FOLIAR DISEASES AND MYCOFLORA ASSOCIATED WITH DISEASED LEAF OF CINNAMON (*Cinnamomum verum* J. Presl) FROM BANGLADESH

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Abstract

Cinnamon (*Cinnamomum verum* J. Presl) is an important spice with medicinal value worldwide. Three foliar diseases, such as small leaf spots, gray leaf blight and leaf blight were recorded from September 2022 to August 2023 at three different locations in Bangladesh. Disease incidence of gray leaf spots recorded from the location of Savar was highest in about 48% and small leaf spots from Dhaka (68%) and leaf blight disease from the location of Savar (88%). A total of six species of fungi such as *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium* sp., *Rhizomucor miehei* and *Colletotrichum gloeosporioides* were recorded from small leaf spots symptom. Highest frequency of occurrence of the fungus was *R. miehei* (66.67%) followed by *C. gloeosporioides* was 62.50%. Four fungal species such as *A. flavus*, *A. fumigatus*, *A. niger* and *Pestalotiopsis palmarum* were isolated from gray leaf spots symptom of the leaves of *Cinnamomum verum*. Highest frequency recorded from *P. palmarum* was 83.33 %. Five fungal species were isolated from leaf blight disease symptoms of *C. verum* were *A. fumigatus*, *A. niger*, *C. gloeosporioides*, *Fusarium* sp. and *R. miehei*. Frequency percentage of *C. gloeosporioides* was highest 75.00% and *Fusarium* sp. was lowest 8.33%. Among the fungi isolated from three disease symptoms, three fungi such as *C. gloeosporioides*, *P. palmarum* and *R. miehei* were capable to produce symptoms of leaves. Seven species of fungi *viz*. *A. flvaus*, *A. niger*, *C. gloeosporioides*, *Colletotrichum* sp., *Fusarium* sp., *P. palmarum*, *Rhizopus* sp. and one sterile fungus were recorded from associated with the different disease symptoms of cinnamon leaf in moist chamber methods.

Key words: Foliar disease; Cinnamon; Pathogenic potential; Bangladesh.

INTRODUCTION

Cinnamon (*Cinnamomum verum* Presl, Syn. *Cinnamomum zeylanicum* Blume), commonly known as 'darchini' in Bangla, is an important spice crop with medicinal value worldwide which belongs to the family Lauraceae. The most common species are *Cinnamomum zeylanicum*, *C. loureirii*, *C. burmanni* and *C. aromaticum*. In Bangladesh, since ancient time it has been used as condiments, medicinal and culinary uses. It contains many notable derived compounds, antioxidants, minerals and vitamins that are essential for good health (Gruenwald *et al*. 2010, Wadud *et al*. 2017). The bark of *C. aromaticum* and *C. zeylanicum* has high demand for commercial use. Cinnamon bark powder is used in chocolate manufacturing industries, spicy candies, tea, hot cocoa and liqueurs worldwide. It also has medicinal value and use for the treatment of diabetes and Chinese naturopathic medicine (Modak *et al*. 2007, Qin *et al*. 2010). Cinnamom, although a hardy plant, is attacked by a variety of pests and diseases during its different growth stage (Rajapakse and Kumara 2007). Diseases are responsible for considerable reduction in the yield of cinnamon bark and also for reducing the quality of other products, such as leaf oil, bark oil. Leaf blight caused by *Pestalotia palmarum* from Indian bay leaf (*Cinnamomum tamala*) caused 90 % leaf damage in India (Karunakaran *et al*. 1993). The

important diseases are leaf spot and die back, seedling blight, gray leaf spots/blight, black sooty mould, algal leaf spots, stem and root diseases, rough bark disease, stripe canker, pink disease, brown root rot (Khan *et al*. 2020). Among the diseases, the gray leaf spot/blight is a major disease of cinnamon widely distributed worldwide. Rajapakse and Kumara (2007) reviewed the important diseases of cinnamon and reported that the leaf spot/blight is caused by *Colletotrichum gloeosporioides,* the gray leaf spot/blight by *Pestalotia cinnamomi*, the stripe canker by *Phytophthora cinnamomi*, the pink disease by *Corticium salmonicolor* affecting stems of cinnamon and *Phellinus lamaensis* causing the brown root rot. Wadud *et al*. (2017) reported the gray leaf blight disease of bay leaf (*Cinnamomum tamala*) from Bangladesh. There has been no significant work done on the diseases of cinnamon in Bangladesh and little information is available on the disease of cinnamon and epidemiological aspects in the disease development under different weather conditions. Hence, the present study was undertaken with the following objectives: (i) to identify foliar diseases of cinnamon leaf; and (ii) to identify the mycoflora associated with diseased cinnamon leaf.

MATERIAL AND METHODS

Sample collection

Different types of foliar diseases were noticed on cinnamon trees located at Mohakhali DOHS at Dhaka, Birulia at Savar and Rajshahi College at Rajshahi district. Diseased leaf samples were collected from the top, central and lower part of the plants that were considered to be approximately equal for mycological analysis. The samples were placed in separate plastic bags lined with tissue paper and brought to the laboratory for study. Cinnamon plants had been kept under observation weekly for recording the foliar diseases during the months from September 2022 to August 2023. Twenty leaves were selected randomly from each foliar disease symptoms of cinnamon (*Cinnamomum verum*) and recorded the severity of each disease. Disease severity (DS) of foliar diseases of *Cinnamomum verum* was recorded following the scale stated by Shahzad and Bhat (2005). The percent disease incidence was calculated as below:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves examined}} \times 100$$

For visual estimation of severity, the disease intensity of all foliar diseases of *Cinnamon zeylanicum*, 0 to 5 scale was used for rating of all foliar diseases studied. The disease intensity was assessed as follows: 0 = healthy leaf, 1 = 10- 20% leaf area infected, 2 = 20- 40% leaf area infected, 3= 40- 60% leaf area infected, 4 = 60- 80% leaf area infected, and 5 = above 80% leaf areas infected. Percent disease intensity (PDI) was calculated by using the following formula:

$$PDI = [\Sigma (n \times v)/ N \times G] \times 100$$

where, Σ=Summation; n=Number of leaves in each category; v=Numerical value of each category; N=Total Number of leaves examined; G=Maximum numerical value.

Isolation of fungi associated with the disease symptoms

The fungi were isolated from the samples following the "Tissue planting method" on PDA (Potato Dextrose Agar) medium and "Blotter method" (CAB 1968). The diseased leaf samples were washed and then cut into pieces (22 mm). Then the cut pieces were sterilized in 10% Chlorox for one minute and then washed again in sterilized water thrice. After washing, the cut pieces were placed on the PDA medium in Petri plates at 3 pieces per plate for "Tissue planting method". A moist chamber was prepared by placing 3 layered of moist blotting paper (Whatman No.1) in bottom part of Petri plates at 5 pieces per plate for "Blotter Method". The Petri plates with inoculated cinnamon leaves were incubated for 5 - 7 days at 25 ± 2°C. The fungi grew from the diseased leaves were transferred to separate PDA plates and PDA slants for further studies and preservation. Then the isolated fungi were identified based on morphological characteristics observed under a compound microscope following standard literatures (Barnett and Hunter 1998, Ellis 1971, 1976, Ellis and Ellis 1997, Sutton 1980). The prevalence (%) of fungi associated with cinnamon leaf was also recorded. The percentage of frequency of the occurrence of the fungal isolates was calculated following Spurr and Welty (1972):

$$\text{Frequency (\%)} = \frac{\text{No. of inocula from which a fungal isolate was obtained}}{\text{No. of inocula cultured}} \times 100$$

Pathogenicity test

Pathogenicity of the isolated fungi was tested following modified detached leaf assay technique (Shamsi *et al*. 2013). Koch's postulates were performed on surface-sterilized healthy leaves of cinnamon. For this purpose, leaves removed from the tree and surface disinfested by immersion in 10% bleach solution (0.5% sodium hypochlorite) for 2 min, rinsed in sterilized water, and then air-dried in a laminar flow hood. The leaves were placed in a humid chamber at room temperature (25°C). The leaves inoculated with mycelial disks (3 mm diameter) isolated from the diseased leaves grown at 25°C from 5 to 7 days were putt reversely on leaf surface directly. Non-inoculated leaves (only plugs of PDA medium), served as control. After symptom expression, isolation was carried out in order to confirm the genus identification with subsequent pathogen re-isolation in PDA medium to fulfill Koch's postulates and to identify the species morphologically.

RESULTS AND DISCUSSION

Different types of symptoms of disease observed on leaves and spots are frequently coalesced to cover maximum leaf areas. Small leaf spots appeared in early May as small brown scattered spots that gradually enlarged in size and attained maximum diameter. Three different foliar disease symptoms, including small leaf spots, gray leaf spots and leaf blight disease, were seen on cinnamon leaves from June to September 2022 (Fig. 7). The highest disease was recorded for leaf spot in the month of June, gray leaf spot in the month of May to July, and in the case of leaf blight disease, maximum disease ratting was found in the month of June to August. Disease ratting was found lower in the winter season (December to February) for all disease symptoms (Fig. 1).

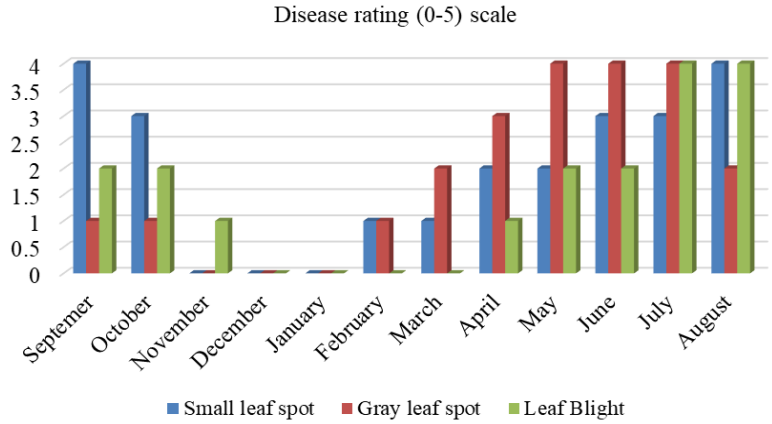


Fig. 1. Foliar disease of cinnamon recorded on disease rating (0-5 scale) during 2022-2023.

Disease severity and the disease incidence of leaf spots, gray leaf spots and leaf blight of cinnamon were recorded during the study periods. The highest disease incidence was recorded in 68, 48, and 88% from small leaf spots, gray leaf spots, and leaf blight of cinnamon, respectively (Fig. 2). The highest disease severity was also recorded at 36, 29.6, and 51.2%, respectively, from the small leaf spots, grey leaf spots and leaf blight disease of cinnamon from Dhaka, Savar and Rajshahi (Fig. 3).

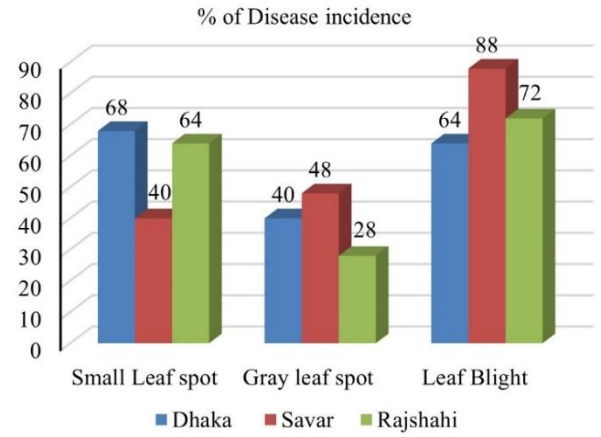


Fig. 2. Disease incidence of foliar disease of cinnamon recorded in three locations.

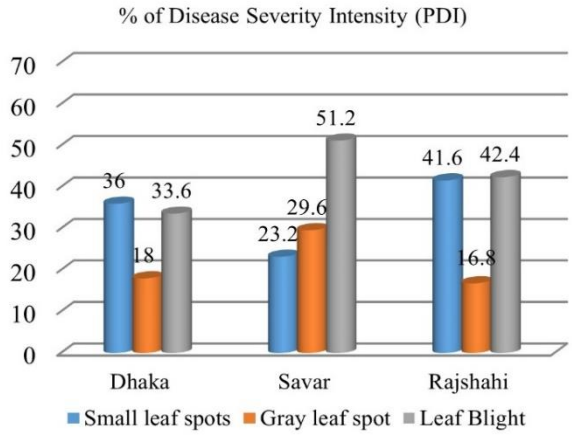


Fig. 3. Disease severity of foliar disease of cinnamon recorded in three locations.

Description of foliar disease symptoms

Small Leaf spots (*Colletotrichum gloeosporioides*)

Leaf spot disease is caused by *Colletotrichum gloeosporioides*. Small deep brown specks appear on the leaf lamina, which later coalesce to form irregular patches. In some cases, the affected portions are shed leaving shot holes on the leaves and later the entire lamina is affected. Small, brownish leaf spots on older leaves can be seen, especially in shaded areas. These small specks coalesce to form large necrotic blotches giving scorching appearance. Karunakaran and Nair (1980) reported leaf spot and die back disease of *Cinnamomum zeylanicum* caused by *Colletotrichum gloeosporioides*.

Gray leaf spots (*Pestalotiopsis palmarum*)

The gray leaf spots of cinnamon caused by the pathogen *Pestalotiopsis palmarum* were considered as one of the major diseases of cinnamon. It was first observed during mid-June when the temperature was high, and it was near about 34°C. The symptoms appeared as small yellow brown spots appearing on the cinnamon leaves, at first. Later, these spots turned gray with a border and spread to the lamina. In later stage these became whitish to grey with a dark brown border. Dark acervuli were produced in older lesions appearing as black dots in the center. The spots were oval in shape initially and later formed necrotic patches. In advanced stages of infection, a major portion of the leaf lamina dried up.

Leaf blight (*Colletotrichum gloeosporioides*)

The leaf blight caused by a fungus *Colletotrichum gloeosporioides* appears in all stages of the plant growth from seedling stage to maturity and the entire foliage can be affected in severe infections. In severe infections, lesions may be larger than the half of the leaf. Small brown blackish spots generate and after some period the spots coalesce and form irregular brown blotches. Under favorable condition the necrotic lesions may cover more than half of the leaves. The spots mature and start covering the leaf lamina and become papery white with reddish brown borders. During the later stage the pathogen produces its fruiting body acervuli which can be seen at the central region of the necrotic spot as black raised dots.

Mycoflora associated with the different disease symptoms

A total of five species of fungi belonging to four genera of Deuteromycetes were found to be associated with small leaf spots disease of cinnamon. The isolated fungi were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium* sp. and *Colletotrichum gloeosporioides*. The highest frequency percentage of occurrence was observed by the fungus *Colletotrichum gloeosporioides* (62.50%) followed by *Aspergillus fumigatus* and *A. niger* (60.01%), isolated from small leaf spots of cinnamon following the Tissue planting method on PDA (Potato Dextrose Agar) medium (Fig. 4).

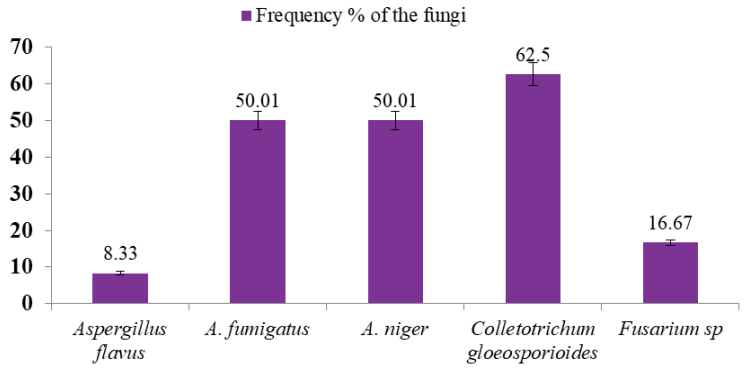


Fig. 4. Frequency % of fungi associated with small leaf spots of cinnamon.

Four fungal species were isolated from the leaves of cinnamon showing gray leaf spots symptom. The isolated fungi were *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *Pestalotiopsis palmarum*. Frequency of association of *P. palmarum* was highest (83.33 %) and *A. fumigatus* was lowest (8.33%) (Fig. 5).

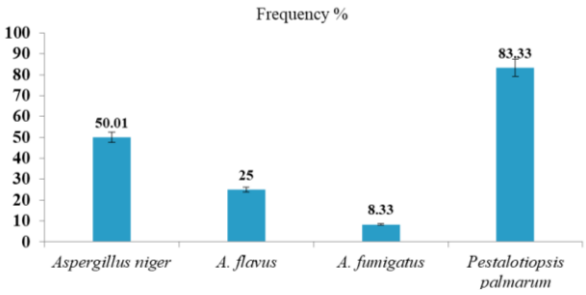


Fig. 5. Frequency (%) of fungi associated with gray leaf spots of cinnamon.

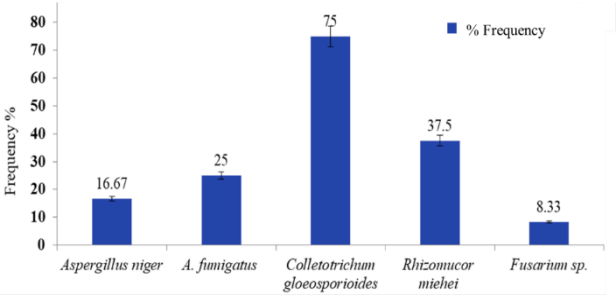


Fig. 6. Frequency (%) of fungi associated with leaf blight of cinnamon.

In the present study five fungal species were isolated from Leaf blight disease symptom of *C. zeylanicum* following Tissue planting method on PDA medium. The isolated fungi were *Aspergillus fumigatus*, *A. niger*, *Colletotrichum gloeosporioides*, *Fusarium* sp. and *Rhizomucor* sp. Frequency percentage of *C. gloeosporioides* was highest 75.00% and *Fusarium* sp. was lowest 8.33% (Fig. 6).

In total, seven species of fungi belonging to five genera of the class Deuteromycetes and a sterile fungus were found to be associated with the different disease symptoms of cinnamon leaf in moist chamber methods. The associated fungi were *Aspergillus flavus*, *A. niger*, *Colletotrichum gloeosporioides*, *Colletotrichum* sp., *Fusarium* sp., *Pestalotiopsis palmarum*, *Rhizopus* sp. and a sterile fungus isolated from the infected leaves in the moist chamber are shown in Table 1 and Fig. 8.

Table 1. Fungi recorded from the infected leaves with different disease symptoms of cinnamon leaf in moist chamber.

Name of fungi	Small leaf spots	Gray leaf blight	Leaf blight
Aspergillus niger	+	+	+
Aspergillus flavus	+	+	+
Colletotrichum gloeosporioides	+	-	+
Colletotrichum sp.	+	-	-
Fusarium sp.	-	+	+
Pestalotiopsis palmarum	-	+	+
Rhizopus sp.	-	+	-
Sterile fungus	+	+	+

'+' = Present, '-' = Absent

Pathogenicity test

A pathogenicity test was done by following a detached leaf assay in vitro on apparently healthy, mature cinnamon leaves. After 12 days of inoculation, a dark brown zone developed on the surface of the inoculated leaves, and the healthy leaves have not shown any symptoms. Four fungi, such as *Colletotrichum gloeosporioides*, *Colletotrichum* sp., *Pestalotiopsis palmarum* and *Rhizomucor miehei* were capable of producing symptoms on leaves. The symptoms on inoculated leaves were very similar to those of a natural infection. Re-isolated fungi showed the same morphological characteristics as original isolates, thus fulfilling the pathogenicity test (Table 2, Fig. 7).

Table 2. Pathogenic potentiality of the associated fungi of the different types of leaf spots.

Name of fungi	Pathogenic potentiality
Aspergillus niger	Non-pathogenic
Aspergillus flavus	Non-pathogenic
Colletotrichum gloeosporioides	Pathogenic
Colletotrichum sp.	Pathogenic
Fusarium sp.	Non-pathogenic
Pestalotiopsis palmarum	Pathogenic
Rhizopus sp.	Non-pathogenic
Rhizomucor miehei	Pathogenic

Leaf spot, gray leaf spot, and leaf blight disease symptoms were recorded during the survey period. Leaf spot and leaf blight caused by *Colletotrichum gloeosporioides* is the major and common disease of cinnamon. It appears in all growth stages of the plant, and the entire foliage can be affected in severe infections (Anandaraj and Devasahayam 2004). Kumara (1999) reported 18% foliar damage due to leaf spot disease in Matara, Sri Lanka. The symptoms in young seedlings include small brown specks on leaf laminae, which later coalesce to form irregular patches. Bhat *et al*. (1988) reported seedlings of *Cinnamomum zeylanicum* in India affected by a leaf spot disease leading to partial drying of the plants, and those seedlings yielded *C. gloeosporioides* (*Glomerella cingulata*). At severe infections, lesions may be covered more

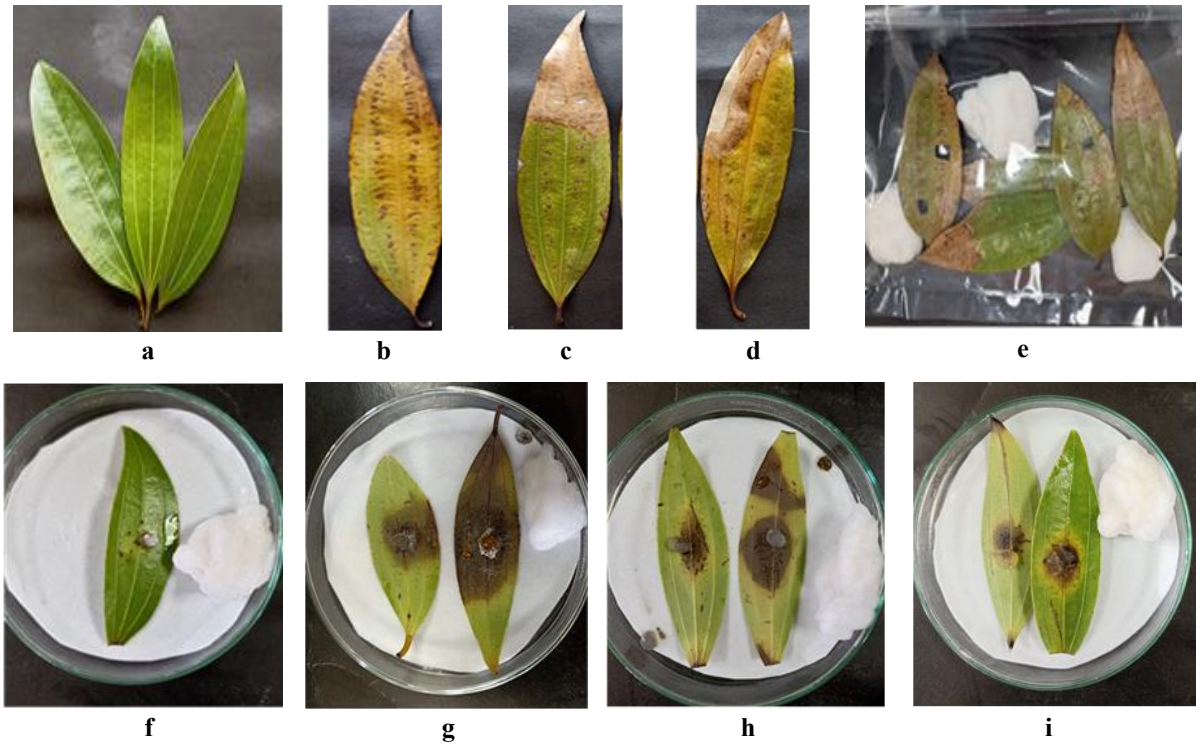
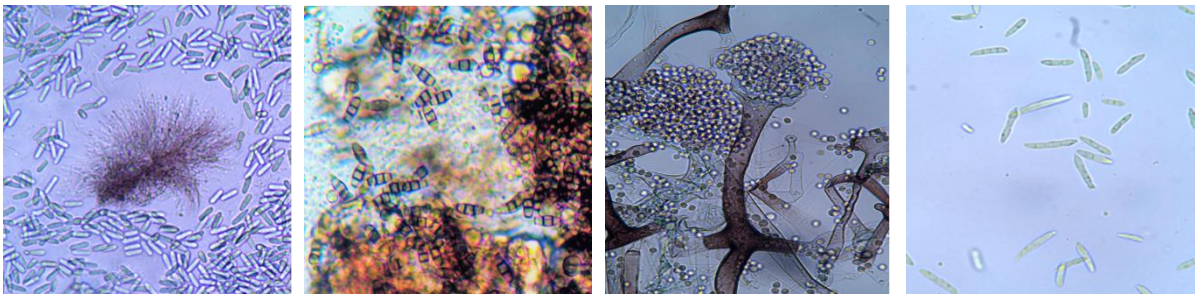


Fig. 7. Photograph showing foliar diseases of *Cinnamomum zeylanicum*: **a.** Fresh leaves; **b.** Leaf spot disease; **c.** Leaf blight disease; **d.** Gray leaf spot disease; **e**. Diseased leaves of *C. zeylanicum* in moist chamber; **f.** Control leaf; **g.** Diseases symptom produced on cinnamon leaf by *Colletotrichum gloeosporioides*; **h**. Diseases symptom produced on cinnamon leaf by *Pestalotiopsis palmarum*; and **i.** Diseases symptom produced on cinnamon leaf by *Rhizomucor* sp. *in vitro*.

than the half of the leaf. In some seedlings, the infection extends to the stem, causing a dieback (Karunakaran and Nair 1980). Karunakaran *et al*. (1993) reported gray blight disease of cinnamon which causes foliar damage. The symptoms appeared as small yellow-brown spots which later became whitish to gray with a dark brown border. This disease can cause severe damage and defoliation. In India, the disease was reported to be caused by *P. palmarum* on *C. verum,* causing foliar damage up to 90% (Karunakaran *et al*. 1993). *Pestalotia furierea* causing similar leaf spot symptoms was reported from the Dominican Republic and also from Pakistan (Ciferri 1926, Ciferri and Fragoso 1927). Fu and Chang (1999) also reported brown to black spots on *C. verum* leaves in Taiwan. These spots later coalesced and the infected leaves were shed.



a **b** **c** **d**

Fig. 8. Photograph showing fungi associate with foliar diseases of mycoflora: **a**. *Colletotrichum gloeosporioides*; **b**. *Pestalotiopsis palmarum*; **c**. *Rhizomucor miehei*; and **d.** *Fusarium* sp.

The study recommends early pathogen diagnosis and adoption of control techniques for leaf spot, leaf blight and gray leaf spot diseases in order to reduce the spread of these diseases, prevent financial losses and boost cinnamon yield.

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