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FIRST REPORT OF *GEOTRICHUM CANDIDUM* FROM PAKISTAN CAUSING POSTHARVEST SOUR ROT IN LOQUAT (*ERIOBOTRYA JAPONICA*)

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ABSTRACT

Post-harvest diseases of fruits are the major threat to fruit quality and cause heavy economic losses. During a survey of the local fruit market in Lahore in the summer of 2014, several samples of rotted loquat fruits were collected to identify the causal organism. *Geotrichum candidum* (FCBP1335) was isolated, purified and identified initially on the basis of morphological characteristics. Further confirmation of identified fungus was carried out by sequencing the Amplified Internal Transcribed Spacer (ITS) sequence (ITS1-5.8S rDNA-ITS4 region) from total genomic DNA of the isolated causal fungus. Re-isolation of same fungal pathogen from the artificially innoculated loquat fruits fulfilled the Koch's postulates. To our knowledge, *G. candidum* is reported for the first time, causing rotting in loquat fruit not only from Pakistan but also from the world. Present investigation will be very helpful for management of this post-harvest disease.

Key words: Loquat, sour rot, *Geotrichum candidum*, Pakistan.

INTRODUCTION

Loquat (*Eriobotrya japonica*) native to China is a sub-tropical fruit tree which is cultivated in many other parts of the world, including, India, Pakistan, America and Australia (Hussain *et al*., 2007). Besides being lovely and luscious, it is very healthy. It contains natural vitamins, nutrients (phosphorus and calcium) and sugars (Karadeniz, 2003). Therefore, it is valued not only as a fresh fruit but used with other fruits and in salads. The fruit is also employed in making jam, jelly and chutney (Pareek *et al*., 2014). Fruit and leaves of loquat have been regarded to have great medicinal value (Wee and Hsuan, 1992). These are used to cure epidermis illnesses and reducing discomfort and coughing (Sakuramata *et al.,* 2004). Loquat seeds extract has an inhibitory impact on liver problems (Hamada *et al.*, 2004).

Postharvest corrosion is a serious problem that reduces storage and shipping of fresh produce, leading to serious economic failures. *Geotrichum candidum* is cosmopolitan fungus that belongs to Ascomycota and causes sour rot of fresh-market fruits such as peach and nectarine (Michailides *et al.*, 2004). The fungus ability to tolerate broad range of pH (5-7) and temperature (5-38 °C) make it commonly occurring contaminant on wide ranges of host (Medveďová *et al*., 2008). Sour rot of peach and nectarine is mainly associated with injured fruit and clean fruit with split pits (Adaskaveg and Crisosto, 2006). The disease mainly occurs on ripe fruit but may also occur on strictly harmed premature fruit. Symptoms include a brown, watering, soft corrosion with a slim layer of white mycelial growth on the fruit surface (Wells, 1977).The fungus is believed to be part of normal human skin and gut flora. It causes respiratory tract infections in human beings (Verghese and Ravichandran, 2003). Some strains of *G. candidum* are reported to have proteolytic, peptidolytic and lipolytic potential and hence used extensively in food industry (Medveďová *et al*., 2008).

The purpose of present investigation was to isolate and identify the pathogen associated with sour rot of loquat fruit and to confirm its pathogenicity on host.

MATERIALS AND METHODS

Survey, sampling and isolation of pathogen: A survey was conducted to the local fruit market of Lahore, Pakistan during summer 2014. Loquat fruits contaminated by an unknown fungus with brown watery soft decay with a thin layer of white mycelium on the fruit surface (Fig. 1) were collected in sterilized polythene bags and brought to First Fungal Culture Bank of Pakistan (FCBP) for pathogen detection. Five different fruits were selected randomly for the identification of pathogen. A small piece of infected fruit flesh from the deeper layer of fruit was transferred aseptically onto the Malt Extract Agar growth medium (Dhingra and Sinclair, 1995). Inoculated petri plates were incubated at 25 °C for 3-4 days or until fungal growth started. Emerging fungal mycelium was transferred to fresh growth medium for purification of pathogen to be used for morphological observations.

Morphology based identification of pathogen: Morphological characterization was done on 7 days old pure fungus culture. Complete description of macroscopic characters of the fungus colony as well as microscopic features was carried out. Colony characters include; color, size, zonation, presence of exudates, type

of mycelium and conidiophores. Under the compound microscope shape, size, ornamentation of conidia and its attachment with the conidiophores was recorded (Domsch *et al*., 1980).

Genetic analysis: Morphology based identification of the pathogen was verified by analysis of Internal Transcribe Spacer (ITS) nucleotide sequence. Total genomic DNA from fungal pathogen was isolated (Wiegand *et al.,* 1993). Using the genomic fungal DNA as template, ITS region of the genome was amplified by a universal primer pair ITS1 forward (5′- TCCGTAGGTGAACCTGCGG-3′) and ITS4 reverse primer (3′-TCCTCCGCTTATTGATATGC-5′) (White *et al.,* 1990). The amplified gene product was sent for nucleotide sequencing. The resulting DNA sequence was explored by employing different Bioinformatics tools and also deposited in the GenBank.

Pathogenicity test: To confirm the pathogenicity of isolated fungus, 20 healthy loquat fruits were selected, surface sterilized with alcohol soaked cotton. A loopful inoculum from the culture of one week old isolated fungal pathogen was inserted inside the pulp of ten fruits with the help of sterilized needle while for control, the rest of the 10 fruits were mock-inoculated with the sterilized needle only. All two sets of fruits (inoculated and mock-inoculated) were placed in separate sterilized glass containers, incubated at 25 °C for 7 days and observed for rotting symptoms. Three independent experiments using three different isolates were run to confirm the finding.

RESULTS AND DISCUSSION

Geotrichum candidum, a filamentous yeast-like fungus, is the inhabitant of a wide range of substrates including air, water, soil, milk and milk products (Ruas-Madiedo *et al*.,2006); Brien *et al*., 2005). *G. candidum* is an important post-harvest pathogen that causes sour-rot in ripe and over-ripe fruits and vegetables (Pitt and Hocking, 2009; Tournas, 2005).

Morphological characteristics: Colonies were white and non-aerial with off white reverse. Conidiophores absent, conidia single celled, arthrosporous, terminal, aerial on agar surface, hyaline, sub-globose to cylindrical in shape. Conidia size ranges 4.5 - 12.5 x 2 - 5 µm. Chlamydospores sub-globose borne on hyphae, 4.0-5.3 µm in diameter (Fig. 2). Based on morphology, isolated fungus was identified as *Geotrichum candidum*. A vial of pure pathogen culture was deposited to First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab, Lahore, under the accession FCBP1335.

Internal Spacer Sequence (ITS) analysis: Primer pair, ITS1/ITS4 amplified the ITS1–5.8S rDNA-ITS4 region of fungal genome and a single compact amplified DNA band of approximately 550 bp sizes was observed on 1 % agarose gel (Fig. 3). The nucleotide sequence of amplified PCR product was BLAST using the National Center for Biotechnology Information (NCBI) and European Bioinformatics Institute (EBI) bioinformatics websites and deposited in GenBank database (KM979207). BLASTn searches using this ITS nucleotide sequence as query, showed99% similarity to *G. candidum* strain L19PD (KF713519.1) isolated from ovine milk and strain TOMYEAST (KF112070.1) isolated from tomato fruit.

Koch's postulates confirmation: After 7 days of infection, all the ten inoculated loquat fruits were found to be rotted showing the same disease symptoms as observed on the infected fruits collected from the market. However, mock-inoculated fruits remained healthy. *G. candidum* was re-isolated from the infected fruits that confirmed its pathogenicity towards loquat fruit.

Fig. 1. Loquat fruits infected with sour rot.

Due to high content of sugar and pectin the *G. candidum* could be susceptible to many post-harvest fungi including *G. candidum*. Previously *Alternaria alternata* has been reported to cause fruit rot in *E. japonica* in Greece (Tziros, 2013) and by *Alternaria* species in Taiwan (Ko *et al*., 2010). There are reports of *G. candidum* causing sour rot of peach and nectarine fruits in California (Michailides *et al.,* 2004) and in Florida (Yaghmour *et al*., 2012).

To the best of our information, this study reports *G. candidum* for the first time from Pakistan causing sour rot in loquat fruit. The findings of this research will help in controlling this rot hence reducing the economic loss caused by this pathogen.

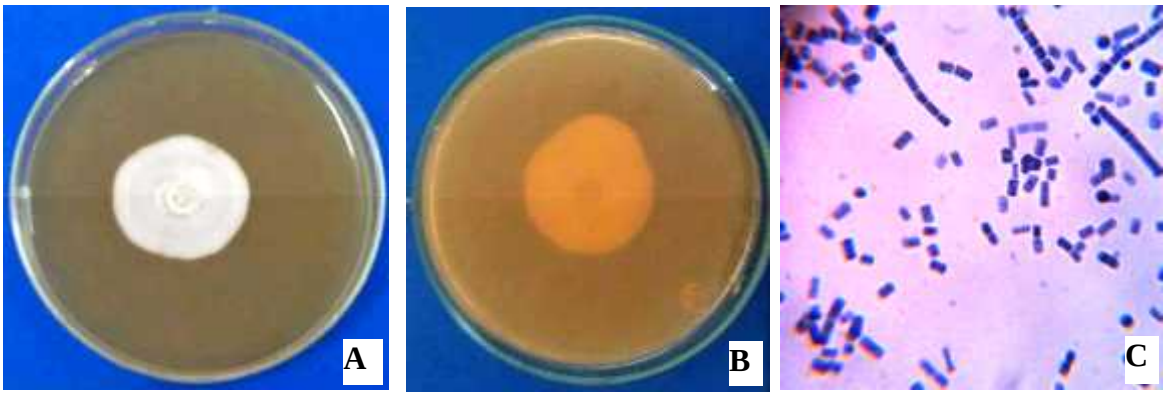

Fig. 2: *Geotrichum candidum*. A: Colony; B: Reverse of fungal growth; C: Conidia.

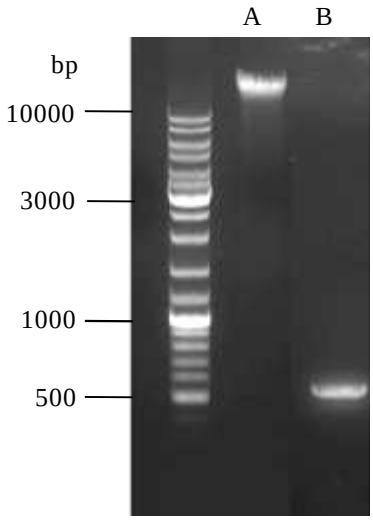

Fig. 3: (A) Total Genomic DNA of *G. candidum* and (B) amplified Internal Transcribed Spacer (ITS) region (GenBank Accession No. KM979207) using this genomic DNA as template and ITS1/ITS4 primer pair. 1kb DNA marker was run as standard

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