

FIRST REPORT OF *COLLETOTRICHUM ACUTATUM* ON *MANGIFERA INDICA* IN SRI LANKA

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ABSTRACT

Colletotrichum acutatum is known to have a wide host range and has become an increasingly important pathogen on many economic crops worldwide. This is the first report of *Colletotrichum acutatum* on mango in Sri Lanka. *Colletotrichum gloeosporioides* together with *C. acutatum* are considered as causal agents of this disease. *Colletotrichum acutatum* was characterized by fusiform conidia and white to orange colonies with slight shades of light mouse grey aerial mycelia. *Colletotrichum gloeosporioides* produced grey colonies with a dark mouse grey centre and conidia were cylindrical. The other differences between the mango isolate of *C. acutatum* and *C. gloeosporioides* were the slower growth rate and extremely high tolerance of *C. acutatum* to the fungicide carbendazim.

Key words: *Colletotrichum gloeosporioides*, *Hevea brasiliensis*, carbendazim

INTRODUCTION

Colletotrichum acutatum (Simmonds ex Simmonds) is pathogenic on a number of economically important fruit and tree crops worldwide. The pathogen is declared as a quarantine pest in member countries of the European community (EC) and has now become an increasingly important plant pathogenic fungus.

Mangoes are an important export fruit for many tropical and sub-tropical countries. Anthracnose is one of the most widespread and common diseases, which causes premature fruit drop and direct reduction in quality of ripe fruits shortening the storage lifetime (Dodd *et al.*, 1992). During epidemics it affects young leaves resulting severe spots and blackening of tips (Fig. 1a). Affected flowers fall off causing lowered fruit set. The most conspicuous symptom of the disease is the circular, dark, sunken anthracnose lesions on ripe fruits (Fig. 1b).

The anthracnose spreads throughout Sri Lanka during monsoons resulting in a considerable economic loss in many crops. With the discovery of *C. acutatum* as the main cause of rubber anthracnose in Sri Lanka (Jayasinghe *et al.*, 1997), an island-wide survey was conducted to re-investigate the pathogens responsible for anthracnose diseases of crops

cultivated in and around the rubber plantations. The present study was undertaken to confirm the identity of *C. acutatum* on mangoes.

MATERIALS AND METHODS

Isolation of the pathogen

The pathogen was isolated from symptomatic mango leaves collected from several locations in the Kalutara district after surface sterilization with 70% ethanol. Pure cultures were obtained and single spore isolates were maintained on potato dextrose agar (PDA).

Koch's postulates were proved using tender mango leaves and fruits. Drops (0.02ml) of an aqueous conidial suspension (1×10^5 spores/ml) prepared from 7-day old cultures were used for inoculations. They were incubated at room temperature (RT) $28 \pm 2^\circ\text{C}$ in humid chambers (Rh- 100% approx).

Identification of *Colletotrichum* spp.

The two species were identified based on criteria described by Jayasinghe and Fernando (1998) and the identify of one of the isolates was confirmed as *C. acutatum* by CABI, UK.

Culture morphology (colony colour), growth rate and the conidial morphology were observed using 6-day-old cultures grown on PDA which were incubated at RT under continuous

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fluorescent light. Sensitivity of the isolates to different concentrations of carbendazim (Bullet 50% a.i., Agrosesca, Spain) to distinguish the two species (Jayasinghe and Wijesundera, 1995; Jayasinghe and Fernando, 1998).

Pathogenicity tests

Pathogenicity of the two *Colletotrichum* spp. (MA 1, MA 2, & MG 1, MG 2) was tested on young mango leaves. Drops (0.02ml) of an aqueous conidial suspension (1×10^5 spores ml^{-1}) prepared from 7-day-old cultures of each isolate were placed on copper brown leaves and six

leaves were inoculated with each isolate. Later cross infection ability of the two isolates was tested on young detached leaves of *Hevea* rubber (clone RRIC 121). Six drops of conidial suspension (0.02 ml, 1×10^5 spores ml^{-1} prepared from 7-day-old cultures) were placed on either side of the midrib on the lower surface of each leaf. Inoculated leaves were incubated at $28 \pm 2^\circ\text{C}$ (RT) in humid chambers. Specimens inoculated with sterile distilled water drops served as controls.



Figure 1. (a) Mango leaves affected with *Colletotrichum* leaf disease showing spots and blackening of tips, (b) Circular, dark sunken anthracnose lesions on ripen fruits, (c) Conidia of *Colletotrichum acutatum* fusiform – tapered to a point in both ends and (d) Conidia of *Colletotrichum gloeosporioides* cylindrical with rounded ends.

RESULTS

Isolates obtained from the affected leaves of mango, consistently produced two types of colonies. *Colletotrichum gloeosporioides* produced dark grey colonies and formed typically cylindrical conidia with rounded ends (Fig. 1d). The other colonies were white to orange in colour, with slight shades of pink and light mouse grey aerial mycelium. On the reverse side, the centre was dark orange to pink and the conidia produced were fusiform (Fig. 1d) (tapered to a point in both ends). *C. acutatum* from both hosts showed significantly slower growth rate of the colonies compared to *C. gloeosporioides* isolates.

Observations on the sensitivity of the two species to fungicides *in vitro* showed that *C. gloeosporioides* (both mango and rubber isolates) were extremely sensitive to carbendazim whereas more than a 1000 fold increase of fungicide concentration was needed to obtain 90-100% growth inhibition (EC₉₀₋₁₀₀) in *C. acutatum* (Table 1). One of the isolates identified as *C. acutatum* was sent to CABI, UK and authenticated as *C. acutatum* (IMI 391758). The specimen has been deposited in the IMI culture collection.

Table 1. Concentrations (ppm) of carbendazim required to inhibit 90 – 100% mycelial growth in *C. acutatum* and *C. gloeosporioides* isolates.

Isolate	Concentration of fungicide required to obtain EC ₉₀₋₁₀₀ (ppm)
RA 1	> 4000
RA 2	> 4000
MA 1	> 4000
MA 2	> 4000
RG 1	< 25
RG 2	< 25
MG 1	< 25
MG 2	< 25

RA 1 & RA 2 – Rubber isolates of *C. acutatum*

MA 1 & MA 2 – Mango isolate of *C. acutatum*

RG 1 & RG 2 – Rubber isolates of *C. gloeosporioides*

MG1 & MG 2 – Mango isolates of *C. gloeosporioides*

All mango leaves inoculated with either *C. gloeosporioides* or *C. acutatum* developed typical anthracnose lesions. In cross inoculation studies, rubber isolates (RA 1, RA 2, & RGI, RG 2) produced lesions on both rubber and mango leaves. Mango isolates (MA 1, MA 2 & MG 1,

MG 2) too produced lesions on both mango and rubber but the size of the lesions on rubber was comparatively smaller.

DISCUSSION

This is the first report of *C. acutatum* causing anthracnose of mango in Sri Lanka (CMI, 1965-1988 & CAB PEST CD, 1989-2007). *Colletotrichum gloeosporioides* was believed to be the only cause of mango anthracnose in Sri Lanka. (Alahakoon & Brown, 1994),

The culture and reproductive characteristics that have been previously utilized by various workers have been employed to distinguish between the two species (Adeskaveg & Hartin, 1997; Jayasinghe *et al.*, 1997). The insensitivity of *C. acutatum* isolates against carbendazim has been utilized by the same authors to distinguish isolates of *C. acutatum* from *C. gloeosporioides* from rubber and *Flacourtia inermis* (Jayasinghe & Fernando, 1998; 2004). Further; high tolerance of *C. acutatum* to this group of fungicides compared to *C. gloeosporioides* has been shown by various workers for strawberry, peach, almond, apple, pecan and citrus isolates (Adaskaveg & Hartin, 1997; Bernstein *et al.*, 1995; Sonada & Pelosi, 1988). Our observations confirm that insensitivity to carbendazim, slower growth rate, fusiform conidia are reliable characteristics to distinguish mango isolate of *Colletotrichum acutatum* from *C. gloeosporioides*.

Based on our findings, we propose that both *C. acutatum* and *C. gloeosporioides* should be considered as causal agents of mango anthracnose. Therefore we also recommend re-investigation of anthracnose pathogens already reported as *C. gloeosporioides* on all fruits in Sri Lanka as the species *C. acutatum* has been shown to be an increasingly important pathogen of fruits worldwide.

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