Isolation And Characterization Of Post Harvest Fungal Pathogens Of Citrus Varieties From The Domestic Markets of Rawalpindi And Islamabad

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ABSTRACT-Citrus fruit health is equally important at national and international markets for attracting customers as well as receiving better prices. Post-harvest diseases of citrus minimize the fruit quality and quantity and cause heavy losses, thus it does not meet the required standard and it causes economic loss in international markets due to the considerable differences between export and local prices. Post-harvest diseases caused by bacteria, yeast and fungi develop on fruits and other plant products between harvesting and consumption. Citrus markets of Rawalpindi and Islamabad were assessed for Post-harvest fungal diseases. The studies were accomplished for the isolation and identification of fungal pathogens from the three varieties of citrus viz. Kinnow, Malta and Musami. A survey was conducted during February 2013 to evaluate the status of major post harvest diseases like stem end rot, mold, citrus canker and alternaria brown spot on citrus fruit. The study illustrated that the citrus variety Kinnow was more prone to fungal diseases than Malta and Musami. The results of the study also revealed that fruit samples were almost 3-5 days old and proper storage facilities were not available at the market. As an initial step to improve the quality of citrus and to meet its enhancing demand in the foreign and domestic markets, there is a dire need to understand the Post harvest fnugal diseases and their pathogens prevailing in the country for later management strategies. Improper packing, transport and market storage facilities can contribute towards food spoilage. The present study found that the Geotrichumcandidum, Diplodianatalensis, Penicilliumsp, Trichodermaviride, Fusarium sp., Alternariaalternata, Aspergillusniger, Aspergilllusfumigatus, and Aspergillusochraceous were the fungal pathogens involved in sour rot, stem end rot, green mold, Trichoderma rot, Fusarium rot, black rot, and black mold rot diseases and these were major post-harvest diseases that damage the citrus fruit. As far as, it was also observed that MEA was the best media for the growth of Diplodianatalensis, and Aspergillusochraceous.

Index terms- Study, citrus disease, fungal pathogens, isolation and identification of Post harvest diseases.

1.INTRODUCTION

Citrus belongs to a family of Rutaceae of tribe Citrae. All the members are fruit bearing, possessing juice filled vesicles known as *hisparidium* [1-2]. The term Citrus may be referred to a family of trees that are grown in warm region for their edible fruit, thick red and pulpy flesh. High juice contents, nutritional values and distinct taste have made the Citrus fruit unique among all other fruits.The present day Citrus is delectable, juicy, seedless, and is of great nutritional significance [3]. Citrus fruits act as a great source of vitamin C and a wide range of essential nutrients required by the body [4].

The optimum temperature range for Citrus growth is commonly considered to be 13–35°c [5]. The suitable climate for citrus is tropical and sub-tropical humid regions of the world. Most citrus species require full sunlight to grow well and produce fruit [6].

Citrus is the most important fruit crops of Pakistan. Among citrus, kinnow mandarin is primarily adjustable and mainly grown in Punjab [7]. The total world production of citrus is estimated at 36 metric tons [1-2]. While total production in Pakistan of citrus is estimated at about 94 percent of citrus production area is in the Punjab, 2.3 per cent in the Sindh, 2.4 percent in the Khyber Pakhtun Khawa,

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and 1.3 per cent in Baluchistan [8].

At the present time, Pakistan ranks among the top ten citrus cultivated countries in the globally [Pakistan (2004)]. Pakistan as a nation produces about 3 to 4 percent of the world citrus fruits but sells out only about 0.8 percent of its harvest abroad. Pakistan is the sixth largest producer of Kinnow and oranges in the world, with 2.1 million tons. According to an estimate nearly 95 percent of the total Kinnow produced all over the world is grown in Pakistan [9] .In spite of the high demand of citrus fruits, its production level is low due to pests and diseases. Many microorganisms have been known to cause various diseases of citrus trees. These include many genera of fungi, bacteria and viruses. The quality and quantity of citrus fruits is severely affected by fungal diseases, such as melanose, greasy spot, and stem-end rot, and brown rot, green and blue moulds. The fungal pathogens *Diplodiana talensis* are responsible for post-harvest stem-end rot. Some other important post-harvest fungal diseases and disorders of citrus include brown rot caused by *Phytophthora spp*., green mold caused by *Penicillium digitatum*, blue mold caused by *Penicillium italium*. Fruits are however affected by a wide array of microorganisms causing its decay. These microorganisms, under the influence of environmental factors, pose a serious threat to fruits production. Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture [10].Present investigation involves the study of various fungal pathogens responsible for the post harvest, decay and deterioration of economically important citrus fruit from the domestic markets of Rawalpindi and Islamabad.

The objectives of the present study are to isolate and identify the post harvest fungal pathogens of citrus varieties from the domestic markets of Rawalpindi and Islamabad and to compare the growth of different fungal pathogens on general and specific media's and to determine the status of post harvest fungal diseases of citrus in the domestic markets of Rawalpindi and Islamabad. All these investigations will be ultimately very helpful for the management of post-harvest diseases of citrus.

2.MATERIALS AND METHODS

A survey was conducted in the domestic markets of Rawalpindi and Islamabad during the month of February 2013 and sampling was done. The citrus were randomly picked from wooden box or pile of fruits. Fruit samples were graded first and then collected in paper bags which were kept in polyethylene bags and labeling was done by permanent marker on each fruit as well as on bag. Survey was based on the interviews of sales men at selected markets.

The sampling was completed with general protocol from the domestic markets as described earlier. After sampling, citrus samples were brought in Environmental Mycology and Ecotoxicology Laboratory, Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi and categorized according to the symptoms of post-harvest diseases.

The spoiled or diseased oranges were identified by visual examination of diseased symptoms. Various types of spoiled oranges were selected, including those that were mechanically wound or bruise, with purplish to dark brown rot as well as those with black lesions on them. Petri plates, media bottles, distilled water; syringes were sterilized in autoclave. After autoclaving all sterilized material will be dried in an oven at 90˚C.Three different Medias are used for the isolation of fungal pathogen from citrus varieties. The general purpose medium namely Potato Dextrose Agar (PDA), as well as specific media e.g. Corn Meal Agar (CMA) and Malt Extract Agar (MEA) were used for fungal cultures growth. After the autoclaving of the prepared medium flasks, the mediums were then poured into the sterilized Petri plates inside the laminar flow.

The samples which are apparently diseased were cut from the advancing edges of lesion with a sterilized knife. The cut portion of the lesion were then disinfect with ethanol of 85% concentration for 2 minutes. Plates of already prepared media containing 0.5ml/L streptomycin to prevent the growth of bacteria were inoculated with 4mm infected portion from samples and incubated at ambient room temperature (25 – 30oC) for 4 days. After 4 days, growth of fungal colonies on the media plates were counted and recorded.

Identification and morphological characterization were based on the conidia shape and measurements on hyphal color , septation ,concentric zone, pigmentation , fruiting bodies or any other visible structures by observing under compound microscope at the magnification of 10X , 400X, and 1000X. The cultures were identified at genus level on the basis of macroscopic (colonial morphology, color, texture, shape and appearance of morphology) and microscopic characteristics [11]. Isolated fungal data were presented as total number of fungi, fungal characters, and comparison of fungus growth on different media, disease symptoms and isolated fungi.

3.RESULTS

The results of market survey showed that fruit samples were almost 3-5 days old and proper storage facilities were not available at the market. According to the owners of shops, all citrus fruit consignments were coming directly from orchards after harvesting even then problems occur in their fruit. Due to the little knowledge, they were unfamiliar with the post-harvest losses and they were asking solutions such as how they can protect their citrus after arriving from the orchard and how is possible to keep them without diseases.

The results from this study have shown that many fungi can cause the citrus fruit spoilage that can serve as a source of raw materials for our food industries. Symptoms were observed on the diseased citrus fruits and there brownish black and necrotic patches were present on the skin of the citrus fruit. A mass of mycelia growing on the surface of the fruits was also observed. Different types of post-harvest fungi were isolated from the diseased citrus fruits which were identified as *Aspergillusniger, Geotrichum candidum, Diplodiana talensis, Trichodermaviride, Penicillium sp, Fusarium sp, Alternaria alternata, Aspergillus ochraceous* and *Aspergillusfumigatus.*

The Table 1 shows the total fungi isolated from the general PDA and specific mediums MEA and CMA.

The Table 2 describes the fungi and diseases, fungal characters, measurements, shape of spores and the colony features on the media. In the table the fungi, their diseases on citrus, their morphological, mycological characters and spore measurements were described in detail.

TABLE NO 1: FUNGI ISOLATED FROM GENERAL AND SPECIFIC MEDIUMS

Fungal isolate	PDA	MEA	CMA
Aspergillusniger	+	+	-
Alternariaalternata	+	-	-
Penicillium sp.	+	+	+
Goetrichumcandidum (red blood)	+	+	-
Goetrichumcandidum (kinnow)	+	+	+
Trichdermaviride	-	+	-
Aspergillusochraceous	-	+	-
Diplodianatalensis	-	+	+
Aspergillusfumigatus	+	-	-

+ present, - absent

TABLE NO 2: CHARACTERIZATION OF ISOLATED FUNGAL PATHOGENS FROM DISEASED CITRUS FRUITS

Disease	Isolated Fungus	Colony Characteristics	Spore Characters	Spore size
Black mold rot	*Aspergillus niger*	The colony consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Conidial heads are large, globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septatemetulae. Conidia are globose to subglobose, dark brown to black and rough-walled.	Length = 19 – 30 um Width = 3.5 – 6 um Average = 24 x 5 um Conidiophore Length = 200 – 223 um Width = 5 – 10 um Average = 208 x 7.6 um
Black rot	*Alternaria alternata*	Colonies are fast growing, black to olivaceous-black or grayish, and are suede-like to floccose.	Multicelled conidia are produced from simple, sometimes branched, short or elongate conidiophores. Conidia are obclavate, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical beak, pale brown, smooth-walled or verrucose.	Length = 11 - 28 um Width = 8 - 15 um Average = 19.5 x 11.5 um Hyphae Length = 33.5 – 52 um

				Width = 4 – 5 um Average = 43 x 4.5 um
Green mold, Blue mold	*Penicillium sp.*	The colonies are usually green, blue-green, or grey green, but can be white, yellow or pinkish. Colonies are mostly velvety to powdery in texture.	The chains of single-celled conidia (ameroconidia) are produced in basipetal succession from a specialized conidiogenous cell called a phialide. Conidiophores are hyaline and may be smooth or rough walled. Spores are globose, ellipsoidal, cylindrical or fusiform, hyaline or greenish, smooth- or rough- walled and green in color.	Length = 11 – 30 um Width = 7 - 26 um Average = 23 x 14 um Stipe Length = 275 - 309 um Width =1.5 - 3 um Average = 268 x 2.8 um
Sour rot	*Goetrichum candidum*	The colonies are white to cream, dry and finely suede-like with no reverse pigment.	This produce chains of hyaline, smooth, one-celled, subglobose to cylindrical, slimy arthroconidia (ameroconidia) by the holoarthric fragmentation of undifferentiated hyphae	Length = 5 - 8 um Width = 4 – 8 um Average = 6.5 x 6 um
Fusarium rot	*Fusarium sp.*	Colonies are usually fast growing, pale or brightly colored and may or may not have a cottony aerial mycelium. The color of the thallus varies from whitish to yellow, brownish, pink, reddish or lilac	Typically produce both macro- and microconidia from slender phialides. Macroconidia are hyaline, two- to several-celled, fusiform- to sickle-shaped, mostly with an elongated apical cell and pedicellate	Microconidia Length = 3 - 7 um Width = 1.5 – 3.5 um Average = 5.8 x 2 um

		shades.	basal cell. Microconidia are 1- to 2-celled, hyaline, pyriform, fusiform to ovoid, straight or curved.	Macroconidia Length = 15 - 22 um Width = 1.5 – 3.7 um Average = 18 x 2.5 um
Trichoderma rot	*Trichderma viride*	The colonies are at first white and downy, later developing yellowish-green to deep green compact tufts, often only in small areas or in concentric ring-like zones on the agar surface.	Conidiophores are repeatedly branched, irregularly verticillate, bearing clusters of divergent, often irregularly bent, flask-shaped phialides. Conidia are mostly green, sometimes hyaline, with smooth or rough walls and are formed in slimy conidial heads (gloiospora) clustered at the tips of the phialides	Length = 3 – 4.8 um Width = 3 – 4 um Average = 3.9 x 3.5 um
Aspergillus rot	*Aspergillus ochraceous*	Colonies growing restrictedly, yellow-orange, ochraceous or buff.	Conidial heads radiate, splitting into several columns with age. Conidiophore stapes brownish, with roughened walls. Vesicles spherical, thin walled and hyaline, Conidia spherical to sub spherical, smooth walled to finely roughen. Sclerotia pink to vinaceous-purple, and irregular in shape.	Length = 14.9 - 37 um Width = 6.7 - 11 um Average = 25 x 9 um
			The immature conidia were hyaline and aseptate while the	Length = 22 - 37 um

Stem end rot	*Diplodia natalensis*		mature were thick walled, dark-brown, 2-celled (septate), and Longitudinally striated.	Width = 7 - 13 um Average = 30 x 9.5 um
Damping off	*Aspergillus fumigatus*	The colonies show typical blue-green surface pigmentation with a suede-like surface consisting of a dense felt of conidiophores.	Conidiophores are short, smooth-walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia are produced in basipetal succession forming long chains and are globose to subglobose, green and rough-walled.	Length = 19 – 26 um Width = 4 – 6 um Average = 22 x 5 um Conidiophore Length = 200 – 230 um Width = 5 – 8 um Average = 211 x 6.5 um

Figure 1.Comparison of fungal growth isolated from the location of Rawalpindi

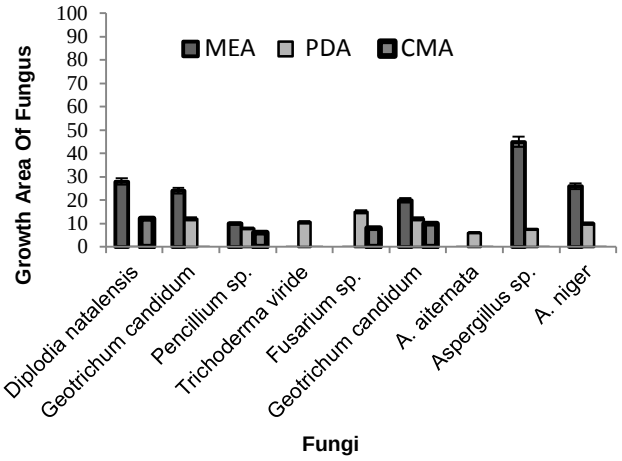
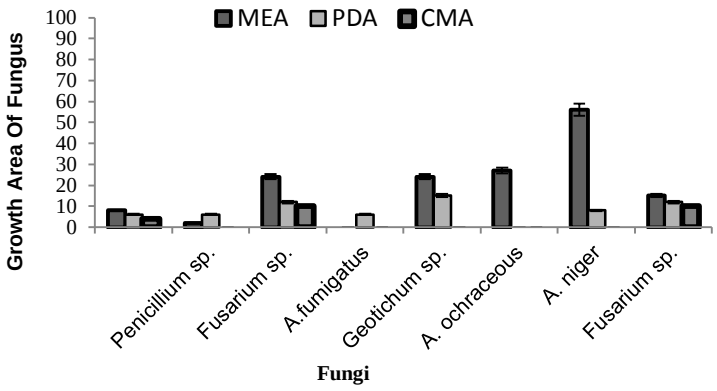


Figure 2.Comparison of fungal growth isolated from the locations of locations of islamabad



The comparison of fungal growth on different media isolated from the citrus fruits of the local markets of Rawalpindi and Islamabad were described in the figures1 and 2.

F**igure 1** shows the comparison of fungal growth isolated from the locations of Rawalpindi and it was found that MEA was the best media for the growth of *Diplodia* that is responsible for the Stem end rot, Geotrichum showed growth on the MEA and PDA but the growth was higher on the MEA, *Penicillium* showed growth on all mediums and best growth on MEA, PDA was the best medium for the growth of *Trichoderma* as it does not showed growth on the other two mediums, *Fusarium* sp. does not showed growth on MEA while its growth was best on PDA, *Alternaria alternata* showed growth only on the PDA while the other two mediums do

not showed any growth, MEA was the best medium for the growth of *Aspergillus sp*. as there was little growth on PDA and no growth on CMA and *Aspergillus niger* showed growth on the MEA and PDA but no growth on CMA.

Figure 2 shows the comparison of fungal growth isolated from the locations of Islamabad and it was found that *Geotrichum* showed growth on all the three mediums but the best was on the MEA and then on PDA I and very less on CMA, *Penicillium sp*. showed growth on MEA and PDA with best growth on PDA but no growth on CMA was observed, *Fusarium* growth was observed on all the three media's with good growth on MEA II then on PDA and less on CMA, *Aspergillus fumigatus* showed growth only on PDA but no growth was observed on MEA and CMA, *Geotrichum candidum* showed growth on the MEA and PDA but the growth was good on the MEA, *Aspergillus ochraceous* showed growth only on the MEA, and *Aspergillus niger* showed growth on the MEA and PDA but no growth on CMA.

So the results of the present study show that PDA was the best media for the growth of *Geotrichum candidum, Penicillium* sp, *Trichodermaviride, Fusarium* sp, *Alternaria alternata* and *Aspergillus fumigatus.*

The *Aspergillus ochraceous* showed best growth only on the MEA and it does not grow on any other media. *Diplodiana talensis* also showed growth on MEA. However it was found that on CMA only certain type of fungi has shown the growth and it was best for the *Diplodia natalensis, Geotrichum candidum, Penicillium sp*. and *Fusarium* sp.

4. DISCUSSION

Present study was focused on assessment of post-harvest fungal diseases of citrus in domestic markets of Punjab. Post-harvest fungal

diseases are one of the major causes of citrus decline throughout the citrus growing areas of the world as well as in Pakistan.Due to poor quality Pakistani citrus receives low price as compared to other well managing orchard countries though it is popular due to its taste in both local as well as international levels markets. To identify, assess and monitor these diseases at the domestic markets and export delivery became a dire need to improve the quality of our citrus for receiving better prices. The Asiatic variety is common in Pakistan, which may be a threat for the citrus industry of Pakistan as well as for the neighboring countries.

There is no previous report in Pakistan regarding the assessment of post-harvest diseases of citrus from domestic markets. Forty locations were targeted for survey of citrus collection which was categorized into two groups (1) Domestic market of Rawalpindi (2) Domestic market of Islamabad during February 2013. It was found that no specific conditions were available for citrus storage. Fresh fruits were coming from orchard within 4-5 days. Present observations indicate that lack of training, knowledge, awareness and storage conditions as well as major constraint and cause of fruit spoilage. Post-harvest losses intensify in Pakistan due to improper management, harvesting techniques which are traditional and packaging is not proper. Distribution of fruit to local markets take long enough, these all factor contribute to damage to economy. Citrus fruit is harvested manually 99% which causes physical damage, injuries and bruising as well as unavailability of optimum temperature and dumping of the wooden boxes irregularly in the market. Packing in the wooden boxes forcibly itself adds to the issues of bruising from where the pathogen of post harvest rots easily enter and spoil the whole lot of fruits. The transports of citrus in van, truck and shehzore without controlled environment have also contributed towards the diseases. The transport time was another factor affecting the incidence of the diseases. In the markets close to the orchard, the incidence of the post-harvest diseases was not much high as compared to the distant markets. Thus the maximum time taken without optimum packing and transport facilities increased the incidence of the diseases. In this study different types of post-harvest fungal pathogens were found associated with deterioration of Citrus which are identified as *Geotrichumcandidum, Diplodianatalensis, Penicillium*sp, *Trichodermaviride, Fusarium* sp, *Mucor*sp, *Alternariaalternata, Aspergillusniger*, *A fumigatus, A ochraceous,* and *A* sp.

In a study, 83% of the citrus fruit samples showed fungal growth at levels ranging from 25 to 100% of tested fruits and *Fusarium sp*. were the most common fungi in citrus fruits[12].

Similarly previous researchers had studied citrus for fungal decay in storage and its relation to shop (local storage places) and a number of

Aspergillus sp., *Aspergillus fumigatus*, *Aspergillus niger*, had been isolated[13].

The preponderance of the isolated moulds from Citrus belongs to *Aspergillus* species and other genus, and this confirms their prevalence in foods and fruits exposed to tropical humid climate thus constituting potential health risks to consumers of this fruit and it's by products.

Aspergillus niger was found associated with spoilage of citrus this is in line with the work of [14].who reported that *Aspergillus sp*. is the predominant organism associated with the spoilage of citrus.

Important information generated through this study is that most post harvest rot appear in market during storage and poor illiterate people handling are unfamiliar with the diseases appearing reducing the fruit quality. The present investigation also revealed that *Alternaria* Brown Spot (ABS) and Mold are the more prevalent diseases in the domestic markets. These result are in line with the[15-16] who carried similar studies in Bhakkar region , Faisalabad and Karachi. The observed difference in the incidence and severity of diseases among the varieties could have been due to different resistant capabilities in the citrus species. This is in line with the work of [17] who worked on varietals reactions of citrus, which showed varietals resistance and also proved that, fungi inoculated into the seedlings were more pathogenic or caused more extensive diseases on citrus before infected or weakened by an agent than on healthy plants. [17].Also said that, the reasons for differences in disease resistance among varieties of a single plant species remain largely obscure; that resistances frequently reside in a physiological or biochemical differences between the resistant and susceptible varieties. It is impossible and uneconomical to completely eliminate post-harvest losses; it is possible and desirable to shrink them by 50%.

CONCLUSION

On the basis of results, from present research it is concluded that the infective marketing practices i.e. improper packing, no specific storage facilities, transport and market storage facilities can contribute towards fruit spoilage. The markets far from orchards have more incidence of post harvest disease. The awareness might be created among growers and customers to produce and quality fruit at all locations. This study also showed that mechanical injuries such as bruises or cuts that occur during harvesting or post-harvesting, grading and packing could provide infection sites for spoilage pathogens. It was also concluded that malt extract agar was the best media for the isolation of *Diplodia natalensis,* and *Aspergillus ochraceous.*

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