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Pathogenic variability of *Colletotrichum* sp. from chilli anthracnose and their tolerance to carbendazim

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Abstract

Twelve isolates of *Colletotrichum capsici* and *Colletotrichum gloeosporides* were isolated from anthracnose infected chilli fruits from different areas of Mymensingh. Isolated pure fungal isolates were grouped on the basis of their morphological characters *viz.* colony color and compactness, size, shape and number of conidia. The white colored isolates were identified as *C. capsici* and showed faster growth on PDA medium. In contrast, grey colored fungal isolates were identified as *C. gloeosporides* and comparatively slow growth on PDA medium. All the fungal isolates were pathogenically active and developed typical symptoms on both green and ripe fruits of chilli. The isolates of *C. capsici* collected from Kalibari showed the highest infection (74.99%) on fruit surface followed by Muktijoddhar bazar (61.83%). Differential tolerance was observed as fungal growth was different against 0.05% and 0.1% carbendazim while 0.2% carbendazim was lethal against all fungal isolates. The results indicate that severity of anthracnose of chilli is different may be due to aggressiveness of fungal and their tolerance against common fungicide like carbendazim.

Introduction

Chilli (*Capsicum annuum* L.) is one of the most important spice crops belongs to the family Solanaceae grown widely in Bangladesh. The average yield of dry chilli is 1,22,848 MT in 2009–2010 (BBS, 2011). This is quite low to meet up the spices requirement of Bangladesh. Chilli suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also due to abiotic stresses. Among the fungal diseases damping off, anthracnose or fruit rot, powdery mildew and leaf spots are the most prevalent ones (Alam *et al.* 2014).

Anthracnose is mainly a problem on mature fruits, causing severe losses due to both pre- and post harvest fruit decay. *Colletotrichum* is one of the most important plant pathogens worldwide causing the economically important disease in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits. Chilli infected by *Colletotrichum* usually develops under high humid conditions when rain occurs after the fruits have started to ripen reported to loss up to 84 % (Thind and Jhooty, 1985).

In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose of the same host (Simmonds, 1965; Freeman *et al.*, 1998; Cannon *et al.*, 2000). Anthracnose of chilli has been shown to be caused by more than one *Colletotrichum* species including *C. acutatum* (Simmonds), *C. capsici* (Syd.) Butler and Bisby, *C. gloeosporioides* (Penz.) Penz. and Sacc., and *C. coccodes* (Wallr.) S. Hughes (Simmonds, 1965; Johnston and Jones, 1997; Kim *et al.*, 1999; Nirenberg *et al.*, 2002; Voorrips *et al.*, 2004; Sharma *et al.*, 2005; Pakdeevaraporn *et al.*, 2005; Than *et al.*, 2008).

Correct and accurate identification will thus ultimately lead to more effective disease control and management, e.g., selecting appropriate fungicides, or long lasting resistant cultivars (Whitelaw-Weckert *et al.*, 2007). The disease can be controlled by seed treatment and foliar spray with azoxystrobin, chlorothalonil, copper, difenoconazole, famoxadone, iprodione, procymidone, tolylfluanid and carbendazim (OEPP/EPPO, 2000). Carbendazim is a broad spectrum benzimidazole carbamate fungicide with systemic activity. Moreover, selection of resistant varieties is an important tool for developing sustainable integrated disease management package. Considering the above facts, the present investigation was undertaken - to study the morpho-physiological characters of the isolates of *Colletotrichum* spp. collected from anthracnose infected chilli of different locations and to investigate whether carbendazim is still effective at its recommended doses against the isolates of *Colletotrichum* spp. from different locations.

Materials and Methods

The experiments were conducted in MS Lab and Seed Pathology Centre (SPC), Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Diseased chilli fruits of 12 local and hybrid varieties (Table 1) were collected from the farmers' from different locations. The samples were stored in zip-lock bags in refrigerator for further study.

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Table 1. Name and locations of Collection of samples

SL. No.	Name of infected chilli sample	Location
1.	Balujhuri	Kalibari
2.	Bindu	Goupondi Bazar
3.	Balujhuri	Fulpur Bazar
4.	Bindu	Kalibari Bazar
5	Lomba morich	Boirar Char
6.	Bindu	Bharotir Bazar
7.	Bindu	Chander Bazar
8.	Bindu	Rashidpur Bazar
9.	Bindu	Muktijoddhar Bazar
10.	Angur	Rajgong Bazar
11.	Balujhuri	Bhangamari Bazar
12.	Bindu	Morichar Char

For isolation of fungi from diseased plant materials small inocula were cut and was surface sterilized with 10% NaOCl solution for 10 second and subsequently washed with distilled water for three times. The inocula were then placed in blotter paper for drying and then placed in PDA medium and incubated at 28 ± 2^{o}C. After 3 days of incubation, mycelial growth of *Colletotrichum* spp. was observed. To obtain pure culture of *Colletotrichum* spp. a bit of fungal growth was picked up by sharp pointed needle and transferred on PDA medium. As the growth of fungi started on PDA medium, they were sub-cultured again on PDA medium following the similar procedure.

Morphological characterization

Morphological characterization was done through the observation of five characters *viz.* radial mycelial growth (mm), size of conidia (mm), number of spores/mL, colony color and compactness of colony of the isolates. Colony diameter of every culture was recorded daily until the mycelium touches the petri-dishes (for 6 days). Mycelial color and appearance of 18 days old growth culture were recorded (Jahan *et al*. 2013). Number of spores per milliliter was counted by using hemacytometer. Fifty spores of each isolate were selected randomly for measurement of width and length, using a calibrated ocular micrometer and stage micrometer (Vengadaramana and Costa, 2014).

Pathogenicity test

For pathogenicity test all isolates of *Colletotrichum.* spp were used to make disease in mature green chilli fruit. The fruits were surface sterilized with 10% chlorox solution for 30 sec followed by consecutive washing in sterilized water for 4 times. Four small incisions were made on the surface of chilli fruit with sterilized needle to facilitate the entrance of the pathogen. One cm mycelial block was taken into wet cotton swab and place them on the surface of chilli and only cotton swab was used as control and incubated at 28 ± 2^{o}C. After 3 days of inoculation the inoculated chillies were observed and visually estimate the diseased area. The observation was continued until the chillies rotten completely.

Fungicide tolerance test

Fungicide tolerance were evaluated against different isolates of *Colletotrichum* spp. on PDA medium considering 0.05 %, 0.1 % and 0.2 % carbendazim by following poisoned food technique (Daoubi, *et al.* 2005). Required amount of fungicide was mixed thoroughly with PDA medium before pouring into petridish for solidification. After solidification, 5 mm of mycelial block from each isolate was placed at the middle of chemical impregnated PDA medium. After properly wrapping with parafilm the plates were kept for incubation at room temperature (28 ± 2^{o}C). The radial mycelial growth of the fungus was measured 2, 3, 4, 5 and 6th days after inoculation.

Statistical Analyses

The data were analyzed following completely randomized design (CRD) with three replications by using the M-STAT C statistical software.

Results

Morphological characterization of *Colletotrichum* spp. isolates

Morphological characters *viz.* radial mycelial growth, colony color and compactness of colony of the isolates of *Colletotrichum* spp. on PDA medium were recorded (Table 2). Two types colony color were found such as white and grey. Isolates from Kalibari, Fulpur Bazar, Goupondi Bazar, Chander Bazar, Bharotir Bazar, Boirar Char and Kalibari Bazar developed white colored fungal colony on PDA medium while the isolates from Rajgong Bazar, Bhangamarir Bazar, Morichar char, Rashidpur Bazar and Muktijoddhar Bazar produced grey colored colony. The white colony was loose considering compactness whereas grey colored colonies were compact. Considering radial mycelia growth, isolates from Kalibari was fast growing (8.5 cm) to cover the 9 cm PDA plate followed by Goupondi Bazar (7.5 cm) and Fulpur Bazar (7.4 cm). On the other hand, grey colored isolates showed slow growth on PDA. Isolates from Morichar Char showed the least growth (5.3 cm) on PDA (Table 2).

Table 2. Morphological characters of *Colletotrichum* spp. collected from different locations

Sl No.	Location	Radial mycelial growth (cm) Days After Inoculation (DAI)					Colony color	Colony Texture
		2nd	3rd	4th	5th	6th		
1.	Kalibari	2.15	3.90	5.65	6.80	8.50	White	Loose
2.	Goupondi Bazar	2.25	3.65	5.25	6.65	7.65	White	Loose
3.	Fulpur Bazar	2.25	3.80	5.55	6.50	7.40	White	Loose
4.	Kalibari Bazar	1.35	3.25	4.60	5.35	6.70	White	Loose
5.	Boirar Char	1.75	2.35	3.60	4.25	6.10	White	Loose
6.	Bharotir Bazar	1.75	2.65	3.85	4.95	6.00	White	Loose
7.	Chander Bazar	1.60	2.75	3.80	4.65	5.75	White	Loose
8.	Rashidpur Bazar	1.85	2.85	3.85	4.90	5.75	Grey	Compact
9.	Muktijoddhar Bazar	1.60	2.55	3.50	4.52	5.63	Grey	Compact
10.	Rajgong Bazar	1.75	2.60	3.70	4.50	5.60	Grey	Compact
11.	Bhangamari Bazar	1.40	2.50	3.66	4.45	5.55	Grey	Compact
12.	Morichar Char	1.50	2.45	3.55	4.25	5.30	Grey	Compact

Identification of the pathogen

In general 2 types of *Colletotrichum* spp. are associated with anthracnose of chilli *viz. C. capsici* and *C. gloeosporioides*. Further characterization was done by measuring the size of the conidium and no. of spore/ml (Table 3). Size of conidium of *C. capsici* was 3×0.5 mm^2 which was slightly larger than *C. gloeosporioides* (2.5×1.5 mm^2), (Mordue, 1971; Sutton, 1992). The shape of conidium of *C. capsici* was crescent; while it was observed rod shaped in case of *C. gloesporioides*. Spore production capacity was higher in case of *C. capsici* (1.62×10^5 spore/mL suspension) while it was 1.25×10^5 spore/mL suspension (Table 3).

Table 3. Identification of the pathogen

Isolates	Length (mm)	Breadth (mm)	No. of spores /ml
Colletotrichum capsici	3	0.5	1.62×10^5
Colletotrichum gloeosporioides	2.5	1.5	1.25×10^5

Pathogenic variability of the *Colletotrichum* isolates

To examine the virulence of the isolates pathogenicity test was conducted on both mature green and ripe fruit of chilli.

On mature green fruits

Pathogenicity test of all the isolates were conducted on mature green chilli fruits considering the similar variety Bindu (Table 4 and Plate 3). Significant variations in fruit infection were observed at 3rd, 4th and 5th DAI. At 3rd DAI the highest (74.00 %) fruit infection was caused by the isolates from Kalibari followed by the isolates from Muktijoddhar Bazar (61.83 %). Moreover, isolates from Rajgong Bazar (39.47 %) and Bhanghamarir Char (39.30 %) showed fruit infection at 5th days after inoculation (Fig. 1).

On ripe fruits

Pathogenicity test of all the isolates were also conducted on ripe fruits considering the similar variety Bindu (Table 5 and Plate 4). All the isolates showed significantly different pathogenic reaction at different DAI. The isolates collected from Kalibari showed the highest fruit infection at 3rd, 4th and 5th days after inoculation. On the other hand, higher fruit infection was also observed by the isolates collected from Kalibari (74.99 %), Muktijoddhar Bazar (61.25 %). Isolate collected from Morichar Char showed the lowest fruit infection at 3rd (6.32 %), 4th (15.28 %) and 5th (9. 27.38 %) respectively.

Table 4. Pathogenicity test of *Colletotrichum* spp. isolates on mature green fruits of chilli (Var. Bindu)

Name of isolates		% Area infected Days After Inoculation (DAI) 3rd	4th	5th
Kalibari		17.25 (4.22) a	37.25 (12.41) a	74.00 (23.52) a
Goupondi Bazar		13.66 (4.55) ab	30.33 (10.11) a	51.15 (19.05) a
Fulpur Bazar		13.01 (4.34) ab	30.13 (10.04) a	48.43 (16.14) a
Kalibari Bazar		12.66 (4.22) ab	28.66 (9.55) a	46.66 (15.55) a
Boirar Char	*Colletotrichum capsici*	12.00 (4.00) ab	28.13 (9.38) a	53.54 (17.84) a
Bharotir Bazar		14.27 (4.76) a	29.30 (9.76) a	48.88 (16.29) a
Chander Bazar		11.33 (3.78) b	27.72 (9.24) a	46.92 (15.25) a
Rashidpur Bazar		9.25 (3.08) b	25.31 (8.44) ab	45.76 (20.25) a
Muktijoddhar Bazar		14.33 (4.78)a	35.33 (11.78) a	61.83 (20.61) a
Rajgong Bazar		8.43 (2.81) ab	19.33 (6.44) ab	39.47 (13.15) a
Bhangamari Bazar	*Colletotrichum gloeosporioides*	11.50 (4.83)a	26.41 (8.91) ab	52.51 (17.50) ab
Morichar Char		9.80 (3.27) ab	21.75 (7.25) ab	39.30 (13.10) ab
Control		0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
P value		*(0.0205)	**(0.0106)	**(0.0011)

In a column figures with same letter do not differ significantly (P<0.05) (as per Tukeys-Kramer HSD). Figures in the parentheses are arcsin transformed value

Table 5. Pathogenicity test of *Colletotrichum* spp. isolates on ripe fruits of chilli (Var. Bindu)

Name of isolates		% Area infected		
		Days After Inoculation (DAI)		
		3rd	4th	5th
Kalibari	*Colletotrichum capsici*	23.42 (7.80) a	49.10 (16.36) a	74.99 (24.99) a
Goupondi Bazar		12.81 (4.27) abc	33.33 (11.11) ab	59.16 (19.72) a
Fulpur Bazar		12.49 (4.19) abc	27.29 (9.09) abc	50.59 (16.86) a
Kalibari Bazar		13.85 (4.61) abc	26.25 (8.75) abc	54.75 (18.25) a
Boirar Char		12.50 (4.16) abc	28.32 (9.42) abc	49.92 (16.64) a
Bharotir Bazar		10.72 (3.57) abc	22.12 (7.37) abc	36.66 (12.22) ab
Chander Bazar		11.51 (3.83) abc	24.24 (8.08) abc	49.15 (16.38) a
Rashidpur Bazar	*Colletotrichum gloeosporioides*	8.90 (2.96) abc	18.33 (6.11) bc	29.16 (9.72) ab
Muktijoddhar Bazar		17.63 (5.47) ab	35.50 (11.83) ab	61.25 (20.41) a
Rajgong Bazar		6.13 (2.04) bc	13.95 (4.65) bc	29.75 (9.91) ab
Bhangamari Bazar		12.17 (4.05) abc	23.27 (7.75) abc	45.00 (15.00) ab
Morichar Char		6.32 (2.10) bc	15.28 (5.09) bc	27.38 (9.12) ab
Control		0.00c	0.00c	0.00b
P value		*(0.0030)	*(0.0018)	*(0.0010)

In a column figures with same letter do not differ significantly (P<0.05) (as per Tukeys-Kramer HSD). Figures in the parentheses are arcsin transformed value

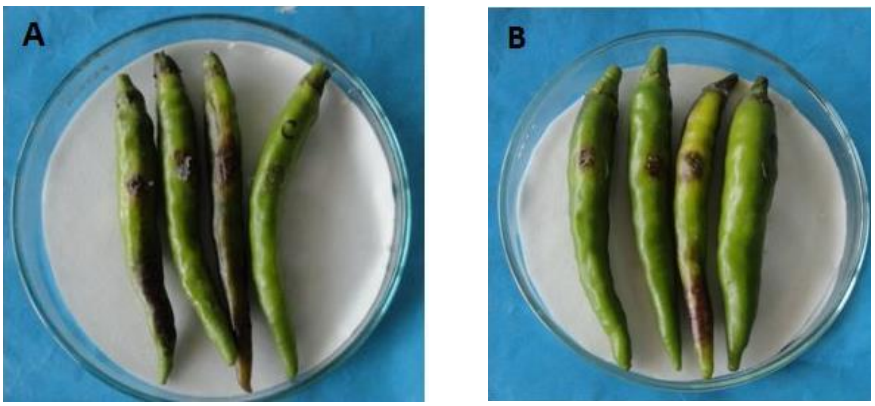

Plate 2. Pathogenicity test on mature green fruits. (A) lesions developed on mature green chilli of Rajgonj bazaar (B) Goupondi bazar

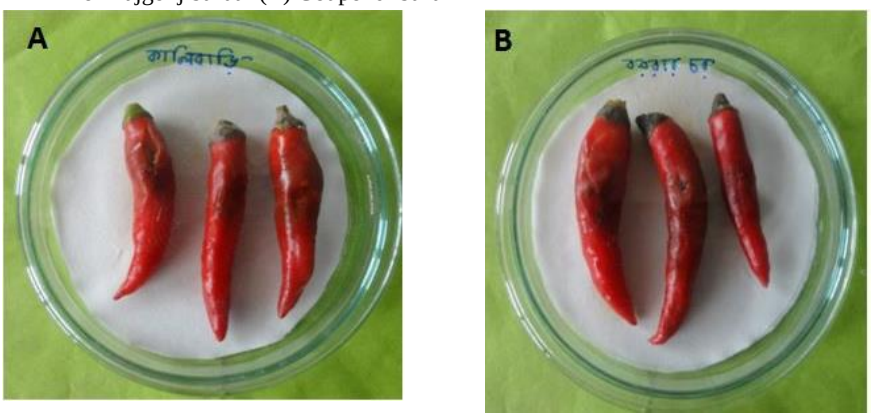

Plate 3. Pathogenicity test on ripe fruits. (A) lesions developed on ripe chilli of Kalibai (B) Boirar char

Fungicide tolerance test against carbendazim

Fungicide tolerance test was conducted against 0.05 %, 0.1 % and 0.2 % carbendazim. In order to conduct this experiment, pure culture of all isolates were inoculated on chemical impregnated PDA medium. Radial mycelial growth was measured until the Petridis completely cover by the growth of mycelia. All the isolates started to grow from 2nd day after inoculation against 0.05 % carbendazim. At 6th day after inoculation the Petri dishes were almost covered with mycelia growth. The isolates from kalibari and Goupondi Bazar showed the highest (7.20 cm) mycelial growth against 0.05 % Carbendazim followed by the isolates collected from Rashidpur Bazar (7.00 cm), Bhanghamari Bazar (6.80 cm) (Fig. 1). On the other hand, all the isolates were fairly grown against 0.1 % Carbendazim. The isolates from Kalibari ,Goupondi Bazar and Muktijoddhar Bazar showed the highest (6.50 cm) mycelial growth against 0.1 % Carbendazim followed by the isolates collected from Kalibari Bazar, Boirar Char, Rashidpur Bazar and Rajgong Bazar (5.50 cm) (Fig. 2). In both cases, the isolates belonged to *C. capsici* (Kalibari, Goupondi Bazar, Fulpu Bazar, Kalibari Bazar, Boirar Char, Bharotir Bazar and Chander Bazar) showed more tolerance and showed comparatively faster growth than rest of the isolates which belonged to *C. gloeosporioides*. But none of the isolate could grow PDA medium against 0.2 % Carbendazim. The growth of the fungi was completely arrested by 0.2% carbendazim (Table 6).

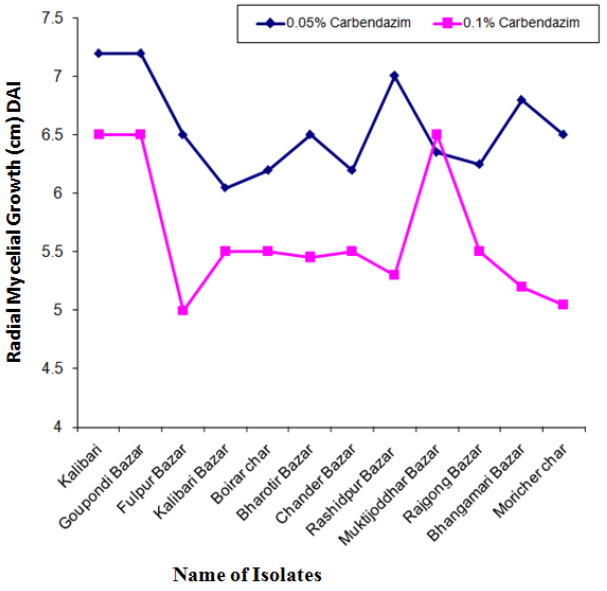

Fig. 1. Fungicide tolerance test against carbendazim

Table 6. Fungicide tolerance test against 0.2 % carbendazim

Sl. No.	Name of isolates		Radial mycelial growth (cm) Days After Inoculation (DAI)				
			2nd	3rd	4th	5th	6th
1.	Kalibari	*Colletotrichum capsici*	0	0	0	0	0
2.	Goupondi Bazar		0	0	0	0	0
3.	Fulpur Bazar		0	0	0	0	0
4.	Kalibari Bazar		0	0	0	0	0
5.	Boirar Char		0	0	0	0	0
6.	Bharotir Bazar		0	0	0	0	0
7.	Chander Bazar		0	0	0	0	0
8.	Rashidpur Bazar	*Colletotrichum gloesporioides*	0	0	0	0	0
9.	Muktijoddhar Bazar		0	0	0	0	0
10.	Rajgong Bazar		0	0	0	0	0
11.	Bhangamari Bazar		0	0	0	0	0
12.	Morichar Char		0	0	0	0	0

Discussion

Several researchers studied on anthracnose of chilli and reported that *C. capsici* and *C. gloeosporioides* are associated with anthracnose of chilli (Kim *et al* 1999). Many researchers were characterized variation in different places and countries are different due to high host range (Embaby *et al*. 2009). All of the isolates were categorized into four morphological groups. The white colored isolates were identified as *C. capsici* and showed faster growth on PDA medium. In contrast, grey colored fungal isolates were identified as *C. gloeosporides* and comparatively slow growth on PDA medium. The similar grouping was done by where 38 isolates were divided based on conidial morphology into three groups (Vithanage *et al.* 2014).

The findings reported that, observations and measurements of radial mycelial growth (mm), size of conidia (mm), number of spores/mL, have usually been made within the species compared that *Colletotrichum capsici* was significantly ($p<0.05$) fast growing, enlarged conidial size (crescent shape) and having maximum number of spores/mL, whereas *Colletotrichum gleosporidies* was slow grower, smaller conidia (rod shaped) and mycelial color variation. In related studies, according to (Abera *et al*. 2016), *C. gloeosporioides* produced symptoms and the shape or size of conidia

(6.0-10×2.0-2.5 µm) was slightly different from those found on white fleshed species in Okinawa Prefecture.

The fungal isolates belong to both *C. capsici* and *C. gloeosporioides* can cause infection on mature green and ripe fruits of chilli. *C. capsici* causes more infection on ripe fruits of chilli but *C. gloeosporioides* causing infection was more frequent on mature green chilli. Hong and Hwang (1998) and Kim *et al.* (1999) reported that *C. capsici* is widespread in red chilli fruits, where as *C. gloeosporioides* was more prevalent on both young and mature green fruits of chilli. Among the isolates Kalibari, Muktijoddhar Bazar and Goupondi Bazar showed the highest diseased area. When the pathogen inoculated in ripe chilli the isolates from Kalibari, Muktijoddhar Bazar, Goupondi Bazar also showed highest diseased area.

To investigate, whether this pathogenic variability is linked to fungicide tolerance experiments were conducted considering all the isolates against 0.05%, 0.1% and 0.2% carbendazim. All the isolates of *C. capsici* and *C. gloeosporioides* grow fairly well against 0.05 % carbendazim. Radial mycelial growth of the isolates were slightly retarded but can still grow against the recommended dose of carbendazim (0.1 %). On the other hand, the growth of the mycelia was completely inhibited against 0.2 % carbendazim. The present findings were supported by Cook and Perija (1976). The results revealed that the recommended dose (0.1% w/v) of carbendazim is no longer effective to control anthracnose of chilli. These results also indicated that some *Colletotrichum* isolates are getting tolerance upto 0.1% carbendazim that might be due to indiscriminate use of carbendazim for long time.

Conclusion

Two species of *Colletotrichum viz. C. capsici* and *C. gloeosporioides* are responsible for anthracnose of chilli in the nearby areas of Mymensingh district. Both *C. capsici* and *C. gloesporioides* can tolerate and grow fairly on culture medium against 0.1% carbendazim. These findings indicate that the recommended dose of carbendazim is no longer effective to control anthracnose of chilli. These results also indicated that *Colletotrichum* isolates are increasing their tolerance level which is different in different morphological group. Indiscriminate use of fungicide may be one of the main reasons to develop tolerance against carbendazim.

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