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New report of *Curvularia pallescens* Boedijn. as leaf spot pathogen in *Manilkara zapota* (L.) P. Royen in India

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Abstract

Manilkara zapota (L.) P. Royen, commonly called as *'Cheekoo, Chikku'*, 'Sapota' or 'Sapodilla', belonging to the family Sapotaceae, is one of the fleshy fruits with high export value in India. The sapota tree is susceptible to many fungal diseases. Many fungi like *Phaeophleospora indica, Pestalotia sapotae* L., and *Glomerella cingulata* are reported to cause leaf spot disease in *M. zapota*. In the present study, the leaf spot disease of *M. zapota* found in Kumaranellur area of Palakkad district of Kerala was subjected to isolation and identification of the causative organism. The disease was characterized by small reddish-brown spots on the upper surface of the leaves. The circular spots gradually enlarge radially producing necrotic lesion at the centre. The margins of the spot showed yellowish or orange color initially. The entire leaf lamina becomes brown and shed off in advanced stages. The lesions were prominent over the upper surface of the leaves. The organism from the diseased sample was isolated and characterized. The pathogenicity of the isolate was proved and identified as *Curvularia pallescens* Boedijn. This is the first report of incidence of leaf spot disease on *M. zapota* by *Curvularia pallescens* Boedijn.

M*anilkara zapota* (L.) P. Royen is one of the economically important crop with high export value for its fruits. The genus *Manilkara* includes 30-32 species, most of which are economically important and commercially used as source of fruit, timber and latex[4]. It has its origin in Mexico and is native to Central America, although it is also cultivated in Asian countries including India[5]. In 2016-17, the area of cultivation of sapota in India was about 97,000 hectares (Ha.) with an annual production of 11,76,000 Metric Ton (MT)[2]. *M. zapota* is susceptible to many diseases. Leaf spot caused by *Phaeophleospora indica* Chinn. is the most serious disease[1]. Rajendran[8] reported *Pestalotia* species as causative organism of leaf spot disease. Highest disease incidence of the disease was noted during October to December by Pathak[7]. Patel[6] reported *Pestalotia sapotae* L. as the reason for foliar disease in Gujarat.

Khalequzamman *et al*.,[3] studied the yearly incidence of leaf spot disease of *M. zapota* in Bangladesh. They reported highest percentage of incidence of leaf spot and diseased area of leaf in the month of December. They also reported that low temperature, low rain fall and dry weather will increase the intensity of the disease. Thammaiah *et al*.,[10] also reported *Phaeophleospora indica* as leaf spot pathogen in sapota. Another pathogen that was reported to cause leaf spot disease in sapota was *Glomerella cingulata*[9]. The disease is a serious problem in the states of Karnataka, Tamil Nadu and Maharashtra[10]. No records from Kerala are available on the leaf spot disease in *M. zapota*. The present investigation was to isolate and identify the pathogen of leaf spot disease in *M. zapota* from Kerala. In the present study, the leaf spot diseased samples of *M. zapota* collected from Kumaranellur area of Palakkad district, Kerala, were subjected to study the causative organism.

Collection of diseased samples and isolation of pathogen :

The diseased leaf samples of *M. zapota* were collected from Kumaranellur area of Palakkad District, Kerala. All the symptoms noted on the diseased plants were studied in detail and recorded. The samples were collected in polybags and brought into the plant pathology laboratory of The Zamorin's Guruvayurappan College, Kozhikode, Kerala. The symptoms of the collected leaf samples were studied in detail to record the features of spots on leaf lamina. The leaves were washed thoroughly with tap water to remove the dust particles. Small bits cut from the lesions along with some healthy portions were surface sterilized with 0.1% $HgCl_2$ for 1-2 minutes followed by washing in sterile distilled water for three times. The surface sterilized pieces were kept in sterile blotting paper to remove excess water. It was then transferred onto Potato Dextrose Agar (PDA) in 90 mm sterilized Petri plates and incubated at room temperature for 3 days. The hyphal tips were transferred onto PDA slants for further studies. The fungal cultures thus grown from the specimen were sub cultured from time to time and maintained on PDA slants.

Pathogenicity of the isolate :

The organism isolated from the diseased leaf samples of *M. zapota* was tested to prove Koch's postulates. Detached leaf method was adopted to prove the pathogenicity. Healthy and mature young leaves of *M. zapota* were collected. The leaves were washed with 70% ethyl alcohol followed by sterile distilled water. The leaves were incubated in sterile plastic box containing wet filter paper and moistened cotton. Small pricks were made on leaves using sterile needle. An agar disc cut from the margin of actively growing hyphae of the fungal isolate was placed in contact with pin prick. An agar disc without pathogen was kept as control. The humidity inside the inoculation box was maintained by placing wet absorbent cotton inside. The box was incubated at room temperature for five days. The part of leaf which showed infection was cut along with healthy part and inoculated onto PDA for reisolation of pathogen as per the methods mentioned before.

Identification of the pathogen :

Different media were tested for the sporulation and identification of the pathogen. Agar discs of 5 mm size cut off from the actively growing pathogen were inoculated at the centre of the 90 mm Petri plates containing media like Carrot agar (CA), Potato-Carrot agar (PCA) and Cornmeal agar (CMA). The plates were incubated at room temperature and regularly observed for 21 days at 3 days interval. The fungal hyphae taken onto glass slides under sterile condition were stained with lactophenol cotton blue stain for microscopic analysis. The microphotographs and measurements were taken using digital camera attached to stereo microscope with the help of software 'IS Capture' Version 2.1.

The diseased leaf samples of *M. zapota* collected were analyzed in detail. The symptoms noted were compared with the symptoms mentioned in earlier reports. The spots on leaves were reddish brown (Fig. 1A & B). The size of the spots ranged from 3-6 mm in diameter. In some areas, the spots were coalesced to form a large spot (Fig. 1A & B). The lesions were prominent over the upper surface of the leaves. The lower surface was characterized by reddish brown colored lesions (Fig. 1A). An orange-red colored margin was noted in large spots (Fig. 1B). The necrotic area was prominent in central part of the large spots which was grey in color (Fig. 1A). The lesions were also found on midrib (Fig.1B). In the advanced stages of the disease, the entire leaf lamina became yellow and showed senescence and leaf fall. Growth of the fungal mycelium from the inoculated leaf pieces started only after 48 hours in PDA plates. The actively growing hyphal tips excised from the margin developed a greyish white colony which later turned into black color after 120 hrs of growth. The sub cultured plates were kept for further studies.

Detached leaf method was used to prove Koch's postulates. No lesion was noted for 48 hrs in and around the region of injury made with sterile needle. A black colored dark spot surrounded by reddish brown border was noted to develop after 72 hrs. The size of the lesion was found to increase after 96 hrs (Fig. 1C). The infected area which was black colored was found to reach up to midrib after 120 hrs of incubation. The midrib was also later found to be infected by the pathogen. The marginal region of dark colored lesion on infected leaf was incised, surface sterilized and inoculated on to PDA. A fungal colony was found to grow after 48 hrs of inoculation. No other fungal or bacterial organisms were found to grow from the inoculated materials. The subcultured fungal colony from this showed similar morphological and cultural characteristics of the fungus that was inoculated to prove pathogenicity in *M. zapota* leaf.

The cultural and morphological characters of the pathogen were noted and recorded. It produced only vegetative hyphae on PDA even after 3 weeks of incubation. Hence other media like CA, PCA and CMA was tested to induce sporulation of this pathogen. The colony of this pathogen starts as grey colored and forms a uniformly spreading colony. Later it turns to black color at its centre and greyish at its margin. Concentric pattern of colony morphology was noted in PCA. The isolate was found to

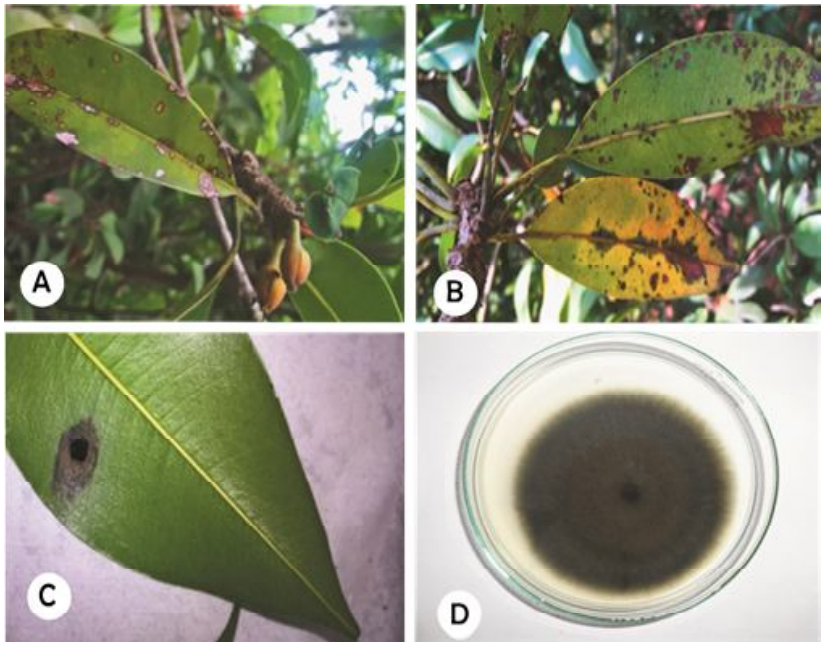

Figure 1. A & **B**. Different stages of leaf spot disease in *M. zapota*. **C**. Pathogenicity test by detached leaf method. **D.** Colony morphology of pure culture of *C. pallescens.*

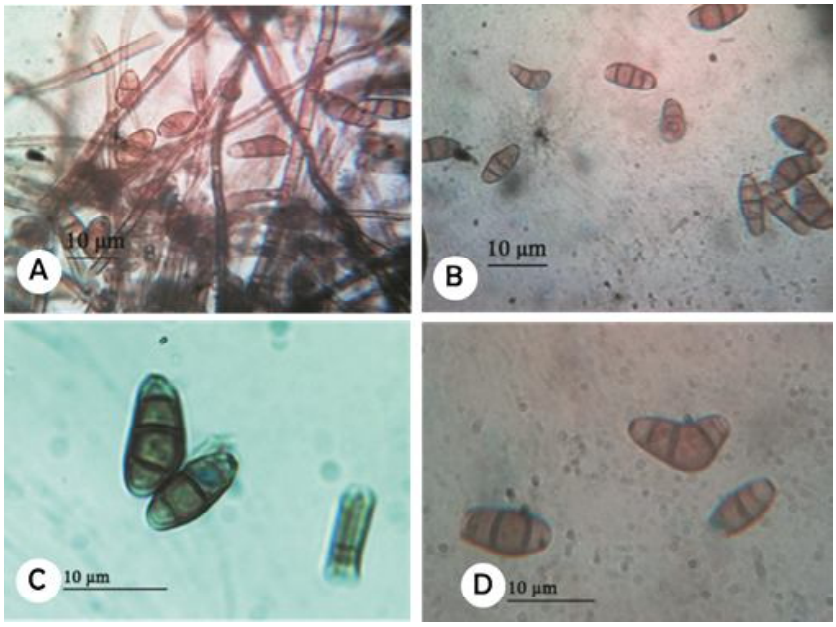

Figure 2. Photomicrograph showing the morphology of *C. pallescens.* **A**. Fungal mycelium with conidiospores **B. - D.** Conidia of *C. pallescens*

produce spores after six weeks of inoculation. The conidia were seen clustered around the conidiogenous hyphae in fungal mycelium grown in PDA and water agar. The fungal mycelia were anastomosing in clusters. The fungal conidia were seen sympodially on conidiogenous cell. Condia were ellipsoid to oboval, slightly curved with 3-4 septa (Fig. 2D). The median cell was large and slightly curved (Fig. 2D). The two terminal cells are relatively smaller. The size of the conidia ranged from 12 - 28 µm X 6-10µm (Fig. 2C). The pathogen was later identified and confirmed as *Curvularia pallescens* Boedijn. from Agharkar Research Institute (ARI), Pune. This is the first report of incidence of leaf spot disease on *M. zapota* by *Curvularia pallescens* Boedijn. in India.

The leaf spot disease in *M. zapota* reported earlier were caused by some other pathogens. There are a few earlier reports of fungal pathogens like *Phaeophleospora indica* Chinn.[1,10] and *Pestalotia sapotae*[6] associated with the leaf spot disease in *M. zapota*. Another pathogen reported to cause foliar disease in sapota was *Glomerella cingulata*[9]. The symptoms of the present disease are almost similar to symptoms of leaf spot diseases caused by other pathogens. Many authors have reported reduction in yield due reduction in photosynthetic area of leaves[3,7]. The management of the disease will be effective only after the accurate identification of the pathogen. The pathogen reported in the present study has not been reported from *M. zapota* as a leaf spot pathogen from India till the date. Hence, it is a new report of *C. pallescens* from India to cause leaf spot disease in *M. zapota.*

Many pathogens have been reported to be associated with leaf spot disease in *M. zapota*. Most of them are fungal pathogens. The leaf spot disease in *M. zapota* in the present study reports *C. pallescens* as causative organism in the samples collected from Palakkad District of Kerala. This is a new report of this pathogen associated leaf spot disease in *M. zapota* in India.

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