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Colletotrichum gloeosporioides causing leaf spot disease on *Ixora coccinea* in West Bengal

Arghya Banerjee, Saidul Islam and Rahamatulla Middya

Abstract
Occurrence of leaf spot disease of *Ixora coccinea* (family - Rubiaceae) is first time observed during mid August to October, 2017, from nursery garden of Birbhum district, West Bengal, India. Typical small to large sized light grey spots surrounded by irregular dark brown zonate margin with numerous production of concentric brown to black dot like structures are observed on the upper surface of leaves. Based on cultural, morphological, vegetative and reproductive structures, the causal pathogen of disease is identified as *Colletotrichum gloeosporioides* which is being considered as new record from West Bengal. Spores of *Colletotrichum gloeosporioides* produced on host are hyaline, 1-celled, smooth walled, eguttulate, cylindrical with rounded ends measuring 11.2 – 12.8 (av. 12.0) x 3.1 – 5.3 (av. 4.2) µ. Acervuli are pale brown to black, 130.1 – 161.5 (av.147.7) µ in size with numerous, black to dark brown, 1 – 3 septate, unbranched, 36.6 – 59.1 (av. 50.6) x 2.8 – 4.4 (av. 3.7) µ sized pointed setae.

Keywords: *Ixora coccinea*, Leaf spot, *Colletotrichum gloeosporioides,* West Bengal

Introduction
Ixora coccinea, known as jungle geranium, flame of the woods, or flame of the forest, is a shrub in the Rubiaceae native to Eastern India and is widely cultivated for its showy red globose inflorescences. The flowers, leaves, roots, and the stem are used to treat various ailments in the Indian traditional system of medicine, the Ayurveda, and in various folk medicines. Phytochemical studies indicate that the plant contains the phytochemicals lupeol, ursolic acid, oleanolic acid, sitosterol, rutin, lecocyanadin, anthocyanins, proanthocyanidins, glycosides of kaempferol and quercetin which have some important antimicrobial, anti-inflammatory, anti-ulcer, anti-asthmatic, anti-plaque, antioxidant, hepatoprotective and cardioprotective effect. On literature surveyed, it has been found that the genus *Ixora* is attacked by several fungal diseases *viz.* leaf spot by *Macrophoma* (Sacc.) Berl & Vogl, *Coniothyrium* Corda, *Botryodiplodia* Sacc., *Phyllosticta ixorae* Rangel, *Pestalotia ixorae* Rangel (from Pune), *Leptostromella* Sacc. (from Punjab), *Phomopsis ixorae* (from MP), *Myrothecium medium* Sacc. & Went (from UP), *Cercospora ixorae-parviflorae* Patw. & Sathe, *Pseudocercospora ixoricola* (from Brazil, Alves and Barreto, 2010) [1]; spotted anthracnose by *Spaceloma ixorae* Thirum. and Narasimhan. Sp. nov. from Maharastra (Narasimhan *et al.*, 1969b) [7], anthracnose by *Colletotrichum* Corda., *Colletotrichum ixorae* Giff & Maubl (from Punjab), *Colletotrichum ixorae-parviflora* Patwardhan, *Colletotrichum dematium, Colletotrichum gloeosporioides* (from Bihar) [*c.f.* Sohi, 1990] [10]; white thread blight by *Ceratobasidium stevensii* from Brazil (Benchimol *et al.*, 2001) [2]; Alternaia blight by *Alternaria alternata* (Devi *et al.*, 1995) [3] and *A. tenuissima* from Punjab (Saini *et al*., 1989) [9]; *Vizella oleariae* from Maharastra (Dubey and Moonnambeth, 2013) [4]; *Asterinella ixorae* (Kumar and Verma, 1987) [6], *Meliola ixorae* Yates var. *psychotriae* Hosagoudar & T.K. Abraham; *Armillaria* root rot and sooty mold (homeguides.sfgate.com/). Out of this, on *Ixora coccinea,* leaf spot disease caused by *Colletotrichum ixorae* Griff & Maubl. is not common and has been collected from Patiala (Punjab). Prasad and Acharya (1967) [8] reported *C. ixorae* from Bihar. They also reported *C. dematium* and *C. gloeosporioides* on *I. coccinea*. But there is no record on occurrence of this disease from West Bengal. From mid-August to October, 2017, it has been found that the plant is suffering severely from leaf spot disease. A detailed study on this disease along with its causal agent has been conducted during present investigation. Objective of this study is to know the intensity and time of occurrence of diseases, pathogenicity establishment and characterization of the pathogen on this plant.

Materials and Methods
Collection of diseased sample
Diseased leaves were collected from nursery garden of Birbhum district, West Bengal, India.

Typical small to large sized light grey spots surrounded by irregular dark brown zonate margin with numerous production of concentric brown to black dot like structures are observed on the upper surface of leaves. In severe case, several spot coalesced and total leaves dried up basipetally.

Pure culture Isolation

Diseased leaves were cut into small pieces, surface sterilized in 1% sodium hypochlorite for 45 - 60 seconds, rinsed three times in sterile distilled water, plated on water agar (Fig. E) and then incubated at 28˚C for 5 days. Hyphal tips from the margin of developing colony were picked up and transferred to potato dextrose agar (PDA) medium for pure culture preparation. Peptone sucrose agar (PSA) medium was used for artificial sporulation (Fig. F).

Morphological observations

Series of slides were prepared from cultures or infected parts for morpho-metric studies of fungal spores, spore bearing and other structures. Micro-photograph of all fungal structures was taken with help of Leica Binocular Microscope and or Karl Zeis Phase Contrast Microscope and by using Canon Powers Shot A640 camera. Dimensions of conidia, conidiophore, acervuli with setae were measured using AxioVision (Rel. 4.8) software.

Establishment of pathogenicity

A pathogenicity test was performed by inoculating healthy leaves with six-day-old purified active fungal culture under controlled laboratory condition. Mycelial and conidial plugs (5 mm diameter) were taken from peptone sucrose agar (PSA) and placed on detached leaves. The inoculated leaves were kept in moistened plastic bags in the dark for two days and then in natural light at 20°C. Although the method of inoculation was somewhat artificial, the symptoms were similar to those observed in natural condition and the fungus was reisolated from infected parts. The control leaves, inoculated with PSA plugs, remained healthy.

Results and Discussion

The infection began as small, round to oval brown spots with light grey centres, which generally appeared close to leaf margin and middle portion of leaf (Fig. A). As disease progressed, the leaves showed characteristic leaf spot symptoms with light brown to grey coloured necrotic areas bordered by dark brown wavy margins. Upon binocular observation, acervuli were epiphyllous, dark, punctiform, subepidermal, 130.1 – 161.5 (av.147.7) µ in size with numerous, black to dark brown, 1 – 3 septate, unbranched, 36.6 – 59.1 (av. 50.6) x 2.8 – 4.4 (av. 3.7) µ sized pointed setae (Fig. C). Spores produced on host (Fig. D) were hyaline, 1-celled, smooth walled, eguttulate, cylindrical with rounded ends measuring 11.2 – 12.8 (av. 12.0) x 3.1 – 5.3 (av. 4.2) µ. On PDA medium the fungus produced cottony white mycelial growth which was slightly convex and dense. Upon observation from below greyish coloured mycelia could be observed. Fluffy mycelial growth covered the media completely with no productions of acervuli. Peptone Sucrose agar (PSA) medium was suited best for sporulation and acervuli production. Size of conidia (Fig. G) produced on PSA, was more or less similar to conidia produced on host tissue, but size of acervuli and setae length were varied. The above mentioned description of isolated pathogen was verified with the description of *Colletotrichum ixorae* Griff & Maubl, *C. dematium* and *C. gloeosporioides* given by Prasad and Acharya (1967) [8] reported on *I. coccinea*. Maximum similarity was matched on *C. gloeosporioides.* So, the causal fungus of presently described leaf spot disease of *Ixora coccinea* being proposed as *Colletotrichum gloeosporioides* from West Bengal.

Fig A: Disease symptom

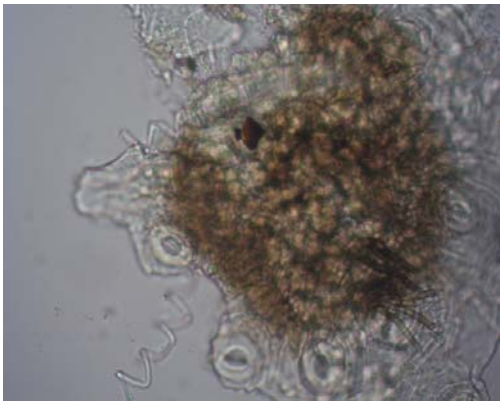

Fig B: Acervulus on host

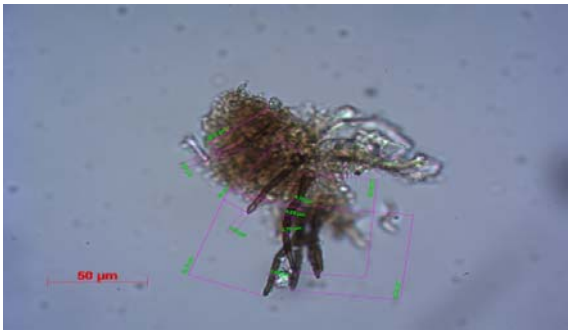

Fig C: Setae produced on host

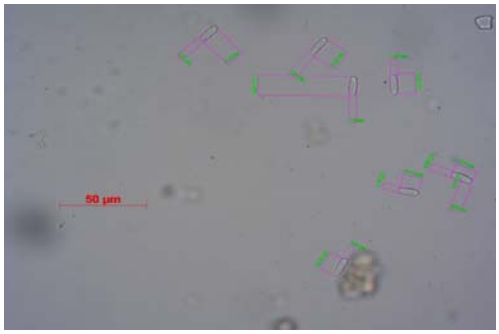

Fig D: Conidia produced on host

Fig E: Isolation of pathogen

Fig F: Purification

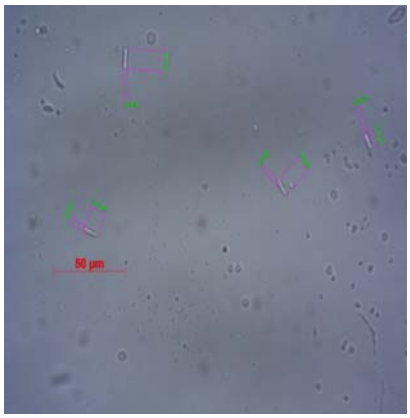

Fig G: Conidia on PSA

Acervuli dimension
161.32
156.91
161.49
137.13
139.25
130.1
Av. 147.7

Spore length(µm)	Spore breadth (µm)
12.77	4.13
12.64	3.53
11.75	4.16
12.4	4.34
13.75	4.38
12.76	4.04
12.26	3.97
11.46	3.5
12.18	3.92
11.5	3.19
11.69	3.97
11.69	4.31
11.79	4.51
12.77	4.13
12.54	5.27
11.22	4.34
12.45	4.13
10.22	3.74
11.79	5.49
12.12	4.38
12.54	4.34
11.39	4.84
Av. 12.03	**4.20**

Setae length (µm)	Setae breadth (µm)
55.44	3.58
63.78	4.35
59.12	4.09
36.67	2.79
38.21	3.85
Av. 50.64	3.73

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