

## CHARACTERIZATION AND PATHOGENICITY OF FUNGI ASSOCIATED WITH ANTHRACNOSE OF CHILLI (*CAPSICUM FRUTESCENS* L.)

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### Abstract

A total of nine fungal species were isolated from the anthracnose infected chilli fruits. The fungi associated with infected chilli fruits were *Alternaria alternata* (Fr.) Keissler, *A. tenuissima* (Kunze ex Pers.), *Aspergillus flavus* Link, *A. niger* van Tieghem, *Colletotrichum dematium* (Pers.) ex Fr., *C. gloeosporioides* (Penz.) Sacc., *Curvularia clavata* Wakker, *Fusarium semitectum* Berk. & Rav. and *Rhizopus stolonifer* (Ehrenb.) Vull. Among the isolated fungi, *Colletotrichum dematium* (49.03), *C. gloeosporioides* (18.06) and *Fusarium semitectum* (20.12) showed the higher per cent frequency. Out of nine fungal species, *Colletotrichum dematium*, *C. gloeosporioides* and *Fusarium semitectum* were found to be pathogenic to chilli fruits.

### Introduction

Chilli (*Capsicum frutescens* L.) is one of the most important spices as well as vegetable crops in the world. It is used in many cuisines but also found to have many medicinal properties. Chilli crop suffers from about more than 40 fungal diseases (Rangaswami 1979). Major diseases of chilli are anthracnose, downy mildew, powdery mildew, white mold etc. Wilt and anthracnose are the most destructive diseases of chilli. In Bangladesh, several scientists worked on wilt disease of chilli caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium annuum* (Ahmed and Hossain 1985). So far, few research works have been done on anthracnose disease of chilli fruit in Bangladesh. Anthracnose disease caused by *Colletotrichum* spp. restricting chilli production, resulting in up to 40 % yield loss (Than *et al.* 2008). Anthracnose disease of chilli drastically reduces the quality and yield of chilli fruits resulting in low returns to farmers. So, for the sake of economy more information is needed on this regard. Considering the importance of chilli, the present investigation was undertaken to identify the fungi associated with infected chilli and to record their pathogenic potentiality.

### Materials and Methods

Samples with anthracnose symptoms of chilli fruits were collected from Karwan bazar, Ananda bazar and Badalgachhi (Naogaon) during the tenure of April to December 2017. Fungi associated with the diseased chilli were isolated separately following 'Tissue planting' method (CAB 1968). The microscopic observations of the isolated fungi were recorded under a digital camera. Morphological structures of the fungi were drawn in detail with the help of camera lucida. Identifications of the isolates were determined following the standard literature (Thom and Raper 1945, Raper and Thom 1949, Booth 1971, Ellis 1976, Ellis and Ellis 1997 and Barnett and Hunter 1972). Occurrence of the fungal isolates was calculated by adopting the formula of Spurr and Welty (1972).

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Pathogenicity test was done according to the method described by Islam *et al.* (2018) with slight modification. Chilli fruits were washed thoroughly in running tap water and dipped in 10% Chlorox solution for surface sterilization for three minutes. Then chilli was washed three times in sterilized distilled water to remove Chlorox from the surface. Excess water from the surface of the chilli was removed by pressing them between two sheets of previously sterilized filter paper. Sterilized cotton was placed in a corner of sterilized Petri plates. Before spraying, the fruits were wounded by previously sterilized needle. Then fruits were inoculated by spraying conidial suspension ( $10^5$  conidia/ml) of isolated fungi with an atomizer and placed into separate Petri plates. In control set, sterilized distilled water was used instead of conidial suspension on the sterilized chilli fruits. The inoculated fruits within the Petri plates were incubated at  $25 \pm 2^\circ\text{C}$  for 7 days. Observation was made after 7 days, when the artificially inoculated chilli had developed symptoms and compared with the previous one which showed the anthracnose symptoms naturally on chilli fruits.

### Results and Discussion

A total of nine species of fungi belongs to six genera were isolated from the infected fruits of *Capsicum frutescens*. The isolated fungi were *Alternaria alternata*, *A. tenuissima*, *Aspergillus flavus*, *A. niger*, *Colletotrichum dematium*, *C. gloeosporioides*, *Curvularia clavata*, *Fusarium semitectum* and *Rhizopus stolonifer* (Tables 1-3).

Nine fungi were isolated from the infected chilli fruits collected from Karwan bazar, Dhaka. The highest mean per cent frequency of fungi associated with diseased chilli fruits was *Colletotrichum dematium* (33.37) which was followed by *C. gloeosporioides* (18.63), *Alternaria alternata* (11.76), *A. tenuissima* (10.62), *Fusarium semitectum* (7.57), *Curvularia clavata* (5.35), *A. niger* (4.03), *Rhizopus stolonifer* (3.95) and *A. flavus* (2.27). *Colletotrichum dematium* was very common in each and every isolations. On the other hand *A. flavus*, *A. niger*, *Curvularia clavata*, *Fusarium semitectum* and *Rhizopus stolonifera* was isolated once out of four isolations (Table 1).

**Table 1. Per cent frequency of fungi associated with diseased chilli collected from Karwan Bazar at different intervals.**

Name of fungi	% frequency of fungi at different intervals				Mean
	19 April	27 May	15August	29 August	
<i>Alternaria alternata</i>	15.78	-	16.12	15.15	11.76
<i>A. tenuissima</i>	21.05	21.42	-	-	10.62
<i>Aspergillus flavus</i>	-	-	-	9.09	2.27
<i>A. niger</i>	-	-	16.12	-	4.03
<i>Colletotrichum dematium</i>	26.31	57.14	25.80	24.24	33.37
<i>C. gloeosporioides</i>	21.05	-	32.25	21.21	18.63
<i>Curvularia clavata</i>	-	21.42	-	-	5.35
<i>Fusarium semitectum</i>	-	-	.	30.30	7.57
<i>Rhizopus stolonifera</i>	15.78	-	-	-	3.95

‘-’ represents no growth of respective fungus.

Seven fungi were isolated from the anthracnose infected chilli fruits collected from Ananda Bazar, Dhaka. The highest mean per cent frequency of fungi of diseased chilli was detected in *Fusarium semitectum* (20.12) which was followed by *C. dematium* (18.18), *C. gloeosporioides* (15.95), *Alternaria alternata* (14.09), *Aspergillus flavus* (9.09), *A. tenuissima* (8.93) and *A. niger*

(7.97). *Alternaria alternata* was very common in every isolations. *Aspergillus flavus* and *Colletotrichum dematium* was isolated once from the infected chilli fruits (Table 2).

**Table 2. Per cent frequency of fungi associated with diseased chilli collected from Ananda bazar at different intervals.**

Name of fungi	% frequency of fungi at different intervals			
	08 May	27 May	23 April	Mean
<i>Alternaria alternata</i>	12.50	18.18	14.28	14.99
<i>A. tenuissima</i>	12.50	-	14.28	8.93
<i>Aspergillus flavus</i>	-	27.27	-	9.09
<i>A. niger</i>	12.50	-	11.42	7.97
<i>Colletotrichum dematium</i>	-	54.54	-	18.18
<i>C. gloeosporioides</i>	25.00	-	22.85	15.95
<i>Fusarium semitectum</i>	37.50	-	22.85	20.12

‘-’ represents no growth of respective fungus.

Six fungi were isolated from the anthracnose infected chilli fruits collected from Badalgachhi, Naogaon, Dhaka. The highest mean per cent frequency of fungi associated with anthracnose of chilli collected from was *Colletotrichum dematium* (49.03) which was followed by *Fusarium semitectum* (14.62), *C. gloeosporioides* (13.57), *Alternaria tenuissima* (11.01), *A. alternata* (6.84) and *A. niger* (4.92). *Alternaria tenuissima* was isolated every time in all isolations (Table 3).

**Table 3. Per cent frequency of fungi associated with diseased chilli collected from Badalgachi, Naogaon at different intervals.**

Name of fungi	% frequency of fungi at different intervals			
	17 July	30 July	08 August	Mean
<i>Alternaria alternata</i>	-	6.25	14.28	6.84
<i>A. tenuissima</i>	16.66	2.08	14.28	11.01
<i>Aspergillus niger</i>	3.33	-	11.42	4.92
<i>Colletotrichum dematium</i>	70.00	77.08	-	49.03
<i>C. gloeosporioides</i>	6.66	8.33	25.71	13.57
<i>Fusarium semitectum</i>	3.33	6.25	34.28	14.62

‘-’ represents no growth of respective fungus.

All the isolated chilli fungi were tested for pathogenicity following spore suspension technique described by Islam *et al.* (2018). Among the isolated fungi, *Colletotrichum dematium*, *C. gloeosporioides* and *F. semitectum* fulfilled Koch’s postulates and found to be pathogenic to chilli whereas control set remains fresh without showing any fungal growth (Fig. 1).

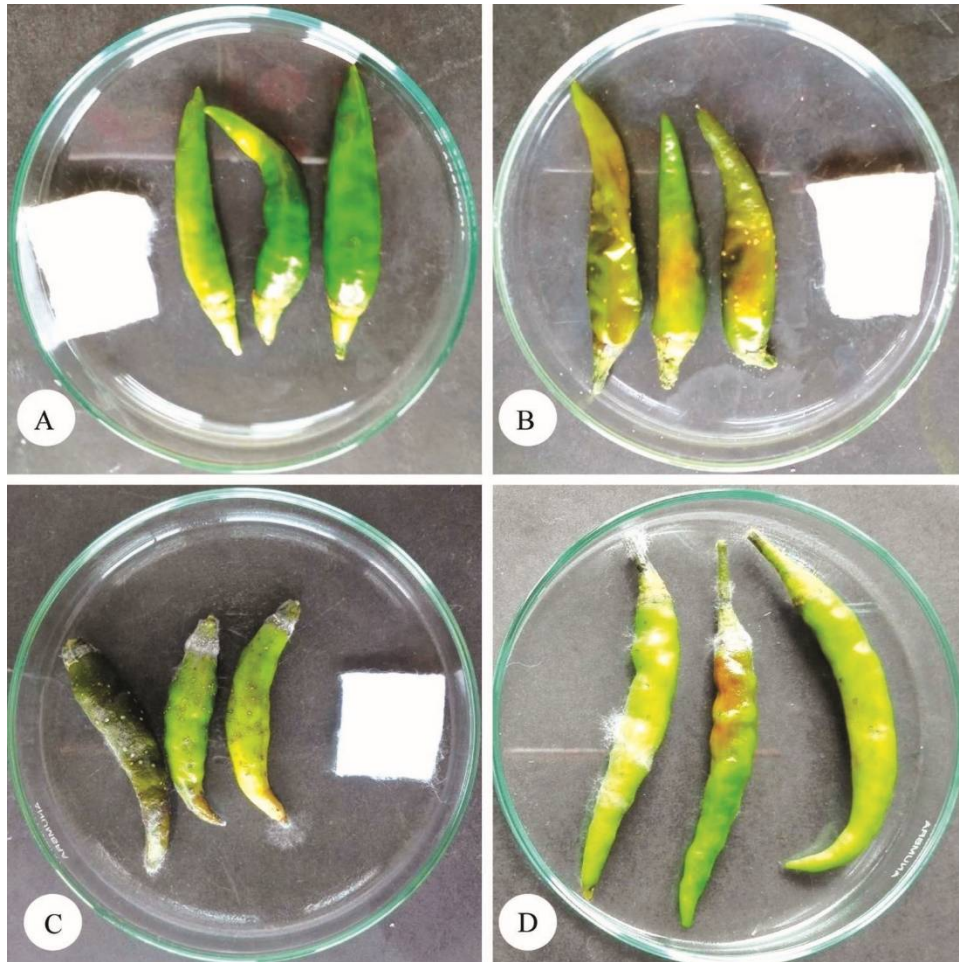


Fig. 1. Photographs of the pathogenicity test of chilli fruits. Fresh and healthy chilli fruits inoculated with: sterile water, Control (A), *Colletotrichum dematium* (B), *C. gloeosporioides* (C) and *Fusarium semitectum* (D).

**The taxonomic description of pathogenic fungi isolated from anthracnose infected chilli fruits is given below:**

**1. *Colletotrichum dematium* (Pers. Ex Fr.) Grove, J. Bot.Lond. 56: 341(1918). (Fig. 2 A-B)**

Colony whitish, reverse brownish. Sclerotia present. Setae dark brown septate, abundant. Conidia hyaline, falcate, aseptate, apices acute,  $18-24 \times 2-3 \mu\text{m}$ . Appressoria abundant, midbrown, clavate to circular, edge usually entire,  $8-11.5 \times 6.5-8 \mu\text{m}$ , often becoming complex and forming long closely branched chain.

*Specimen examined:* Isolated from diseased *Capsicum frutescens* L. Karwan bazar, Dhaka, MAR Rony 12, 19 April 2017.

**2. *Colletotrichum gloeosporioides* (Penz.) Sacc., Fung. Agrum. 2: 6 (1882). (Fig. 2. C-D)**

Colony white, grayish with lighter centre at maturity. Sclerotia present, setae absent. Conidia hyaline, straight, aseptate, comparatively large, straight, obtuse at the apex,  $11-22 \times 3-5 \mu\text{m}$ . Appressoria abundant, Pale to medium brown, circular or slightly irregular.

*Specimen examined:* Isolated from diseased *Capsicum frutescens* L. Ananda bazar, Dhaka, MAR Rony16, 29 April 2017.

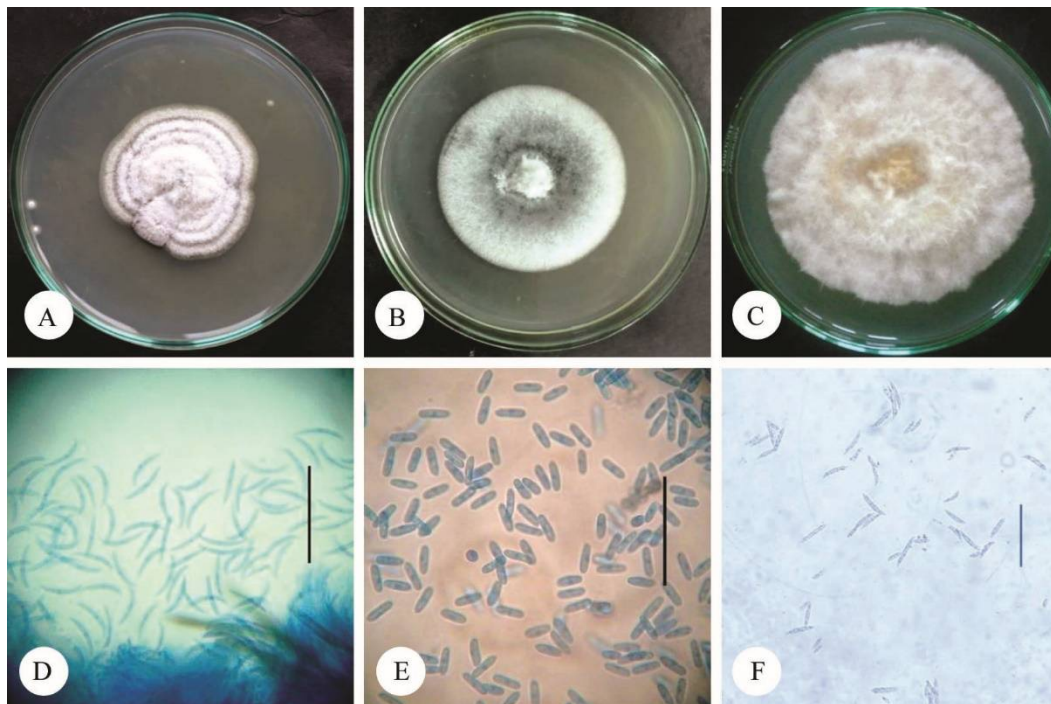


Fig. 2. Colonies and conidia of *Capsicum frutescens*. A-B: *Colletotrichum dematium*, C-D: *C. gloeosporioides* and E-F: *Fusarium semitectum* (Bar = 50  $\mu\text{m}$ ).

**3. *Fusarium semitectum* Berk. & Rav. In Berkeley, Gravillea 3: 98,(1875). (Fig. 2. E-F)**

Cultures at first white peach tinge and reverse peach coloured. Aerial mycelium floccose, peach gradually changing to avellaneous and finally becoming buff brown (14-2 days). Sporodochia absent, brown stromatic. Conidia formed in aerial mycelium from loosely branched conidiophores. Each branch terminates in a conidiogenous cell  $19-24 \times 2-4 \mu\text{m}$ . The conidia hyaline, 3 to 5 septate and are curved with a wedge-shaped but not pedicellate basal cell and pointed apex. 3 septated conidia is  $19-25 \times 3.15-3.6 \mu\text{m}$  and 5 septated conidia is  $21-30 \times 3.15-4.05 \mu\text{m}$ .

*Specimen examined:* Isolated from diseased fruits of *Capsicum frutescens* L. Ananda bazar, Dhaka, MAR Rony 08, 08 May 2017.

Majority of researchers from different countries have implicated that anthracnose disease of chilli is caused by a complex of *Colletotrichum* species, reported as *C. fructicola* and *C. siamense* in India (Sharma and Shenoy 2014), *C. gloeosporioides* in Korea (Kim *et al.* 1999), and Thailand

(Than *et al.* 2008) and Indonesia (Voorrips 2004), *C. truncatum* in Australia, China, India and Thailand (Diao *et al.* 2015); *C. acutatum* from almost all chilli-growing countries, including China, India, Korea, New Zealand, Sri Lanka, Thailand, USA and Indonesia (Than *et al.* 2008, Damm *et al.* 2012) and *C. coccodes* in New Zealand and India (Cannon *et al.* 2012). All the fungi associated with chilli as pathogens or saprophytes deteriorate fruits qualitatively and quantitatively.

Until now, outbreaks of chilli anthracnose have severely affected pepper production. Thus, it is very urgent to accurately identify the chilli anthracnose pathogen for early diagnosis and disease management in the field. Although several researches have been worked out on anthracnose disease of chilli, resistant chilli cultivars for these pathogens have not been commercialized (Park 2007). The findings of the present investigation slightly differed from others. Present results reveal that out of 9 fungal species three *viz.*, *C. dematium*, *C. gloeosporioides* and *F. semitectum* were pathogenic to the diseased chilli. This identification will help in further research work related with management practices in the farmer level.

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