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BIODIVERSITY OF AEROMYCOFLORA IN MAJOR FRUIT AND VEGETABLE MARKETS OF KARACHI CITY, PAKISTAN

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ABSTRACT

Airborne fungi one of the major components of aeromycobiota known to produce several fungal diseases in fruits and vegetable as well as allergic disorders in human being. The total 17 genera *Alternaria, Aspergillus, Botrytis, Curvularia, Drechslera Cladosporium, Colletotrichum, Fusarium, Monilia, Nigrospora, Mucor, Phoma, Penicillium, Rhizopus, Rhizoctonia, Trichoderma* and *Stachybotrys* were isolated during the study period of January to December 2018 from fruit and Vegetable markets of Karachi. The range of average temperature of the Karachi city was in between 20ºC to 33ºC and the humidity ranged from 33 to 70 percent during study period. The maximum fungal spores were recorded in the month of August whereas minimum noted in January 2018. Some isolated genera such as *Aspergillus, Alternaria, Fusarium, Rhizopus* and *Penicillium* cause rottenness of fruit and vegetables in local markets of Karachi. Most contaminated site with airborne fungal spores was the Sabzi Mandi followed by Saddar, Landhi, Nazimabad and Gulestan-e-Johar vegetable markets.

Key words: airborne fungi, fruit and vegetable markets, gravity setting technique, Karachi

INTRODUCTION

In Pakistan, few workers have undertaken aerobiological studies in fruit and vegetable markets. Hasnain *et al*. (2012) isolated *Aspergillus*, *Alternaria*, *Cladosporium*, *Curvularia*, *Penicillium* and *Stemphylium*. They also found that a number of fungal spores exist in the atmosphere of Karachi throughout the year. Farooq *et al*. (2015) conducted aerobiological survey and reported 30 species of fungi – dominant fungal species were *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium chrysogenum*, and *Fusarium* spp. Afzal and Mehdi (2002) identified 53 species of fungi belonging to 21 genera in Karachi city, out of which species of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* were the most frequently isolated. They also reported that the concentration of airborne spores in Karachi was greater at 5m height atmosphere. Some of the isolated fungi are known to cause allergic diseases (Nasir and Jalaluddin, 2005).

The left over totting fruits and vegetables are the habitat for various pathogens which cause different diseases in fresh fruits and vegetables and also affect the working atmosphere of the market. The concentration and range of occurrence of these microorganisms depends on various factors such as the season and time, rain fall, moisture, temperature and wind (Sharma *et al*; 2017). On the other hand, airborne pathogenic fungi could cause allergies or infections and become a severe health risk for both consumers as well as workers on the markets (Vermani *et al*., 2014). *Aspergillus* spp. and *Alternaria* spp. are reported to cause asthma in adult (Fairs *et al*., 2013; Zubair *et al.,* 2014). Airborne fungi are involved to cause the respiratory system infection by *Bipolris spicifera* (Buzina *et al*., 2003). Pande *et al.,* (2012) concluded the adverse effect of inhaled mold spores on the immune system on human beings. Some airborne *Aspergillus* conidia were responsible for the prevalence of bronco-pulmonary aspergilloses (Haque, 2004). Airborne fungi are involved in the damage to foodstuffs and the deterioration of organic materials as well as stored products (Pyuri and Kapsanaki, 2007). According to Singh *et al*., 2014 most predominant spores of fungi in the air were *Alternaria* spp., *Aspergillus* spp. *Curvularia* spp., *Fusarium* spp. and *Rhizopus* spp. Many human health disorders can be caused by *Alternaria* species which grow on skin and mucous membrane, sinus cavities, nails and respiratory tract (Vennealad *et al*., 1999). Airborne fungi occur as single units, spores and occasionally as hyphal fragments, conidiophore, associated with inorganic particle or as bio aerosol. The number and type of airborne fungi vary with time, conditions of the surrounding areas, weather and seasonal fluctuations, climatic conditions and the presence of a local source of spores.

The aim of this investigation was to document different species of fungi present in fruit and vegetable markets to know the disease risk factor due to fungal exposure. It may be helpful in identifying association between fungal sensitization and seasonal allergic diseases as well as to prevent the postharvest fungal diseases of fruit and vegetables.

MATERIALS AND METHODS

***Study area*:** The aero-mycological investigation was undertaken in the five fruit and vegetable markets of Karachi city viz., Gulistan-e- Johar, Landhi, Nazimabad, Saddar Empress market and new Sabzi Mandi. The experiment work was carried out for one year from January2018 to December 2018.

***Preparation of media and sampling method*:** The samples of airborne fungi were collected on potato dextrose agar medium plates (supplemented with chloramphenicol (1mg/liter) by gravity setting technique (Toqeer *et al*., 2009). The sampling was done while the market was the most active during morning hours (9am to 12pm). The PDA plates were taken to the market in pre-sterilized container.

Petri plates were exposed to air at a height of 1 meter at multiple locations for 10 minutes in each market. Petri plates were returned to the laboratory in pre sterilized bags, after exposure and stored in the incubator for 5 days at 28 ± 2ºC. The colonies of airborne fungi after incubation were observed and counted as well as fungus isolated sub-cultured on PDA slants and identified subsequently. The identification was based on colony morphology and microscopic characteristics with the help of key given by Barnet and Hunter (1972) and Ellis (1971). The data were reported as the percentage of abundance of each airborne fungus calculated according to formula.

$$Incidence\ of\ airborne\ fungi = \frac{\text{Total no. of colonies of any genus}}{Total\ no. of\ colonies\ in\ all\ replicates} \times 100$$

Statistical Analysis

The data was 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) was used for analysis.

RESULTS

***Meteorological* parameters:** Fungal growth is influenced by weather pattern such as temperature, humidity, wind pressure and wind speed. The sampling was conducted during throughout the year January to December 2018 by collecting the samples at the sites of five different markets of fruit and vegetable of Karachi city. The minimum average temperature (20 ºC) was observed in the month of January and December whereas maximum temperature was found during the month of May (33ºC). Air pressure, rainfall and wind speed were also recorded during the sampling period (Table 1). The highest fungal contamination of fungi during the month of August has been recorded which was (16.51%) while lowest was observed in the months of January and January (2.98%) (Fig. 1).

***Fungal contamination*:** In all thirty-one fungal species belonging to seventeen genera were recovered from five different fruit and vegetable markets of Karachi. The genera isolated as airborne fungi were *Aspergillus, Alternaria, Botrytis, Colletotrichum, Curvularia, Cladosporium, Drechslera, Fusarium, Monilia, Mucor, Nigrospora, Phoma, Penicillium, Rhizopus, Rhizoctonia*, *Trichoderma* and *Stachybotrys* (Table 2). On the individual basis the total of 10 genera and 12 species of fungi were isolated from Gulestan-e-Johar market (Fig 2). The dominated fungi were *Alternaria alternata, A. flavus, A. niger, Botrytis cinerea, Fusarium oxysporum, Rhizopus stolonifer* and *Monilia fructicola*. The highest airborne mycoflora was observed in the month of July (14.12%) whereas lowest in January (3.35%) (Fig 3). In Nazimabad market fifteen species of fungi belonging to eight genera were isolated *Aspergillus niger* was the higher mean value compare to all fungi. *Fusarium moniliforme* only isolated from this site. The highest percentage of fungal contamination was documented in the month of August (17.08) whereas lowest in January (1.79%) (Fig 3). At the site of Landhi market eleven genera and seventeen species of aeromycoflora were isolated. *Rhizopus stolonifer* was frequently isolated with highest mean vale whereas *Rhizoctonia solani* only isolated from this site (Table 2). The peak concentration was noticed in the month of August whereas least in January. Total eleven genera comprising twenty species were isolated from fruit and vegetable market of Saddar. The highest mean value was notice for *Aspergillus niger* followed by *Rhizopus stolonifer* and *Alternaria alternata*.

The highest fungal contamination was observed in the month of August (17.09%) and lowest in January (3.41 %) from this site (Fig 3).

Table 1. Record of temperature, humidity and air pressure (Jan –Dec. 2018).

Months	Average temperature (ºC)	Average humidity (%)	Average wind speed (kmph)	Average air pressure (mbar)
January	20	50	12.2	1015
February	23	52	13.3	1015
March	27	50	19.7	1011
April	30	55	22.7	1088
May	33	51	25.6	1004
June	31	66	27.9	1000
July	30	66	28	998
August	29	70	26.8	1001
September	29	67	24.3	1007
October	29	51	13.9	1012
November	26	43	11.4	1015
December	20	33	12	1017

Table 2. Comparative analysis of aerial mycobiota of five fruit and vegetable markets of Karachi.

S. No.	Name of fungi	Gulestan-e-Johar	Nazimabad	Landhi	Saddar	Sabzi Mandi
1	*Alternaria alternata*	+	+	+	+	+
2	*A. solani*	-	+	-	-	+
3	*A. porri*	-	-	-	-	+
4	*A. tenuissima*	-	+	+	+	+
5	*Aspergillus candidus*	+	+	+	+	+
6	*A. flavus*	+	+	+	+	+
7	*A. fumigatus*	-	-	-	+	+
8	*A. orchareus*	-	-	-	-	+
9	*A. nidulans*	-	-	+	+	+
10	*A. niger*	+	+	+	+	+
11	*A. terreus*	-	+	+	-	+
12	*Botrytis cinerea*	+	+	-	+	+
13	*Cladosporium herbarum*	+	-	+	+	+
14	*Colletotrichum acutatum*	-	-	+	+	+
15	*C. gloeosporioides*	+	-	+	+	+
16	*Drechslera hawaiiensis*	+	-	+	-	+
17	*Curvularia lunata*	-	-	-	-	+
18	*Fusarium oxysporum*	+	+	+	+	+
19	*F. semitactum*	-	+	-	+	+
20	*F. solani*	-	-	-	+	+
21	*F. moniliforme*	-	+	-	-	-
22	*Mucor racemosus*	-	+	+	+	+
23	*Monilinia fructicola*	+	+	-	-	-
24	*Nigrospora spp*	-	-	-	-	+
25	*Phoma beta*	-	-	-	-	+
26	*Penicillium digitatum*	+	+	+	+	+
27	*P. expansum*	-	-	-	+	+
28	*Rhizopus stolonifer*	+	+	+	+	+
29	*Rhizoctonia solani*	-	-	+	-	-
30	*Stachybotrys spp*	-	-	-	+	+
31	*Trichoderma harzianum*	-	-	+	-	+

Table 3. Analysis of Variance

	SS	df	MS	F	Sig.
Months	890.909	52	17.133	1.539	.008
	43086.713	3870	11.134		
	43977.622	3922			
replicates	592.875	52	11.401	9.291	.000
	4749.002	3870	1.227		
	5341.878	3922			
localities	366.570	52	7.049	3.499	.000
	7796.177	3870	2.015		
	8162.747	3922			
fungi	27732.300	52	533.313	5.232	.000
	394548.442	3871	101.924		
	422280.742	3923			
Treatments	397.614	52	7.646	3.964	.000
	7462.988	3869	1.929		
	7860.602	3921			

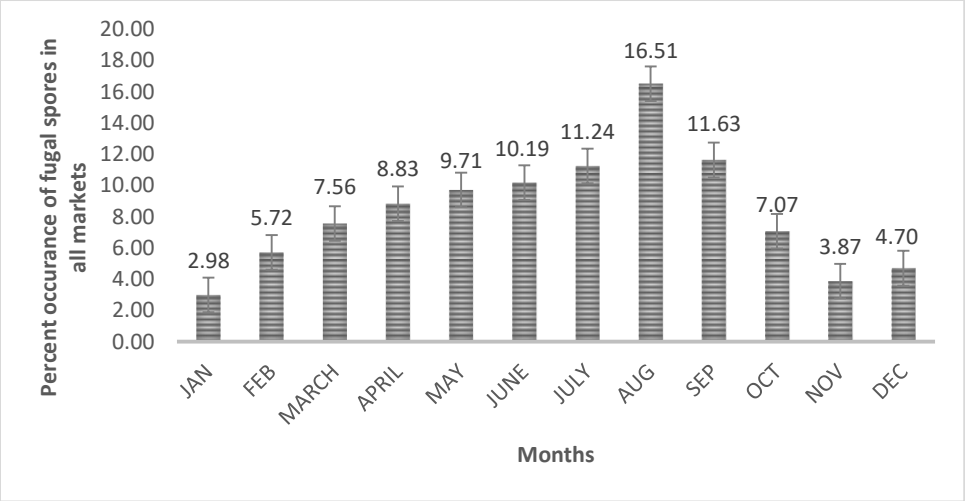

Fig 1. Monthly concentration of aeromycoflora during the period of January to December 2018.

The results indicate that in all five fruit and vegetable market, the Sabzi Mandi were found more contaminated with aeromycoflora. Sabzi Mandi which is the biggest fruit and vegetable wholesale market of Karachi. The terrain elevation of this market is above sea level is 65m (Latitude: 24°59'53.52" and Longitude: 67°9'22.69"). Twenty-eight species of fungi belonging to fifteen genera were reported from this site. The dominated fungi *were Alternaria alternata, Aspergillus niger, A. flavus, Botrytis cinerea, Curvularia lunata, Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer* (Table 2). Two fungi occupied highest mean value which was *Aspergillus niger* followed by *Rhizopus stolonifer*. Some fungi which only associated with Sabzi Mandi were *Alternaria solani, A. porri, Aspergillus orchareus, Curvularia lunata*, *Nigrospora* spp. and *Phoma beta*. In the month of August high contamination of fungi was found and least recorded in January. The results indicate that fungal contamination was gradually increased from the month of January till August, after August decline was observed till December (Fig. 2) in the fungal spores' pattern. The results also indicated that some fungi viz., *Alternaria alternata, Aspergillus candidus, A. flavus, A. niger, Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer* were present in all the months in all fruit and vegetable markets (Table 2). The maximum contribution of airborne mycoflora were found in Sabzi mandi (30.43%) followed by Saddar, Landhi, Nazimabad and Gulestan e johar (Fig. 4).The analysis of variance to compare all fruit and vegetable market throughout the year at 0.01 level showed significant difference

at all levels ($P \leq 0.001$) (Table 3). The study of airborne fungi become important to find out the status of numerous types of allergic and pathogenic spores at several spaces in the air and their role in causing health threats to humans as well as decay of fruit and vegetables.

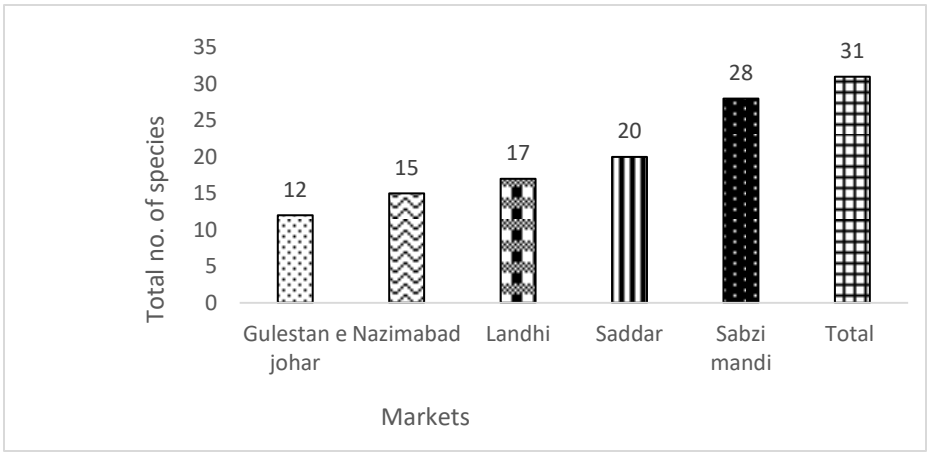

Fig 2. Airborne mycoflora isolated from all fruit and vegetable markets.

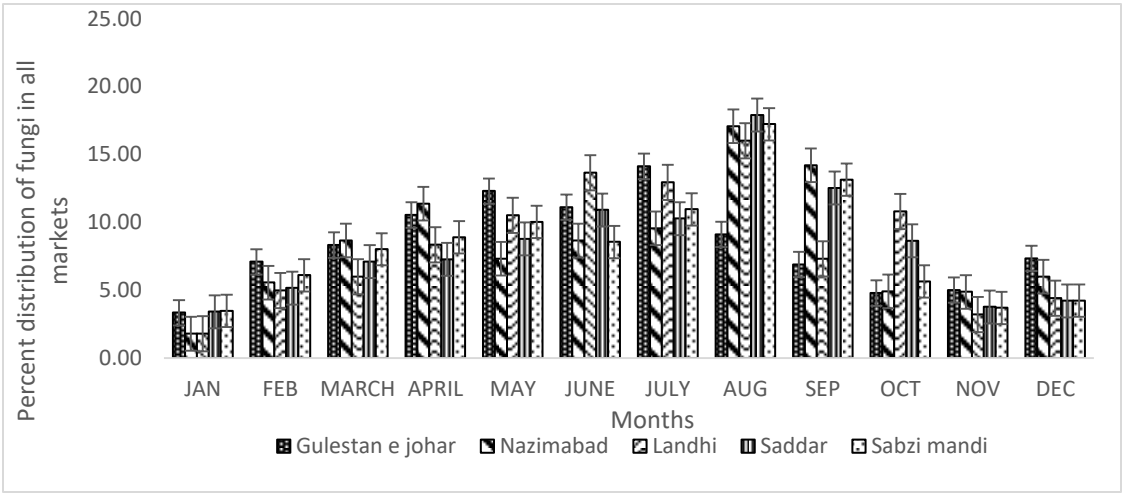

Fig 3. Percent distribution of fungi in all markets in the whole year 2018.

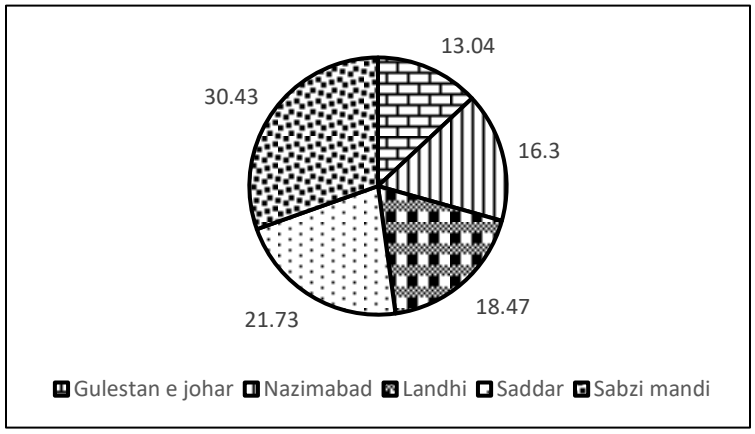

Fig 4. Total % contribution of fungi in all fruit and vegetable markets.

DISCUSSION

Knowledge of airborne fungi in fruit and vegetable market is essential in analyzing contamination and management of different allergic diseases. In this study total seventeen genera and thirty one species of airborne fungi were identified; however the concentration of these airborne fungi were different from market to market due to variations in weather pattern along with equal and uneven organic waste amount spreading at different places of fruit and vegetable markets. There were no big difference observed in metrological parameters such as temperature and humidity in all sites. The dominated aeromycoflora isolated were *Alternaria alternata, Aspergillus flavus, A. candidus, A. niger, Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer*. Our results also conformity with Toqeer *et al*. (2009) who isolated allergic microorganism viz., *Alternaria solani, Aspergillus candidus, A. terreus, A. wentii, A. flavus, A. fumigatus, A. niger, Drechslera dertioiclea*, *Curvularia clavata,* and *Penicillium notatum* at different site of Karachi. They also reported that the contamination of airborne fungi more in summer than in winter season in Karachi.

In our results it was found that species of *Aspergillus niger* was the most dominated fungi which got highest contamination position in the environment of fruit and vegetable markets of Karachi followed by *Rhizopus stolonifer* which are responsible for allergic diseases. Haque (2004) undertook aerobiological survey in Pakistan and studied that the conidia of *Aspergillus* were responsible for the prevalence of bronco-pulmonary aspergilloses. Some of the isolated fungi from Karachi reported to cause allergic diseases such as *Alternaria* spp., *Aspergillus* spp. and Fusarium spp. (Nasir and Jalaluddin, 2005). Shah and Bashir (2008) studied that *Aspergillus niger* with 24.08% was recorded as dominated airborne fungus at the place of Rohtas fort in Pakistan. According to Garg and Singh (2016) *Alternaria alternata* was dominated fungi which was present all the months and produce a number of mycotoxins. Bavaji *et al*. (2012) studied the aeromycoflora and reported *Aspergillus* as dominant species in India. In our research it was observed that incidence of airborne fungi is closely related with climatic condition of Karachi as high contamination was noticed in the month of August. It was observed that fungal contamination was gradually increased from the month of January and highest peak was noticed in the month of August 2018. It was due to moderate temperature and high relative humidity which favor the growth of fungi in the month of August and September. These environmental factors may have significantly contributor in the summer season at Karachi to abundant mycelial growth of airborne fungi. The reason in low fungal counts in the winter months could have been due to low humidity, which is unfavorable to fungal growth. Humidity was high throughout the year, with most humid months in the Karachi city from July to September, this is the reason between occurrence of aeromycoflora and fruit and vegetable diseases at market environment of Karachi. Our results was in accordance with the observation made by Pande *et al.* (2012) who found that airborne fungi were copious during summer and monsoon season while in January month they occurred with low concentration. He also reported fifty-one fungal species were *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp. and *Curvularia* spp. These fungal spores were settled down on the surface of fruits and vegetables which cause rot and reduced the economic value. In addition, various workers considered them responsible for causing major diseases in different fruit and vegetable markets worldwide. (Afzal *et al*., 2004; Ravesha, 2015; Sri indrasutdhi, 2015; Shafa and Fatema, 2015). Ahire and Sangle (2012) recorded seventeen species of airborne mycoflora during the one year in India such as *Cladosporium* spp., *Alternaria* spp., *Curvularia* spp., *Aspergillus* spp. and *Penicillium*. He also studied that atmospheric condition such as temperature and humidity which influence the growth of fungal spores. According to Singh *et al*. (2014) most prevalent fungal spores typed in the air were *Alternaria* spp., *Aspergillus* spp., *Curvularia* spp., *Rhizopus* spp. and *Fusarium* spp.

In our research, spore types like *Alternaria, Aspergillus, Rhizopus, Fusarium* etc. were trapped all fruit and vegetable markets of Karachi city which known as allergen during the whole year and exposure to verity of such aeromycoflora may result in serious asthma, respiratory infection and chronic obstructive pulmonary diseases. *Aspergillus* spp. is the agent of aspergilloses that has ability to invading the arteries of the lung and brain (Marcoux *et al.,* 2009; Baxter *et al*., 2011). *Rhizopus* spp. may cause rhino cerebral, thoracic and gastro internal problem while ergot alkaloid is also produced which is toxic to human (Mulac *et al*., 2011). Further species of *Penicillium* may damage the lung (Rick *et al*., 2016).It is observed that in all fruit and vegetable markets, Sabzi Mandi is one of the main wholesale market of Karachi city was found heavily contaminated by air borne microorganisms through the year such as *Alternaria alternata, Aspergillus niger, A. flavus, Botrytis cinerea, Curvularia lunata, Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer*. Garg and Singh (2016) isolated *Alternaria alternata* which produce a number of mycotoxins from Sabzi Mandi in India. In our research it is also noticed that the predominance of such airborne mycoflora in all fruit and vegetable markets is due to variation in weather pattern such as moderate temperature and high humidity along with poor sanitation condition of fruit and vegetable markets. Results showed that in the month of August and September 2018 high contamination was observed due to average moderate temperature (29 ºC) and high humidity (70%) which was suitable for fungal sporulation whereas in the

winter season low humidity was noticed such as in the months of December (33%) and January (50%) which was the reason for decreasing in fungal contamination in all fruit and vegetable markets. The lowest fungal count observed in the market of Gulistan-e-Johar due to good sanitation condition compare to other fruit and vegetable markets of Karachi while higher fungal contamination was recorded in Sabzi Mandi due to rotten fruits and others organic matter were scattered on ground caused bad odor. There were people loading up vegetables everywhere on racks attached to motorcycles and wheelbarrows as well after winning their bids. The high concentration of fungi in fruit and vegetable market indicate the unhygienic condition in the markets. According to Kakde and Kakde (2012) various things were present in fruit and vegetable markets such as rotten fruits and vegetables, un wanted paper bag and packaging material etc. as a substrate for dispersing the saprophytic and pathogenic spores of fungi in the atmosphere .Studies on atmospheric fungal spores concentration over the fruit and vegetable market certainly would help to understand the dissemination of post-harvest pathogens prevailing in the air and evolve a forecasting system for storage diseases of fresh fruit and vegetables. Achieved data would serve as a base in formatting diseases forecasting system for the effective control of fruit and vegetable damage in the market of Karachi. This kind of information could be useful to allergologists and aero biologists.

The spore of *Aspergillus niger* and *Rhizopus stolonifer* were the largest contributor of the total airborne fungal spores as well as *Aspergillus, Alternaria, Rhizopus, Penicillium* and *Fusarium* species were also the common component of aeromycoflora at fruit and vegetable markets of Karachi during the study period.

Conclusion

The investigations showed that the variety of air-borne fungi contaminated the survey sites in the fruit and vegetable markets. *Aspergillus niger* and *Rhizopus stolonifer* were the most prevalent fungal species. Besides, *Alternaria, Botrytis, Cladosporium, Mucor* and *Fusarium* species were also associated with post-harvest fruit and decaying vegetable.

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