

## FUNGAL DISEASES OF COTTON PLANT *GOSSYPIUM HIRSUTUM* L. IN BANGLADESH

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### Abstract

Seven types of symptom were recorded in four varieties such as CB3, CB9, Hill cotton 1 (HC-1) and 2 (HC-2) of cotton (*Gossypium hirsutum* L.) during July, 2005 to June, 2008. The symptoms were Anthracnose, Alternaria leaf spot, Boll rot, Cercospora leaf spot, Rust, Sclerotium rot and Wilting. Present study revealed that association of 30 species of fungi which belonged to 21 genera such as one in Zygomycetes, one in Ascomycetes, one in Basidiomycetes, 18 in Deutermycetes and one in sterile fungus. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Arthrinium* sp., *Ascochyta* sp., *Aspergillus flavus*, Link., *Aspergillus niger* Van Tiegh, *Aspergillus* sp., *Botryodiplodia* sp., *Cercospora* sp., *Chaetomium* sp., *Chaetophoma* sp., *Cladosporium* sp., *Colletotrichum coffeanum* Noack, z. f. Pflanzl., *Colletotrichum* spp., *Curvularia clavata* Jain, *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliforme*, Wr. and Reink, *Fusarium* spp., *Nigrospora* sp., *Pestalotia* sp., *Penicillium* sp., *Phoma* sp., *Pteroniconium* sp., *Puccinia* sp., *Rhizopus stolonifer* (Ehrenb. Ex. Fr) Lind, *Sclerotium rolfsii* Sacc., *Trichoderma viride* Pers. ex Fries. and one sterile fungus. *Fusarium* spp. and *Colletotrichum* spp. were frequently associated with all the four cotton varieties examined. *Botryodiplodia* sp. was associated with Hill Cotton 1 (HC-1) and Hill Cotton 2 (HC-2). *Sclerotium rolfsii* was found associated with CB-3 cotton variety.

### Introduction

Cotton plant (*Gossypium* sp.), belongs to Malvaceae. It is a shrub native to tropical and subtropical regions around the world, including America, India and Africa. However, virtually all of the commercial cottons cultivated today world-wide are grown from varieties of the native American species *Gossypium hirsutum* and *G. barbadense*. Cotton is cultivated in several distinct geographical areas, including Africa, Arabia, Australia and Mesoamerica. During the past several centuries, people native to these regions developed four distinct species of cultivated cotton including upland cotton (*Gossypium hirsutum* L. the primary species grown in the United states. In 2009 five leading cotton producing countries are United States, India, Uzbekistan, Brazil and Australia. The cultivar grown in Bangladesh is known as American cotton (*Gossypium hirsutum*). *G. arboreum*, locally called Comilla Cotton, is grown in hilly regions of Chittagong, and the Chittagong hill tracts. In Bangladesh *G. hirsutum* is grown in about

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35,000 ha and annual yield is approximately 35,000 mt. Locally produced cotton meets about 16% of the country's cotton need. The Cotton Development Board of the Ministry of Agriculture is in charge of cotton cultivation in the country.

The yield of cotton plant is affected by fungal disease along with bacteria, virus, nematodes and pests. Fungal diseases are anthracnose caused by *Colletotrichum gossypii* Southworth/*Glomerella gossypii* Edgerton, areolate mildew caused by *Ramularia gossypii* (Speg.) Cif. Ascochyta blight caused by *Ascochyta gossypii* Woromichin, black root rot caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris, charcol rot *Macrophomina phaseolina* (Tassi) Goidanich, *Colletotrichum gossypii*, *Glomerella gossypii*, Fusarium wilt caused by *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *vesinfectum* (Atk.) W. C. Synder & H. N. Hans., leaf spot caused by *Altenaria macrospora*, A. Zimmerm. *A. alteraata*, *Cercospora gossypina* Cooke, *Mycosphaerella gossypina* (Atk.) Earle *Cochliobolus spicifera* R.R. Nelson, *Bipolaris spifceera* (Bainier) Subramenium, *Myrotheceum roridum* Tode : Fr, *Rhizoctonia solani* Kuhn, *Stemphylium solani* G.F. Weber, lint contamination caused by *Aspergillus flavus*, *Nematospora*, spp. *Nigrospora oryzae*, *Phymatotrichum* root rot caused by *Phymatotrichopsis omnivora* (Duggar) Hennebert, powdery mildew caused by *Leveillula taurica* (Lev) G. Arnaud, *Salmonia malachrae* (Seaver) Blumer & E. Muller, cotton rust caused by *Puccinia scheedonnardi* Kellerm. & Swingle, Southern cotton rust caused by *Puccinia cacabata* Arth. & Holw. in Arth., tropical cotton rust caused by *Phakospora gossypii* (Lagerh.) Hiratsuka, Sclerotium stem and root rot caused by *Sclerotium rolfsii*, *Athelia rolfsii* (Curzi) Tu & Kimbrough, seedling disease complex caused by *Colletotrichum gossypii*, *Fusarium* spp., *Pythium* spp., *R. solani*, *T. basicola*, *Chalara elegans* Nag Raj & Kendrick, stem canker caused by *Phoma exigua* Desmaz and *Verticillium*, wilt caused by *Verticillium dahliae* Kleb. Boll rot disease of cotton is one of the notorious disease of cotton. *Ascochyta gossypii*, *Colletotrichum gossypii*, *Fusarium* spp. *Lasiodiplodia theobrome* (Pat.) Griffon & Maubl, *Botryosphaeria rhodina* (Cooke) Arx, *Phytophthora* spp. and *Rhizoctonia solani* have been reported from boll rot symptom. *Sclerotium rolfsii* was reported on stem and root of infected cotton but information regarding the association of fungi and the causal agent is inadequate. From Bangladesh *Sclerotium rolfsii* was isolated from boll for the first time.<sup>(1-4)</sup>

Compared to other cotton growing countries of the world, cotton diseases were less prevalent in Bangladesh. But with changed environment and climate presently cotton plants are becoming susceptible and attacked by various fungi and different types of symptom. In Bangladesh information regarding the association of fungi and the causal agent is inadequate. The present study was therefore undertaken: (i) to record the various symptoms on cotton plant, (ii) to isolate the fungal organisms associated with the plant, (iii) to characterize the isolates and (iv) to identify the associated fungi.

### Materials and Methods

Seventy five samples were collected from Sripur, Gazipur, Chittagong hill tracts and Botanic Garden, Curzon Hall Campus, University of Dhaka during July, 2005 to January, 2008. All these samples showed a number of associated fungi. Thirty nine samples were examined from healthy and infected seed, stem, leaf, fruit and infected root of *Gossypium hirsutum*.

The fungi were isolated on Potato Dextrose Agar (PDA) medium following "Tissue Planting" method. From a particular sample 30 inocula, each measuring 2 square mm were cut and kept in a sterile Petri plate. The inocula were washed in sterile water and then surface sterilized by dipping in 10% Chlorox for 3 - 5 min and transferred to a sterile Petri plate containing sterile blotting paper to remove the surface water. These three inocula were placed in each plate and incubated for 5 - 7 days at  $25 \pm 1^\circ\text{C}$ . Fungi growing out of the inocula were transferred to separate plates and slants for further studies and storage. Percentage of association of the fungi was also recorded. Two hundred seeds were incubated per variety per year. Seeds were surface sterilized with 10% Chlorox for 10 min and isolated following 'Blotter' method. Ten seeds were inoculated per plate.

For microscopic observations fungal structure like mycelia, spore bearing structures and spores were scrapped off from the surface with a scalpel or a 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 the isolates was made following the standard literatures.<sup>(5-9)</sup> All the specimens were preserved in the Salar Khan Herbarium, Mycology and Plant Pathology section, Department of Botany, University of Dhaka, Bangladesh.

### Results and Discussion

In the present study seven types of disease symptoms were recorded on four varieties of cotton plant. The symptoms were:

1. *Boll rot*: Small brown to black lesions on infected bolls and the entire boll eventually may become blackened and dried (Fig. 1A). Causal organism: *Fusarium* spp., *Colletotrichum* spp., *Botrydiploia* sp., and *Sclerotium rolfsii*.

2. *Wilting*: Small (2 mm) brown lesions on leaves that enlarge into irregular dead areas surrounded by a dark brown border. Lesion centre may crack and fall out. The stunted plant may fruit early. The yellowing spreads inward and is followed by necrosis leading to defoliation at the bottom of the plant which spreads to the top resulting in progressive wilting of leaves and stem, defoliation and finally death of the plant occurred (Figs 1B and 2A). Causal organism: *Fusarium* spp.



Fig. 1A. Boll rot infected cotton boll, B. Wilt infected cotton boll and C. Infected cotton boll with *Sclerotia*.

3. *Anthraxnose*: Infected leaves show pinkish-brown spots that appear specially on lower surfaces. Large areas of the tissues around the veins become yellowish to brownish and eventually dry out. The fungus causes pink boll rot (Fig. 2B). Causal organism: *Colletotrichum* spp.

4. *Rust*: The initial symptoms are yellow spots on leaf surfaces. The spots develop into oval to elongate reddish-brown powdery and elevated lesions that contain a powdery mass of orange to reddish-brown spores (pustules) on the upper and lower leaf surfaces (Fig. 2E). Causal organism: *Puccinia* sp.

5. *Cercospora leaf spot*: Infected leaf showed tan to brown spots with curled leaf margin and later on it withers. As the disease progresses, the leaflets turn yellow and also with curled margin. Spots on the leaf petioles, stems and flower parts become elongated and have dark brown colour. On heavily infected plant, defoliation may occur (Fig. 2D). Causal organism: *Cercospora* sp.

6. *Alternaria leaf spot*: *Alternaria* produces leaf spots that start as tiny, dull brown, circular lesions, which may enlarge to spots 1/4 to 1/2 inch in diameter. Because of higher humidity, the lower leaves are more commonly affected. Spots also may occur on bolls (Fig. 2C). Causal organism: *Alternaria alternata*.

7. *Sclerotium rot*: Infected bolls were rotten and covered with numerous golden brown sclerotia. Cent per cent *S. rolfsii* was isolated from the infected samples (Fig. 1C). Causal organism: *Sclerotium rolfsii*.

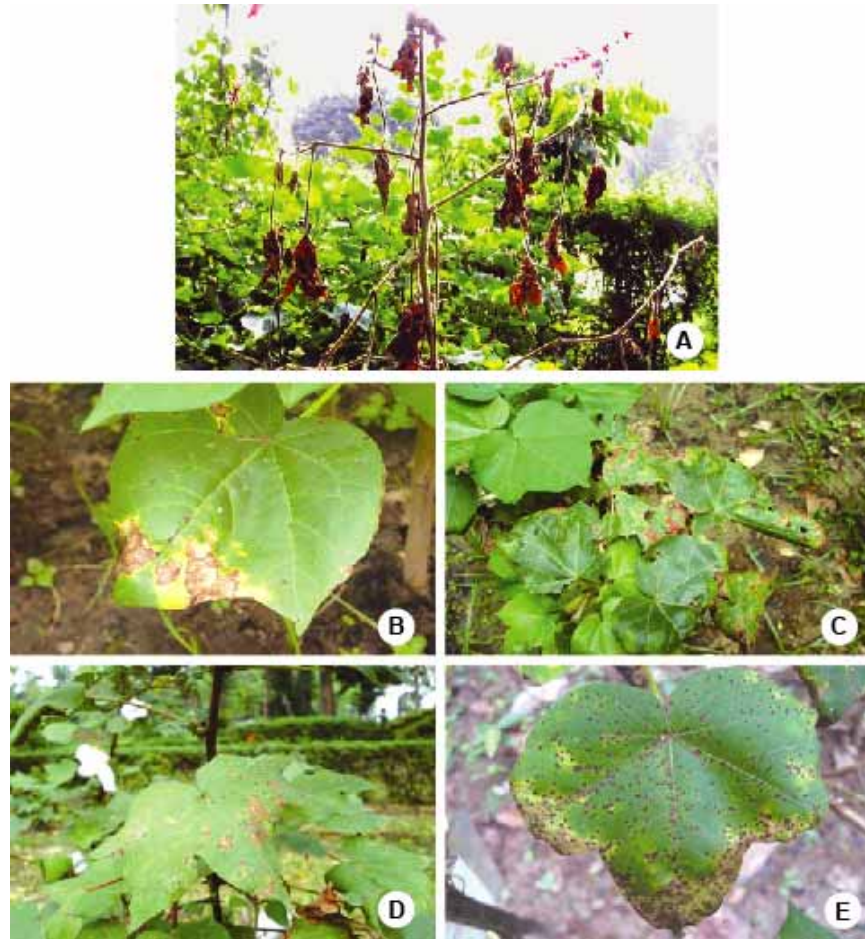


Fig. 2A. Cotton plant with wilting symptom, B. Anthracnose disease of cotton, C. Alternaria leaf spot of cotton, D. Cercospora leaf spot of cotton and E. Rust symptom on cotton leaf.

From four varieties of cotton plant 30 species of fungi were found. The isolated fungi were *Alternaria alternata*, *Arthrinium* sp., *Ascochyta* sp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp., *Botryodiplodia* sp., *Cercospora* sp., *Chaetomium* sp., *Chaetophoma* sp., *Cladosporium* sp., *Colletotrichum Coffeanum*, *Colletotrichum* spp., *Curvularia clavate*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium* spp., *Nigrospora* sp., *Pestalotia* sp., *Penicillium* sp., *Phoma* sp., *Pteroniconium* sp., *Puccinia* sp., *Rhizopus stolonifer*, *Sclerotium rolfsii*, *Trichoderma viride* and one sterile fungus.

The percentage association of fungi with CB-3 varied in different years. The percentage association of *Fusarium* spp. was higher (12.69) in the year 2005 than that of other seasons. The percentage of association of *Colletotrichum* sp. was 10.44 in the year 2006. Frequency of percentage of *Fusarium* spp. was higher (12.69) and *Curvularia* sp., *Pteroniconium* sp. and *S. rolfsii* was lower (0.23) (Table 1).

**Table 1. Frequency (%) mean of different fungi with four varieties of diseased cotton plant during 2005-2008.**

Fungi	CB3				CB9				HC-1				HC-2			
	2005	2006	2007	2008	2005	2006	2007	2008	2005	2006	2007	2008	2005	2006	2007	2008
<i>Alternaria alternata</i>	0	0.45	0	0	1.34	0	1.99	0.23	0	0	0	0	0	0	0	0
<i>Arthrinium</i> sp.	0	1.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascochyta</i> sp.	8.0	0	0.89	0.89	0	0	4.67	4.67	0	16.67	0	0	0	0	0.45	0
<i>Aspergillus flavus</i>	0.14	2.67	0	0.45	2.34	1.56	1.56	0.89	4.00	0	0	0	3.34	10.00	0	4.00
<i>A. niger</i>	6.0	3.11	8.22	0	8.83	6.67	8.23	8.23	8.67	0	0	1.56	9.34	0	8.87	0
<i>Botryodiplodia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	11.67	0	0	0
<i>Cercospora</i> sp.	0	0	0	0.23	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cladosporium</i> sp.	3.47	0	3.11	0.89	13.17	3.34	3.34	0	4.00	0	0	0	0	0	6.00	1.78
<i>Chaetomium</i> sp.	0	0	0.23	0	0	0	5.11	5.11	0	0	2.23	2.23	0	0	0	1.17
<i>Chaetophoma</i> sp.	0	0	3.34	3.34	0	0	0	4.67	0	0	0	0	0	2.67	0.23	0
<i>Colletotrichum</i> spp.	0	10.44	0	4.45	0.34	7.33	0	8.44	2.00	0.67	0	4.00	1.47	10.67	0	0
<i>Curularia</i> spp.	5.34	0.23	0.23	0.45	8.00	0	0.45	0.45	7.89	0	0.67	0	0	0	0	0
<i>Fusarium</i> spp.	12.67	3.78	2.0	2.0	22.8	4.00	4.00	1.11	6.14	5.34	2.23	0	8.5	0	0.23	0
<i>Nigrospora</i> sp.	0	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pestalotia</i> spp.	0	0	0	0	0	0.23	0.23	0	0.94	0	0	0.67	0	2.00	0	0.23
<i>Penicillium</i> sp.	9.74	3.34	3.34	3.77	5.50	2.67	2.67	2.23	0.03	1.34	1.11	1.11	2.5	0	0	5.11
<i>Phoma</i> sp.	0	0	1.11	1.11	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pterocotium</i> sp.	0	0	0.23	0.23	0	0	0.23	0.23	0	0.23	0.23	0	0	0	1.33	1.33
<i>Puccinia</i> sp.	0	0	0	0.23	0	0	0.45	0.23	0	0	0	0	0	0	0	0
<i>Rhizopus stolonifera</i>	0.27	0.89	2.0	0	0.67	2.34	0.67	0	1.87	0	0.23	0.23	0	0	0.23	0
<i>Sclerotium rolfsii</i>	0	0.23	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichoderma viride</i>	0	0	0	3.77	0	0	0.23	0.23	0.28	0	1.11	1.11	0	0	0.67	0
Sterile	0	9.77	9.77	3.77	0.34	4.34	5.11	5.11	0	6.00	0	3.34	0	0	6.45	0

Mean calculated from 8 samples examined per variety per year.

The percentage of association of fungi with CB-9 varied in different years. The percentage of association of *Fusarium* spp. was higher (22.8) in the year 2005 than that of other seasons. The percentage of association of *Cladosporium* sp. was 13.17 in the year 2005. Frequency of percentage of *Fusarium* spp. was higher (22.8) and *Alternaria alternata*, *Pestalotia* sp., *Pteroconium* sp. and *Trichoderma viride* was lower (0.23) (Table 1).

The percentage of association of fungi with HC-1 varied in different years. The percentage of association of *Ascochyta* sp. was higher (16.7) in the year 2005 than that of other seasons. Frequency of percentage of association of *Ascochyta* sp. was higher (16.7) and *Pteroconium* sp. and *Rhizopus stolonifer* was lower (0.23) (Table 1).

The percentage of association of fungi with HC-2 varied in different years. The percentage of association of *Botryodiplodia* sp. was higher (11.67) in the year 2006 than that of other seasons. The percentage of association of *Chaetophoma* sp., *Fusarium* spp., *Pestalotia* sp., *Rhizopus stolonifer* was lower (0.23) (Table 1).

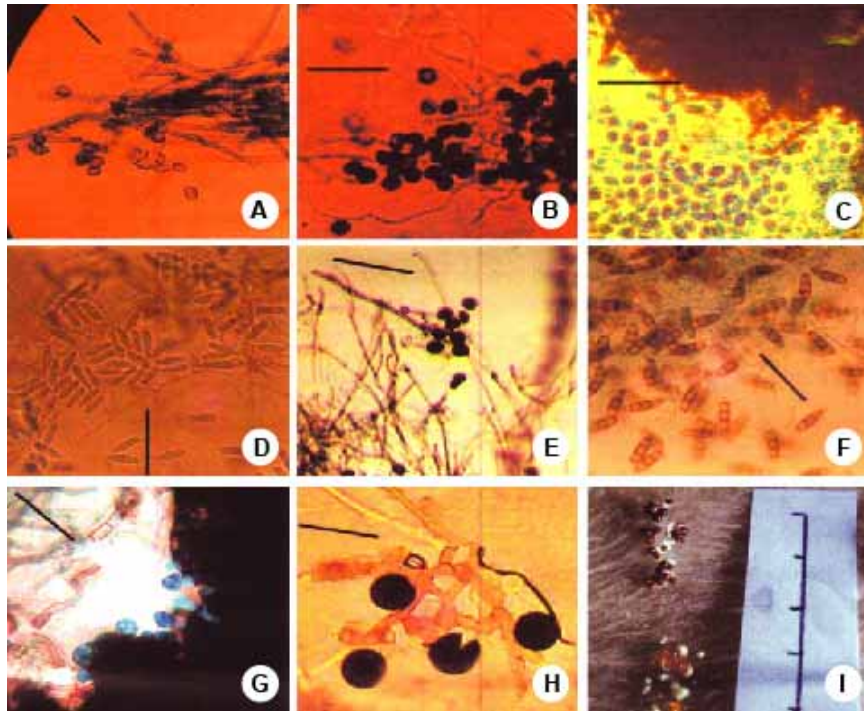


Fig. 3. Microphotograph showing conidiophores and conidia of A. *Chaetomium* sp., B. *Arthrinium* sp., C. *Chaetophoma* sp., D. *Colletotricum coffeanum*, E. *Nigrospora* sp., F. *Pestalotia* sp., G. *Phoma* sp., H. *Pteroconium* sp. (Bar = 50  $\mu$ m) and I. Sclerotia of *Sclerotium rolfsii* on PDA plate.

The present report is the first record of association of *Chaetomium* sp., *Arthrinium* sp., *Chaetophoma* sp., *C. coffeanum*., *Nigrospora* sp., *Phoma* sp., and *Pteroconium* sp. with cotton. *S. rolfsii* was first time isolated from cotton bolls (Fig. 3A-I).

The present study revealed the presence of 30 species of fungi belonging to 21 genera, associated with four varieties of cotton plant. The associated fungus were *Alternaria alternata*, *Arthrinium* sp., *Ascochyta* sp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp., *Botryodiplodia* sp., *Cercospora* sp., *Chaetomium* sp., *Chaetophoma* sp., *Cladosporium* sp., *Colletotrichum coffeanum*, *Colletotrichum* spp., *Curvularia clavate*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium* spp., *Nigrospora* sp., *Pestalotia* sp., *Penicillium* sp., *Phoma* sp., *Pteroniconium* sp., *Puccinia* sp., *Rhizopus stolonifer*, *Sclerotium rolfsii*, *Trichoderma viride* and one sterile fungus. Seven types of symptom were recorded on four varieties of cotton plant. The symptoms were Anthracnose, Alternaria leaf spot, boll rot, Cercospora leaf spot, rust, Sclerotium rot and wilting.

*Fusarium* spp. and *Colletotrichum* spp. were frequently associated with all the four cotton varieties examined. *Botryodiplodia* sp. was associated with Hill Cotton 1 (HC-1) and Hill Cotton 2 (HC-2). *Sclerotium rolfsii* was found associated with CB-3 cotton variety.

The present finding clearly indicates that wilting, Anthracnose and boll rot is the main diseases causing the damage of cotton plant and boll in Bangladesh. Present study will be helpful for designing the proper control measures of cotton plants.

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