SHORT COMMUNICATION

FIRST REPORT OF *CLADOSPORIUM* INFECTION OF MANGO INFLORESCENCE IN THE MID-COUNTRY OF SRI LANKA

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ABSTRACT

Inflorescences with moderate to abundant green-gray mycelial growth were observed in several mango cultivars during 2008 and 2012 in Peradeniya-Kandy area. Fungal growth was first observed when inflorescences were in full bloom and heavy fungal colonization could be seen at the time of fruit set. The infected flowers, pedicels and small fruits became completely covered with the fungal mycelium, turned brown and dropped as the disease progressed. The fungus was isolated from diseased inflorescences and identified as *Cladosporium* sp. by reproductive and colony morphology. Original disease symptoms were reproduced after artificial inoculation and the same fungus was re-isolated from inoculated inflorescences suggesting that *Cladosporium tenuissimum* may be the causal organism.

KEYWORDS: *Cladosporium* infection, inflorescence disease, *Mangifera indica L.*

INTRODUCTION

Asia is the largest mango (*Mangifera indica* L.) producer, representing more than 75% of global production (FAOSTAT, 2012). According to the FAO's 2009 Food Market Analysis of Tropical Fruits, mangoes dominated world production at 31.5 million metric tons, comprising 40% of global tropical fruit output. Present extent under mango cultivation in Sri Lanka is about 12,160 ha (Mankotte, 2006) producing 86,580 tons in 2010 (FAOSTAT, 2012).

Flower and fruit abortion are two major problems that hamper global mango production. A few hundreds to over a thousand of mango flowers are formed on branched apical inflorescences. Initially hundreds of fruits can be set

on each flowering inflorescence. The tree thins the crop by self-shedding fruit throughout the fruit development period. The critical phases that directly affect mango production are flowering and fruit set.

All parts of the mango tree, trunk, branch, twig, leaf, petiole, flower and fruit, are attacked by a number of pathogens. Various fungi infect the mango flower and fruit before maturity. The most important floral diseases reported worldwide are blossom blight or anthracnose (*Colletotrichum gloeosporioides*), powdery mildew (*Oidium mangiferae*), inflorescence malformation (*Fusarium mangiferae*) and sooty moulds (Ploetz, 2003). Powdery mildew is of great economic importance as it causes heavy losses in mango production. A few affected flowers may lead to a widespread epidemic under favorable climatic conditions and ultimately lower crop production. However, inflorescence diseases are not given much attention and as a consequence, losses occur when conditions conducive to infection are present or proper management practices are not adopted.

Guillén-Sánchez et al. (2007) reported for the first time *Cladosporium tenuissimum* causing severe damages to mango inflorescence in Mexico. Very similar disease symptoms were observed in Peradeniya-Kandy area during flowering seasons in 2008 and again in 2012. The objective of the present study was to establish the cause of the disease observed in inflorescences of several mango cultivars.

MATERIALS AND METHODS

The investigation was carried out in Peradeniya-Kandy (7° 17' 4.15"N, 80° 38' 14.08"E) area in the Central Province of Sri Lanka. While '*Vellaicolomban*', *'Gira'*, *'Peterpasand'* and *'Dampara'* are the cultivars recommended by the Sri Lanka Department of Agriculture for the cultivation in the wet zone, other cultivars including *'Karuthacolomban'*, *`Kohu*' and *'Willard'* are also grown predominantly as home garden trees in this area.

Five inflorescences each randomly selected from 30 trees of mango cultivars, *`Vellaicolomban*', *`Kohu*', *'Gira'*, *'Peterpasand'*, *'Dampara'*, *'Karuthacolomban'* and *'Willard'*, were examined in the tree during the flowering season, January to early March and September-November in 2008 and again in February-March in 2012. Blighted inflorescences with grayish green mycelial

growth at different stages of disease were collected and examined in the Plant Pathology laboratory at the Department of Botany, University of Peradeniya. Symptoms and symptom progression were recorded. Segments with mycelial growth were examined under the microscope and morphological characteristics of the fungus were recorded.

Isolation of the pathogen

Five panicle segments containing 2-3 flowers were excised from each diseased inflorescence. The segments were surface-sterilized by submerging in 1% sodium hypochlorite and then in 70% ethanol for 1 min each. The segments were rinsed well in sterile distilled water and placed on sterile filter paper to remove excess water before plating on Potato Dextrose Agar (PDA). Four or five replicate plates were prepared per diseased inflorescence and the plates were incubated at room temperature (26±2 °C). After 7 days of incubation, the plates were examined and the fungal colonies that emerged from diseased segments were sub-cultured on PDA.

To prepare single conidia cultures, a suspension of conidia (10 conidia/µl) was prepared in 10 ml of sterile distilled water in a clean glass tube. An aliquot (0.1 ml) of conidia suspension was poured and spread over a water agar plate and the excess suspension was discarded. The seeded plates were incubated in an inclined position at RT (30-40°C) for 24 h. The agar surface was scanned under the low power of the light microscope to locate individual germinated conidia. Small squares (0.5 X 0.5 cm^2) of agar pieces containing a single germinated conidium were cut and transferred to fresh PDA and the plates were incubated as described previously.

Colony characteristics and fungal morphology

Colony growth rate was determined daily by measuring the diameter at two perpendicular positions of colonies grown on PDA in the dark at 25 °C from 3 to 10 days. Six replicate plates were used. The colony radius vs time was plotted and radial growth rate (mm day^{-1}) was obtained from the slope by linear regression (Dantigny et al., 2002).

Microscopic observations were made and images of fungal structures were captured using a light microscope with digital camera (Olympus CX31 fixed with Olympus Microscope Digital Camera DP20 and Olympus application software DP2-BSW build 2738, Olympus Corporation, 2006). Length and width of 300 randomly selected conidia were measured at high power (X400) and averaged.

Pathogenicity test

Ten healthy inflorescences were selected on a mango tree (cv. '*Kohu*') and disinfected by spraying 1% sodium hypochlorite and thoroughly washing with sterile distilled water. Conidia from 12-14 days old cultures were used for inoculation. A suspension of conidia was prepared by pouring 20 ml of sterile distilled water into a 12-14 day old *Cladosporium* sp. culture. The mycelium was rubbed gently with a sterile glass spreader to dislodge conidia and the suspension was filtered through glass wool. The suspension (2-3 ml) of conidia (10^6 conidia/ ml) was evenly sprayed using a fine hand sprayer on each panicle. The inoculated inflorescences were kept covered with thin polythene (120 gauge) bags to provide moist conditions for 2 days. Ten control panicles on the same experimental tree were similarly disinfected and kept covered with thin polythene bags, sprayed with sterile distilled water, to provide moist conditions as controls and kept covered with polythene bags. The inoculated panicles were observed daily and once the symptoms appeared, the pathogen was re-isolated from inoculated panicles on PDA plates. The experimental design was a Completely Randomized Design (CRD) and the experiment was repeated twice.

RESULTS

Cladosporium disease was observed in the inflorescences of 30% - 40% of 30 trees examined for disease. The frequency of occurrence of *Cladosporium* sp. in inflorescence collected in the study with slightly and severely infected flowers was 71% and 96% respectively. *Cladosporium* sp. was not isolated from healthy inflorescences collected from any cultivar in the study.

Disease symptoms

Symptoms were first observed when panicles were in full bloom and heavy fungal colonization could be seen at the time of fruit set. The mycelium completely

covered the infected fowers, pedicels and small fruits. Infected fowers, pedicels and small fruits turned brown and dropped as disease progressed. Olive green–gray extensive mycelial growth on the infected organs at later stages was characteristic to infected inforescences (Plate 1).

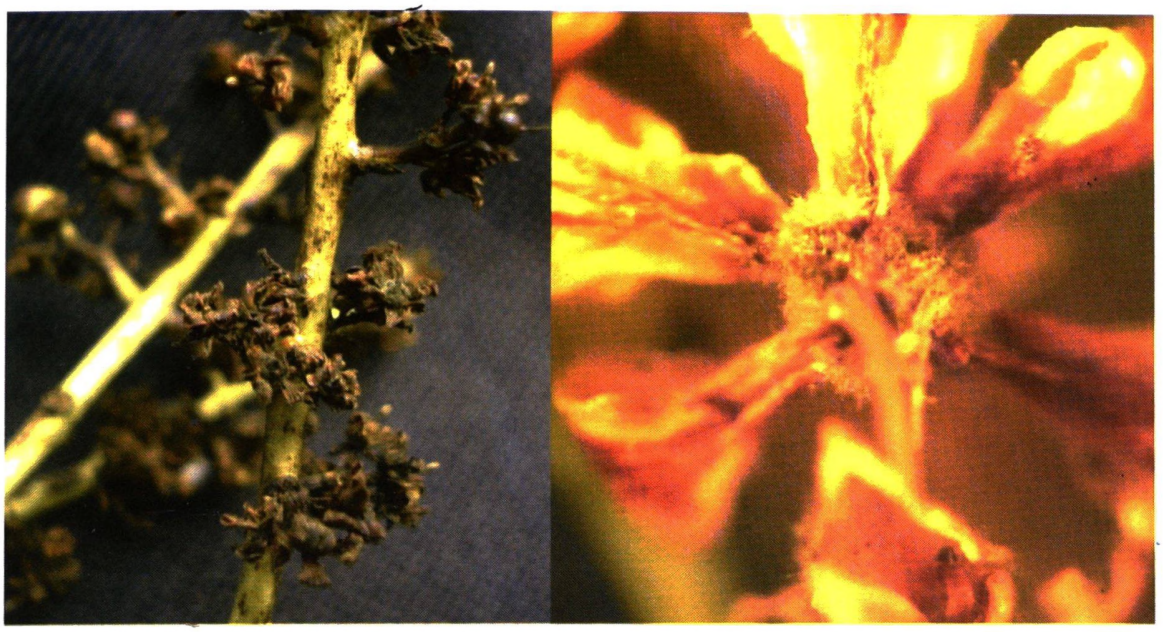

Plate 1. Infected inforescence (left) and fowers (X 10) (right) showing symptoms characteristic to *Cladosporium* disease, i.e. extensive olive green-gray mycelium over fowers and small fruits.

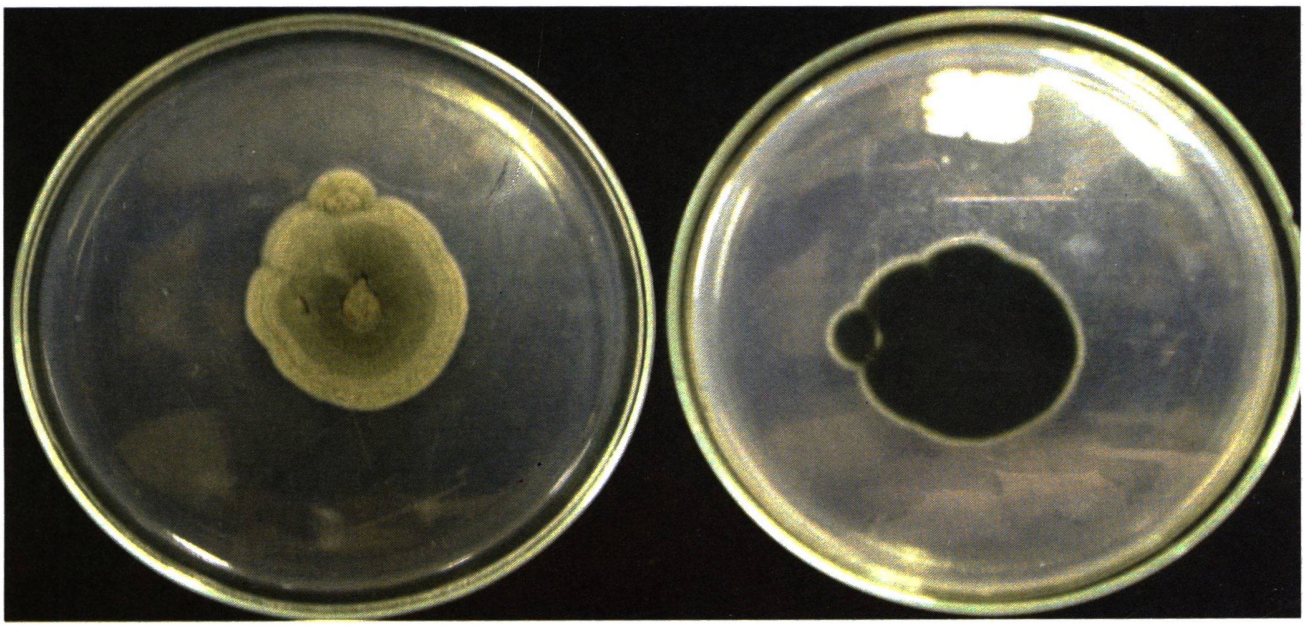

Plate 2. A fourteen day old *Cladosporium* colony isolated from a diseased inforescence, (a) upper and (b) lower surface

Pathogen

Colonies on PDA were green to olive green in colour with a narrow whitish outer margin and with time the colour turned into dark green and black (Plate 2). Reverse of the colonies was greenish to black with radial furrows. Radial growth rate was 1.20 mm day^{-1}. Aerial mycelium was abundant, high, fluffy, grey and dense, immersed and superficial, hyphae branched, septate, sometimes constricted at septa, sub-hyaline to pale brown, with swellings and constrictions. Conidia were pale olive to brown colour, smooth, variable in size, 8.94 x 5.06 µm (2.48 - 22.56 x 2.48-7.57 µm) and shaped, from cylindrical to ellipsoidal, sub spherical or limoniform and oblong to fusiform (Plate 3i). Ramo-conidia are borne apically or sub-apically, pale olive–brown, cylindrical to clavate, slightly inflated at the apex, and bearing 2–3 flat thickened scars and dentical shape projections and the dimension are 22.78x7.30 µm with variations 13.43-50.04 x 5.56-8.8 µm. Conidiophores were pale brown in colour, paler distally, geniculate and sympodially elongated, thick walled, septate, straight and moderately rigid or somewhat flexuous, smooth, cylindrical, gradually tapering toward the apex, mostly simple, occasionally with a lateral branches (Plate 3ii).

Pathogenicity test

Pathogenicity test reproduced disease symptoms similar to those of natural infection 1-2 weeks after inoculation (Plate 4) of healthy inflorescences. The pathogen was re-isolated and the characteristics were compared well with the original isolate. Hundred percent of the inoculated flowers showed disease symptoms. Control inflorescences did not show any infection symptoms. The re-isolation resulted in *Cladosporium* sp. with the same morphological features as described earlier.

DISCUSSION

Cladosporium species are reported to be pathogens on various crops such as *C. fulvum* on tomato (Joosten and De Wit, 1999), *C. alli-cepae* on onion and leek (Kirk and Crompton, 1984), *C. variabile* on spinach and *C. cladosporioides* on *Carica papaya* (Kodikara et al., 1996).

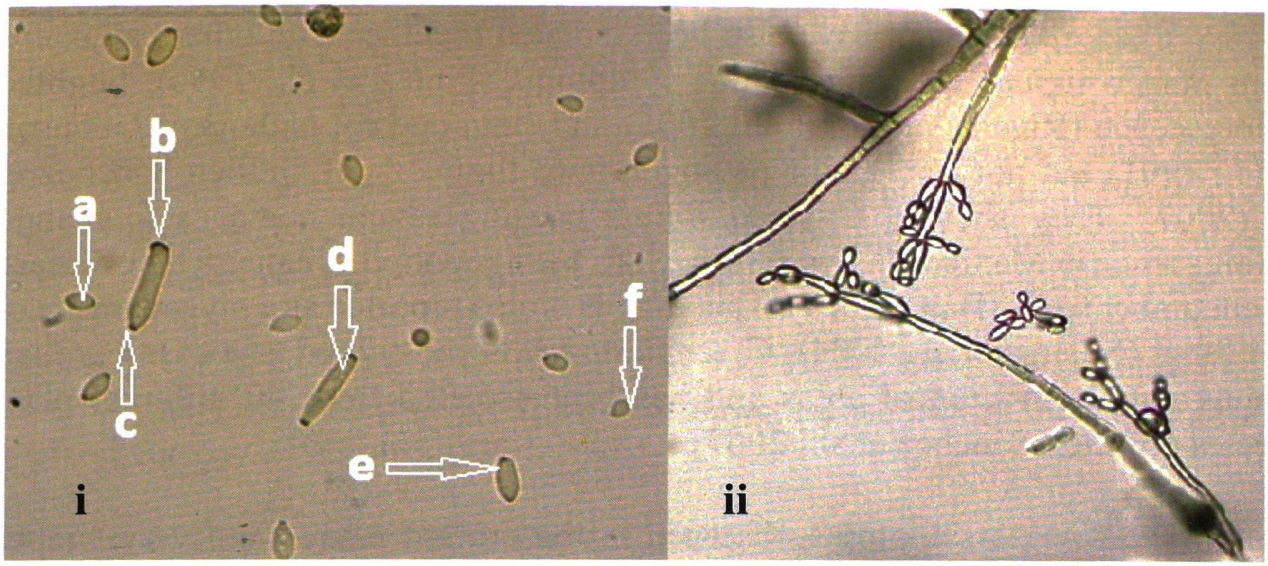

Plate 3. Conidia (i) and conidiophores (ii) of *Cladosporium* sp. observed in single spore culture grown on PDA (X 400). (a). Lemon-shaped conidia (b). Denticle shaped projections (c). Conidia scars (d). Ramo conidia (e). Fusiform conidia (f). Sub-spherical conidia

Plate 4. Symptom expression following artificial inoculation with *Clodosporium* sp. Control inflorescence (left), Inflorescence artificially inoculated with *Cladosporium* sp. showing extensive green-gray mycelia growth (right)

Cladosporium sp. have also been isolated together with other pathogens from mango tissues showing decline symptoms (Ploetz et al., 1996), blighted inflorescence (Rivera-Vargus et al., 2006) and stem-end rot pathogens (Johnson et al., 1991) but not as a major pathogen until the work of Guillén-Sánchez et al. (2007). They showed *C. tenuissimum* causing severe damages to mango inflorescence in Mexico. *C. tenuissimum* has been reported to cause a new disease on cucumber fruits (Batta, 2004). This fungus is also reported as a mycoparasite of rust fungi (Nasinia et al., 2004). *C. tenuissimum* is considered as a cosmopolitan fungus mainly in tropical areas and has been isolated from 40 different plant species (Guillén-Sánchez et al., 2007).

The disease symptoms observed in the current study were very similar to the symptoms described in Guillén-Sánchez et al. (2007). The pathogenicity was confirmed by completing Koch's postulates when the repeated inoculation and subsequent re-isolation of *Cladosporium sp.* from mango inflorescences were done successfully. The morphological characteristics observed for the *Cladosporium* sp. in this study matched with *C. tenuissimum* except the colony growth rate (1.20 mm day^{-1}) which was lower than the earlier reported value, 4.6 mm day^{-1} (Guillén-Sánchez et al., 2007). Relative humidity, temperature, media components and other such factors could contribute towards the rate of growth of a fungus. Therefore, the species confirmation should be carried out by further studies. The distribution of this pathogen in mango growing areas of the country is worthwhile to be studied in order to establish the importance of this pathogen in causing crop losses and develop control strategies.

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