

Aeromycoflora of fruit markets of Baroda, India and associated diseases of certain fruits

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Abstract Fungal organisms cause diseases in plants, animals, and human beings. They are responsible for deterioration of organic objects. Scientists have found that it is not the occurrence but quantum of inoculum or number of spores per unit area which has a direct effect on development of diseases in fields as well as in markets. An aerobiological survey of three fruit markets of Vadodara, India was undertaken during 2006 to determine various fungal pathogens present in the atmosphere using the petriplate exposure method. Isolations were also done from six fruits available during summer and rainy seasons. The surface washing technique yielded 12 micro fungi from *Prunus avium*. Direct correlation was observed between percentage rot of fruits and occurrence of fungal spores in different months.

Keywords Aeromycoflora · Cherry · Fruit rots · Fruit and vegetable markets · India

1 Introduction

Bio-pollutants like fungi, yeast, and bacteria may cause diseases in agriculture fields, orchards, or in

vegetable markets. They may attack during harvesting, packaging, or during transport to the fruit markets. Fungi constitute a major share of bio-pollutants as well as contaminants present in our atmosphere. They usually reproduce by sexual and asexual means, and their spores as well as conidia form components of aerospora; their presence in the environment may pose a threat to plants, animals, and human beings. Gregory (1973) recorded spread of fungi and bacteria by the action of wind and rain splash. In tropical and sub tropical climates diseased plants can act as sources of biopollution.

Of all the losses caused by plant diseases, those which occur after harvest are most costly whether measured in monetary terms or in man hours. World food shortage demands for reducing postharvest losses and developing improved systems for transporting and distribution of produce. International organizations responsible for monitoring food resources have recognized that the most economically feasible and expedient means to increase the world food supply is to reduce losses in food crops that occur after they have been harvested (Kelman 1984). Postharvest losses in fruits and vegetables are greater because of their high moisture content.

Postharvest losses of perishable crops in developing countries range from 5–50% or even more (Salunkhe and Desai 1984). A major reason for this has been the lack of awareness at all stages from fruit production to fruit consumption. Harvested fruits and vegetables become vulnerable to attack by

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microorganisms because of the following: (1) high moisture content; (2) high sugar and nutrients; (3) harvesting, packing and transportation facilitate entry of certain pathogens; and (4) during storage.

Fungi have been identified as a major cause of biodeterioration of all kinds of stored products throughout the world leading to both qualitative and quantitative losses. Eckert (1978) has enumerated the following less evident consequences of postharvest deterioration of perishables.

1. Partial or total loss of consumer packages with only one or several diseased units
2. Reduced postharvest life of the product due to accelerated ripening
3. Possible contamination of the edible product with mycotoxins
4. Softening of processed fruits by heat tolerant pectolytic enzymes of postharvest pathogens, e.g. *Rhizopus* sp. and *Sclerotinia* (Strand et al. 1981)

Propagules of the pathogenic fungi causing post-harvest fruit rot are found abundantly in the atmosphere (Meredith 1961) and even on the surface of fruits as they mature in the orchards (Dennis 1983). Thus postharvest diseases may be influenced by the location of orchards and the husbandry of the fruit trees. Some literature is available on the surface microflora of freshly harvested fruits and their possible role in spoilage and marketing (Singh and Kainsa 1983; Gupta et al. 1983a, 1983b; Kalafatolgu and Karapinar 1989). Singh and Sumbali (1997, 1998) reported post harvest fungal diseases of Indian jujube. Arya (2004) reported different fruit rots in Baroda markets.

Quantification of air borne biological particles forms the basis of aerobiological research related to agriculture, horticulture, forestry, and air borne

shall have to go molecular way to understand not only the taxa, genus, species but also virulence, avirulence and frequency of type of pathogen if at all, this knowledge is to be used for management.”

2 Materials and methods

Baroda is situated in the western part of the country on the Delhi–Mumbai train route. It is an important city in Gujarat, India. The place is situated 72°45′–74°30′ E and 21°45′–23°0′ N with an altitude of 35 m. Three markets were selected for study. Khanderao market, situated in the heart of city, is a wholesale market of fruits and vegetables. Kadak bazaar is another market situated near the railway station. Iskon temple market is the third market, situated on the northern side of the city, and is a comparatively small market.

2.1 Isolation of aeromycoflora from different fruit markets

Isolation of aeromycoflora was done at 8:30 am on the 1st and 15th of every month; sampling was done from the 15th of May to the 1st of August 2006. Five petriplates with PDA medium were taken to the fruit market and exposed at a height of 1 m. The petriplates were exposed at different places in the markets for 10 min. After exposure, these were brought to the laboratory and kept at $25 \pm 2^\circ\text{C}$ for 7 days. Number and type of the individual colonies were counted and the fungi were identified after subculturing them.

Percentage abundance of each fungal isolate was calculated as follows:

$$\text{Percentage of abundance} = \frac{\text{Total number of colonies of a particular fungal species in all the replicates}}{\text{Total number of colonies of all the replicates}} \times 100$$

pathogens of plants, animal, and humans. Fruits and vegetables are important constituents of our diet. Postharvest spoilage may further reduce their availability to the common man at economical rates. According to Mayee (2005), “Aeroflora diagnostics

To observe the effect of different time of a day the petridishes were exposed at three different times in a day, and data were recorded on the 2nd of November and the 20th of December 2006. Five petri dishes were exposed at 1 m height each time.

2.2 Isolation of surface mycoflora

For isolation of the surface mycoflora associated with red cherry fruits, samples were subjected to the surface washing technique. The experiment was performed on June 1, 2006. In this method, 25 g of whole fruit were taken in an Erlenmeyer flask (500 ml) containing sterilized distilled water and given horizontal shaking for 30 min. The liquid was then centrifuged at 3,000 rpm for 20 min, and the residue thus obtained was poured into sterilized petriplates at the rate of 1 mL/plate. PDA medium was modified by adding Streptomycin sulphate (0.06 g/L), and five replicates were maintained. The experiment was repeated three times. The inoculated petriplates were incubated at $25 \pm 2^\circ\text{C}$ for 7 days, and the resulting fungal colonies were subcultured on PDA slants and identified.

2.3 Isolation of fungi from diseased fruits

Fifty fruits of six varieties of mango, papaya, cherry, sapota, plum, and banana were collected from three markets. Percentage rot was calculated on the basis of visual symptoms of whole fruit and cut fruits. Samples of two different varieties of mango, i.e. Alphonso and Totapuri, and two varieties of banana, i.e. Sona and G9, as well as Chhatti variety of *Achras sapota* L. were collected from three different markets. These were collected from the fruit markets in clean polyethylene bags and brought to the laboratory. The experiment was performed during May and August 2006. The organisms responsible for different fruit rot were isolated, purified by subculturing, and pure cultures were maintained on PDA slants. Fungal isolates were identified on the basis of their macro and micro-morphological characters, using the relevant literature and keys.

3 Results and discussion

3.1 Fungal spores in fruit markets

A large proportion of aerobiological studies are devoted to study air borne spores in the markets of different cities and towns by employing viable plate counts or gravity slides, and a few have used air

samplers. Irrespective of place, duration, and technique of sampling the predominance of deuteromycetes fungi in air spore has been confirmed by all the investigators with *Cladosporium* most predominant at all locations. The total number of spore types identified varied from 35–65 (Vittal 2005).

Percentage abundance of species comprising the mycoflora is depicted in Table 1. *Aspergillus niger* van Tiegh, *A. flavus* Link, *Rhizopus stolonifer* (Ehrenb. Fr.) Lind, and *Fusarium solani* (Mart) Sacc. were the most prevalent components of the aeromycoflora. Other frequently isolated species were *Alternaria alternata* (Fr.) Keissler, *Cladosporium oxysporum* Berk., *Curvularia lunata* (Wakker) Boedijn, *Penicillium expansum* Thom. Of these fungi, *Alternaria alternata*, *A. niger*, and *P. expansum* were found responsible for spoilage of cherry during the postharvest period, thus indicating that propagules of these fungi possess the potential to cause rot of mature fruits. Fungi which were absent from the environment during the period July 1 through August 1, 2006 included *Aspergillus ochraceous*, *Chaetomium globosum*, *Drechslera* sp., *Emericella nidulans*, and *Monascus ruber*. Spores of *Myrothecium roridum*, *P. expansum*, *Thielaviopsis paradoxa*, and *Trichoderma viride* were absent on July 15th and August 1st. May and June were hot summer months with maximum temperature reaching 42°C , and July and August were rainy months.

Occurrence of aeromycoflora in Kadak bazaar was recorded at three different times in a day (Table 2). It was found that the fungal spores were maximum during the morning followed by evening and the least around noon (1:00 pm). The lesser market activities were recorded during mid day, which may initiate poor dispersal of fungal propagules.

3.2 Effect of environmental conditions on occurrence of fungal spores

Abundance of fungal spores in the markets may be due to unhygienic conditions, which prevail in the market area. Rotten fruits, vegetables, decaying paper bags from take-away food, and packing materials in the market are again substrates for fungal growth and become the chief source of dissemination of spores in the air. Furthermore, a semi enclosed environment of the crowd and nearness of the sampling sites to the

Table 1 Percentage abundance of fungal organisms present in aeromycoflora of fruit markets of Baroda from May–August 2006

Fungi isolated	Percentage abundance of fungi					
	15th May	1st June	15 June	1st July	15 July	1st August
<i>Alternaria alternata</i>	2.3	2.0	2.1	1.5	1.5	2.5
<i>Aspergillus niger</i>	7.8	7.0	8.9	12.0	15.5	10
<i>A. flavus</i>	5.7	7.5	9.2	10.0	8.5	8.5
<i>A. ochraceus</i>	1.6	1.6	–	–	–	–
<i>A. tamarii</i>	1.5	–	1.2	–	1.0	2.5
<i>Chaetomium globosum</i>	0.16	0.5	0.5	–	–	–
<i>Cladosporium oxysporum</i>	6.8	6.0	7.2	2.0	5.0	–
<i>Colletotrichum gloeosporioides</i>	1.4	2.0	1.8	2.0	2.5	5.0
<i>C. capsici</i>	5.4	6.6	6.6	6.0	5.5	2.5
<i>Curvularia lunata</i>	5.9	6.0	6.4	5.9	5.0	2.5
<i>Drechslera sp.</i>	8.7	–	7.3	–	–	–
<i>Emericella nidulans</i>	3.5	2.0	2.6	–	–	–
<i>Fusarium moniliforme</i>	6.9	5.0	7.2	2.2	2.2	–
<i>F. oxysporum</i>	5.3	5.0	2.6	2.0	5.0	–
<i>F. solani</i>	6.5	2.5	5.4	6.5	6.0	2.5
<i>Lasiodiplodia theobromae</i>	1.6	2.5	1.3	1.5	1.5	2.5
<i>Monascus ruber</i>	–	0.5	0.5	–	–	–
<i>Mucor sp.</i>	8.6	8.5	5.2	2.0	2.0	2.5
<i>Myrothecium roridum</i>	0.4	1.5	0.5	1.5	–	–
<i>Penicillium citrinum</i>	1.3	1.5	1.0	2.5	1.0	2.5
<i>P. expansum</i>	–	–	1.3	1.5	–	–
<i>P. oxalicum</i>	–	–	1.2	1.5	2.0	–
<i>Phoma herbarum</i>	0.2	–	–	2.0	2.0	–
<i>Rhizopus stolonifer</i>	9.7	8.0	8.4	10.0	10.0	10.0
<i>Syncephalastrum racemosum</i>	–	–	–	1.5	2.0	1.5
<i>Thielaviopsis paradoxa</i>	–	–	1.5	0.5	–	–
<i>Trichoderma oryzae</i>	–	–	2.0	2.0	1.0	–
<i>T. viride</i>	–	1.5	1.4	1.4	–	–
Unidentified	2.3	5.0	5.0	2.5	5.0	2.5

Data based on five petridishes (100 mm dia) exposed for 10 min

source of contamination may have accounted for the higher spore concentrations. In most cases, maximum colonies were encountered during the rainy season (July–August). This was probably due to the congenial climate, including high rainfall with corresponding increased humidity and moderate temperature, which may have contributed to the growth and sporulation of these microorganisms. In a real sense, a micro climate can exist between fruit and packing boxes with no air circulation, because heat and humidity encourage pathogens to flourish and disperse when fruits are taken out for sale. In this study, the numbers of fungal species were 21 on May

15th, 22 on July 1st, and 25 on June 15, 2006. The percentage abundance of fungal species was more on the 1st and 15th of July, which may be explained on the basis of increased humidity in the environment.

3.3 Fungi associated with cherry fruit surface

A plethora of fungal species were isolated from the fruit surface of cherry. Members of the genus *Aspergillus* were predominant followed by *Penicillium* and *Fusarium* species. Other genera isolated by the fruit washing technique as components of the

Table 2 Study of aeromycoflora in Kadak bazaar at different times during the day

Sampling day	November 2, 2006		December 20, 2006	
Time	Fungi isolated	Number of colonies	Fungi isolated	Number of colonies
Morning	8:30 am	29	8:30 am	31
	<i>Aspergillus awamori</i>	1	<i>Penicillium</i> sp.	2
	<i>Aspergillus niger</i>	10	<i>A. niger</i>	12
	<i>Aspergillus flavus</i>	4	<i>A. flavus</i>	6
	<i>Aspergillus fumigatus</i>	6	<i>A. fumigatus</i>	–
	<i>Cladosporium</i> sp.	2	<i>Cladosporium</i> sp.	3
	<i>Mucor hiemalis</i>	5	<i>M. hiemalis</i>	7
	<i>Fusarium oxysporum</i>	1	<i>Fusarium</i> sp.	1
Noon	1:00 pm	24	1:00 pm	22
	<i>Aspergillus flavus</i>	2	<i>A. flavus</i>	6
	<i>Aspergillus fumigatus</i>	5	–	–
	<i>Aspergillus niger</i>	11	<i>A. niger</i>	10
	<i>Cladosporium</i> sp.	2	<i>Cladosporium</i> sp.	2
	<i>Mucor hiemalis</i>	4	<i>M. hiemalis</i>	3
			<i>Fusarium</i> sp.	1
Evening	6:00 pm	26	6:00 pm	26
	<i>Aspergillus flavus</i>	2	<i>A. flavus</i>	3
	<i>Aspergillus niger</i>	14	<i>A. niger</i>	11
	<i>Cladosporium</i> sp.	2	<i>Cladosporium</i> sp.	2
	<i>Mucor hiemalis</i>	8	<i>M. hiemalis</i>	4
			<i>Fusarium</i> sp.	2
			<i>Penicillium</i> sp.	1
			<i>Rhizopus</i> sp.	3

Data based on average of five petri dishes exposed for 10 min

mycoflora included, *Cladosporium oxysporum*, *Myrothecium roridum*, *Nigrospora oryzae*, *Phoma herbarum*, and *Rhizopus stolonifer*. A total of 12 different fungi were isolated. A greater number of colonies encountered included those of *A. niger*, *C. oxysporum*, and *R. stolonifer*. The data are recorded in Table 3.

Fungal spores may settle on fruit surfaces by impaction or sedimentation under the influence of gravity, and during rain by splash droplets. The presence of saprophytic mycoflora may influence the pathogenicity of an organism by an antagonistic effect. The nutritional status of particular fruit and available moisture has direct correlation with occurrence of disease. Postharvest losses can be reduced by monitoring the aeromycoflora in different markets, and by providing the conditions so that pathogenic fungi can not come in direct contact with fruits.

Table 3 Fungi isolated from fruit washing of cherry (*Prunus avium*) on June 1, 2006

Fungi associated with fruit surface	Number ^a
<i>Alternaria alternata</i>	2
<i>Aspergillus niger</i>	5
<i>A. flavus</i>	1
<i>A. tamarii</i>	2
<i>Cladosporium oxysporum</i>	5
<i>Fusarium moniliforme</i>	1
<i>F. oxysporum</i>	1
<i>Myrothecium roridum</i>	1
<i>Nigrospora oryzae</i>	1
<i>Penicillium expansum</i>	2
<i>Phoma herbarum</i>	1
<i>Rhizopus stolonifer</i>	5

^a Colonies recovered by washing 25 g fruit in sterile distilled water

Table 4 Percentage fruit rot recorded from May–August 2006

	Fruit	Percentage fruit rot					
		15 May	1st June	15 June	1st July	15 July	1st August
Percentage rot calculated on the basis of 50 fruits collected from three different markets in Baroda in each case	<i>Mangifera indica</i> L.	2.6	10	15	15	7	2
	<i>Carica papaya</i> L.	2	2	2.6	10	15	10
	<i>Prunus avium</i> L.	7	7	2.6	5	5	5
	<i>Achras sapota</i> L.	5	5	2	—	—	—
	<i>Prunus domestica</i> L.	—	5	5	2	5	—
	<i>Musa paradisiaca</i> L.	5	7	7	10	15	10

Wattal et al. (2003) reported consumer losses due to postharvest diseases of cherry. Isolation of *Cercospora cercumcsissa*, *Wilsionomyces carpophyllum*, *Penicillium expansum*, and *Tricothecium roseum* was recorded from cherry fruits for the first time. The studies further revealed that, except for *C. cercumcsissa*, all species were pathogenic. Earlier studies have shown *Alternaria alternata*, *A. dendritica*, *F. semitectum*, and *Geotrichum candida* to be associated with cherry fruits.

3.4 Postharvest diseases of certain fruits

Percentage of fruit rot of six different fruits was calculated every fortnight and data are presented in Table 4. It is evident from the table that percentage rot was more in *Musa paradisiaca* and *Carica papaya* followed by *Mangifera indica* and *Prunus avium*. The losses were less in sapota and plum. Banana showed infection due to *Gloeosporium musarum*, *Aspergillus niger*, *Lasiodiplodia theobromae* and *Thielaviopsis paradoxa*. *Fusarium*, *Colletotrichum*, and *Aspergillus* rots were common on papaya, while mango suffered most due to *Aspergillus*, *Colletotrichum* and *Rhizopus* rots. Sapota had infection due to *Aspergillus*, *Pestalotiopsis*, and *Phomopsis* sp. *Prunus domestica* had infection due to *Aspergillus*, *Penicillium*, and *Colletotrichum* sp. *P. avium* showed infection due to species of *Aspergillus*, *Penicillium*, and *Myrothecium roridum*. Presence of most of these fungi was recorded during sampling from three markets of Baroda.

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