

IDENTIFICATION AND PATHOGENIC POTENTIALITY OF FUNGI ASSOCIATED WITH *HOULTTUYNIA CORDATA* THUNB.

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Abstract

A total of 17 species of fungi representing 11 genera were found to be associated with *Houttuynia cordata* Thunb. The isolated fungi were *Aspergillus flavus* Link, *Aspergillus niger* Van Tiegh., *Cercospora houttuyniicola* Goh et Hsieh, *Colletotrichum coffeanum* Noack, *Colletotrichum dematium* (Pers. Ex Fr.) Grove, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Curvularia brachyspora* Boedijn., *Curvularia fallax* Boedijn., *Curvularia pallescens* Boedijn., *Fusarium* sp., *Nigrospora sacchari* (Speg.) Mason., *Penicillium* sp., *Pestalotiopsis* spp., *Syncephalastrum* sp., *Trichoderma viride* Pers. Ex Fries. and a member of Zygomycetes. *Cercospora houttuyniicola* is a new record for Bangladesh. All the isolated fungi were tested for pathogenicity.

Introduction

Houttuynia cordata Thunb. is the sole species in the genus *Houttuynia* of the family Saururaceae. It is a traditional medicinal herb and known as "Chameleon Plant". Its local name is "Aistya Gachh". This plant is native to Japan, Korea, Southern China and Southeast Asia. In Bangladesh it is found in Sylhet and Narail districts.⁽¹⁾

The whole plant is antibacterial, antiinflammatory, antimicrobial, antiphlogistic, antiviral, depurative, diuretic, emmenagogue, febrifuge, hypoglycaemic, laxative and ophthalmic. Plant decoction is used internally in the treatment of many ailments including cancer, coughs, dysentery, enteritis and fever. Its use is said to strengthen the immune system. Externally, it is used in the treatment of snake bites and skin disorders. The leaf juice is antidote and astringent. A root extract is diuretic. The root is also said to be used in medicinal preparations for certain diseases of women. This plant is antitumorous and is a special medicine for lung cancer.⁽²⁻³⁾

A lot of research has been done on phytochemical, pharmacological and biochemical aspects of *H. cordata*. But research about its fungal disease is inadequate. So far *Cercospora houttuyniicola* has been reported on this plant from Taiwan.⁽⁴⁻⁵⁾

Two types of symptoms were found during the present investigation. These are anthracnose and leaf spots of which the former is the major one. The present studies were undertaken (i) to find out the association of the fungi with the healthy and infected plants and (ii) to detect the pathogenic potentiality of the associated fungi.

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Materials and Methods

Samples from healthy and infected leaves of *Houttuynia cordata* Thunb. were collected from the Botanical Garden, Curzon Hall campus, Dhaka University during November, 2008 to August, 2009. All the collected samples showed a number of associated fungi. Fourteen samples were examined.

The fungi were isolated from samples following the "Tissue Planting" method and "Blotter" method.⁽⁴⁾

In case of tissue planting method, 50 inocula each measuring 2 square mm were cut with a sterilized scalpel from a particular specimen and kept in a sterile Petri plate. The inocula were washed in sterile water and then surface sterilized by dipping in 10% Clorox for 3 - 5 min. Then the inocula were transferred to a sterile Petri plate containing sterile blotting paper to remove the surface water. Then three inocula were placed in each plate containing sterilized Potato Dextrose Agar (PDA) medium and incubated for 5 - 7 days at $25 \pm 2^{\circ}\text{C}$.

In "Blotter" method, moist chambers were made by placing two layers of filter paper on the bottom of the Petri plates, moistened with sterilized water, covered with upper lids and were sterilized (autoclaved at 15 lbs pressure and 120°C). Square 2 mm sized inocula were prepared from the leaf samples, surface sterilized with 10% chlorox for 3 - 5 min. A total number of 50 inocula were transferred in ten Petri plates and incubated for 5 - 7 days at $25 - 28^{\circ}\text{C}$. The fungi growing out of the inocula were transferred to separate plates and slants for further studies and storage. Percentage association of the fungi was also recorded.

For microscopic observations fungal structures like mycelia, spore bearing structures and spore were scrapped off from the surface with a scalpel or blade or picked up with a needle and was mounted in lacto phenol over a clean slide for microscopic observation. In case of hyaline structures, a little amount of aniline blue was added to the mounted fluid.

Identification of isolates were determined by following the standard literatures.⁽⁷⁻⁹⁾ All the specimens were preserved in the Herbarium, Mycology and Plant Pathology section, Department of Botany, University of Dhaka, Bangladesh.

The pathogenicity of all the isolated fungi were tested on detached leaf technique (modified).⁽¹⁰⁾ Moist chamber was prepared by placing small cotton bar at the corner of Petri plate and autoclaved. Six treatments with three replications for each fungus was used as follows: control for unpricked and pricked leaves; dorsally inoculated unpricked and pricked leaves; and ventrally inoculated unpricked and pricked leaves.

All the test fungi were also inoculated on stem. The pricked and unpricked portion of stems were inoculated with 2 mm square mycelial block. Here four treatments with

two replications for each fungus were used as follows: control for unpricked and pricked stems; and unpricked and pricked inoculated stems.

Results and Discussion

A total of 17 species of fungi representing 11 genera were found to be associated with *Houttuynia cordata*. Fungi were isolated from healthy and infected leaves (Fig. 1A-C).



Fig. 1. A. Healthy plants of *Houttuynia cordata*, B. Anthracnose symptom and C. Leaf spot symptom.

Ten fungal species were isolated from healthy leaves of *Houttuynia cordata* of which the percentage of association of *Pestalotiopsis* sp.1 was highest (11.43).

In case of anthracnose symptom of the plant, 13 fungal species were isolated. Frequency of association of *Colletotrichum gloeosporioides* was highest (32.59%; Table 1).

Table 1. Frequency (%) of association of fungi with leaves of *Houttuynia cordata*.

Name of isolates	Healthy leaves	Diseased leaves (anthracnose)
<i>Aspergillus flavus</i>	0.95	3.33
<i>Aspergillus niger</i>	0.48	4.29
<i>Colletotrichum coffeanum</i>	7.62	7.62
<i>Colletotrichum dematium</i>	-	5.71
<i>Colletotrichum gloeosporioides</i>	8.10	32.59
<i>Curvularia brachyspora</i>	10.48	3.81
<i>Curvularia fallax</i>	-	3.33
<i>Curvularia pallescens</i>	4.76	2.86
<i>Fusarium</i> sp.	-	1.90
<i>Nigrospora sacchari</i>	1.90	-
<i>Penicillium</i> sp.	0.48	-
<i>Pestalotiopsis</i> sp. 1	11.43	3.33
<i>Pestalotiopsis</i> sp. 2	10.48	12.86
<i>Syncephalastrum</i> sp.	-	1.90
<i>Trichoderma viride</i>	-	1.90

- = Not isolated.

Cercospora houttuyniicola was frequently associated with leaf spot symptom but did not isolated on culture plates. From leaf spot symptoms four fungal species were isolated. Frequency of *Colletotrichum gloeosporioides* was highest (40.0%; Table 2).

Table 2. Frequency (%) of association of fungi with infected leaves (spot) of *Houttuynia cordata*.

Name of fungi	Diseased leaves (leaf spot)
<i>Cercospora houttuyniicola</i>	13.34
<i>Colletotrichum gloeosporioides</i>	40.0
<i>Fusarium</i> sp.	10.0
One unidentified lower fungus	3.34

The present report is the first record of *Cercospora houttuyniicola* from Bangladesh.

Taxonomic enumeration of the newly recorded taxon

Cercospora houttuyniicola Goh *et* Hsieh.

(Fig. 2A-D)

Leaf spots circular or irregularly suborbicular, 1 - 8 mm (mostly 2 - 4 mm) in diameter, greyish brown in the center with a dark reddish brown margin, dead tissues may drop out leaving the leaf with holes. Fruiting amphigenous. Secondary mycelium absent. Stromata absent or a few brown cells. *Conidiophores* arise singly or 2 - 4 (rarely up to 10) in a fascicle, deep olivaceous brown to pale brown, paler towards the apex,

irregular in width, curved, not branched, multiseptate, sometimes slightly constricted at the septa, $60 - 180 \times 4 - 7 \mu\text{m}$. Conidial scars conspicuously thickened. *Conidia* acicular, hyaline, curved or undulate, indistinctly multiseptate, subacute at the apex, truncate at the base with a thickened hilum, $100 - 300 \times 3 - 4 \mu\text{m}$.⁽⁵⁻⁶⁾

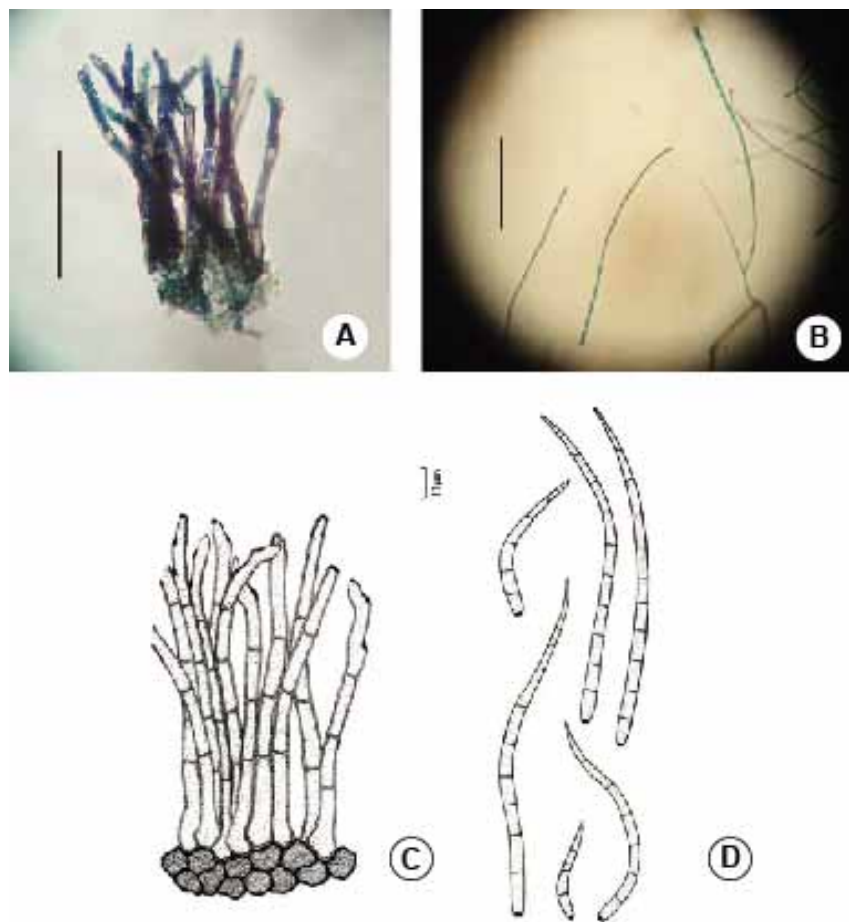


Fig. 2. A. Conidiophores, B. Conidia of *Cercospora houttuynicola*. (Bar = $50 \mu\text{m}$),
C. Conidiophores and D. Conidia of *Cercospora houttuynicola*.

All the isolated fungi were tested for pathogenic potentiality. Pathogenicity test following detached leaf inoculation technique revealed that *Colletotrichum coffeanum*, *C. dematium* and *C. gloeosporioides* produced symptoms on *Houttuynia cordata* (Fig. 3 and Table 3).

Colletotrichum coffeanum, *C. dematium*, and *C. gloeosporioides* were also capable of causing characteristic symptoms on inoculated stems (Fig. 4 and Table 4).

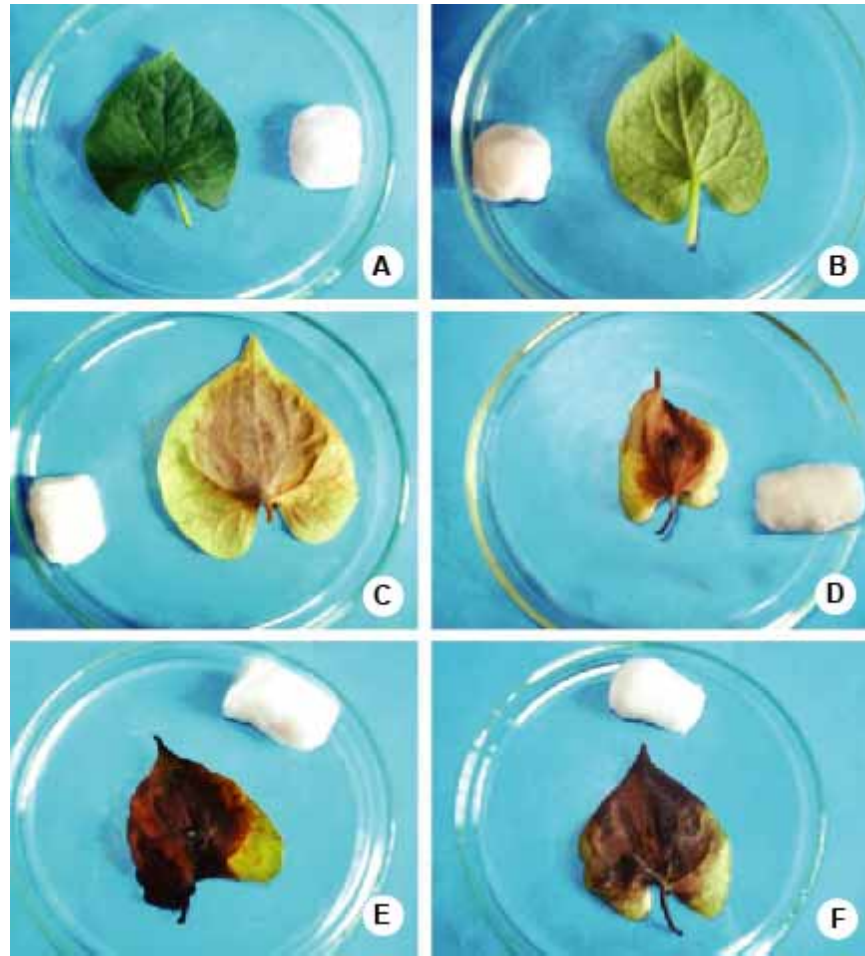


Fig. 3. A. Uninoculated dorsally pricked leaf of *Houttuynia cordata*, B. Uninoculated ventrally pricked leaf, C. Symptom produced by *Colletotrichum coffeanum* on ventrally inoculated leaf, D. *Colletotrichum dematium* on ventrally inoculated leaf, E. *Colletotrichum gloeosporioides* on dorsally inoculated leaf and F. *Colletotrichum gloeosporioides* on ventrally inoculated leaf.

Table 3. Response of the fungi to produce symptoms after inoculation on leaf.

Inoculation type	Response of the fungi		
	<i>Colletotrichum coffeanum</i>	<i>C. dematium</i>	<i>C. gloeosporioides</i>
T1 = Unpricked	-	-	-
T2 = Pricked	-	-	-
T3 = Unpricked dorsally	-	-	-
T4 = Pricked dorsally	+	+	+
T5 = Unpricked ventrally	-	-	-
T6 = Pricked ventrally	+	+	+

+ and - indicate positive and negative response, respectively.

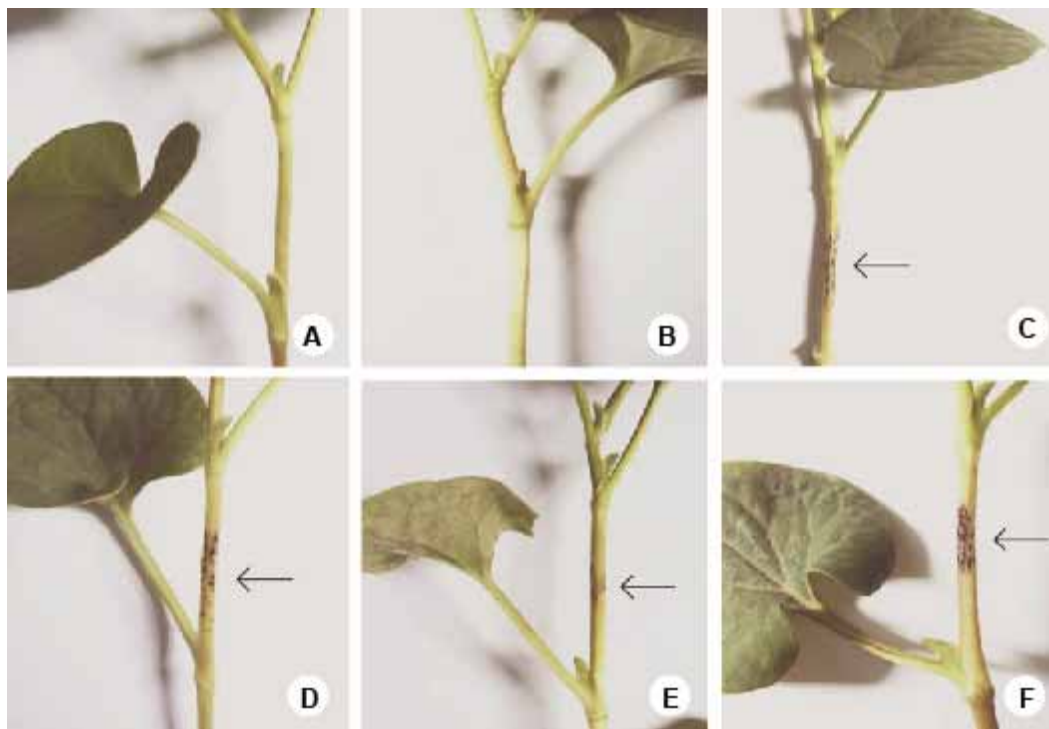


Fig. 4. A. Uninoculated unpricked stem of *Houttuynia cordata*, B. Uninoculated pricked stem, C. Symptom produced by *Colletotrichum coffeanum* on pricked stem, D. *Colletotrichum dematium* on pricked stem, E. *Colletotrichum gloeosporioides* on unpricked stem and F. *Colletotrichum gloeosporioides* on pricked stem.

Table 4. Response of the fungi to produce symptoms after inoculation on stem.

Inoculation type	Response of the fungi		
	<i>Colletotrichum coffeanum</i>	<i>C. dematium</i>	<i>C. gloeosporioides</i>
T1 = Unpricked	-	-	-
T2 = Pricked	-	-	-
T3 = Unpricked inoculation	-	-	+
T4= Pricked inoculation	+	+	+

+ and - indicate positive and negative response, respectively.

Uninoculated unpricked and pricked leaves and stems (control) did not produce any symptom. It was revealed from the result that test fungi were capable of causing infection on pricked leaves and stems. In case of *C. gloeosporioides* only, unpricked stems inoculated by the fungus showed characteristic symptom.

The disease symptoms produced by *Colletotrichum* spp. were similar to those symptoms that were observed in nature. Each test fungus was reisolated from the artificially inoculated leaves and stems.

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