International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Prevalence of post-harvest rot of fruits and vegetables by *Penicillium* species

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Abstract

Post-harvest diseases caused by *Penicillium* species develops on fruits and other plant products between harvesting and consumption. The threat of post-harvest disease influences the way most horticultural crops are handled. Therefore the accurate identification of the causal pathogen is essential before appropriate treatment can be made to control the pathogens. In the present study a number of (24) *Penicillium* species viz., *Penicillium aurantiogriseum, P. bilaii*, *P. chrysogenum*, *P. claviforme, P. crustosum, P. digitatum, P. echinulatum, P. expansum, P. fennelliae, P. granulatum, P. implicatum, P. islandicum, P. italicum, P. janthinellum, P. melinii, P. miczynskii, P. olsonii, P. oxalicum, P. paxilli, P. rubrum , P. simplicissimum, P. spinulosum, P. verrucosum var. cyclopium* and *P. viridicatum* are responsible for postharvest deterioration of fresh fruits and vegetables were isolated and identified. It has been also revealed that following fourteen species of *Penicillium* are new records from Pakistan; *Penicillium aurantiogriseum, P. bilaii*, *P. claviforme, P. crustosum, P. echinulatum, P. fennelliae*, *P. granulatum , P. melinii*, *P. miczynskii, P. olsonii, P. paxilli, P. simplicissimum, P. verrucosum var. cyclopium* and *P. viridicatum.* Although previous records of *Penicillium chrysogenum, P. digitatum, P. expansum, P. implicatum, P. islandicum, P. italicum, P. janthinellum, P. oxalicum, P. rubrum* and *P. spinulosum* exist from Pakistan, these are new isolates from fruits and vegetables.

Keywords: Fruits, Vegetables, *Penicillium*

Introduction

Pakistan agro-climatic environment ranging from tropical to temperate allow growing 40 different kinds of vegetables and 21 types of fruits (Raja, M.B. and K.M. Khokhar, 1993). Major vegetables grown in Pakistan include potato, onion, chilli, melons, cucurbits, tomato, turnip, okra and pea, whereas citrus, dates, mango, guava, apple, banana, apricot, grapes, almonds, peach, plum and pomegranate are the major fruit crops. In developing countries Post-harvest diseases destroy 10-30% of the total yield of crops; they destroy more than 30% of the crop yield in some perishable crops (Agrios, 2005; Kader, 2002). The quality of fruits and vegetables is influenced by post-harvest handling, transportation, storage and marketing because they are highly fragile products. Decay and production of microorganisms, which become activated because of the changing physiological state of the fruits and vegetables, may result at some stage in improper handling, packaging, storage and transportation (Wilson *et al*., 1991). Low pH, higher moisture content and nutrient composition make fruits very susceptible to attack by pathogenic fungi. In addition to causing rots, mycotoxins production may also make them unhealthy for consumption (Philips, 1984; Moss, 2002; Stinson *et al.* 1981). The genus *Penicillium* is very important among the known fungal biota. The genus *Penicillium* contains approximately 200 species of an impressive diversity and they are consistently found all over the world. *Penicillium* is ubiquitous and cosmopolitan. It is also considered an Opportunistic fungus. Some species are acidophilic, alkalinophilic, psychrophilic, thermophilic and xerophilic (Andersen and Frisvad, 2002).

Penicillium is the major cause of degradation of agricultural products during pre harvesting and post harvesting stages, thus *Penicillium* cause substantial economic losses due to spoilage. *Penicillium* also cause Blue-eye mold in corn (Albert, 2003).Three *Penicillium* species, namely *P. crustosum, P.expansum* and *P. solitum,* are known to be destructive pathogens on pome fruits. *Penicillium glabrum* is a filamentous fungus frequently involved in food contamination (Nevarez *et al*. 2008). *Penicillium expansum, P. carneum, P paneum* and *P. griseofulvum* recovered from the fruits of ripped service tree (*Sorbus domestica* L.)(Roman et al., 2005). *Penicillium expansum* is the most important cause of blue mould rot, a major post-harvest disease of apples worldwide (Katleen et al., 2008).

Penicillium species are commonly found as contaminants in foods while drying and subsequent storage . Thus, accurate identification of *Penicillium* at the species level is essential. *Penicillium* is not easy to identify to the species level. To further complicate things, the taxonomy of genera still needs work. Although molecular, biochemical and physiological methods are important for systematic of *Penicillium* species, morphological properties are used common for identification.

Correct species identification will indicate the mycotoxins which may be present in given samples under given environmental conditions and indicate ways to prevent mycotoxin production.

The present work describes the role of *Penicillium* species in postharvest rot of fresh fruits and vegetables from Lahore, Pakistan. The purpose of this list is to record the *Penicillium* species isolated from fruits of Lahore, PAKISTAN. The database will make the Pakistani literature on the subject available to an international audience. It will also give future researchers information on whether a species is a new record for Pakistan.

Materials and Methods

Sample collection

Fresh fruits like, apple (*Malus domestics* Mill.), grapes (*Vitis vinifera* L.), lemon (*Citrus limon* (L.) Burman.f.), sweet orange (*Citrus sinensis* Osbeck), pomegranate (*Punica granatum*) and vegetables like Onion (*Allium cepa*), Ginger (*Gingiber officinale)* and Garlic (*Allium sativum*) showing the deterioration and rotting were collected from different markets/ shops of Lahore like Lahore University Campus, Gulshan-e-Iqbal, Gulistan-e-Jouhar, MalirLiaquat Market, Liaquatabad, Gulbahar and Empress Market Saddar. Samples were kept at 4°C until the identification and isolation were made within 48 hours.

Isolation of fungi from diseased tissues

A bit healthy tissue adjacent to diseased tissue was cut with the help of a sharp razor and transferred onto malt extract agar (MEA) plates containing penicillin (100000 units/L) and streptomycin (0.2 g/L) after surface sterilization with 1% $Ca(OCl)_2$. Plates were incubated for 5 days at 28°C under 12 hours light and dark conditions. Fungi that grew were identified.

Identification of fungi and long-term preservation

The isolated fungi were identified to the genus and species level on the basis of macro (Colony size, colony shape, colony color, colony reverse, texture and exudates) and micro (Conidiophores, metulae, phialides and conidia) morphological characteristics using the most updated keys for identifications (Pitt 1979; Domsch *et al*., 1980). Identified *Penicillium* species were preserved by direct lyophilization of the fungal mat grown in 2% Malt Extract (ME) broth for 7-10 days. Each fungal mat was washed three times with distilled sterilize water and dried in paper towel. Fungal mat was transferred in sterile falcon tubes and tubes were covered with nylon mesh (200µm). Falcon tubes were placed in the vacuum bottles and the samples were lyophilized in a freeze dryer (TFD5505, Ilshin, Korea) under vacuum at ≤ 50 °C for 6-8 hours. Samples were removed from vacuum bottles when pressure reached at 25 bars and lyophilized samples were kept at 4 ^{o}C and room temperature.

Results

Penicillium species isolated were identified as *Penicillium aurantiogriseum (Allium cepa, Gingiber officinale* and *Allium sativum), P. bilaii* (*Punica granatum*), *P. chrysogenum* (*Allium cepa* and *Citrus limonia*), *P. claviforme* (*Citrus limonia), P. crustosum (Citrus limonia* and *Lycopersicon esculentum), P. digitatum* (*Citrus limonia), P. echinulatum (Lycopersicon esculentum), P. expansum* (*Allium*

sativum), *P. fennelliae* (*Citrus limonia), P. granulatum* (*Malus domestica*), *P. implicatum* (*Punica granatum* and *Allium cepa*), *P. islandicum (Citrus lemon), P. italicum* (*Allium sativum* and *Citrus lemon*), *P. janthinellum* (*Citrus lemon), P. melinii* (*Allium cepa*), *P. miczynskii* (*Citrus limonia*), *P. olsonii* (*Allium cepa*), *P. oxalicum (Lycopersicon esculentum), P. paxilli (Citrus limonia) P. rubrum* (*Punica granatum*), *P. simplicissimum* (*Allium cepa, Vitis vinifera and Gingiber officinale*), *P. spinulosum* (*Punica granatum*), *P. verrucosum var. cyclopium* (*Citrus lemon*) and *P. viridicatum (Allium sativum, Malus domestica* and *Citrus limonia)* (Table1).

Table: 1. *Penicillium* species isolated from deteriorated fruits and vegetables collected from different market of Lahore.

S. No.	Host		*Penicillium* species
	Scientific name	Common name	
1.	*Allium cepa*	Onion	*P. aurantiogriseum, P. chrysogenum, P. implicatum, P. melinii, P. olsonii, P. simplicissimum*
2.	*Allium sativum*	Garlic	*P. aurantiogriseum, P. expansum, P. italicum, P. viridicatum*
3.	*Citrus limon* L.	Lemon	*P. islandicum, P. italicum, P. janthinellum, P. verrucosum var. cyclopium*
4.	*Citrus limonia*	Orange	*P. chrysogenum, P. claviforme, P. crustosum, P. fennelliae, P. miczynskii, P. paxilli, P. viridicatum, P. italicum, P. digitatum*
5.	*Gingiber officinale*	Ginger	*P. aurantiogriseum, P. simplicissimum*
6.	*Malus domestica*	Apple	*P. granulatum, P. viridicatum, P. expansum*
7.	*Lycopersicon esculentum* Mill.	Tomato	*P. crustosum, P. echinulatum, P. oxalicum*
8.	*Punica granatum* L.	Pomegranate	*P. bilaii, P. implicatum, P. rubrum, P. spinulosum*
9.	*Vitis vinifera* L.	Grapes	*P. simplicissimum*

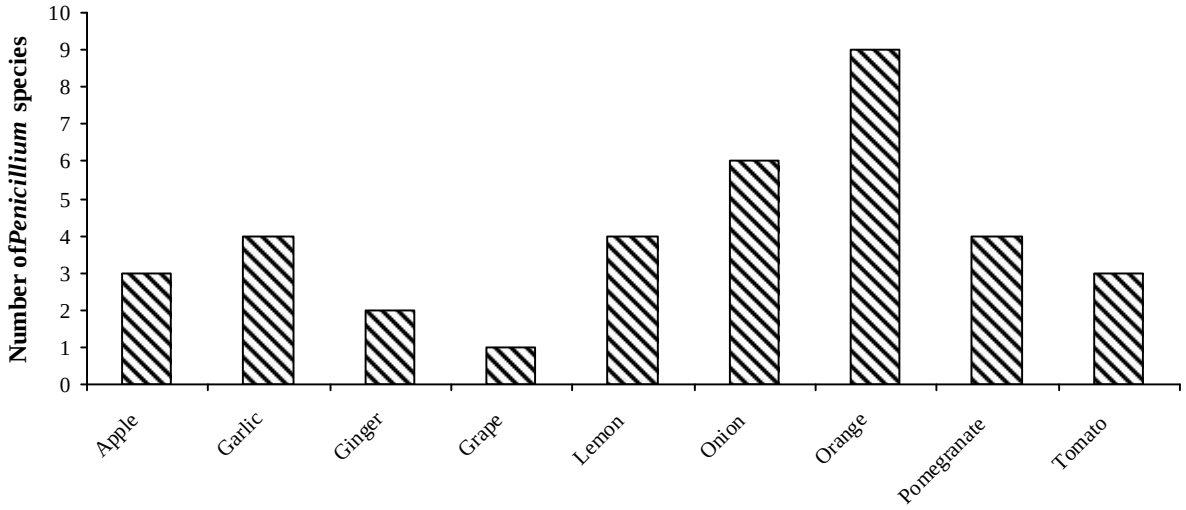
It has also been observed that *P. aurantiogriseum, P. italicum, P. simplicissimum* and *P. viridicatum* found to be predominant species with 8.57 %age of incidence, while *P. chrysogenum, P. expansum* and *P. implicatum* showed 5.71%. *P. bilaii, P. claviforme, P. crustosum, P. digitatum, P. echinulatum, P. fennelliae, P. granulatum, P. islandicum, P. janthinellum, P. melinii, P. miczynskii, P. olsonii, P. oxalicum, P. paxilli, P. rubrum, P. spinulosum*, and *P. verrucosum var. cyclopium* were isolated with incidence of 2.86% only from the samples (Table2).

Table 2. Occurrence (%) of *Penicillium* species from fruits and vegetables.

Penicillium species	Occurrence (%)	*Penicillium* species	Occurrence (%)
P. aurantiogriseum	8.57	*P. italicum*	8.57
P. bilaii	2.86	*P. janthinellum*	2.86
P. chrysogenum	5.71	*P. melinii*	2.86
P. claviforme	2.86	*P. miczynskii*	2.86
P. crustosum	2.86	*P. olsonii*	2.86
P. digitatum	2.86	*P. oxalicum*	2.86
P. echinulatum	2.86	*P. paxilli*	2.86
P. expansum	5.71	*P. rubrum*	2.86
P. fennelliae	2.86	*P. simplicissimum*	8.57
P. granulatum	2.86	*P. spinulosum*	2.86
P. implicatum	5.71	*P. verrucosum var. cyclopium*	2.86
P. islandicum	2.86	*P. viridicatum*	8.57

It has been noted that *Penicillium* species on orange, onion, garlic, lemon and pomegranate showed highest % age of incidence i.e. 25.72, 17.14, 11.43, 11.43 and 11.43, respectively. While on apple, tomato, ginger and garlic is 8.57, 8.57, 5.71 and 2.86, respectively. (Figure1)

Figure 1: % age incidence of *Penicillium* species on Fruits and vegetables.



Following fourteen species of *Penicillium* are new records from Pakistan; *Penicillium aurantiogriseum, P. bilaii, P. claviforme, P. crustosum, P. echinulatum, P. fennelliae, P. granulatum , P. melinii, P. miczynskii, P. olsonii, P. paxilli, P. simplicissimum, P. verrucosum var. cyclopium* and *P. viridicatum.* Although previous records of *Penicillium chrysogenum, P. digitatum, P. expansum, P. implicatum, P. islandicum, P. italicum, P. janthinellum, P. oxalicum , P. rubrum* and *P. spinulosum* exist from Pakistan, these are new isolates from fruits and vegetables.

Discussion

In this study, *Penicillium* species have been found involved in post-harvest rot of some vegetables and fruits viz., Apple (*Malus domestica*), Garlic (*Allium sativum*), Ginger (*Gingiber officinale*), Grapes (*Vitis vinifera* L.), Lemon (*Citrus limon* L.), Onion (*Allium cepa*), Orange (*Citrus limonia*), Pomegranate (*Punica granatum* L.) , Tomato (*Lycopersicon esculentum* Mill.). This result is similar with the observation of Asan (2004) who found *Penicillium* species from Apple, apricot, cherry, citrus fruits, fig, grape, grapefruit, lemon, orange, pear, satsuma mandarin, seedling of vegetables, seedling root of vegetables, sweet cherries, tangerine and tomato.

Postharvest fungal decay may cause significant losses to the citrus industry. *Penicillium digitatum* Sacc. and *P. italicum* Wehmer, causal agents of green and blue mould, respectively. These pathogens occur in almost all citrus growing regions of the world (Palou et al., 2001). *Penicillium digitatum* is the major constraint during handling process of tangerine (*Citrus reticulata* Blanco) fruit in Northern Thailand (Smilanick et al., 2005). This pathogen is of main concern as it is responsible for 90% of citrus losses due to diseases occurring during the storage period, and it causes serious damages in commerce (Eckert and Eaks, 1989).

Some fruits, apples among them, are usually stored after harvest. During cold storage losses of economic importance are produced by several decays due to fungal rot. *Penicillium expansum* and *Botrytis cinerea* are well-known postharvest pathogens. They produce blue and gray rots, respectively (J. Calvo et al. 2007). A blue mold rot on tomato fruit caused by *Penicillium oxalicum* has also been observed in Mexico and

Korea (Picos-Munoz et al., 2011; Kwon et al., 2008).

The value of fresh fruits and vegetables increases several-fold because of losses caused by post-harvest diseases are greater than generally realized while passing from the field to the consumer (Eckert & Sommer, 1967). Species of *Alternaria*, *Aspergillus*, *Botrytis, Fusarium, Geotrichum* as well as *Penicillium* to have been reported as common post-harvest fungi (Splittstoesser, 1987; Adaskaveg *et al*., 2002). Some of the moulds could produce mycotoxins while grown on fruits (Stinson *et al*., 1980) even during refrigeration (Tournas & Stack, 2001). Pathogenic fungi, on the other hand, could cause infections or allergies in susceptible individuals (Kurup, 2003). In view of the fact that pathogenic fungi alone caused 10-30% reduction in the yield of major food and cash crops (Agrios, 2005). A number of pre and postharvest technologies have been used to control their decay (Serrano *et al*., 2005). The *Penicillium* species isolated do not appear to be recorded from fruits and vegetables in Pakistan (Sultan *et al*., 1997).

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