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# Isolation, identification and proving the pathogenicity of banana anthracnose pathogen *Colletotrichum musae*

■ RANI R. UNNITHAN* AND N. THAMMAIAH

College of Horticulture, Yalachahally Horticulture Farm, Yelwala, MYSORE (KARNATAKA) INDIA

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***Corresponding author:**

ABSTRACT :

*Colletotrichum musae* was isolated from dark-brown anthracnose lesions on banana (*Musa* sp.) to establish the causal agent of the symptom. The fungus grew fast and producedpale red mycelial growth on PDA when incubated at 28 $^0$C for 7 days. Conidia were aseptate, hyaline, mostly ellipsoid, ranging from 10-18 µm and 5-9 µm (average of 14.5-6.9 µm).The isolates of *C. musae* caused black necrotic lesions on banana fruits byneedle-wound inoculation and orange-coloured sporemasses were produced on the lesions. The control fruits which were not inoculated with the fungus did not show any symptoms of the disease.

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## INTRODUCTION

Banana (*Musa* sp.) belongs to the family *Musaceae* is one of the most important crops in tropical and subtropical countries. In many Asian and African countries, banana is an important staple food crop next to rice, wheat and maize.Anthracnose caused by *Colletotrichum musae* (Berk. and Curt.) Arx. is one of the most important and widely distributed diseases of ripening and ripe bananas and is particularly associated with wastage following injuries in the form of scratches and other wounds sustained by the fruits during handling and transport (Wardlaw, 1934). There are also losses in local markets because of disease infection of ripe fruits. The fungus can infect banana fruits at any time during the growing season in the field (Simmonds and Mitchell, 1940). Banana anthracnose usually starts as quiescent infections on green fruit in the field. However, successful penetration of the fungus is restricted by accumulation of phytoalexins as the fruits ripen (Jegger *et al.*, 1995 and Turner, 1995). Therefore, symptoms generally can be seen only in overripe fruits. Anthracnose becomes a serious problem when bananas are shipped as bunches for a long time and ripened under high temperature (Meredith, 1960a). *Colletotrichum musae* is the most important pathogen on wounded green and ripe banana fruits (Meredith, 1960b and Stover and Simmonds, 1987b). Occasionally, the fungus invades necks of green fingers when damaged by flexing (Wardlaw, 1995). Lesions are sunken and covered with salmon-colored acervuli (Sutton and Waterston, 1970). Infections stimulate ripening of

fruits and lesions elongate with ripening. On ripening fruits, sunken brown spots develop with orange acervuli (Stover and Simmonds, 1987). This study was conducted to identify the casual agent associated with anthracnose lesions on banana fruits and to prove the pathogenicity of the fungal isolates.

## MATERIAL AND METHODS

**Isolation of the pathogen :**

Banana fruits showing typical symptoms of anthracnose were collected from Kittur Rani Chanamma College of Horticulture, Arabhavi during January 2013. Standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues along with healthy portions were surface sterilized with 0.1 per cent mercuric chloride solution for 30 sec and such bits were transferred to petridishes containing sterile water successively for three times and then bits were transferred into petridishes containing 15 ml of potato dextrose agar medium and incubated at 28 °C for 7 days. Pure culture of the fungus was obtained by single spore isolation and hyphal tip isolation method.

**Single spore isolation:**

A loopful of well sporulated culture was taken with help of inoculation needle and suspended in to sterilized water blank, serial dilutions were made so as to obtain 50 spores/ml and spore suspension was mixed with 2 per cent molten agar in the ratio of 1:15, poured into sterile petridishes and allowed to solidify. After solidification, Petridishes was inverted on the stage of the microscope and examined under low power objective. Separated spores were located and marked them with glass marking pencil. Marked area was cut with a help of sterilized scalpel and transferred the blocks into centre of Petri plates containing potato dextrose agar medium and incubated at 28°C for 8 days. Such cultures were used for further studies.

**Identification of the pathogens :**

The cultures were identified based on spore morphology and colony characters :

**Maintenance of culture :**

The culture of *Colletotrichum musae* was maintained at 5°C in the refrigerator and subcultured periodically at an interval of 30 days during the course of investigation.

**Proving the pathogenicity :**

Fungal inocula were prepared in the laboratory by inoculating 5 mm disc to the potato dextrose broth which was cut at periphery of the actively growing culture and incubated at 26±10°C for 7 days. The conidial suspension was prepared by adjusting conidial concentration to 4 x $10^6$ cfu/ml by adding sterile distilled water to the inoculum. Mature banana fruits were brought from the orchard of All India Co-ordinated Research Project on Fruits, K.R. C. College of Horticulture, Arabhavi, Belgaum district. The fruits were thoroughly washed with sterilized distilled water using moist cotton and air dry it . Later inoculation to the fruits was done with the help of atomizer by spraying with spore suspension of (4 x $10^6$ cfu /ml) prepared from seven days old culture in sterile distilled water. The fruits were covered with polythene bags for 48 hours to maintain sufficient humidity and to ensure successful penetration of the pathogen in to the host tissue. Control fruits were maintained by atomizing sterile distilled water in the similar manner. Observations were recorded for the appearance and development of symptoms. After symptom development, reisolation was done from the artificially infected fruits. The isolate obtained was compared with the orginal culture for confirmation.

**Morphological studies:**

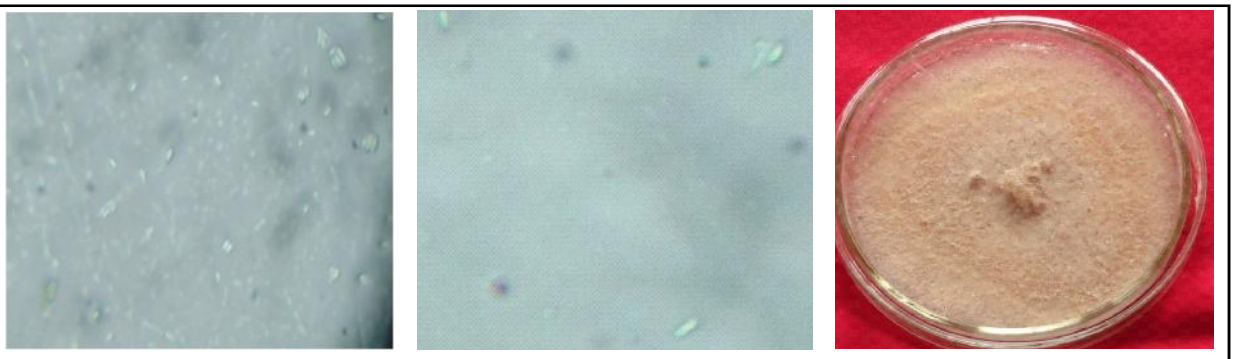
*Spore shape and size*:

Spores of *Colletotrichum musae* were taken from infected banana fruits and mounted on a clean glass slide; spores were mixed with lactophenol thoroughly in order to obtain uniform spread, over which a cover slip was placed. One hundred spores were measured under high power objective using ocular and stage microscope. The average size of the spores was calculated. Photomicrographs were taken to show the typical spore morphology of the fungus.

## RESULTS AND DISCUSSION

Standard tissue isolation technique was followed to obtain *Colletotrichum musae* from banana fruits showing typical symptoms of the disease. Repeated isolation of the fungus was done from the banana fruits collected which yielded a species of *Colletotrichum.*

The description of the fungus isolated from banana

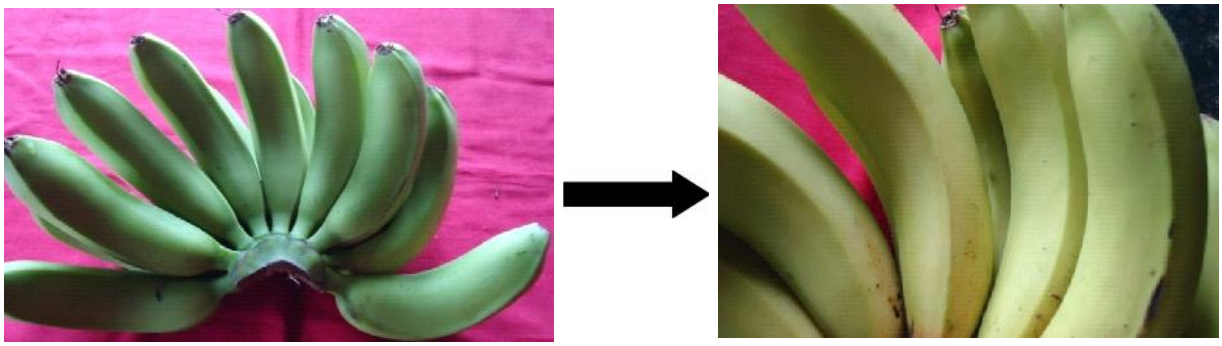


(a) Condia of *Colletotrichum musae*

(b) Pure culture of *Colletotrichum musae*

Inoculated

Uninoculated



(C) Pathogenicity of *Colletotrichum musae*

**Plate 1 : Isolation, identification of the pathogen and proving pathogenicity**

fruits is as follows. The colonies first appeared as pale red mycelial growth on potato dextrose agar when incubated at 28 $^0$C for 7 days. Subculturing was done aseptically on potato dextrose agar medium and incubated at 28 $^0$C. The colony was pale red in colour (Plate 1). Similar results were recorded by Jinyoung Lim *et al.* (2002) and Thangamani *et al.* (2011). Jinyoung Lim *et al*. (2002) reported the isolation of the fungus *Colletotrichum musae* from anthracnose lesions on bananas. After single spore isolation, the isolate ST-01 was selected and details of the characteristics of the isolates were investigated. ST-01 grew well on PDA medium.

Pathogen associated with anthracnose disease was isolated on PDA medium and it was identified as *Colletotrichum musae* (Berk. and Curt.) Arx based on the morphological and cultural characteristics of the fungus (Thangamani *et al.*, 2011).

**Identification of the fungus:**

Conidia of *Colletotrichum musae* isolates were aseptate, hyaline, mostly ellipsoid, ranging from 10-18 µm and 5-9 µm (average of 14.5-6.9 µm). Mycelium colour of *Colletotrichum musae* was whitish to pale red and having a regular or an irregular margin with both submerged or aerial topography depending upon media used for growth. This is in agreement with the results of Mordue (1971); Jinyoung Lim *et al*. (2002); Photita *et al*. (2005) and Thangamani *et al.* (2011).

Mordue (1971) enumerated the morphological characters of *Colletotrichum musae*. The conidia was of ellipsoidal to cylindrical shape, brown colour and 11-17×3-6 mm size and the apperessoria was irregularly lobed with dark brown colour and 6-12 × 5-10 mm size.

Jinyoung Lim *et al.* (2002) studied the cultural and morphological characters of *Colletotrichum musae*. They observed that the colony was loose with white aerial mycelium, which later becomes orange in colour. Several black, acervulus-like masses developed on the culture plates after incubation for 10 days at 25°C with dark-orange drops of conidia. Conidia were aseptate, hyaline, mostly ellipsoid, from10-18 µm and 5-9 µm (average of 14.5-6.9 µm ) in size.

Photita *et al*. (2005) reported that the *Colletotrichum musae* isolates were pathogenic on banana. The cultures were distinct with fast growing sparse aerial mycelium, white, with copious cinnamon conidial masses, elliptical shaped conidia and setae absent in coloured acervuli.

Thangamani *et al*. (2011) reported that all the isolates of *Colletotrichum musae* produced hyaline cylindrical conidia. Significant variations were observed with respect to conidial dimensions among the isolates. The length of conidia ranged from 10.2-14.7 µm.

**Pathogenicity test:**

Pathogenicity test was proved by inoculating the pure culture of *Colletotrichum musae* to the healthy banana fruits cv GRAND NAINE. Symptoms of black circular sunken spots on the peel developed after 5 days of incubation. The results are in line with findings of Griffee and Burden (1974). The reisolation of cultures from these artificially inoculated fruits were similar to that of original culture. The control fruits which were not inoculated with the fungus didnot show any symptoms of the disease (Plate 1). Similar results were recorded while proving pathogenicity by Bhat (1991) and Ekbote (1994) on pomegranate and mango respectively. Kota (2003) proved the pathogenicity of *Colletotrichum gloeosporioides* on mango and banana.

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