**	-	-	1	2.35±0.0	-	-	1	0.06±0.0	2	0.12±0.0	-	-
Penicillium sp.	-	-	1	0.59±0.0	1	0.06±0.0	-	-	1	0.12±0.0	-	-
*Phoma eupyrena**	2	0.12±0.0	1	0.06±0.0	-	-	2	3.54±0.65	1	0.06±0.0	-	-
*P.exigua**	-	-	1	0.06±0.0	-	-	-	-	-	-	-	-
*P.medicaginis**	-	-	1	5.94±70.00	-	-	-	-	-	-	-	-
*Rhizopus arrhizus**	-	-	2	6.47±63.64	-	-	-	-	3	0.71±5.19	-	-
*R.oryzae**	2	6.53±62.93	6	23.00±46.71	1	0.06±0.0	1	1.77±0.0	7	4.12±35.91	-	-
*R.stolonifer**	7	5.47±11.19	8	20.29±42.18	-	-	7	2.82±2.48	10	16.18±36.39	1	0.06±0.0
*Scytilidium lignicola**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Septotrullula bacilligera**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Stachybotrys cylindrospora**	1	0.18±0.0	-	-	-	-	-	-	-	-	-	-
*Staphylotrichum coccosporum**	-	-	-	-	-	-	-	-	-	0.60±0.0	-	-
*Taeniolella exillis**	-	-	-	-	-	-	-	-	1	0.06±0.0	-	-
*Trichocladium opacum**	-	-	-	-	-	-	-	-	1	0.12±0.0	-	-
*Trichoderma hamatum**	-	-	2	0.12±0.0	-	-	-	-	2	0.18±0.71	-	-
*T.harzianum**	-	-	2	0.35±2.83	-	-	1	0.06±0.0	3	0.53±3.46	-	-
*T.viride**	-	-	-	-	-	-	-	-	-	0.06±0.0	-	-

N.SI = Number of infected seed samples, SD = Standard deviation, I% = Infection percentage, *= Newly report from Pakistan

Discussion

Of the 17 seed samples tested, samples collected from Peshawar, Mardan, Abbottabad, Murree, Swabi, Mandibahuddin, Ghotki and Sukkur were found to be infected with pathogenic fungi like *Fusarium* spp, *M.phaseolina,* and *Phoma* spp.

Quantitatively as well as qualitatively, agar plate method was found to the best for the isolation of most the fungi from Pumpkin seeds. Unlike Sultana & Ghaffar (2009) who found blotter and deep-freezing methods most suitable for the seeds of bottle gourd. High incidence of *Aspergillus* species caused retarded growth and decay of seeds and seedlings. *Chaetomium* species were also observed in higher frequency on the seeds, as *Chaetomium* is cellulose decomposing fungus (Domsch *et al.,* 1980) blotter method was found to be good for the isolation of *Chaetomium* species. Various sizes of sclerotia of *M.phaseolina* were observed on seeds causing char-coal rot and decay. Similar results were also observed by Sultana & Ghaffar (2009) on bottle gourd, where the fungi have produced small sized sclerotia and black rot of seeds. Such similar results were also reported by Maholay & Sohi, (1982); Maholay, (1988, 1989), where *M.phaseolina* has produced black rot on the seeds of muskmelon, bottle gourd and squashes. *Fusarium* species are responsible for the seed rot, seedling blight and wilt in number of cucurbitaceous crops (Booth, 1971). Weidenborner (2001) isolated 25 different species belonging to 17 genera from the seeds of pumpkin using different media for isolation which included the species of *Absidia, Alternaria, Aspergillus, Chaetomium, Cladosporium, Epicoccum, Eurotium, Fusarium, Mucor, Phoma, Penicillium, Rhizopus, scopulariopsis* and *Trichoderma* etc. Due to higher infection of fungi, seeds failed to germinate in agar plate method and as fungal infection were low on blotter method, the seeds germinated well on it. Such similar results were also reported by Kaiser *et al*., (1989) on lentil seeds where seeds failed to germinate due to high fungal infection. Overall, good germination of seeds was observed on surface sterilized seeds. Similar results were observed by Odofin (2010) who reported that treatment with bleach has enhanced the germination rate of okra seeds.

These saprophytic as well as pathogenic fungi attack pumpkin before and after harvest causing rot, decay, scab, blight etc of the pumpkin in the field as well as after harvest during the storage of fruit. Mostly the fungi are present as dormant mycelium in the tissues of fruits and seeds and cause infection when the environment is suitable for their germination. From the consumption point of view presence of so many fungi both pathogenic and saprophytic, is not a good sign. Nearly all the fungi isolated hereby, are known to produce mycotoxins. Niaz *et al*., (2012) reported that out of 59 maize seed samples, 50 were found to be contaminated with aflatoxins, while 43 seed samples were contaminated with zearalenone. *Aspergillus* species are responsible for the production of aflatoxins. Aflatoxins are carcinogenic and responsible for the production of aspergillosis and systemic infections in man, animals and birds (Raper & Fennell, 1965). Storage and pathogenic fungi are responsible for the loss of germination and discoloration of seeds (Barton, 1961; Harrington, 1963; Golumbic & Laudani, 1966; Naqvi *et al*., 2012). *Alternaria* spp. produces mycotoxins such as alternariols, altenuens, altertoxins and tenuazonic acid (King & Schade, 1984). Most of the *Chaetomium* species are cellulose decomposing fungi causing soft rot, decay and decomposition of wide variety of hosts besides being food for mites (Domsch *et al*., 1980). Fungi forming fruiting bodies always have high mycotoxins production ability and are more pathogenic. Presence of *Melanospora* sp. and other ascomycetes showed that the seed samples were highly infected with pathogenic fungi.

Studies on the pumpkin seeds showed that pumpkin fruit is highly susceptible to fungal infestation before and after harvest while the seeds are prone to pathogenic as well as saprophytic fungi during storage. Care must be taken while handling the seeds; they must be cleaned and properly washed before drying the seeds for storage to avoid any fungal infection, mites and insects attack. Being agricultural state, proper steps must be taken to avoid diseases and damage to the crop due to fungal mycoflora, for saving economy of the country.

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