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Original Research Article

Penicillium aurantiogriseum Dierckx: First Report on Causing a Postharvest Rot of Onions (*Allium cepa* L.) in Pakistan

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A b s t r a c t

In present study *Penicillium aurantiogriseum* Dierckx. was found one of the cause of postharvest rot of stored onions (*Allium cepa* L.) in Pakistan. Infected onion tissues were cultured on Czapek Dox Agar, 2% Malt Extract Agar, Czapek Yeast Autolysate Agar and 25% Glycerol Nitrate Agar at 25°C. *P. aurantiogriseum* was identified as the pathogen on the basis of morphological and molecular characteristics. Pathogenicity tests conducted on healthy onions under laboratory conditions showed typical rot symptoms after seven to fourteen days. This is the first report of postharvest rot of onions caused by *P. aurantiogriseum* in Pakistan.

K e y w o r d s

Allium cepa
Onions
Penicillium aurantiogriseum
Postharvest rot

Introduction

As are many other liliaceous plants *Allium* crops (Onion and garlic) are badly affected by *Penicillium* decay (Byther and Chastagner, 1993; Frisvad and Samson, 2004; Davis, 2008; Sumner, 2008). Degradation of fruits, vegetables and agricultural products during pre harvesting and post harvesting stages is mainly caused by *Penicillium*. Due to spoilage *Penicillium* causes considerable economic losses. Blue mold on onion is also caused by *Penicillium* species (Schwartz and Mohan, 1995). In all households onion (*Allium cepa* L.) is one of the important condiments extensively used. The total world production of onion was about 86.34 million tonnes and Pakistan occupied 8th position with 2.25% share in production (FAOSTAT, 2011). The world production of dry onion during 2011 were estimated 29490 million US $. Production countries consumed more than 90% of onion. However, the crop is a major export earner for some economies.

During 2011, Pakistan produced about 1.94 million tonnes of onion. The average yield of onion is 13.6 t/ha in the country which is rather low. Poor planting densities, weed infestation, use of inferior quality seed and low use of fertilizer could be the cause of it. Lack of plant protection measures further magnifies the situation in decreasing yields and poor quality.

Materials and methods

Sample collection and fungus isolation

From local markets of Lahore samples of moldy onion have been collected in August 2011 and 2012. Moldy onion samples were examined at laboratory to clarify the

causal agents of those symptoms. A green fungal growth was observed from the necrotic areas of rotted onions. Under light microscope moldy tissues were observed. For surface sterilization 1% Na (O) Cl was used. Rotting tissue of 3 mm from the onions were placed onto 2% malt extract agar (MEA) and incubated at 25°C in darkness for 5 days.

DNA sequencing

The identity of the causal fungus had been proved by using tailored 2% CTAB method for extraction of total DNA (Doyle and Doyle, 1990). ITS1/ITS4 Primers were used to amplify the ITS (Internal transcribed spacer) region of rDNA (White et al., 1990).

Tests for pathogenicity

Healthy onions have used to confirm the pathogenicity of isolated organism. Subcutaneous scales of onions by means of a sterile needle were directly inoculated with conidial suspension (2×10^4 conidia mL^{-1}) from a pure culture of the fungus. Infected onions for 7 to 14 days were incubated at 25 °C.

Results and discussion

From moldy onions a species belonging to the genus *Penicillium* subgenus *Penicillium* was regularly found. Primarily it is characterized by heavy grayish turquoise sporulation, forming crusts, production of a reddish brown or orange to sienna pigment, its relatively slow growth on Czapek-based media and MEA at 25°C and inability to grow at 5 and 37°C on both Cz and MEA, according to key (Raper and Thom, 1949; Pitt, 1979, 1985; Ramírez, 1982; Samson et al., 1995; Pitt and Hocking, 1997). The fungal colonies were sub cultured on on Cz (Czapek Dox Agar), 2% MEA (Malt extract agar), CYA (Czapek Yeast Autolysate Agar) and G25N (25% Glycerol Nitrate Agar) at 25°C (Fig. 1) in order to allow the confirmation of the fungus identity. The description of our fungal specimen is given below.

MEA, 25°C, 7 days

Colonies diameter was from 20-30 mm, radially sulcate, centrally umbonate, moderately sparse with surface texture finely granular to the unaided eye, marginal hyphae entirely subsurface, bearing fascicles of conidiophores. Mycelium usually subsurface then became glaucous blue green (Fig. 1) due to conidiogenesis. Reverse of the colony was pale, yellow or brown.

CZ, 25°C, 7 days

Colonies diameter was from 25-30 mm, conspicuously radially sulcate and surface quality finely granular to the unaided eye. Mycelium white, usually inconspicous then became glaucous blue green and glaucous grey due to conidiogenesis. Reverse was pale, reddish to violet brown. Exudate present typically prominent and pale.

CYA, 25°C, 7 days

Colonies diameter was variable in size from 30-35 mm, conspicuously radially sulcate, moderately deep, with surface texture smooth to granular with the unaided eye. Mycelium white, usually inconspicous then became grayish turquoise to dull green due to conidiogenesis, sometimes in paler or brighter shades at the margins or centrally brown. Reverse of the colony was pale, reddish to violet brown. Exudate present typically conspicuous, pale.

G25N, 25°C, 7 days

Colonies diameter was from 20-25 mm in, radially sulcate, with a granular surface texture. Mycelium white, deep grey green, often with bluish marginal areas or centrally brown due to conidiogenesis. Reverse was pale, yellow or brown.

Conidiophores and conidia

Conidiophores mostly from subsurface hyphae, borne singly or in fascicles, with stipes commonly 200-400 × 3.0-4.0 µm, although sometimes longer or of indeterminate length because of fasciculation. Stipe was conspicuously roughened, bearing terverticillate or biverticillate penicilli. Rami 15-25 × 3.0-3.5 µm. Metulae (Fig. 2) in verticils of 3-4, measuring 10-12 (-15) × 2.8-3.5 µm. Phialides were ampulliform, in verticils of 5-8, 9-10 × 2.5-2.8 µm with undistinguished collula. Conidia smooth walled, subspheroidal to ellipsoidal, 3.5-4.0 × 2.5-3.2 µm.

DNA sequencing

The ribosomal internal transcribed spacer (ITS) region was amplified with ITS1 and ITS4 primers and sequenced. Sequence analysis showed that the ITS sequence of *P. aurantiogriseum* (GenBank Accession No. FN868484) was 100% identical to the ITS sequence of *P. aurantiogriseum* (HG326290).

Fig. 1: ***Penicillium aurantiogriseum.*** **A-C &G, 7-days old Colony at MEA, CZ, CYA and G25N, respectively.D-F and H, Reverse on MEA, CZ, CYA and G25N, respectively. I, Microphotographs (100 X) (bar = 10µm).**

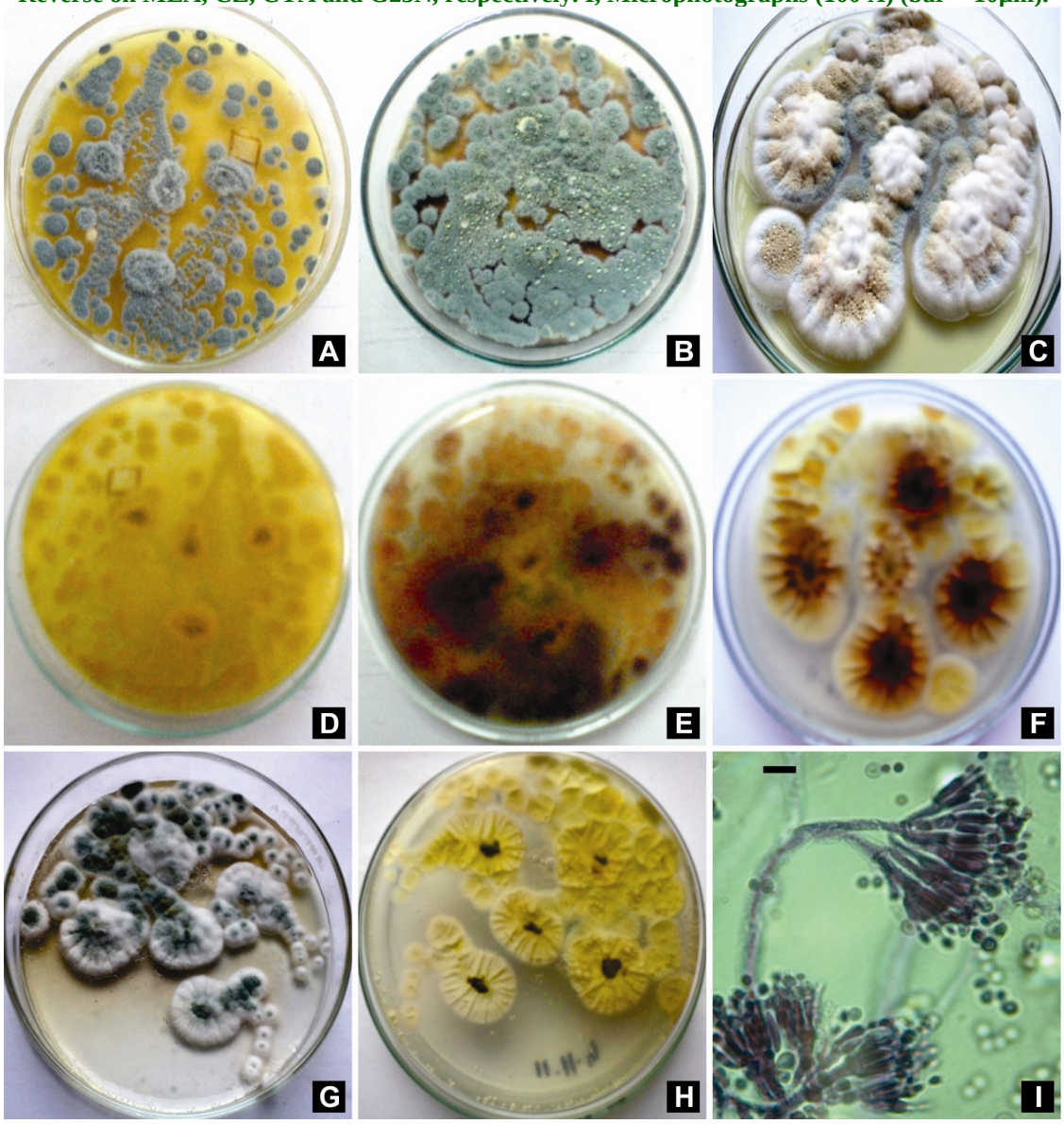
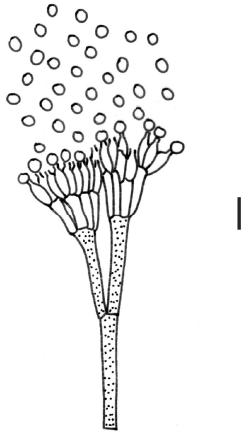


Fig. 2: Conidiophore and conidia of *Penicillium aurantiogriseum* (bar = 10µm).



Tests for pathogenicity

On the inoculated onions after 7 days typical symptoms were produced. From the inoculated onions the pathogen on 2 % MEA medium was re-isolated. The characteristics of the re-isolated fungus were compared with the original pathogen.

Conclusion

The work showed that the post harvest loss caused by *P. aurantiogriseum* is not good for stored onions. So it is necessary to protect the onions from such fungal infection and save our environment. This is also the first

report of postharvest rot of onions caused by *P. aurantiogriseum* in Pakistan.

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