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OCCURRENCE OF LEAF BLOTCH DISEASE (*BOTRYOSPHAERIA* SP.) IN *FICUS RELIGIOSA* IN SRI LANKA

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ABSTRACT

This paper reports the occurrence of a leaf blotch disease in *Ficus religiosa* in Sri Lanka. The disease first appears as small, irregular, yellow specks in leaves at later stages of maturity and with time the infected areas become necrotic and gradually enlarge. At advanced stages the whole leaf turns yellow with brown blotches and is shed. The causal agent was identified as *Botryosphaeria* sp. The necrotic blotches are associated with erumpent fruiting bodies of the fungus, ascomata. Ascomata are solitary or botryose and are found on both sides of leaves. Asci are with numerous pseudoparaphyses and ascospores were observed. Colony developed on water agar medium showed hyaline, aseptate conidia and mature brown conidia. In the presence of leaf blight caused by *Glomerella cingulata*, the leaf blotch symptoms appeared much later, about 10-12 weeks after the new flush. The diseased leaves do not last long as *F. religiosa*, being a deciduous species, sheds leaves seasonally. However, the fallen leaves, unless eliminated, may provide a good source of inoculum for infection of the new flush. Commercial fungicide, BULLET 50, inhibited the *in vitro* growth of *Botryosphaeria* sp. This is the first report on a blotch disease attacking leaves of *F. religiosa* in Sri Lanka.

Key words: sacred fig, fungal disease

INTRODUCTION

Ficus religiosa (Family: Moraceae) is native to India, Nepal, Sri Lanka, Southeast China and Indochina and is widely planted in the tropics (Dassanayake and Fosberg, 1981). This large deciduous tree is sacred to the Hindus and Buddhists, and hence the name 'Sacred Fig' was given to it. In Sri Lanka, this is often the site of Buddhist shrines.

Several fungi are known to cause disease in the foliage, stem and roots of *F. religiosa* tree. The leaf blight caused by *Glomerella cingulata* is the commonest foliage disease in Sri Lanka (Abeygunawardhane, 1969). The disease initiates with the new flush and when severely infected, the trees show reduced vigour. Brown root caused by *Fomes noxius* is the commonest root disease in *F. religiosa* and is often responsible for the mysterious death of individual trees (Abeygunawardhane, 1969).

Leaf blotch, although prevalent in most areas, has not received much attention. Diseases designed as blotch have symptoms that are intermediate between blights, where the entire leaf or shoot dies, and leaf spots, where the necrotic lesions are definitely delimited. Blotches are irregular or indefinite, large necrotic areas on leaves and fruits (Horst, 2008). Leaf blotches are caused by bacteria and numerous fungi which include *Cercospora spp.*, *Cladosporium spp.*, (Schubert *et al*., 2007), *Guignardia spp., Gnomonia spp., Alternaria spp. and Septoria spp*.

There had been no reports on leaf blotch in *F. religiosa* in Sri Lanka, hence this study was undertaken with the aim determining the nature of the disease and its causal agent.

MATERIALS AND METHODS

Study site and plant material

Diseased leaves of *F. religiosa* were collected from Peradeniya and Kandy area (Central Province), situated at elevation of 518 MSL and geographic coordinates; longitude 80^{o} 36' E and latitude 7^{o} 16' N. The area has mean annual temperature of 24.1^{o}C and mean annual precipitation of 2,121 mm. The study was carried out from July 2008 to April 2009. Leaf samples were collected into polythene bags from *F. religiosa* trees at five different locations, representing diseased (flush, young and mature stages), healthy and fallen diseased leaves for examination. Diseased leaves at different maturity stages were examined and symptoms were recorded.

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Isolation and identification of the pathogen

Pieces (1 x 1 cm^2) of infected leaves were cut and surface sterilized with 1% sodium hypochlorite for 2 minutes. The leaf pieces were washed in sterilized deionized water and the excess liquid was removed from the leaf pieces by placing them on a previously sterilized tissue paper. Leaf pieces were transferred under sterile conditions onto PDA medium. The plates were incubated at room temperature (26°C-31°C). To induce sporulation, the pathogen isolated was sub-cultured on water agar medium, and the plates were incubated at 24°C and room temperature (26°C - 31°C). The plates were placed under 6 hour intervals of daylight and fluorescent black light (Blue T10 lamp 365nm) to enhance sporulation for one and a half months. Morphology of fungal colonies was recorded. Fungal mycelium and spores were observed under light microscope and photographed. Microtome sections were taken across lesions on the leaves and examined under microscope.

Pathogenicity test

One and a half month old sporulating culture of *Botryosphaeria* sp. on water agar medium was flooded with Czapek-Dox nutrition solution (Lingppa and Lingppa, 1965), and the mycelia were scraped using a spatula. The ascomata were crushed using a glass rod. The mycelial suspension was passed through glass wool to remove hyphal fragments and the filtrate containing spores was collected. The filtrate was centrifuged at 3000 rpm for 3 minutes and a 10^3 ml^{-1} concentrated conidia suspension was prepared. An *in situ* pathogenicity test was done on fifteen different healthy young leaves of a *F. religiosa* tree near the Department of Botany. Surface of leaves were wiped with 70% ethanol and allowed to air dry. Leaves were separated into 3 groups, each group consisting of 5 leaves. Aliquots (20µl) of conidia suspension were placed on the upper surface of five leaves and lower surface of five leaves separately. The remaining five leaves were maintained as controls by placing drops of sterile deionized water. The leaves were covered with polythene bags and labeled. After three days, polythene bags were removed and daily observations were made on the development of disease symptoms. Leaves showing symptoms were removed and the pathogen in the diseased tissue was isolated on PDA as described previously.

Pattern of disease development

Five selected trees in Peradeniya and Kandy area were observed fortnightly for a period of 10 months (July 2008 – April 2009) in order to collect data on the incidence of leaf disease/s, the stage of disease, foliage (the new flush, defoliation, young or mature leaves) and weather conditions. Trees with more than 60% leaf fall was considered as the complete defoliation stage. The extent of the disease was determined by the number of leaves infected, area of infection and area of black ascomata. The intensity of infection was recorded as low, medium and high by visual observations.

Determination of a suitable fungicide

Systemic fungicide, BULLET 50 (Agro Tecnica Ltd.) was purchased from the market. The active compound is carbendazim and the manufacturer recommended dose is 7 g per 10 l. Three doses of fungicide, 0.05%, 0.07% (manufacturer recommended dose) and 0.09%, were mixed in separate 100 ml portions of melted PDA medium. The control consisted of PDA (100 ml). The medium was poured into sterile Petri dishes. Mycelial plugs (0.5 cm x 0.5 cm) taken from one month old fungal cultures were placed on the centre of each plate. Five replicate plates were made for each fungicide dose. The plates were incubated at 24°C and the radius of the colony was measured daily.

RESULTS AND DISCUSSION

The disease was observed in most *F. religiosa* trees encountered in Peradeniya and Kandy area during the study was identified as leaf blotch. This is the first record of leaf blotch in *F. religiosa*. Initial infections generally occurred in mature leaves, about four weeks after the new flush. The disease first appeared as small, irregular, yellow specks around the veins (Fig. 1a) and with time the infected areas became necrotic and gradually enlarged. The individual infected areas varied in shape, mostly irregular and coalesced to form large blotches. At advanced stages the whole leaf turned to yellow with brown blotches (Fig. 1b) and was shed. Ten to twelve weeks after initial infection, yellow chlorotic areas became dotted with small erumpent, black fruiting bodies. These ascomata were solitary (diameter 1-1.5 mm) or botryose and found on both sides of the infected leaves. Ascomata taken from diseased leaves were many celled thick, black, with papillated ostiole (Fig. 2a). Also asci were clavate, bitunicate with numerous pseudoparaphyses. Ascospores were hyaline and aseptate (Figs. 2b and 2c).

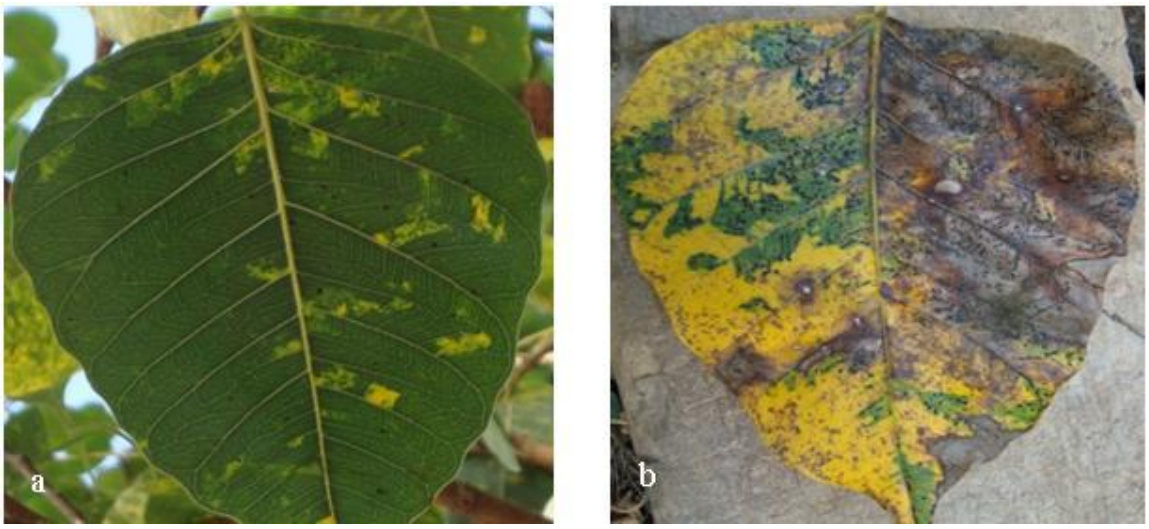

Figure 1. Stages of leaf blotch disease in *Ficus religiosa* at (a) early stage and (b) later stage.

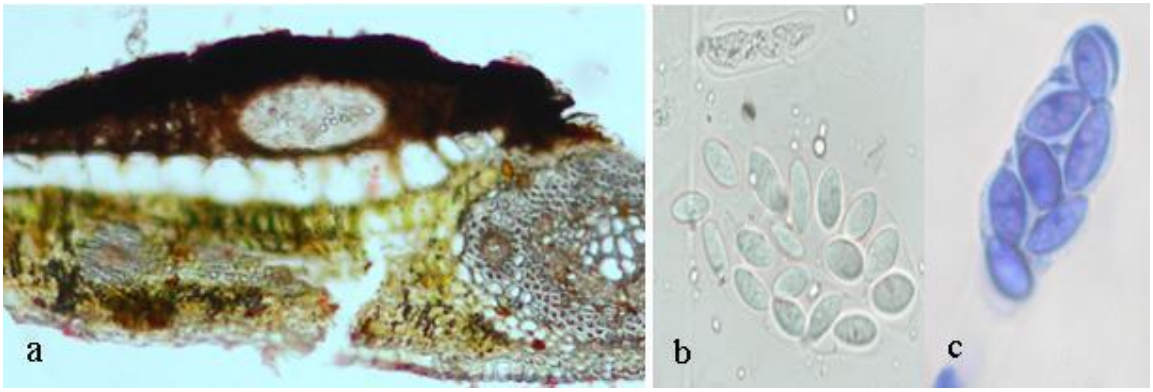

Figure 2. (a) Transverse section of an ascoma taken from a diseased leaf (× 40), (b) Asci and (c) Ascus found in ascoma (×100).

Initially, a colony on PDA showed blackish white to slight grayish color (Fig. 3a) with an evenly dense mat and after three weeks colony changed to black colour (Fig. 3b). However, no sporulation occurred until 3 months and the colony developed in culture medium showed two types of conidia; hyaline, aseptate and mature brown septate conidia (Fig. 4b). The fungus was identified as *Botryosphaeria* sp. This fungus, *Natrassia mangiferae* previously known as *Dothiorella mangiferae* has been recorded previously as the cause of branch die-back and trunk canker in *F. religiosa* in Iran (Mirzaee *et al.*, 2002)**.** *Botryosphaeria* is a species-rich genus with a cosmopolitan distribution (Denman *et al*. 2000). Species occur on a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts, on woody branches, twigs herbaceous leaves, stems and haulms of grasses, and in thalli of lichens (Barr, 1987). Taxa range in habit from being saprobic, parasitic and endophytic (Smith *et al.*, 1996, Denman *et al*., 2000), and can cause die-back and canker diseases of numerous woody hosts (von Arx, 1987). *Botryosphaeria* spp. are rarely primary pathogens, instead cause stress-related diseases or perennial cankers (Schubert, 2007). Some related anamorph genera of *Botryosphaeria* are *Botryodiplodia*, *Diplodia*, *Fusicoccum*, *Lasiodiplodia*, *Macrophoma*, *Macrophomopsis* and *Sphaeropsis*, and these genera are not clearly delimited because the morphological features that separate them are poorly defined (Crous *et al*., 2006).

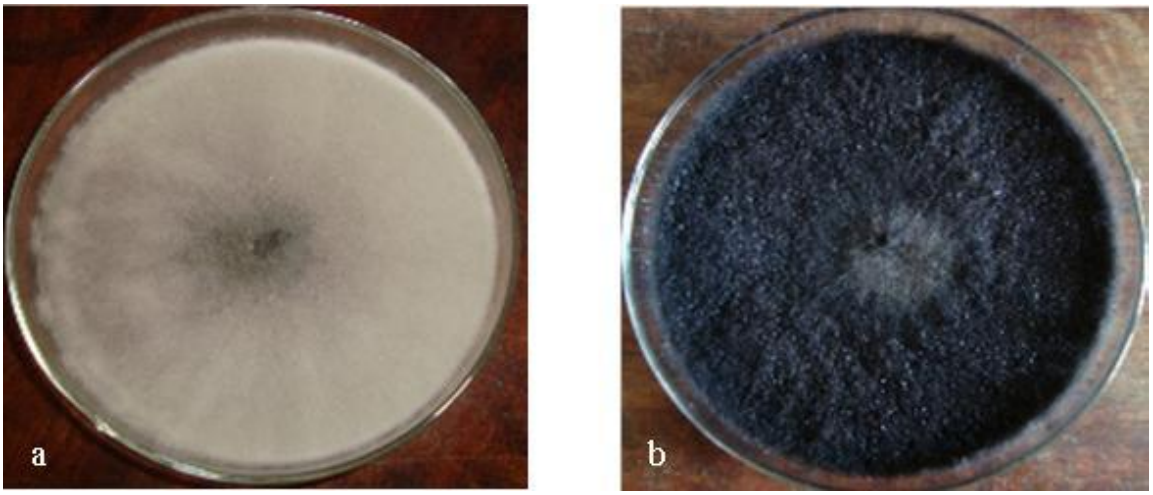

Figure 3. *Botryosphaeria* sp. on PDA (a) 10 day old culture and (b) one month old culture.

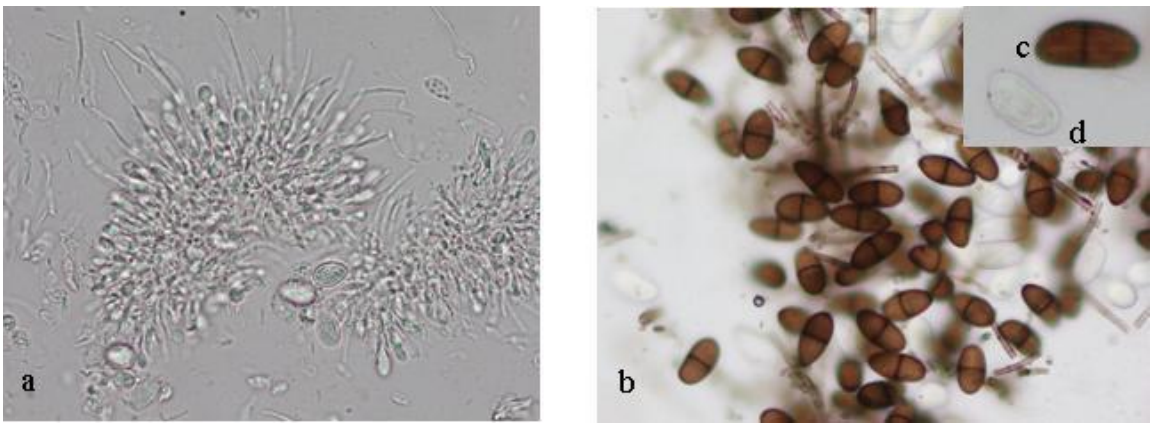

Figure 4. (a) Conidia formation on conidiogenous cells in culture, (b) Conidia in culture (×100), (c) mature brown conidium (×200) and (d) hyaline, aseptate conidium (×200).

Leaves artificially inoculated with drops of conidia suspensions showed initially browning of tissue which developed into yellow irregular chlorotic areas with brown blotch after one week. However, there were no developed ascomata. No symptom development was observed in the controls. The symptoms developed were similar to those generally associated with the leaf blotch in *F. religiosa*. The pathogen that was re-isolated from 3 out of 5 diseased leaves and cultured on PDA had similar culture and morphological characteristics as the original isolate.

The information collected from the field study has shown the pattern of disease development at different locations. The leaf blotch normally appeared four weeks after the young flush of leaves (Fig. 5). However, in the presence of leaf blight disease caused by *Glomerella cingulata*, symptoms of leaf blotch appeared later; 10 – 12 weeks after new flush. This shows that the presence of leaf blight disease has a negative effect on the development of leaf blotch on the same leaf. Also our results indicate that leaf blotch intensity gradually increases with time and when defoliation occurs, the plants were severely infected.

The fallen leaves carry fruiting bodies of the pathogen which may remain and survive even after the leaves have decayed. These can provide a good source of inoculum for infection of the new flush. In order to prevent or reduce the intensity of the disease in the following season, the leaf litter may be collected and destroyed on a regular basis.

The mean radius of *Botryosphaeria* colonies in the control plates was 3.8 cm and with 0.05% fungicide, the mean colony radius was 2.1 cm. However, no growth occurred at 0.07%, the recommended dose of the fungisice (Fig. 6). Carbendazim, the active compound of BULLET 50 (Garcia *et al.*, 2002), inhibits the development of fungi probably by interfering with spindle formation at mitosis phase of cell division. Carbendazim belongs to the benzimidazole group of fungicides (Alpertunge, 2009). *In vitro* tests indicated that the fungicide at the manufacturer recommended dose could completely inhibit the pathogen. The present study could not be extended to test the effectiveness of the fungicide *in vivo* due to several obstacles such as non-availability of machinery to spray large and tall *F. religiosa* trees. However, considering its high degree of effectiveness *in vitro*, in situations where the disease is serious, the affected tree may be treated with this fungicide.

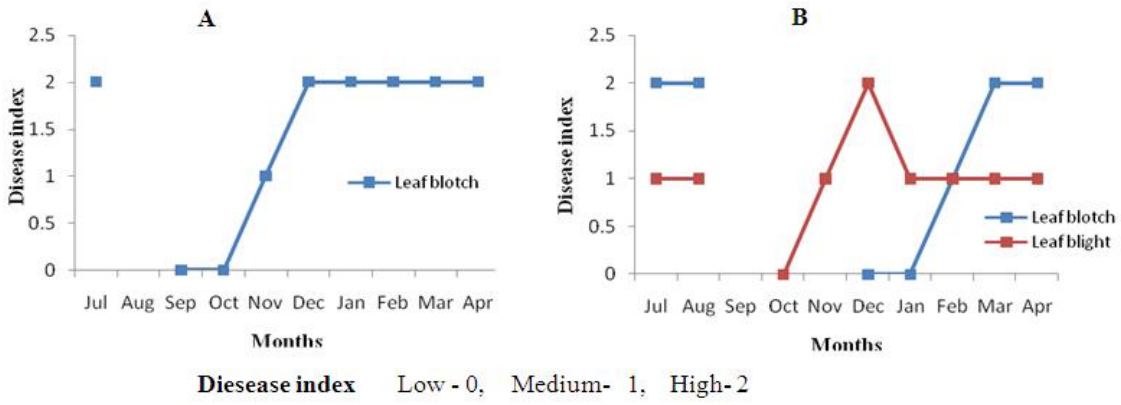

Figure 5. Pattern of disease development in sites A and B.

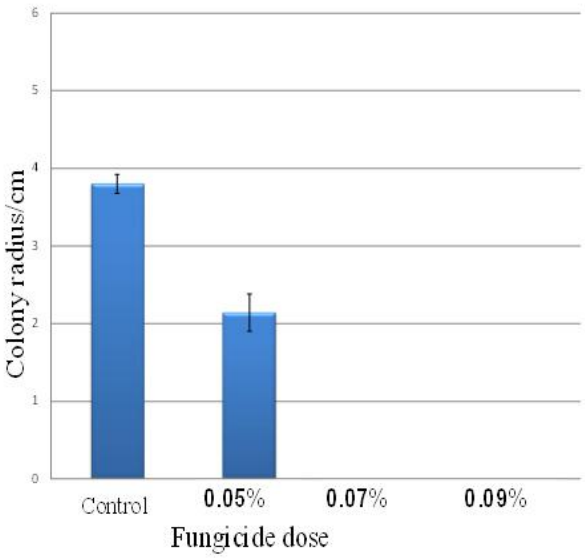

Figure 6. Growth of *Botryosphaeria* sp. on PDA with different fungicide concentrations, 0.05% (low dose), 0.07% (recommended dose) and 0.09% (high dose), after 4 days.

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