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PATTERN OF POST-HARVEST FUNGAL INFESTATION ON VEGETABLES STORED IN VARIOUS VEGETABLE MARKETS OF KARACHI

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Abstract

We isolated and identified 17 genera and 25 species of fungi from five vegetable crops including Bitter gourd (*Casia Momordica*), Garlic (*Allium sativum*), Okra (*Abelmoschus esculentus*), Onion *(Allium cepa*) and Potato (*Solanum tuberosum*) from five major vegetable markets of Karachi *viz,* Gulestan e Johar, Landhi, Nazimabad, New Sabzi Mandi and Saddar Empress Market. Among all isolated fungal flora; the fungi *A. niger*, *Rhizopus stolonifer*, *Colletotrichum gloeosporioides*, *Botrytis cinerea* and *Fusarium oxysporum* were the most common species causing the post-harvest decay of the target vegetables. Blotter paper method yielded more number of mycoflora with higher frequency over the agar plate method. In pathogenicity test, *Aspergillus niger* showed the highest rot diameter of 40.33mm on onion while it appeared as the lowest rot diameter of 7 mm on okra. The higher fungal infestation (34.84%) was recorded on vegetables that were stored or sold in Sabzimandi and the least fungal infestation (3.63%) was found on vegetables stored n Landhi. Vegetables are an essential part of our diets, therefore, we must keep our vegetables clean and free of diseases. Thus we surveyed a few large vegetable markets in Karachi to identify post-harvest mycoflora associated with various commonly used vegetables in our diets. We used blotter paper as well as Potato Dextrose Agar (PDA) methods to isolate and identify the fungal species infecting vegetable crops.

Key words: Vegetables, Mycoflora, Pathogenicity, Blotter paper method, PDA method.

Introduction

Pakistan produces a large variety of vegetables including but not limited to onion, potato, tomato, okra, bitter gourd, eggplant and garlic that are cultivated in diverse climatic conditions. In Pakistan during the year 2016-2017 the total vegetable cultivated area was101197 hector for Kharif crop and 159902 hectors for Rabi crops vegetables (Anon., 2016-17).

The fungal species are the most predominant pathogens causing major losses to fresh vegetables during storage and transportation (Amadi *et al*., 2014; Onuorah & Ifeany, 2015). Approximately 20-25% of the harvested fruits and vegetables also decay by the attack of pathogens during post-harvest handling (Zhu, 2006). Pathogenic fungi alone cause 10-30% yield reduction of major crops. Fruit and vegetable markets are known to contain different species of fungi. Atmospheric air contains many pathogenic fungi that settle on the surface of fruits & vegetables (Vermani & Hussain, 2014; Tsukamoto *et al*., 2018). Fungi *Aspergillus, Botrytis, Colletotrichum, Fusarium, Geotrichum, Gloeosporium, Monilinia, Mucor, Penicillium & Rhizopus* are known the causal agent of blue mold, grey mold, bitter rot, black mold, *Fusarium* rot & *Phoma* rot on post-harvest vegetable crops. Fungal infestation is very common on vegetable crops in all most all parts of the world (Umesh & Kakde, 2012; Masood, 2013; Kahramanoğlu *et al*., 2018; Sobia *et al*., 2016; Goel *et al*., 2018). Common post-harvest diseases resulting from wound infection. Pathogenic fungi associated with post-harvest potato tuber rot are *Alternaria solani* (early blight), *Rhizogospora subtranea* (powdery scab), *Fusarium roseum* & *Fusarium solani* (*Fusarium* rot) and *Helmenthosporium solani* (skin blemishes) (Akinleye *et al*., 2013; Rukaia & Gherbawy, 2013; Markson *et al*., 2014). Fungal species have also been reported as disease agents of onion and garlic worldwide. For instance; *Colletotrichum* spp., *Fusarium* spp., *Botrytis* spp., *Aspergillus* spp caused soft rot, brown rot, basal & neck rot (Daniel *et al*., 2012; Bashir *et al*., 2013; Ushasri & Kumar, 2018). A total of 15 genera and 29 species of fungi were isolated from bitter gourd collected from different areas of Pakistan (Sultana & Gaffar, 2007).

This study was aimed to identify the fungal flora; responsible for post-harvest rot, associated with five vegetable crops including Bitter gourd (*Casia momordica*), Garlic (*Allium sativum*), Okra (*Abelmoschus esculentus*), Onion (*Allium cepa*) and Potato (*Solanum tuberosum)* collected from five major markets in the areas of Gulestan e Johar, Landhi, Nazimabad, New Sabzi Mandi and Saddar Empress Market in Karachi, Pakistan.

Materials and Methods

Collection of samples: One fifty Samples each of bitter gourd, garlic, okra, onion, and potato and showing the symptom of deterioration and rotting like were collected from five major markets of fruits & vegetables in Karachi. Infected and non-infected samples were separately kept inside the clean plastic bags, transferred to the laboratory and stored in a refrigerator (5±1ºC) until mycological analysis.

Mycological analysis (Isolation techniques): The fungal mycoflora were isolated by using the Agar Plate Method (APM) and Standard Moist Blotter Method (SMB) as recommended by International Rules for Seed Health Testing Association (1993).

Isolation of fungi by Agar plate method

Isolation of mycoflora from surface sterilized samples of vegetables: The samples of vegetables were surface sterilized with 5% sodium hypochlorite (NaClO) solution in a sterile beaker for 1-2 minutes and cut into small pieces (1-2 cm). The disinfected vegetable pieces were transferred with sterile forceps into Petri plate (five pieces per Petri plate) containing PDA, (each vegetable sample contained four replicate), PDA was supplemented with 1mg chloramphenicol/L to restrict bacterial growth. Plates were incubated at room temperature at 28±2ºC for 5-7 days.

Isolation of mycoflora from non-sterilized surface samples of vegetables: The non-sterilized samples of vegetables were cut into small pieces (1-2 cm) and transferred with sterilized forceps into Petri plates (five pieces per Petri plate) that contained sterilized PDA. There were 4 replicates of each treatment. All plates were incubated at 28±2ºC at room temperature for 5-7 days.

Isolation of fungi by Blotter paper method

Isolation of mycoflora from sterilized surface samples of vegetables: The samples of vegetables were surface sterilized with 5% sodium hypochlorite solution in a sterilized beaker for 1-2 minutes and then washed with distilled water 3X. Samples of vegetable were cut into small pieces (1-2 cm) then transferred onto three layers of moist blotter paper in sterilized plate by using sterilized forceps, 5 pieces per Petri plate at equal distance (each sample contained four replicates), Petri plates were incubated at 28±2ºC for 5-7 days; after incubation, the samples were examined under the microscope.

Isolation of mycoflora from non-sterilized surface samples: Five pieces of non-sterilized vegetable samples were placed on the layer of moist blotter paper that was kept on a sterilized Petri plate. The plates were incubated at 28±2ºC for 5-7 days; fungi developing on vegetable samples were examined and then transferred to PDA slant for further identification.

Identification of fungi: Isolated fungi were identified using cultural and morphological features illustrated by Barnet & Hunter (1998), Booth (1971) & Ellis (1971).

Pathogenicity test: To determine the pathogenicity of the isolated fungi, freshly harvested uninfected vegetables have been used for the pathogenicity test (Iwuagwu *et al*., 2014). We followed the same procedures. We used 85% ethanol in water to surface sterilized healthy vegetables. Then a 3mm cork borer was used to obtain discs of the mycelium of each identified fungus from the periphery of the 4 to 5 days old PDA culture. These discs were placed in the wound made on a healthy tissue of the test product of vegetables with a cork borer. The rim of the wounded vegetables was sealed with petroleum jelly to prevent contamination. The produce of vegetables was left for 5 to 7 days at 28±2ºC at room temperature. After incubation, the sample of vegetables was examined for any symptom development. For the control, sterile PDA disc was used on the wounded surface of the produce.

Statistical analysis

The data were analyzed by Analysis of variance (ANOVA) using one way ANOVA SPSS 23 version (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp) (Shaukat & Siddiqui, 2005; Ahmed & Shaukat, 2012).

Results

Incidence of post-harvest fungi by blotter paper method: We isolated 17 genera and 25 species of fungi by using the blotter paper method. The isolated genera were identified as *Aspergillus, Alternaria, Botrytis, Colletotrichum, Chaetomila, Curvularia, Cladosporium, Drechslera, Fusarium, Gloeosporium, Macrophomina, Monilinia, Mucor, Phoma, Penicillium, Rhizopus & Urocystis* (Table 1).

Six genera and 7 species of fungi *viz*., *Aspergillus niger, A. fumigatus, Botrytis cinerea, Colletotrichum distructum, Fusarium oxysporum, Rhizopus stolonifer* and *Urocystis cepulae* were isolated from rotten vegetables from Gulestan e Johar vegetable market (Table 1). Four fungal species were isolated from onion; 3 fungal species were isolated from okra, potato & garlic and 2 fungal species were isolated from bitter gourd (Fig. 1). In Nazimabad, vegetable market, 4 genera and 5 species of fungi were isolated, whereas 7 genera and 10 species were recorded for the Landhi vegetable market. In Empress market of Saddar; total 15 genera & 20 fungal species were isolated from vegetables found in Sadar Empress Market and total 17 genera and 24 fungal species were isolated from sabzi mandi; out of which 8, 10, 10, 11 & 13 fungi were recorded on okra, garlic, onion, potato & bitter gourd respectively (Fig. 1). *A. niger* and *R. stolonifer* were found common in all vegetables and accounted highest mean values in blotter paper method (Table 1).

Incidence of post-harvest fungi evaluated by Potato Dextrose Agar method: Twenty-three fungal species belonging to 16 genera were isolated from the rotten samples of vegetables from all five vegetable markets by PDA method (Table 2). Six fungal species were isolated from Gulestan-e-Johar market; out of which 3 fungi were reported from okra, potato and onion, 2 fungal species were isolated from garlic & bitter gourd. From Nazimabad, vegetable market, 4 genera and 5 fungal species were reported. Six genera and 9 species of fungi were isolated from the vegetable market of Landhi. In Empress Market of Saddar, 15 genera and 19 species of fungi were identified; the species of *Chaetomella, C. lunata, F. solani, Gloeosporium* & *P. digitatum* were isolated only from bitter gourd (Table 2).

The highest numbers of fungi were isolated from Sabzi Mandi, the main vegetable market of Karachi. A total of 16 genera and 22 species of fungi were isolated. 11 fungal species were reported on potato,10 on garlic & bitter gourd, 9 on onion & 8 on okra, were recorded (Fig. 2). The highest fungal colonies were obtained by blotter paper for all non-sterilized vegetable samples whereas the lowest number of fungi was recorded on PDA for sterilized samples of all vegetables (Fig. 3).

Table 1. % occurrence and mean value of fungi on Blotter paper method.

Sr. No.	Localities	Name of fungi	Okra		Potato		Garlic		Onion		Bitter gourd		Mean ± S.E	
			B. method		B. method		B. method		B. method		B. method			
			ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST
1.	Gulestan-e-johar	*Aspergillus fumigatus*	0	0	0	0	0	0	60	80	0	0	12±12.0	16±16.0
		A. niger	35	40	0	0	0	0	75	95	55	60	33±14.8	39±18.1
		Botrytis cinerea	15	20	0	0	0	0	0	0	0	0	3±3.0	4±4.0
		Colletotrichum distructum	0	0	15	25	5	10	0	0	0	0	4±2.9	7±4.8
		Fusarium oxysporum	0	0	15	20	10	15	0	0	0	0	5±3.1	6±4.3
		Rhizopus stolonifer	15	25	15	25	10	15	70	80	50	60	32±11.8	41±12.3
		Urocystis cepulae	0	0	0	0	0	0	85	95	0	0	17±17.0	19±19.0
2.	Nazimabad	*A. niger*	20	25	40	50	10	15	70	80	60	65	40±11.4	47±12.1
		A. flavus	10	10	0	0	0	0	65	80	35	40	22±12.4	26±15.3
		Fusarium oxysporum	0	0	0	0	20	30	0	0	0	0	4±4.0	6±6.0
		Macrophomina phaseolina	0	0	20	30	0	0	0	0	0	0	4±4.0	6±6.0
		Rhizopus stolonifer	0	0	10	15	5	10	0	0	40	45	11±7.5	14±8.27
3.	Landhi	*A. niger*	25	30	45	55	45	55	55	60	55	60	45±5.4	52±5.61
		A. terreus	0	0	0	0	10	15	50	60	50	60	22±11.5	27±13.7
		A. flavus	10	15	0	0	0	0	0	0	0	0	2±2.0	3±3.0
		Colletotrichum distructum	0	0	35	40	0	0	0	0	0	0	7±7.0	8±8.0
		C. gloeosporioides	15	20	15	25	15	25	40	50	40	45	25±6.0	33±6.0
		Drechslera hawaiiensis	0	0	0	0	0	0	60	70	10	20	14±11.6	18±13.5
		Fusarium oxysporum	0	0	25	30	20	30	0	0	0	0	9±5.5	12±7.3
		Macrophomina phaseolina	0	0	10	15	0	0	0	0	0	0	2±2.0	3±3.0
		Phoma beta	0	0	0	0	35	40	0	0	0	0	7±7.0	8±8.0
		Urocystis cepulae	0	0	0	0	0	0	50	60	0	0	10±10	12±12.0
4.	Saddar	*A. niger*	35	40	35	40	25	30	50	70	65	70	42±7.0	50±8.36
		A. terreus	0	0	35	40	30	35	0	0	0	0	13±8.0	15±9.21
		A. flavus	35	40	0	0	0	0	30	55	0	0	13±8.0	19±11.8
		A. candidus	0	0	0	0	10	15	0	0	0	0	2±2.0	3±3.0
		Alternaria solani	0	0	0	0	0	0	0	0	15	20	3±3.0	4±4.0
		A. alternata	20	25	35	45	5	10	0	0	0	0	12±6.8	16±8.57
		Botrytis cinerea	10	15	10	15	10	15	0	0	0	0	6±2.4	9±3.67
		Chaetomella spp.	0	0	0	0	0	0	0	0	40	50	8±8.0	10±10.0
		Curvularia lunata	0	0	0	0	0	0	0	0	45	65	9±9.0	13±13.0
		Colletotrichum distructum	0	0	25	35	5	10	0	0	0	0	6±4.8	9±6.78
		Fusarium oxysporum	0	0	15	20	10	15	0	0	0	0	5±3.1	7±4.35
		F. solani	0	0	0	0	0	0	0	0	45	55	9±9.0	11±11.0

Table 1. (Cont'd.).

Sr. No.	Localities	Name of fungi	Okra		Potato		Garlic		Onion		Bitter gourd		Mean ± S.E	
			B. method		B. method		B. method		B. method		B. method			
			ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST
		Gloeosporium spp.	0	0	0	0	0	0	0	0	30	35	6±6.0	7±7.0
		Macrophomina phaseolina	0	0	15	25	0	0	0	0	10	15	5±3.1	8±5.14
		Monilinia fructicola	10	15	10	15	10	15	0	0	0	0	6±2.4	9±3.67
		Mucor racemosus	0	0	0	0	0	0	60	70	25	30	17±11.7	20±13.7
		Penicillium digitatum	0	0	0	0	0	0	0	0	25	30	5±5.0	6±6.0
		Phoma beta	0	0	0	0	10	15	0	0	0	0	2±2.0	3±3.0
		Rhizopus stolonifer	25	30	25	30	10	15	50	70	50	70	32±7.8	43±11.3
		Urocystis cepulae	0	0	0	0	0	0	45	65	0	0	9±9.0	13±13.0
		Aspergillus. niger	45	50	60	50	50	60	70	85	70	85	59±5.09	66±7.96
		A. flavus	15	20	0	0	0	0	40	55	30	40	17±8.0	23±0.90
		A. fumigatus	0	0	0	0	0	0	50	60	25	40	15±10.0	20±12.6
		A. candidus	10	15	5	10	10	20	0	0	0	0	5±2.2	9±4.0
		Alternaria solani	0	0	0	0	0	0	25	35	25	30	10±6.1	13±8.0
		A. alternata	25	35	40	45	10	15	0	0	0	0	15±7.7	19±9.13
		Botrytis cinerea	10	15	20	25	15	25	0	0	0	0	9±4.0	13±5.61
		Chaetomella spp.	0	0	0	0	0	0	0	0	40	55	8±8.0	11±11.0
		Colletotrichum gloeosporioides	20	25	0	0	0	0	30	40	30	40	16±6.7	21±9.0
		Cladosporium cladosporioides	0	0	15	20	15	25	0	0	0	0	6±3.6	9±5.56
		Colletotrichum distructum	0	0	10	15	10	15	0	0	0	0	4±2.4	6±3.67
		Curvularia lunata	0	0	0	0	0	0	0	0	50	70	10±10.0	14±14.0
5.	Sabzi Mandi	*Drechslera hawaiiensis*	0	0	0	0	0	0	20	30	5	10	5±3.8	8±5.83
		Fusarium Oxysporum	0	0	20	25	0	0	0	0	0	0	4±4.0	5±5.0
		F. solani	0	0	25	30	20	30	0	0	0	0	9±5.5	12±7.34
		Gloeosporium spp.	0	0	0	0	0	0	0	0	30	35	6±6.0	7±7.0
		Macrophomina phaseolina	0	0	15	20	0	0	0	0	15	20	6±3.6	8±4.89
		Monilinia fructicola	10	20	15	20	15	20	0	0	0	0	8±3.39	12±4.8
		Mucor racemosus	0	0	0	0	0	0	45	50	45	50	18±11.0	20±12.2
		Penicillium digitatum	0	0	0	0	0	0	20	35	0	0	4±4.0	7±7.0
		P. expansum	0	0	0	0	0	0	0	0	20	35	4±4.0	7±7.0
		Phoma beta	0	0	0	0	15	20	0	0	0	0	3±3.0	4±4.0
		Rhizopus stolonifer	10	15	10	20	10	20	70	80	70	80	34±14.6	43±11
		Urocystis cepulae	0	0	0	0	0	0	50	65	0	0	10±10.0	13±13.0
		Mean	**6±1.3**	**8±1.6**	**10±1.7**	**13±2**	**7±1.3**	**10±1.7**	**20±3.3**	**25±4**	**18±2.7**	**22±3.3**		

ST= Sterilized, **N.ST=** Non-sterilized, **B. method=** Blotter paper method

Table 2. % occurrence and mean value of fungi on PDA (Potato Dextrose Agar) method.

Sr. No.	Localities	Name of fungi	Okra		Potato		Garlic		Onion		Bitter gourd		Mean± S.E	
			PDA method		PDA method		PDA method		PDA method		PDA method			
			ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST
1.	Gulestan-e-Johar	*Aspergillus niger*	25	30	0	0	0	0	90	95	40	45	31±16.6	34±17.5
		Botrytis cinerea	10	15	0	0	0	0	0	0	0	0	2±2.0	3±3.0
		Colletotrichum distructum	0	0	5	10	0	0	0	0	0	0	1±1.0	2±2.0
		Fusarium oxysporum	0	0	10	15	5	15	0	0	0	0	3±2.0	6±3.6
		Rhizopus stolonifer	5	10	10	15	5	10	60	65	35	40	23±10.7	28±10.7
		Urocystis cepulae	0	0	0	0	0	0	90	95	0	0	18±18.0	19±19.0
2.	Nazimabad	*A. niger*	10	15	25	15	10	15	65	70	55	60	33±11.4	35±12.3
		A. flavus	5	10	0	0	0	0	60	65	25	30	18±11.4	21±12.2
		Fusarium oxysporum	0	0	0	0	15	20	0	0	0	0	3±3.0	4±4.0
		Macrophomina phaseolina	0	0	15	10	0	0	0	0	0	0	3±3.0	2±2.0
		Rhizopus stolonifer	0	0	5	10	0	0	0	0	30	35	7±5.8	9±6.70
3.	Landhi	*A. niger*	15	20	30	35	30	35	45	50	45	50	33±5.6	38±5.61
		A. terreus	0	0	0	0	10	20	0	0	45	50	11±8.7	14±9.79
		A. flavus	5	10	0	0	0	0	0	0	0	0	1±1.0	2±2.0
		Colletotrichum distructum	0	0	30	25	0	0	0	0	0	0	6±6.0	5±5.0
		C. gloeosporioides	10	15	10	15	10	15	0	0	30	40	12±4.0	17±6.4
		Fusarium Oxysporum	0	0	20	15	10	15	0	0	0	0	6±4.0	6±3.6
		Macrophomina phaseolina	0	0	5	10	0	0	0	0	0	0	1±1.0	2±2.0
		Phoma beta	0	0	0	0	30	35	0	0	0	0	6±6.0	7±7.0
		Urocystis cepulae	0	0	0	0	0	0	40	55	0	0	8±8.0	11±11.0
4.	Saddar	*A. niger*	25	30	25	30	15	20	50	65	60	65	35±8.5	42±9.5
		A. terreus	0	0	30	25	15	20	0	0	0	0	9±6.0	9±5.56
		A. flavus	25	35	0	0	0	0	0	0	0	0	5±5.0	7±7.
		A. candidus	0	0	0	0	10	20	0	0	0	0	2±2.0	4±4.0
		Alternaria alternata	15	20	30	35	0	0	0	0	0	0	9±6.0	11±7.14
		Botrytis cinerea	5	10	5	10	5	10	0	0	0	0	3±1.2	6±2.44
		Chaetomella spp.	0	0	0	0	0	0	0	0	35	40	7±7.0	8±8.0
		Curvularia lunata	0	0	0	0	0	0	0	0	35	50	7±7.0	10±10.0
		Colletotrichum distructum	0	0	20	25	0	0	0	0	0	0	4±4.0	5±5.0
		Fusarium oxysporum	0	0	10	15	5	10	0	0	0	0	3±2.0	5±3.1
		F. solani	0	0	0	0	0	0	0	0	35	45	7±7.0	9±9.0

Table 2. (Cont'd.).

Sr. No.	Localities	Name of fungi	Okra		Potato		Garlic		Onion		Bitter gourd		Mean± S.E	
			PDA method		PDA method		PDA method		PDA method		PDA method			
			ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST	ST	N.ST
		Gloeosporium spp.	0	0	0	0	0	0	0	0	20	30	4±4.0	6±6.0
		Macrophomina phaseolina	0	0	10	15	0	0	0	0	5	10	3±2.0	5±3.1
		Monilinia fructicola	10	15	10	5	5	10	0	0	0	0	5±2.2	6±2.9
		Mucor racemosus	0	0	0	0	0	0	40	50	15	20	11±7.8	14±9.7
		Penicillium digitatum	0	0	0	0	0	0	0	0	10	15	2±2.0	3±3.1
		Phoma beta	0	0	0	0	10	20	0	0	0	0	2±2.0	4±4.0
		Rhizopus stolonifer	10	15	10	15	5	10	40	60	40	60	21±7.8	32±11.4
		Urocystis cepulae	0	0	0	0	0	0	35	50	0	0	7±7.0	10±10.0
		A. niger	35	40	50	40	20	30	60	70	65	70	46±8.2	50±8.36
		A. flavus	5	10	0	0	0	0	20	30	20	30	9±4.5	14±6.7
		A. fumigatus	0	0	0	0	0	0	25	40	20	30	9±5.5	14±8..7
		A. candidus	5	10	5	5	10	15	0	0	0	0	4±1.8	6±2.91
		Alternaria solani	0	0	0	0	0	0	10	20	10	20	4±2.4	8±4.8
		A. alternata	20	30	30	35	10	15	0	0	0	0	12±5.8	16±7.31
		Botrytis cinerea	10	10	10	15	10	15	0	0	0	0	6±2.4	8±3.39
		Chaetomella spp.	0	0	0	0	0	0	0	0	35	40	7±7.0	8±8.0
		Colletotrichum gloeosporioides	15	20	0	0	0	0	15	35	15	25	9±3.6	16±6.9
		Cladosporium cladosporioides	0	0	5	10	5	10	0	0	0	0	2±1.2	4±2.4
		Colletotrichum distructum	0	0	5	10	5	10	0	0	0	0	2±1.2	4±2.44
5.	Sabzi Mandi	*Curvularia lunata*	0	0	0	0	0	0	0	0	45	55	9±9.0	11±11.0
		Fusarium oxysporum	0	0	10	15	0	0	0	0	0	0	2±2.0	3±3.0
		F. solani	0	0	10	15	10	15	0	0	0	0	4±2.4	6±3.67
		Gloeosporium spp.	0	0	0	0	0	0	0	0	25	30	5±5.0	6±6.0
		Macrophomina phaseolina	0	0	5	10	0	0	0	0	0	0	1±1.0	2±2.0
		Monilinia fructicola	10	15	10	15	15	25	0	0	0	0	7±3.0	11±4.8
		Mucor racemosus	0	0	0	0	0	0	15	20	15	20	6±3.6	8±4.89
		Penicillium digitatum	0	0	0	0	0	0	10	20	0	0	2±2.0	4±4.0
		Phoma beta	0	0	0	0	5	10	0	0	0	0	1±1.0	2±2.0
		Rhizopus stolonifer	5	10	5	10	10	15	60	65	60	65	28±13.0	33±13
		Urocystis cepulae	0	0	0	0	0	0	25	30	0	0	5±5.0	6±6.0
		Mean	**5±1.0**	**6±1.3**	**8±1.3**	**9±1.5**	**5±0.9**	**8±1.25**	**14±3.1**	**17±3.5**	**14±2.4**	**18±2.8**		

ST= Sterilized, **N.ST=** Non-sterilized

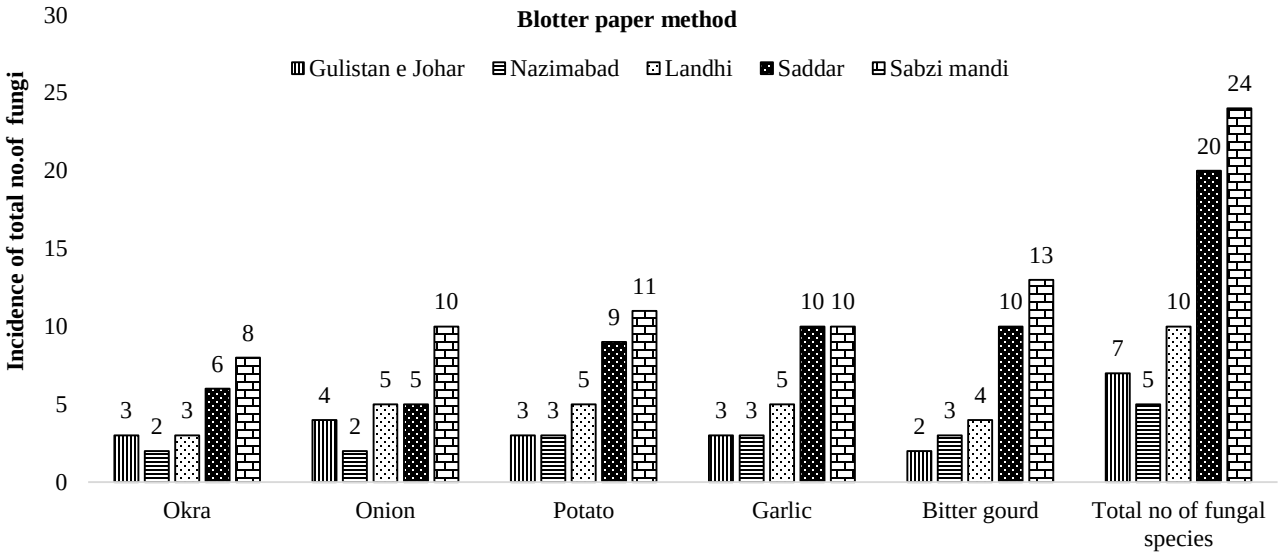

Fig. 1. Incidence of fungi on Blotter paper method.

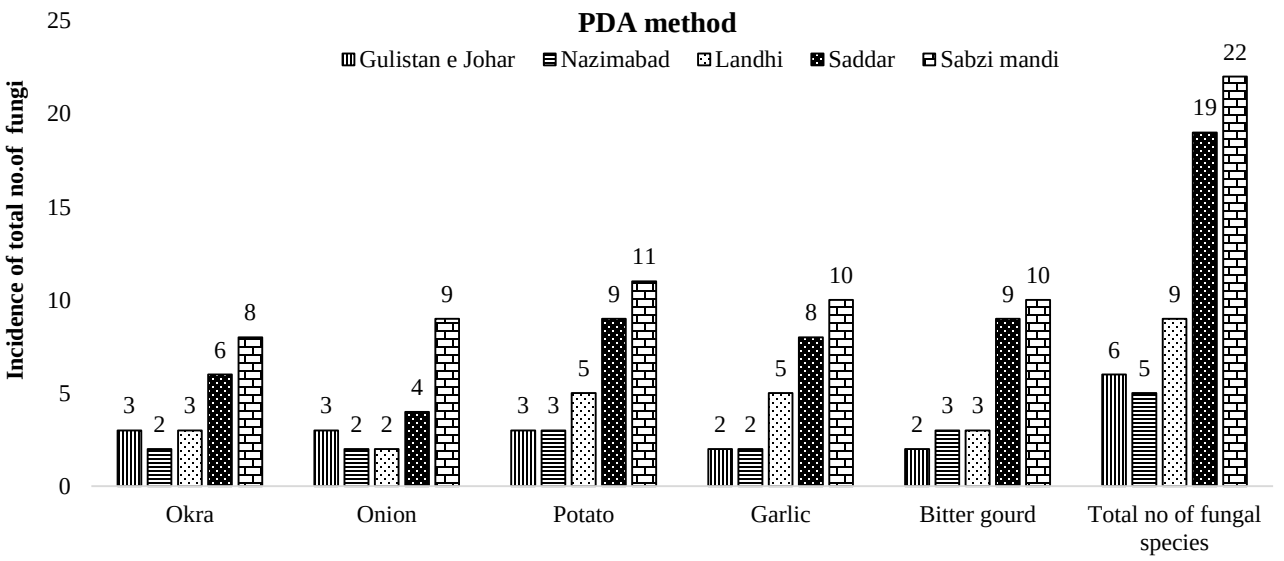

Fig. 2. Incidence of fungi on PDA method.

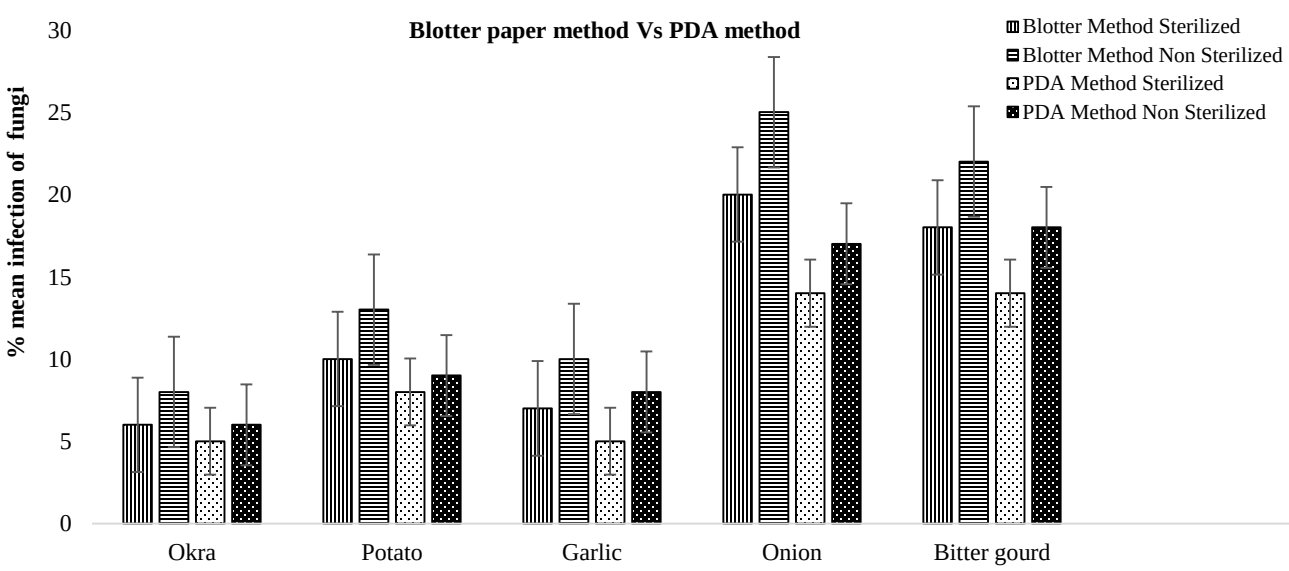

Fig. 3. % Mean infection of fungi on different isolation methods.

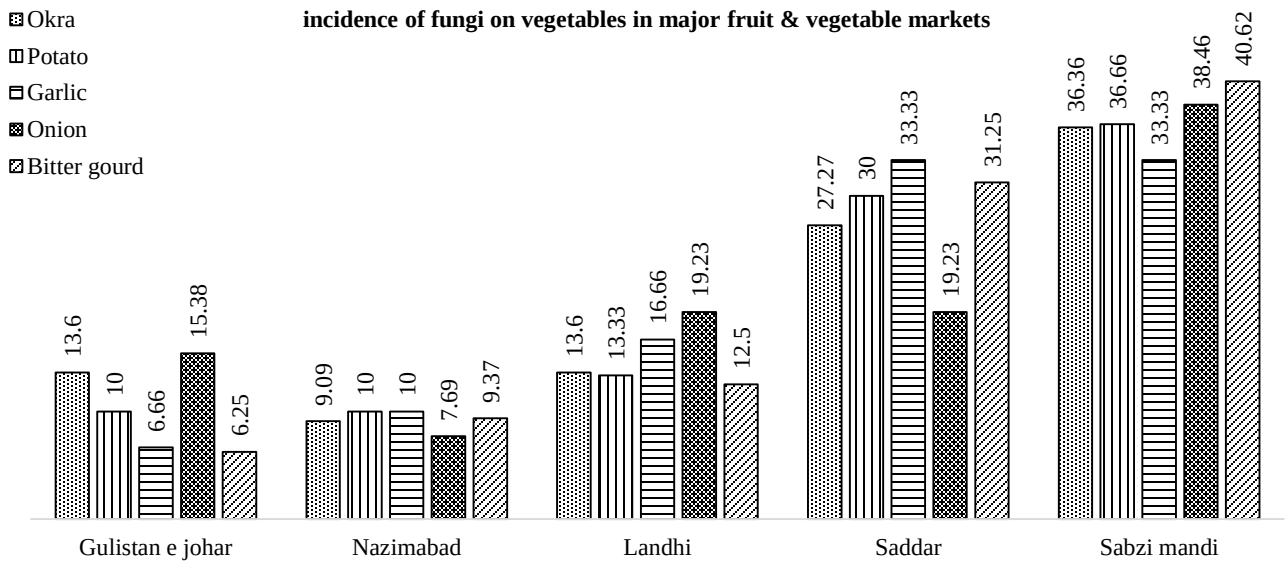

Fig. 4. % incidence of fungi on different vegetable.

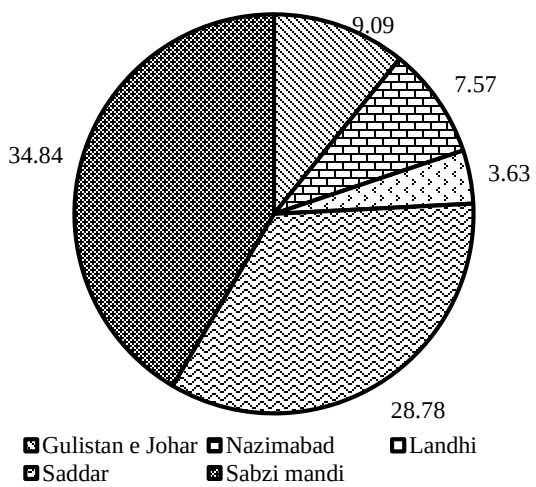

Fig. 5. % occurrence of fungi on different vegetable markets of Karachi.

We noted a higher percentage of fungal infestation on vegetables stored or sold in Sabzi Mandi vegetable market, whereas the other vegetable markets including Saddar, Gulestan-e-Johar, Nazimabad & Landhi showed lower fungal infestation (Fig. 4). Figure 5 shows a higher incidence of fungi on various vegetables.

Pathogenicity of fungal isolates: Pathogenicity test indicates that the isolated fungal species from five vegetables were pathogenic because they were able to produce the same damage in healthy vegetables when they were re-inoculated. Of all fungal species, *A. niger* was found to be highly pathogenic whereas *M. fructicola* was found to be least pathogenic to all vegetable tested, however, *C. distructum* showed pathogenicity to potato only (Table 3).

Discussion

In this report, we have isolated and identified 17 genera and 25 fungal species that were found on rotten okra, onion, potato, garlic & bitter gourd collected from five different vegetable markets of Karachi. We applied two different procedures to determine the efficient method for fungal isolation.

Our study indicates that *Aspergillus, Rhizopus, Botrytis, Colletotrichum* & *Fusarium* may be involved in the post-harvest rot of vegetables. Our result was in well agreement with those previously recorded by Fatima *et al*., 2009 have also reported the presence of eight genera of fungi *viz*., *Aspergillus, Cladosporium, Geotrichum, Alternaria, Fusarium, Phytophthora, Penicillium* and *Drechslera* on the rotten vegetables collected from the vegetable market of Karachi however in our study we isolated seventeen genera of fungi.

We isolated 7 fungal species including *A. niger, A. flavus, A. candidus, A. alternata, B. cinerea, C. gloeosporioides, M. fructicola* & *R. stolonifer* from rotten okra; *Aspergillus niger* was found dominated species However, Sharma *et al*., 2013 identified 11 fungal species from okra. In their report, *Aspergillus* species were found to be the most common species. *Aspergillus niger, A. flavus, Rhizopus* spp., *Mucor* spp., *F. oxysporum* and *M. phaseolina* have been reported to cause fruit rot, root-rot and seed rot in okra (Fagbohun & Faleye, 2012; Zahoor *et al*., 2012; Rahim & Dawar; 2015). We found *A. niger, R. stolonifer, F. oxysporum* & *C. distructum* to be the dominant fungi on potatoes. Interestingly, Ibrahim *et al*., 2014 also isolated *A. niger, A. flavus, Penicillium* spp., *M. racemosus, F. oxysporum* and *A. alternate* from potatoes. Kumar *et al*., 2014 reported *A. niger* & *F. oxysporum* remained the more dominant species on potatoes. Fiers *et al*., (2010) also reported the most represented fungi of potatoes belonging to the genera *Alternaria, Fusarium, Rhizoctonia* and *Penicillium* in France. We isolated 13 fungi including *F. oxysporum, R. stolonifer, A. niger, A. terreus, C. gloeosporioides, P. beta, A. candidus, B. cinerea, C. distructum, M. fructicola, A. alternata, C. cladosporioides* and *F. solani* from garlic. Ghangaonkar 2013 also found *A. niger* and *F. oxysporum* causing severe infestation on garlic during storage in India We isolated *A.*

fumigatus, A. niger, R. stolonifer, U. cepulae, A. flavus, A. terreus, C. gloeosporioides, D. hawaiiensis, M. racemosus, Alternaria solani & *P. digitatum* infesting onion. It is interesting to note that *U. cepulae* which caused smut diseases in onion, were extensively found from 4 vegetable markets except Nazimabad. We found that *A. niger* was the most common fungi on rotten samples of onion in this study. Our data is in agreement with Abdulsalam *et al*., 2015 who reported that *A. niger* had the highest percentage distribution in rotten onion bulb. *Penicillium* spp. infecting onion was also reported by Ibtasam & Bajwa (2014) from Pakistan. On bitter gourd, 17 species we isolated 17 fungal species including *B. cinerea, R. stolonifer, A. niger, A. flavus, A. terreus, C. gloeosporioides, D. hawaiiensis, A. solani, Chaetomella* spp., *Curvularia lunata, F. solani, Gloeosporium* spp., *M. phaseolina, M. racemosus, P. digitatum* & *P. expansum* from bitter gourd. Jianghua & Jiang (2015) also isolated *Cladosporium, Fusarium* & *Penicillium* species on fruit bitter gourd in China. We found that blotter paper method yielded more fungal growth that PDA method. The frequency of fungal species associated with post-harvest rot of vegetables greatly depends on the detection methods. Blotter paper method is more economical and provides reliable results (Fakhr-un-Nisa *et al*., 2006; Dhekle & Bodke, 2013). Mari & Anusree (2015) observed that the incidence of postharvest fungi is greater in blotter paper method than the PDA method. Sobia *et al*., (2016) reported that blotter method yielded more number of mycoflora with higher frequency over the agar plate method.

Table 3. Pathogenicity of fungal isolates.

Sr. No.	Name of fungi	Rot in diameter (mm)				
		Okra	Potato	Garlic	Onion	Bitter gourd
1.	*Colletotrichum gloeosporioides*	11	33.0	10.66	26.66	18.33
2.	*Colletotrichum distructum*	0	36.33	0	0	0
3.	*Botrytis cinerea*	5.33	25	12.66	35	11.33
4.	*Rhizopus stolonifer*	7.66	31.66	14.66	31	21.33
5.	*Aspergillus niger*	10	21.33	20.68	40.33	20
6.	*Monilinia fructicola*	7	17	8.66	18	13

Table 4. F-ratio derived from ANOVA for all treatments.

Source	F-ratio	P-value
Vegetables	240.527	
Localities	8.373	
Treatment	51.998	
Fungi	30.042	0.000
Replicates	11.451	
Localities *vegetables	15.876	
Localities*fungi	4.620	

The analysis of variance to compare blotter paper method and PDA method at 0.01 level showed a significant difference at all levels ($p \leq 0.001$). Interaction between localities & vegetables and fungal pathogen & locality were also found highly significant different at all levels (Table 4). Different genus of fungi was isolated from five rotten vegetables in five Karachi vegetable markets. In all markets, especially in Sabzi Mandi, it is observed that careless handling of vegetables was the main reason for the deterioration of vegetables as well as increase in temperature causes skin breaks and increasing water loss which provides sites for fungal infection in vegetables. All the dominated fungal spores recovered from the infected vegetables collected from the vegetable stock in five vegetable markets. The presence of such mycoflora in the vegetable of Karachi markets indicates that the fungi already present in the field from where these vegetables are transported. Some vegetables can be infected with airborne fungi by the market environment. There is a need to develop good storage facilities to prolong the shelf life of vegetables and avoid preventing fungal infection.

Conclusion

Overall, blotter paper method is better than PDA method. Fungi can change the nutritious value of vegetables by producing enzymes. It can lead to economic losses. In our study, the majority of the destructive microorganisms were pathogenic. *A. niger* and *R. stolonifer* occurred most as the spoilage microorganism. It is concluded that proper management should be adopted to protect the vegetables from decay. Vegetables in markets should not be subjected to long soaking, spraying or washing with contaminated water. Physical damage to the vegetable should be avoided because they cause an entry point of microbes. Unhealthy and contaminated vegetables should be separated from healthy produce and disposed of properly to avoid further contamination. An advisory service must assist farmers and post-harvest handling staff to ensure high quality and toxic-free vegetables.

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