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Research Paper

New fruit rot diseases caused by *Penicillium citrinum* Thom and *Fusarium sambucinum* Fukel. in star gooseberry (*Phyllanthus acidus* (L.) Skeels) from Andhra Pradesh, India

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Abstract. Fruit is used in the diet for humans, so during a routine survey of Star gooseberry trees (*Phyllanthus acidus*) in Andhra Pradesh, India, a pre-harvest fruit rotting was frequently observed during the cold months of November to January from the year 2015 to 2019. Based on the morphological and cultural characteristics, the pathogen responsible for this fruit rot is identified as *Penicillium citrinum* Thom and *Fusarium sambucinum* Fukel. The *Penicillium* fruit rot disease caused by *P. citrinum* developed symptoms like greyish-green spore masses around the decayed area with a musty odour. On the other hand, *Fusarium* fruit rot caused by *F. sambucinum* produced a distinct reddish or purplish rotten tissue in the infected area of mature fruit. Later the decayed or rotten tissue changes to brown lesions with the mycelium growth in tissue infected regions of fruits. For the first time, the pre-harvest fruit rot of star gooseberry by *P. citrinum* was reported from India and also noted it is a new record of disease in Andhra Pradesh.

Keywords: Fruit rot disease, star gooseberry, *Phyllanthus acidus*, *Penicillium citrinum, Fusarium sambucinum.*

INTRODUCTION

Phyllanthus name was coined from the Greek word "phullon" means leaf, "anthos" means flowers, the members of *the Phyllanthus* genus have flowers as dense clusters in leaf axils (Orwa *et al*., 2009). It is intermediate between shrubs and trees, having small, yellow, berries fruit. Fruit is a drupe, oblate, 1 - 1.9 cm × 1.2 - 2.8 cm in size when fresh, 6 to 8 lobed, greenish-yellow to creamy-white coloured; flesh firm, sour taste, with a hard, bony, stone endocarp containing eight smooth seeds (Orwa *et al.*, 2009). The fruit is tart in taste has a greenish-yellow exterior and a white to cream-coloured fleshy interior covering a pit of seeds (Brooks *et al.*, 2020). India has emerged as a major stakeholder in the global horticulture scenario accounting for 10.9% of the world's star gooseberry fruit production, with the total star gooseberry fruit production of 68.7 million tonnes from 5.5 million hectares (Hemalatha, 2010). 100 g of edible star gooseberry contains the following values: 91.9 g Water, 28 kcal energy, 0.155 g Protein, 0.52 g Fat, 6.4 g Carbohydrate, 0.8 g Fiber, 51% Ash, 5 mg Calcium, 2.43 mg Iron, 23 mg Phosphorous, 223.44 mg Potassium, 163.22 mg Calcium, 17.5 mg Sodium, 1.63 mg Zinc, 1.31 mg Manganese, 0.2 mg Copper, 0.019 mg carotene, 8 mg Ascorbic acid, 0.01 mg Thiamine, 0.05 mg Riboflavin, 0.292 mg Niacin (Reddy, 2017; Mahapatra *et al*., 2012). Phytochemical analyses on barks, leaves, roots and fruits of *P. acidus* identified triterpene, diterpene, sesquiterpene, and glycosides as predominant classes of bioactive substances in this plant (Tan *et al.*, 2020). Nelson and Cox (2005) reported that *P. acidus* contains 4-hydroxybenzoic acid, caffeic acid, adenosine, kaempferol and hypogallic acid. Chopra *et al.* (1992) reported that fruits flesh contains ascorbic acid, tartaric acids and tannin, root bark contains 18% tannin, gallic acids, saponins, lupeol. Stem bark includes a phytosterol, different from lupeol (Ghani, 2003). A new triterpene - phyllanacidol B is isolated from leaves

of *P. acidus* (Li 2020). The fresh fruit and dried form were utilized in Ayurvedic medicine as a liver tonic and blood purifier (Kirtikar and Basu, 1987). The methanolic extract of *P. acidus* has shown potential pharmacological activities to justify its use in folkloric medicines (Afrin *et al.*, 2016). Fruits of *P. acidus* were also used in the treatment of several diseases like jaundice, piles, constipation, vomiting, bronchitis, biliousness and urinary concretions (Kirtikar and Basu, 1987). The antioxidants present in the fruit have a hepatoprotective effect on the liver and are used as a laxative (Reddy, 2017; Jain *et al.*, 2011). Previous studies showed that fruits of this plant showed antioxidant, memory enhancing, anti-cholinesterase, astringent, hepatoprotective, cytotoxic and antimicrobial activity (Moniruzzaman *et al.*, 2015). The occurrence of *Alternaria tenuissima* on fruits of *P. acidus* at Madhavnagar constitutes a new host record. Both attached and fallen fruits were affected. Pathogenicity was confirmed experimentally by Patil and Maharaj (1986). Tahitian gooseberry seedlings and fruit of *P. acidus* (L.) Skeels. from Haleiwa, Oahu infected with a rust fungus (Berndt *et al.*, 2007). The rust fungus was identified by the USDA-APHIS-PPQ National Identification Services (NIS) as *Phakopsora phyllanthi* Dietel (Phakopsoraceae). It is the first record for this species in Hawaii and the U.S (Farr and Rossman 2015). The present study aims at occurrence, isolation, identification; cultural characters of fungal pathogens and fruit rot disease identification based on symptoms that appear in fruits of star gooseberries (*P. acidus* (L) Skeels) in Andhra Pradesh, India.

MATERIALS AND METHODS

A survey was conducted in the cold months like November to January from 2015 to 2019 for observing the fruit rot disease-causing fungi in different orchards and Fruit markets of Vijayawada, Krishna district, Andhra Pradesh, India. Around 100 wild fruits were selected in wooden packed fruit baskets and stored fruit baskets in a dark room; for the study of fruit rot diseases. Thirty-seven infected fruits samples are collected for isolating the fruit rot pathogens. Mature infected fruits were collected in sterile polyethene bags. The sample is brought to the laboratory to study symptoms, isolation, identification and cultural characters of fruit rotting fungi.

Isolation of fruit rotting fungi by Blotter Tests Method

The blotter method was used to isolate the fungal pathogens associated with the fruits. This method is also used to determine the healthiness of fruits in transport and storage. The samples were tested according to ISTA (1996). Paper towels are kept inside the Petri dishes, which conserve and maintain the moisture inside the dish so that fungi may get used to it. These plates are autoclaved at 121 lbs pressure for 30 min. three replicates were tested from each sample. The sample with and without treatment then fruit pieces were plated directly on top of three layers of well-soaked blotter paper. Four fruit pieces were placed on a Petri-dish of 9 cm diameter. The plates were incubated at 27°C temperature and relative humidity of 80 to 90% for seven days under darkness. After incubation, each piece was observed for fungal growth. Pathogenic fungi developing on fruit pieces were isolated. Fruit rot pathogen and the microflora associated with fruits were cultured on a potato dextrose agar media slant. The isolated fungi were identified based on the spore characters by staining with lactophenol cotton blue up to species level following the descriptions of Singh *et al*. (1991) and Samson *et al.* (1995).

Identification of fruit rot fungal pathogen

The fungal isolates were observed morphologically and microscopically. For this purpose, a few fungal mycelia were taken from a pure culture grown on PDA and stained with lactophenol cotton blue for visual observation. Sporulating isolates were identified down to species level with the help of standard manuals (Mathur and Kongsdal, 2003). Sterile isolates could not be assigned to any taxonomic group and were sorted into morpho-species based on colony surface texture, hyphal pigmentation, exudates, and growth rates, as described by Suryanarayanan *et al.* (1998). Such fungal forms were identified up to species level.

RESULT AND DISCUSSION

Isolation of fruit rots fungi

The survey was conducted in different orchards and markets of Andhra Pradesh, for the identification of fruit rots causing fungi and they were isolated by using the blotter test method as shown in Plate I Fig C, D, H. Out of 100 wild fruits collected from both packed and stored baskets 80% of fruits were spoiled by *Penicillium* fruit rot whereas the 60% of fruits were spoiled by *Fusarium* fruit rot The fruit rotting fungi isolated from star gooseberry is identified as *P. citrinum* Thom (Figure 1E) and *F. sambucinum* Fukel (Figure 1I). The *P. citrinum* Thom causing *Pencillium* fruit rot and *F. sambucinum* Fukel causing *Fusarium* fruit rot in *P. acidus* fruits was confirmed by pathogenicity test. The symptoms and cultural characters were given below.

Symptoms of *Penicillium* fruit rot

Fruit rot caused by *P. citrinum* Thom is more during fruit maturity, storage, and transportation. The infected area on

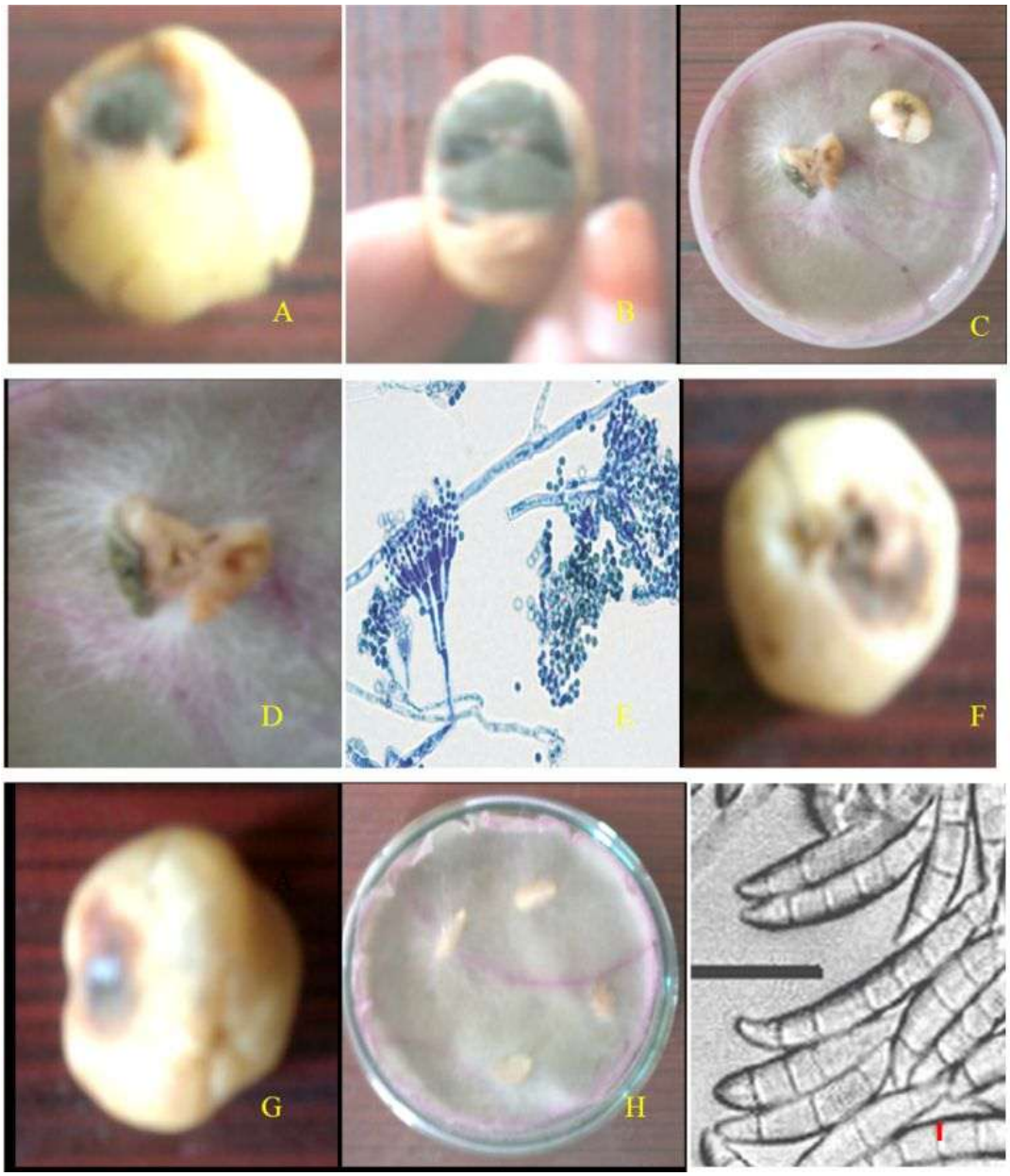

Figure 1. A. *Penicillium* fruit rot showing greyish green to black coloured patch with mycelium and conidia, B. *Penicillium* fruit rot caused by *Penicillium citrinum* producing greyish green coloured conidia on fruit, C. Isolation of fungi from *Pencillium* rot fruit samples by wet plate method, D. Mycelium growth from fruit infected with *Pencillium* for in wet plate method, E. Microscopic view of *Peniicillium citrinum* having conidiophores and conidia, F. Fruit infected with *Fusarium* fruit rot showing pinkish brown patch, G. Pinkish brown patch with mycelium on fruit infected with *Fusarium sambucinum*, H. Isolation of fungi from *Fusarium* rot fruit samples by wet plate method, I. Microscopic view of *fusarium sambucinum* macroconidia.

fruits appears as water-soaked spots, after some time spot turned into purple to dark brown colour due to the rotting of fruit then finally turned greyish-green colour (Figure 1A). The spots size increase due to the decaying of tissue by mycelium with greyish green spore masses (Figure 1B). Decayed tissue is soft, watery and readily separated from healthy tissue, leaving it like a bowl. The decayed tissue gets rotten and shows a musty odour.

Mishra and Hasib (2004) observed the blue or greyish-green spore masses appear on fruit in the decayed area of Aonla (*Emblica officinalis* Gaertn.). The presence of greyish-green spore masses at the decayed area and associated musty odour are the positively diagnostic indication of fruit rot caused by *P. citrinum* (Mishra and Hasib, 2004). In the present study also similar symptoms are observed on *P. acidus* fruit so fruit rot causing fungal

pathogen is identified as *P. citrinum* Thom. It is the first time reported from India on Star gooseberry fruits.

Cultural characters of P. citrinum Thom

Colonies on PDA is 21-28mm diameter, radially sulcate; surface texture floccose; conidiogenesis moderate to heavy, grey blue at the margin, elsewhere dull green; exudates absent; reverse pale brown to yellow-brown. Conidiophores arising from subsurface to surface hyphae, stipes 120-340 µm long, smooth-walled, biverticilate with 3-5 divergent metulae in a whorl; metulae usually uniform, 16-22 × 4-6 µm, bearing 5-10 phialids; phialids ampulliform, 8-10 × 2-3.5 µm; conidia globose to subglobose, 2.5 to 3.5 µm diameter, smooth-walled, produced in long columns (Figure1E).

The cultural characters of *P. citrinum* were studied by Thom (1910), Malmstrom *et al.* (2000) and Philip (2004). In the present study also some of colony characters on PDA medium were observed.

Symptoms of *Fusarium* fruit rot

F. sambucinum produced distinct reddish or purplish rotten tissues in the infected area of mature fruit. Later the decayed or rotten tissue changes to brown lesions with the mycelium growing in mature fruit (Figure 1F, G). The decayed tissues are demarcated from healthy tissues by depression and wound formation. The fungal pathogen enters into the fruit walls through wounds that happened during storage and post-harvesting periods (Figure 1G).

Fruits of *P. acidus* were affected by *Fusarium* rot (*F. roseum* Link). *Fusarium* rot is the fungal disease of this fruit reported for the first time in India (Cherian, 2002). In the present study, the fruit rot of star gooseberry is caused by the fungal pathogen *F. sambucinum*. It is a new record to Andhra Pradesh and the second time reporting from India as a new host record.

Culture characters of F. sambucinum Fukel.1869

On PDA after 10 days: Growth rate of isolates ranges from 8.5 to 9.0 cm length with a mean of 8.51 cm. Aerial mycelium is abundant, dense, white and the pigmentation is brown. Light orange sporodochia are common. Macroconidia: Curved, fusoid, lanceolate, 3-5 septate, 35-54 × 4.2-5.8 µm with pointed apical cell and foot-shaped basal cell (Figure 1I). Microconidia: Very rare, Chlamydospores: Sparse, terminal in hyphae, in chains and clusters, and smooth-walled.

F. sambucinum is a very important species in *Fusarium* taxonomy (Gams *et al.*, 1997). It is characterized by curved, fusoid, macroconidia with pointed apical cell and foot-shaped basal cell and sparse microconidia production and chlamydospores occurrenceimportant in the identification of *Fusarium* (Gams *et al.*, 1997). In the present study, similar characters were observed so it is identified as *F. sambucinum* causing *Fusarium* fruit rot on star gooseberry. *F. sambucinum* is associated with deterioration of avocado fruits (Zauberman and Schiffmann-Nadel, 1977; Darvas *et al*., 1987), fruit blotch of strawberries (Hunter and Jordan, 1974). In the present study, it is observed second time from India and first time from Andhra Pradesh on Star gooseberry fruits. Eggplant, an important vegetable crop in Iraq, faces yield losses due to early blight disease caused by *Alternaria solani* (Matrood *et al.*, 2021). In the present study the Star gooseberry tree is an important medicinal plant having antioxidant properties, rich sources of vitamin C in fruits; which is infected by two fruit rot pathogen like *Penicillium* and *Fusarium* respectively.

CONCLUSION

The study of underutilized fruit crop (Star gooseberry) facing the fruit rotting disease is important as the fruits and their extracts are used in medicine for several diseases like as astringent, cathartic, as an antidote to viper venom, asthma, bronchitis, hypertension, respiratory problems, urticaria, piles, psoriasis, constipation amnesia, diabetes etc. So much important medicinal plant fruit rot disease identification is very much needed. In the present study, the fruit rotting disease was identified as *Penicillium* and *Fusarium* Fruit rot. The causal organisms are isolated and identified as *P. citrinum* and *F. sambucinum.* The management of disease control is very important to increase the crop yield; so at present, there is no approved specific fungicide to control the fruit rot disease. The present study recommends good sanitation practices, such as removing and bagging or destroying infected fruits as soon as symptoms appear. Sanitizing the tools before and after use and keeping the orchard area clean and dry. This study gives preliminary information on fruit rot diseases but it gives wide scope to plant pathologists to find eco-friendly methods and Bio-control methods for controlling these fruit rot diseases.

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