Pak. J. Bot., 54(1): DOI: http://dx.doi.org/10.30848/PJB2022-1(38)

FIRST REPORT OF POSTHARVEST DECAY OF *ACTINIDIA DELICIOSA* CAUSED BY *PENICILLIUM EXPANSUM* IN PAKISTAN

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Abstract

Kiwifruit (*Actinidia deliciosa* A. Chev.) has been known for its high nutritional and medicinal values. It contains several phytoconstituents which belongs to the class of phenylpropanoids, flavonoids, triterpenoids, steroids and quinones. Postharvest decay was observed on Kiwifruit collected from Lahore, Pakistan during November 2016, which were kept for 15 days in storage at 5°C. Blue green fungal mass was observed on infected fruit surface, followed by soft, watery and pale yellow to light brown colored lesions. Based on morphological characteristics the fungus was identified as *Penicillium expansum* Link. For molecular study, the ITS regions of the isolated rDNA was amplified by primer pair ITS1 and ITS4 and the PCR product was got sequenced, and deposited in GenBank with Accession No. LT799972. The pathogenicity of the fungus was verified with Koch's postulates. As per our knowledge, this is the first description of postharvest decay of kiwifruit caused by *P. expansum* in Pakistan.

Key words: Blue mold rot, Cardiovascular disease, Kiwifruit, Phytoconstituents.

Introduction

Kiwifruit is grown in many parts of the world for its excellent food and nutritional value (Nasib *et al*., 2008). The genus Actinidia is broadly distributed on the Asian continent. Presently, this fruit plant is commercially grown in many countries including the Chile, Sri Lanka, France, Greece, Italy, Japan, India and United States (Singletary, 2012). Kiwifruit is not commercially grown in Pakistan. However, it is grown in Mansehra, Pakistan for the research project. Kiwifruit vine is not resistant to frost. It cannot bear very low and very high temperatures. The fruits are small, oval and pulp is yellow, green etc., (Ramzan *et al*., 2018). Kiwifruit has been known for its highly instinctive and medicinal values. It holds quite a lot of phytoconstituents which belongs to the class of phenylpropanoids, flavonoids, triterpenoids, steroids and quinones (Fiorentino *et al*., 2009). It is a good source of nutrients such as potassium, folate and dietary fiber. It is rich in vitamin C and also contains vitamins A and E (Tripthi *et al*., 2017). It has worldwide market due to its medicinal importance as it is helpful in treating cancer, cardiovascular disease, rheumatoid arthritisand hepatitis (Shastri *et al*., 2012).

Blue mold disease, caused by *Penicillium expansum* (Link), is the most economically significant postharvest disease of fruit and vegetables in storage (Errampalli, 2014; Khan *et al.*, 2021). It causes blue mold rot, which is a decay that can lead to substantial economic losses during storage, and can also affect fruit meant for processing due to the production of carcinogenic mycotoxin patulin and citrinin (Amin *et al*., 2017). *Penicillium* species caused post-harvest diseases on fruits and other plant products between harvesting and consumption. Accurate identification of the causal pathogen is necessary before proper treatment can be made to control the pathogens (Khokhar & Bajwa, 2014). The accurate identification of *Penicillium* at species level on morphological basis under light microscope is not easy. Molecular identification of *P. expansum* using DNA probes and PCR (polymerase chain reaction) are of supreme importance for its specific and rapid detection for ensuring both safety and quality of fruits (Yin *et al*., 2017; Khan & Javaid, 2021). In previous studies, *P. expansum* was isolated from the surface of apple fruit in Pakistan (Ilyas *et al*., 2007; Khokhar & Bajwa, 2014) but it has not been reported from kiwifruit previously. The purpose of the current investigation was to isolate and identify the causative agent of postharvest decay of kiwifruit in Pakistan.

Materials and Methods

Sample collection and identification: Diseased samples of kiwifruits were collected during November 2016 from Lahore, Pakistan (Fig. 1). Blue green fungal mass was observed on infected fruit surface which was transformed to soft, watery and pale yellow to light brown colored lesions. Tissues were taken from the healthy and diseased kiwifruits and surface sterilized with 1% sodium hypochlorite (NaOCl) for one minute, washed with sterilized water three times and plated on malt extract agar (MEA) medium. The fungus retrieved from the infected tissue was sub-cultured on fresh MEA plates that produced copious amount of mycelia and conidia after 7 days at 22°C. Microscopic examinations of 8-day-old culture were performed at 4X, 10X and 40X magnifications of light microscope to observe cultural and conidial morphology of the fungal isolates.

Molecular characterization: Fungal DNA was extracted bothfrom mycelia and conidia, and the internal transcribed spacer (ITS) region was amplified by using ITS1 and ITS4 (Innis *et al*., 2012) primers. The sequence obtained through amplification was blasted in GenBank which was 415 bp long and showed 98% similarity to the ribosomal sequences of *P. expansum* Accession No. KJ608113, KJ744356, KM357339 from Italy, Germany and China, respectively. The sequence was submitted in GenBank (Accession No. LT799972).

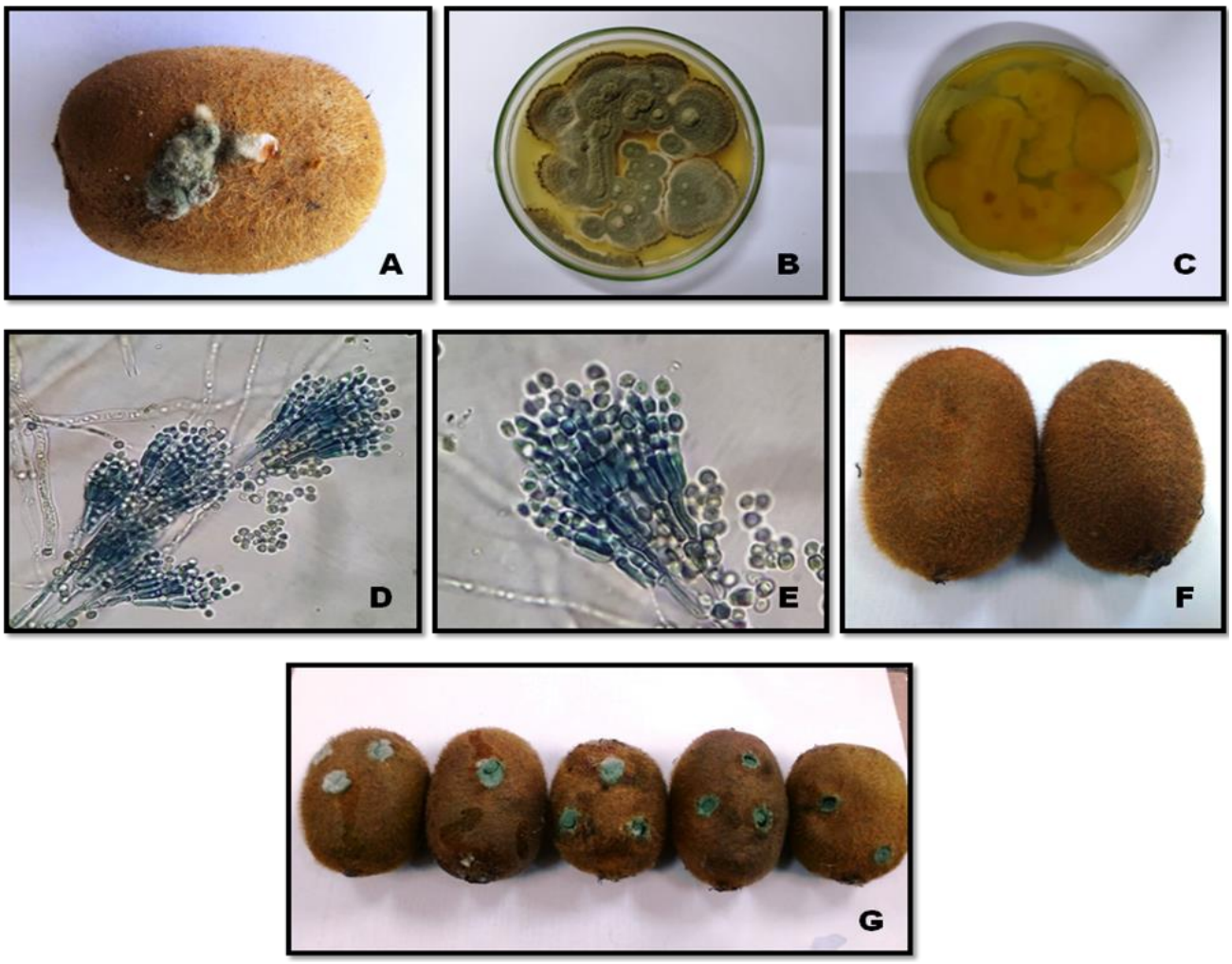

Fig. 1. (A)- Kiwifruit showing symptoms of decay, morphological characterization of *Penicillium expansum*, (B)- Colony morphologyon MEA, (C)- Colony reverse on MEA, (D-E)- Conidia at 40X, (F)- Control kiwifruits which are symptomless, and (G)- Typical symptoms of *P. expansum* decay after inoculation on kiwifruits.

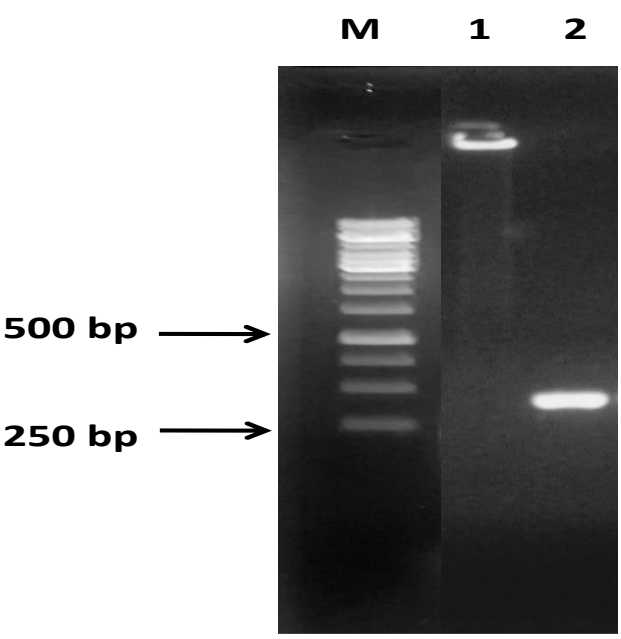

Fig. 2. Agarose gel electrophoresis. 1: Total genomic DNA isolated from kiwi. 2: Amplified PCR product of approximately 415 bp by universal primer pair ITS1/ITS4. M: DNA size marker.

Pathogenicity test: Pathogenicity was tested on 10 ripe fruits, which were surface sterilized by 1% sodium hypochlorite (Okigbo *et al*., 2009). Thereafter, the fruits were inoculated with 5mm discs of freshly grown *P. expansum* on MEA and a control was prepared by placing plain MEA discs. Kiwifruits were placed in autoclaved beakers for complete establishment of *P. expansum*. Fungus was re-isolated on MEA after 10 days, from the inoculated fruits. Further identification of the pathogen was done as described earlier in comparison to symptomless control specimens.

Results and Discussion

Colonies of the fungus were fast growing reached 4–5 cm diameter in 10 days on MEA. Colony appearance was light green, reverse colorless to yellow brown. Conidiophores were typically loosely synnematous (rarely distinctly synnematous), roughened, 400-700 µm long. Phialide tips were thin walled. Conidia were sub-globose to ellipsoidal, smooth walled, 3-3.5 µm in diameter. On the bases of these morphological characteristics, the isolated fungus was identified as *Penicillium expansum* Link ex Grey (Brito *et al*., 2020). Further identification

was confirmed by sequence analysis and the isolated fungal strain was deposited in the First Culture Bank of Pakistan having accession No. FCBP#1541.

Inoculated kiwifruits showed the typical symptoms of *P. expansum* ten days after inoculation. All the inoculated fruits showed symptoms of the disease (Fig. 2). Sequence alignment revealed that all the isolates were similar to each other. BLAST analysis of the ITS region of the isolated pathogen revealed that there was 98% similarity with *P. expansum* sequences KJ608113 from Italy. The internal transcribed spacer (ITS) is the universally accepted genetic barcode which provides a greater taxonomic resolution towards the different fungal genera due to elevated success rate of sequencing and amplification. Therefore, being a highly conserved region, it can be easily investigated in phylogenetic studies by using PCR amplification of ribosomal DNA (Li *et al*., 2019). Phylogenetic analysis was performed using neighbor-joining method in MEGA 6 version 6.0 (Tamura *et al*., 2012). In the phylogenetic tree, the characteristic isolate was sited within a clade including reference isolates of *P. expansum* (Fig. 3). These results indicated that *P. expansum* is the causal agent of the postharvest decay of kiwifruit. In agricultural systems, the correct pathogen identification is necessary for its effective control.

As per our information, this is the first description of postharvest decay of kiwifruit caused by *P. expansum* in Pakistan.

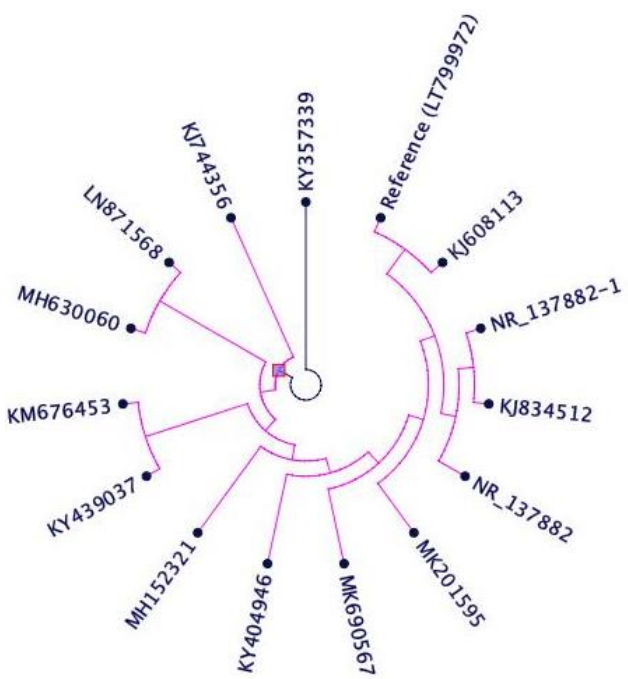

Fig. 3. The ITS1gene sequence of the isolate from this study was aligned with reference sequences of *P. expansum* isolates from GenBank using Clustal W© program. The phylogenetic tree was constructed using the neighbor-joining method in MEGA 6 version 6.0 (Tamura *et al.,* 2012).

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(Received for publication18 April 2020)