

## DISEASE SEVERITY AND MYCOFLORA ASSOCIATED WITH ANTHRACNOSE ON LEAVES OF FIVE ANGIOSPERMS

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### Abstract

Severe anthracnose symptom was recorded on the leaves of five angiosperms during September to November, 2010. In total nine species of fungi belonging to four genera of the class deuteromycetes and a sterile fungus were found to be associated with the infected leaves of *Abelmoschus esculentus* (L.) Moench, *Cucurbita maxima* Duchesne, *Datura metel* L., *Gossypium hirsutum* L. and *Vigna sinensis* L. The associated fungi were *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. nidulans* (Eidam) G. Winter, *A. niger* van Tiegh, *Cercospora* sp., *Colletotrichum capsici* (Syd.) Butler & Bisby, *Colletotrichum dematium* (Pers.) Grove, *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., *Curvularia clavata* Jain, and a sterile fungus.

**Key words:** Mycoflora, anthracnose, leaves, five angiosperm

### Introduction

Anthrachnose is a major and most common fungal disease of different angiospermic plants throughout the world. It can spread very quickly in warm (80<sup>0</sup>F), wet weather, especially if air circulation is poor. This disease first appears as small, variously colored, circular spots on the older leaves, though it eventually spreads to younger leaves, stems, pods and fruits. The spots enlarge and merge, getting darker until the leaves drop off and the plant is defoliated and dies. In some cases symptoms appear as off-white, transparent lesions on leaves and other infected parts. *Colletotrichum* spp. are the causal organism of anthracnose disease (Douglas 2011).

Species of *Colletotrichum* produce spores in tiny, sunken, saucer-shaped fruiting bodies known as acervuli. Symptoms include sunken spots or lesions (blight) of various colours in leaves, stems, fruits, or flowers, and some infections form cankers on twigs and branches. Anthracnose causes the wilting, withering, and dying of tissues, though the severity of the infection depends on both the causative agent and the infected species and can range from mere unsightliness to death (Thurston 1998).

Many economically important crop plants, for example, cotton, cucurbits, tomato, banana, mango, onion and pepper are affected by the disease. Shade trees such as sycamore, ash, oaks, and maple are also susceptible. Lot of research had been carried out on anthracnose disease of economically important plants (Helene 1988, Thurston 1998, Latunde-Dada 1990 and Mukerji and Bhasin 1986). In Bangladesh a few research had been done on anthracnose disease of cotton and cow pea (Lutfunnessa and Shamsi 2011 and Shamsi *et al.* 2012). The present investigation

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was undertaken to record the disease severity, pathogen and other associated fungi with anthracnose symptom of five angiosperms those were severely affected in the year of 2010 in some vegetable gardens of the Dhaka city.

### Materials and Methods

Disease severity (DS) of anthracnose of leaf of five angiospermic plants *viz.* *Abelmoschus esculentus* (L.) Moench (Okra), *Cucurbita maxima* Duchesne (Pumpkin), *Datura metel* L. (Datura), *Gossypium hirsutum* L. (American cotton) and *Vigna sinensis* L. (Cowpea) was recorded following the scale stated by Ghos *et al.* (2009). These plants were grown at Botanical Garden of Curzon Hall Campus, Dhaka University and vegetable gardens near Mohakhali and Sobhanbag of Dhaka city during the month of January to December, 2010.

For visual estimation of disease severity, 0 - 9 point scale was used for rating all foliar disease studied (Ghos *et al.* 2009).

The associated fungi were isolated from symptomatic leaves following “Tissue Planting” method on PDA medium except *Cercospora* sp. was isolated following “Blotter” method.

Fifty plants were selected (ten from each angiosperm) randomly and 25 leaves from each plant were examined in each month.

Percentage of frequency of the occurrence of the fungal isolates was calculated following Spurr and Welty (1972):

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

The isolated fungi were purified following serial dilution technique and identified on the basis of morphological characteristics recorded under a compound microscope using standard key books (Barnett and Hunter 1998, Ellis 1971, 1976, Ellis and Ellis 1997, Sutton 1980).

Pathogenicity of the isolated fungi was tested following modified detached leaf assay technique (Azad and Shamsi 2011 and Shamsi *et al.* 2013).

### Results and Discussion

Anthracnose symptom on the leaves of five angiosperms are presented in Plate 1A-E. The highest disease severity (DS) of anthracnose (score 9) was recorded on okra, cowpea and datura in the month of November. The highest DS (score 8) was recorded on American cotton and pumpkin in the same month. The minimum DS (score 1) was recorded on all the angiospermic plants studied in the month of April (Table 1).

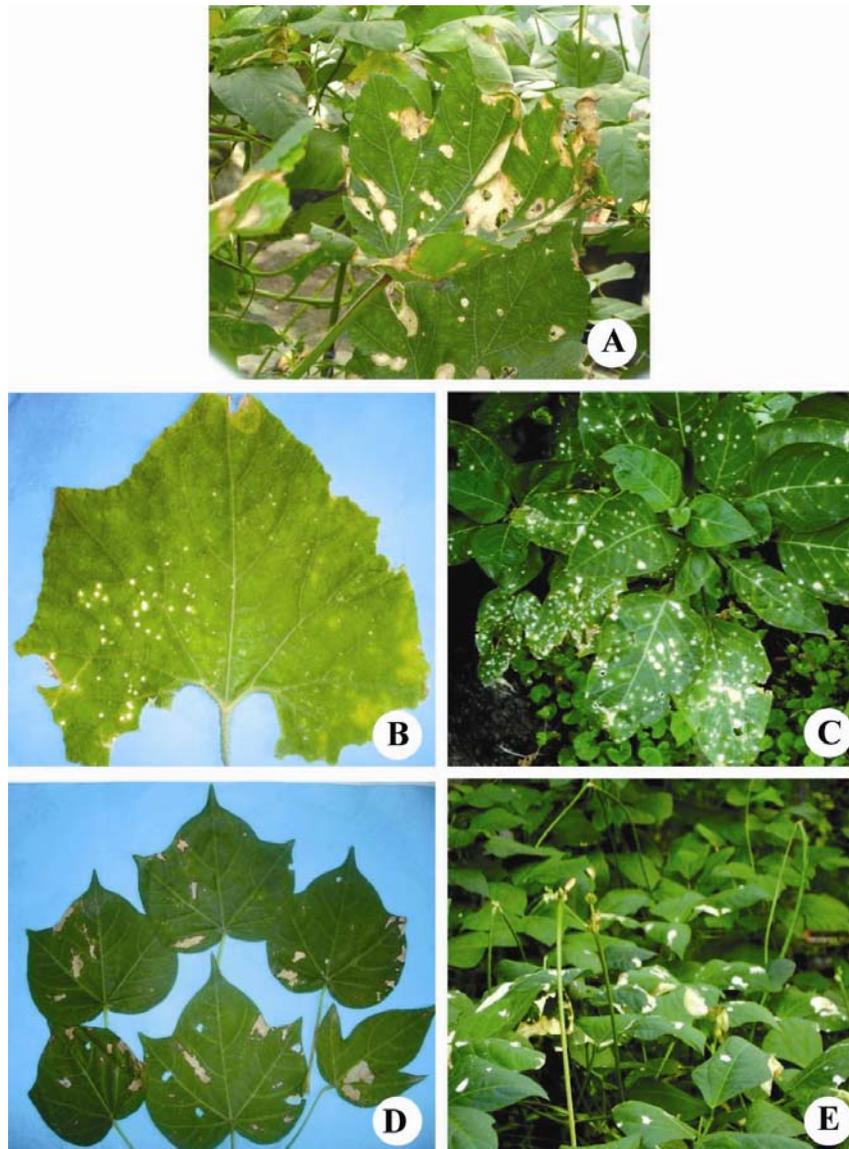


Plate 1. Anthracnose symptom on: (A) *Abelmoschus esculentus* (Okra); (B) *Cucurbita maxima* (Pumpkin); (C) *Datura metel* (Datura); (D) *Gossypium hirsutum* and (E) *Vigna sinensis* (Cow pea).

In total, nine species of fungi, belonging to four genera of the class deuteromycetes and a sterile fungus was found to be associated with the infected leaves of okra, pumpkin, datura, A. cotton and cowpea showing anthracnose symptom. The associated fungi were *Aspergillus flvaus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *Cercospora* sp., *Colletotrichum capsici*, *C. dematium*, *C. gloeosporioides*, *Curvularia clavata* and a sterile fungus (Table 2).

Table 2 shows that from symptomatic leaves of okra, six species of fungi and a sterile fungus were isolated. Per cent frequency of occurrence of *A. flavus* was highest (46.66) followed by sterile fungus (40) and it was lowest in *C. clavata* (6.66). Per cent frequency of occurrence of *C. capsici* and *C. gloeosporioides* were 13.33 and 6.66, respectively.

**Table 1. Disease severity of anthracnose recorded on five angiosperms during 2010.**

Name of plants	Months											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec
Okra	0	0	0	1	3	4	5	6	7	8	9	Plant died
A. cotton	0	0	0	1	2	3	4	5	6	7	8	''
Pumpkin	0	0	0	1	3	4	5	6	7	7	8	''
Datura	0	0	0	1	3	4	5	6	7	8	9	''
Cowpea	0	0	0	1	3	4	5	6	7	8	9	''

For visual estimation of severity, 0 - 9 point scale were used for rating of all foliar diseases studied

No infection - 0	40 - 50% leaf area infected - 5
0 - 10% leaf area infected - 1	50 - 60% leaf area infected - 6
10 - 20% leaf area infected - 2	60 - 70% leaf area infected - 7
20 - 30% leaf area infected - 3	70 - 80% leaf area infected - 8
30 - 40% leaf area infected - 4	80 - 90% or more leaf area infected - 9.

**Table 2. Per cent frequency of associated fungi with anthracnose symptomatic leaves of five angiospermic plants.**

Name of fungi	Name of angiospermic host				
	Okra	A. cotton	Pumpkin	Datura	Cowpea
<i>Apergillus flavus</i>	46.66	-	46.66	-	26.66
<i>A. fumigatus</i>	6.70	-	-	-	-
<i>A. nidulans</i>	6.70	-	-	-	-
<i>A. niger</i>	-	0.5	6.66	-	-
<i>Cercospora</i> sp.	-	-	10.00	-	-
<i>Colletotrichum capsici</i>	13.33	20.00	33.33	41.66	60.00
<i>C. dematium</i>	-	30.00	-	-	-
<i>C. gloeosporioides</i>	6.66	20.00	60.00	41.66	40.00
<i>Curvularia clavata</i>	6.66	52.00	13.33	16.66	20.00
Sterile fungus	40.00	20.00	6.66	25.00	6.66

- = Not found.

Mukerji and Bhasin (1986) reported *Colletotrichum capsici*, *C. gloeosporioides* and *C. hibisci* from okra.

In Bangladesh 16 diseases of okra have been recorded, among them anthracnose is one of the major disease. Anam *et al.* (2002) reported five seed borne fungal diseases in okra field. Foot and Root rot, Anthracnose and die-back, *Cercospora* leaf spot, *Corynespora* leaf spot and blight

caused by *Fusarium oxysporum*, *Colletotrichum dematium*, *Cercospora abelmoschi*, *Corynespora cassicola* and *Macrophomina phaseolina*, respectively.

Table 2 shows that four species of fungi and one sterile isolate were associated with symptomatic leaves of American cotton (*G. hirsutum*). Percentage frequency of occurrence of *C. clavata* was highest (52) followed by *C. dematium* (30), *C. gloeosporioides* and a sterile isolate (20). Lowest per cent frequency of occurrence was recorded in *A. niger* (5).

The family Malvaceae is large and consists of important agricultural crops (cotton, okra, kenaf etc.). The family, as a whole is affected by several anthracnose diseases. Three main species of *Colletotrichum* have been recognized as the causal agents of anthracnose, *Colletotrichum gossypii*, *C. malvarum* and *C. gloeosporioides*. *Colletotrichum gossypii* is the main causal organism of anthracnose of cotton (Bailey *et al.* 1996). Mukerji and Bhasin (1986) reported *C. capsici* and *C. indicum* on *G. hirsutum* from India.

Six species of fungi and a sterile isolate were associated with symptomatic leaves of pumpkin (*C. maxima*). Per cent frequency of association of *C. gloeosporioides* was highest (60) followed by *A. flavus* (46.66), *C. capsici* (33.33), *C. clavata* (13.33) and *Cercospora* sp. (10). Lowest per cent frequency of association was recorded in *A. niger* and a sterile fungus (6.66) (Table 2).

Anthracnose is a common fungal disease of pumpkin in fields, greenhouses, and high tunnels. The disease can develop on all above ground parts of pumpkin plants. In pumpkin anthracnose is caused by the fungus *Glomerella lagenarium* (*Colletotrichum orbiculare*). The symptoms of anthracnose vary somewhat on different hosts. On cucumber leaves the spots start as water soaked areas and expand into brown spots which are roughly circular (Wasilwa *et al.* 1993). Mukerji and Bhasin (1986) reported *C. capsici*, *C. lagenarium* and *C. orbiculare* on *C. maxima* from India.

Table 2 shows that three species of fungi and one sterile isolate were associated with symptomatic leaves of datura. Frequency percentage of association of *C. capsici* and *C. gloeosporioides* were highest (41.66) followed by sterile fungus (25). Lowest frequency percentage of association was recorded in *C. clavata* (16.66). Mukerji and Bhasin (1986) also reported *C. capsici* on *Datura* spp. from India.

In Bangladesh three species of fungi (*Colletotrichum* sp., *Pseudocercospora* sp. and *Trichoderma* sp.) found to be associated with anthracnose symptom of *Datura metel* and *Vigna catjang* (Shamsi *et al.* 2012).

Table 2 also shows that from anthracnose symptomatic leaves of cowpea (*V. sinensis*) four species of fungi and one sterile isolate were isolated. Per cent frequency of association of *C. capsici* was the highest (60) followed by *C. gloeosporioides* (40), *A. flavus* (26.66) and *A. fumigatus* (20). Lowest per cent frequency of association was recorded in sterile fungus (6.66).

The major fungal pathogen of cowpea crop was reported as the *Colletotrichum destructivum*. (Akinbode and Ikotun 2008, Emechebe and Lagoke 2002). Latunde-Dada (1990) reported anthracnose disease of five cowpea cultivars: Farin Juda-C, Ife Brown, Market Brown, IT82E-60

and TVX 3236 caused by *C. lindemuthianum*. Mukerji and Basin (1986) also reported *C. capsici*, *Colletotrichum dematium* and *C. lindemutheanum* on *V. sinensis* from India.

Difference may occur in result of the experiment due to variation of time, place/locality, weather conditions, plant varieties used in the experiment and ability of the fungi to attack the specific plants.

#### Details on the isolated pathogens are as follows

##### 1. *Colletotrichum capsici* (Syd.) Batler & Bisby (Plate 2A - B)

Colonies grayish. Hyphae brown, septate, branched, conidiophores are 3 - 45 × 2 - 6 µm, hyaline, cylindrical, unicellular or septate. Setae dark brown. 3 - 4 septate, 61 - 75 µm × 3.5 - 4.0 µm. Conidiogenous cells are 6 - 10 µm × 2.5 - 4 µm wide, hyaline, ellipsoidal to subglobose, conidia are 17 - 24 µm × 2.5 - 3.5 µm, one-celled, gluttulate, hyaline, fusiform with both ends pointed.

*Specimens examined:* Isolated from infected leaves of *Vigna sinensis*. Sobhanbag, Dhaka. S. Shamsi, 30 38, 30 June, 2010.

##### 2. *Colletotrichum dematium* (Pers.) Grove (Plate 2C - D)

Colonies dark, cottony, with black shining aervuli. Hyphae brown, septate, branched superficial. Setae dark brown. 3 - 4 septate, 62 - 72 µm × 3.5 - 4.0 µm. Conidiophores pale brown, 14 - 22 × 1.8 - 2.7 µm. Conidia solitary, curved, hyaline, 20 - 34 × 3 - 4 µm. Appressorium present, brown, small irregular, 4.8 - 6.0 × 4.0 - 48 µm.

*Specimens examined:* Isolated from infected leaves of cotton varieties CB6, Botanic garden, Dhaka University, Dhaka. S. Shamsi, 30 48, 13 July, 2010.

##### 3. *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. (Plate 2E-F)

Colonies grayish. Hyphae brown, septate, branched superficial. Setae dark brown. 3 - 4 septate, 59 - 72 µm × 3.5 - 4.0 µm. Conidia are straight, obtuse at the apex, (6.2) 11 - 27 to 2.2 - 3.4 (5) µm. Appressoria are 8.4 - 11.2 to 4.2 - 8.0 µm, clavate or irregular, sometimes becoming complex.

*Specimens examined:* Isolated from infected leaves of cotton varieties CB6, Botanic garden, Dhaka University, Dhaka. N. Naher, 75, 23 July, 2010.

Present results indicate that per cent frequency of association of anthracnose pathogen *C. capsici* was the highest (60) in *V. sinensis* followed by 41.66 and 33.33 in *D. metel* and *C. maxima*, respectively. Per cent frequency of association of the fungus was the lowest 13.33 in *A. esculentus*. Per cent frequency of association *C. dematium* was 30 in *G. hirsutum*. Per cent frequency of association of *C. gloeosporioides* was the highest (60) in *C. maxima* followed by

41.66, 40.00 and 20.00 in *D. metel*, *V. Sinensis* and cotton, respectively. Per cent frequency of association of the fungus was the lowest 6.66 in *A. esculentus*.

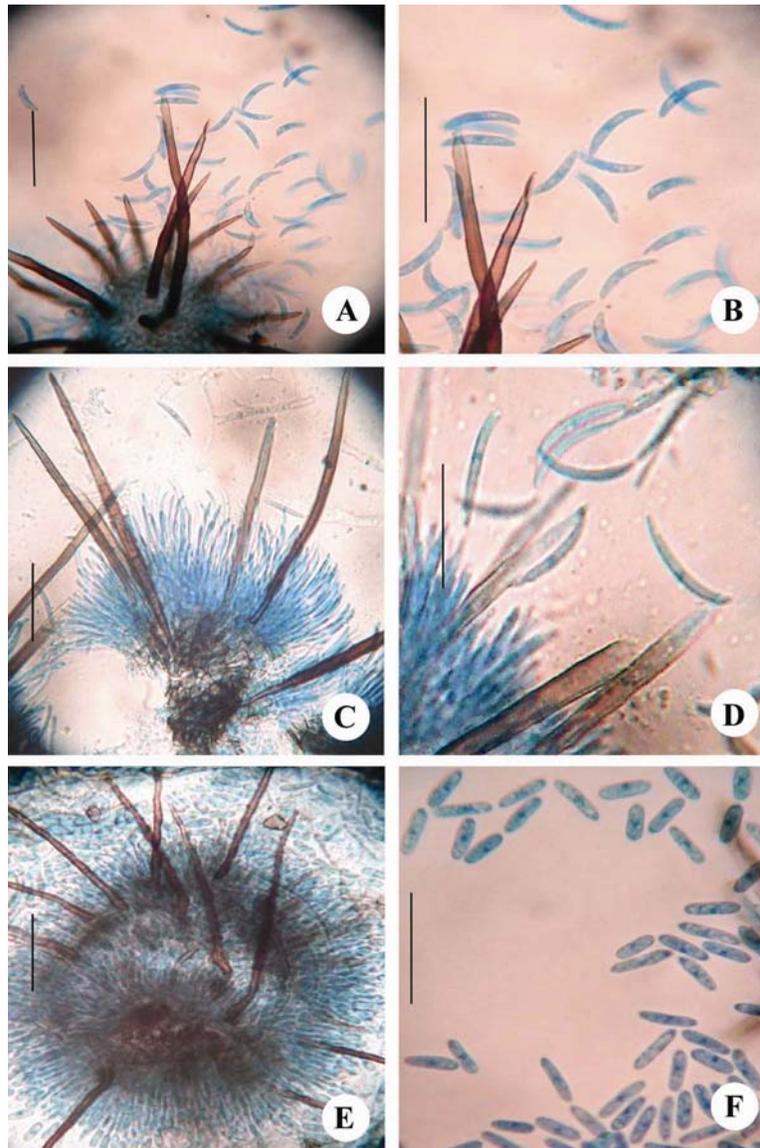


Plate. 2. Acervulus of: (A) *Colletotrichum capsici*; (B) Conidia and setae of *Colletotrichum capsici*; (C) Acervulus of *Colletotrichum dematium*; (D) Conidia and setae of *Colletotrichum dematium*; (E) Acervulus of *C. gloeosporioides* and (F) Conidia of *C. gloeosporioides*. (Bar = 50  $\mu$ m).

Pathogenicity test following modified detached leaf technique revealed that *Colletotrichum capsici* was pathogenic to *A. esculentus*, *C. maxima*, *D. metel*, *G. gossypium* and *V. sinensis*. *Colletotrichum dematium* was pathogenic to *G. gossypium* and *C. gloeosporioides* was pathogenic to *A. esculentus*, *C. maxima*, *D. metel*, *G. gossypium* and *V. sinensis*.

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