Field Resistance of Chilli Cultivars Against Anthracnose Disease Caused by *Colletotrichum capsici*

M.S. Rahman[1], M.S. Akhter[1,*], M.A. Maya[2], A.H.M.A. Rahman[3] and A.M. Akanda[3]

[1]*Bangladesh Agricultural Research Institute, Bangladesh*
[2]*United Graduate School of Agriculture, Gifu University, Japan*
[3]*Department of Plant Pathology, BSMRAU, Gazipur-1706*

**Corresponding author. Email: sakhter09@gmail.com*

Abstract

Anthracnose of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici* is a major fungal disease in Bangladesh. In this study, the causal pathogen was identified based on symptoms, morphological characteristics including fruiting bodies, conidia as well as pathogenicity test. Among the eleven chilli cultivars tested in this study all the cultivars except V_8 /comilla-2 were found to be susceptible by *C. capsici*. However, the incidence of anthracnose was varied from 2.06-17.17% depending on the cultivar while the highest incidence were found in V_9 /kustia followed by V_4 /Jamalpur and V_1/BARI Marich-1. Moreover, the fruit infection at the matured stage was recorded as 2.53 to 11.57% while highest was in V_6 /chandpur and the lowest was in V_8 /comilla-2. Due to anthracnose infection plant height and canopy diameter reduction were recorded as 0 to 36.39% and 0 to 35.74%, respectively while lowest was in V_8 /comilla-2 and highest was in V_1/BARI Marich-1. Marketable yield lose were observed as 2.53 to 11.57% after *C. capsici* infection where highest was recorded in V_6/chandpur and lowest was in V_8/comilla-Based on the result of field performance it seemed that field resistance was found in V_8/commilla-2 variety against anthracnose.

Keywords: Chilli, Anthracnose, *Colletotrichum capsici*

Introduction

Chilli (*Capsicum annuum* L., family Solanaceae) is an important spice and vegetable crops on the basis of its high consumption, nutritional and cash value to the farmers and consumers in the tropical and sub tropical area. Chilli fruits are consumed as fresh, dried or processed products, table purpose, spice and condiments. Moreover, the green fruit of chilli is one of the richest sources of vitamin C and A (Howard, 2000). In Bangladesh, chilli is grown in large scale commercial field as well as in small kitchen garden. In Bangladesh, the crop is cultivated in an area about 89268 ha with a total production of 109337 tons dried chilli in 2009 (FAOSAT, 2011). But the production in terms of yield (12248 hg ha^{-1}) is not satisfactory as compared to the other chilli growing countries in the world (Anonymous, 2007).

Chilli is infected by several fungal, bacterial and viral diseases which incite severe economic losses. Among the diseases anthracnose/die back or ripe fruit rot caused by *Colletotrichum capsici.* (Syd.) Butler and Brisby (Tel: Glomerella cingulata (Stonem) Spauld and Schrenk) is considered to be the major constrain of chilli cultivation in Bangladesh because its frequent year round incidence of varying intensities which intern inflicts considerable quantitative and qualitative losses both in the field and storage condition. However, four *Colletotrichum* species, namely *C. capsici C. gloeosporioides*, *C. acutatum* and *C. coccodes* have been reported as causal agents of chilli anthracnose

and the major species are *C. capsici* and *C. gloeosporioides*. At the present time the disease is mostly managed by using chemical fungicides. The continuous and indiscriminate use of chemicals resulting accumulation of harmful residues in the soil, water and grains which break down the ecological balance by killing the beneficial and/or antagonistic microorganisms. Therefore, the present study was undertaken to identify the casual pathogen of chilli anthracnose/die back or ripe fruit rot including its incidence under field condition, marketable yield loss and screen the chilli cultivars to find out the resistant source which is the pre-reqisite before development and deployment of resistant varieties.

Materials and Methods

This study was conducted at research farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh during November 2006 to May 2007. The soil of the experimental field belongs to Salna series under the Agroecological Zone (AEZ)-28: Madhupur Tract. The pH of the soil ranges from 6 to 6.5. Popularly cultivated ten loal cultivar namely Chittagong (V_2), Comilla-1 (V_3), Jamalpur (V_4), Gazipur (V_5), Chandpur (V_6), Pusa jawla (V_7), Comilla-2 (V_8), Kustia (V_9), Bogra (V_{10}) and Balujhuri (V_{11}) along with one Bangladesh Agricultural Research Institute (BARI) released variety BARI (V_1)/Marich-1 were tested in this study.

Isolation and Identification of the Causal Pathogen

The fungus was isolated from infected plant parts and fruits of chilli following standard phytopathological procedures (Dasgupta, 1981; Agostini and Timmer, 1992). The infected plant parts were cut into small pieces (1 cm^2) and then surface sterilized by 1% NaOCl solution for 2-3 minutes then washed three times with sterile de-ionized water to remove the NaOCl. The cut pieces were then placed onto sterilized water agar (20 mL) in glass Petri dishes and incubated at room temperature until acervuli formation. Conidia produced in acervulus came out in the form of ooze and were placed onto PDA and incubated at room temperature for 7 days. The pathogen was identified following specialized published literature and (Sutton, 1980)

Inoculation of Chilli Plants and Fruits with *C. casici*.

Chilli plants grown in the net house at seven weeks old were inoculated with the spore suspension (5×10^5 conidia mL^{-1}) through micro sprayer (Mian, 1995). About 0.1 mL of Tween-20 added to the spore suspension before spraying the inoculums. The inoculated plants were then placed under polyethylene covering and watered periodically through sprayer to maintain high humidity up to 48 hours of inoculation. The plants were inspected every day for two weeks to observe symptom development. The symptomatic leaves were collected and carried to the laboratory for isolation and identification of the pathogen to confirm either the disease was caused by the inoculated pathogen or not. In case of fruit inoculation, healthy ripe fruits of chilli were surface sterilized with 70% ethanol followed by washing with sterilized distilled water for three/four times. Samples were injured softly by flame sterilized multipointed needles (Daykin and Millholland, 1984; Winch et al., 1984; Eastburn and Gubler, 1990). Spore suspension were then dropped carefully by Pasteur pipette onto injured sample and incubated at room temperature for 5 days. After symptoms development reisolation was done for confirmation either the disease was caused by inoculated pathogen or not.

Disease Incidence

Disease incidence were calculated by using the following formula

$$\text{Diseases incidence (\%)} = \frac{X_1}{X_2} \times 100$$

Where, X_1= Total no of infected plants, X_2= Total no of plants.

Measurement of Canopy Diameter (cm)

Canopy diameter of plants was measured by average length of North-South and East-West coverage of fully matured chilli plants.

Percent Reduction of Growth and Yield Contributing Characters

Percent reduction of the growth and yield contributing characters were calculated by using the following formula

$$R = \frac{Y - Y_1}{Y} \times 100$$

Where R = Percent reduction of growth per yield contributing character.

Y = Growth per yield contributing characters of healthy plants.

Y_1 = Growth per yield contributing characters of infected plants.

Data Analysis

Data were analyzed by MSTAT-C program and means were compared according to Duncan's Multiple Range Test (Gomez and Gomez, 1984). Before analysis, data were transformed as and when necessary following Arcsine transformation.

Results and Discussion

Symptoms of Disease and its Morphological Characteristics of the Causal Fungi

Plant symptoms were initially expressed as water-soaked, slightly sunken, dark dot like lesions on leaf blade. Within 2 to 3 days the lesions increased rapidly and most of the leaves were infected and the infected plant started to die from the top. The leaves and flowers of infected plants became soft and dropped from the plants. Within 7 to 10 days the disease became very severe and infected plants will die (Figure 1A-D). Fruit symptoms initially developed as water-soaked lesions that became soft, slightly sunken, and became tan. The lesions could cover most of the fruit surface and multiple lesions occurred (Figure 2). Black, minute spots might also be developed on seed of infected fruits. The asexual fruiting bodies/structures called acervuli appeared as numerous black dots on the lesions.

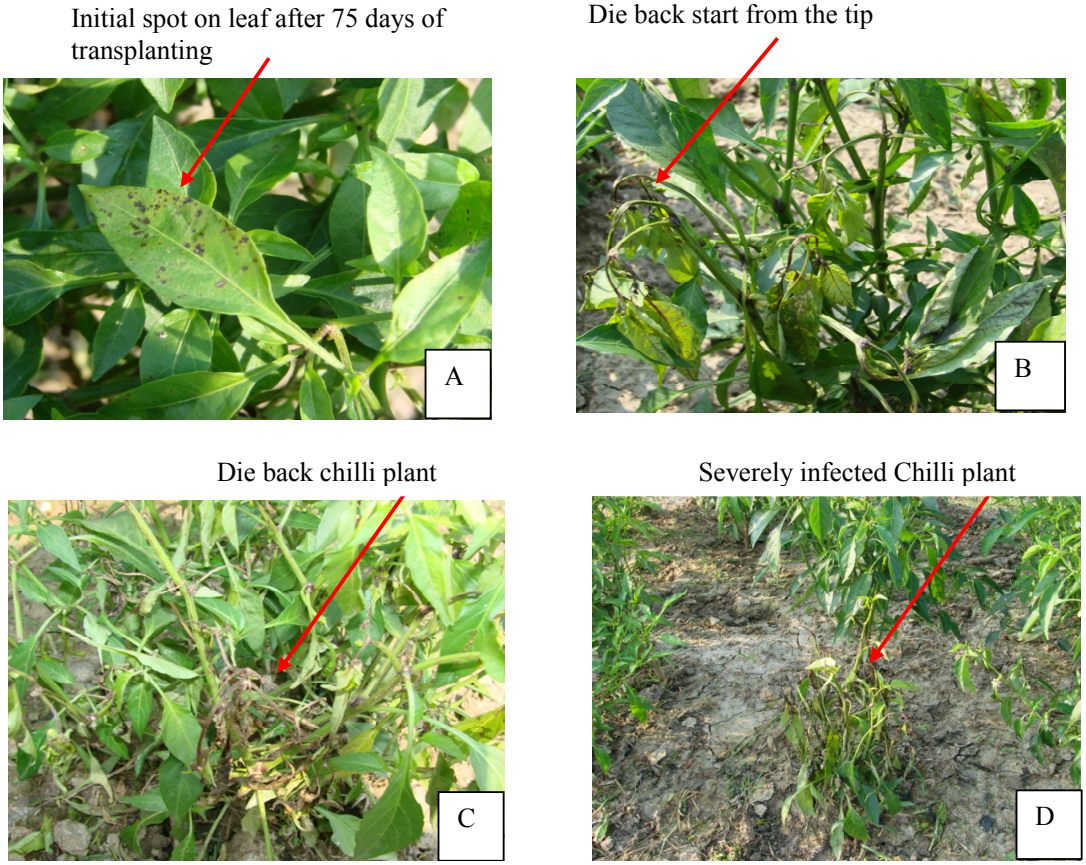

Figure 1 Symptoms of anthracnose disease of chilli caused by *Colletotrichum capsici* A. initial symptom on leaves, B and C. die back of chilli plant D. severely infected chilli plant.

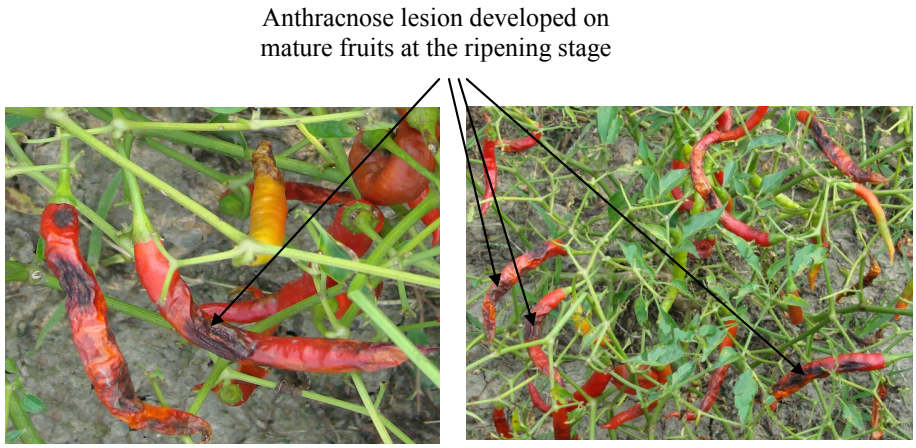

Figure 2 Mature chilli fruits showing multiple lesions of anthracnose under field condition.

The isolation and identification of causal pathogen of chilli anthracnose was done based on the characteristic symptoms appeared on infected plant, morphology of causal organism, pathogenicity and host specificity. The photographs of the field symptoms and causal organism under compound microscope are given in Figures 1-3. The results of this study indicated that *Colletotrichum capsici* was capable of causing disease on almost all parts of the chilli plants during any stage of plant growth.

The characteristic whitish gray colony was appeared on PDA after culturing the pathogen. The asexual fruiting bodies/structures called acervuli were present with abundant setae. The setae were mostly pointed, rarely blunt, elongate, strait or slightly curved, aseptate, smooth and dark brown. Conidiophores were short, bearing with fusiform and falcate conidia. Conodia were hyaline with one or both end curved and pointed as well as sudden or gradual tapering towards the both ends (Figure 3). This morphological features of the causal organism including seate, conidiophores and conidia were similar with the findings of (Mordue, 1971; Sutton, 1980 and Azad et al., 2005). Perithecia had not been observed. The conidial dimension of *C.capsici* was found 16.5-25.5 × 3.5-4.2 µm while (Azad *et al.* 2005) reported that the conidial dimension of *Colletotrichum capsici* as 17-24 × 3.8-4.4 µm. Upon inoculation on healthy ripe fruits of chilli, similar symptoms were observed as incase of natural infection. The morphological characteristics of reisolated pathogen were similar with the pathogen isolated from naturally infected chilli. The symptoms, colony color, acervuli production, pathogenicity test, setae characteristics, conidiophores and conidial dimension proved that the pathogen was *Colletotrichum capsici.*

Incidence of the Anthracnose Disease in Different Chilli Cultivars

The results as presented in the Figure 4 indicating the incidence of the disease both in plants and fruits of chilli under field condition. Among the eleven chilli varieties the highest incidence of anthracnose (*Colletotrichum capsici*) was recorded in V_9/kustia (17.17%) followed by V_4/Jamalpur (16.16%) and V_1/BARI Marich-1 (15.00%). The incidence in rest of the varieties were found to be ranges from 2.06 to 10.30 %. However, in the field condition none of the plant of Commila-2/V_8 was infected by the causal pathogen. In case of fruit infection the highest prevalence of anthracnose was recorded in V_6/chandpur (11.57%) followed by V_7/pusa jawla (11.19 %), V_{11}/balujhuri (11.16 %), V_9/kustia (10.42%) and V_4/jamalpur (10.18%) as shown in (Figure 4) and the lowest fruit infection was recorded in variety V_8/comilla-2(2.53%). The results revealed that the varieties were distinctly different from one another in respect of anthracnose prevalence under natural field condition. Though there were no infection found at the vegetative stage of variety V_8/comilla-2 a minimum infection was recorded on the fruits at the ripening stage. It indicated that the ripe fruits might be susceptible to *C.capsici* as compared to the plants and green fruits. . Based on the result of field performance it might be seemed that field resistance was found in V8/Commilla-2 cultivar

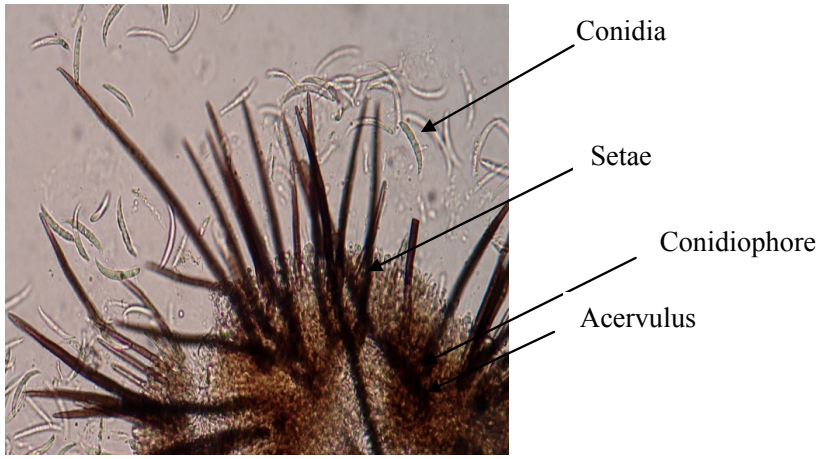

Figure 3 Acervulus, setae, conidiophores and conidia of *Colletotrichum capsici* under compound microscope.

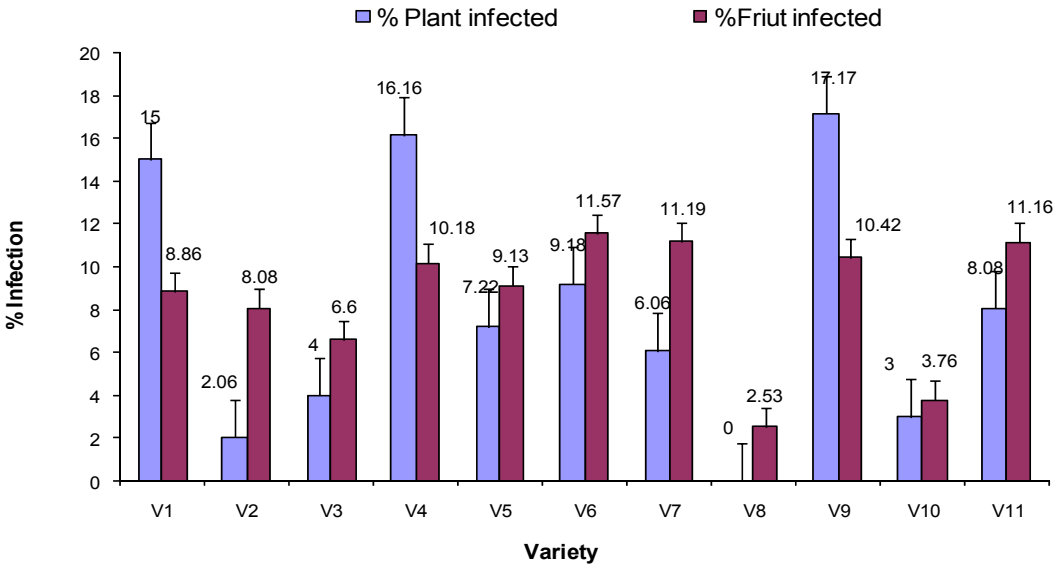

Figure 4 Incidence of anthracnose disease on eleven chilli cultivars in filed condition.

against anthracnose. Moreover, the other chilli varieties showed variability in respect of incidence of anthracnose.

Effect of Anthracnose Disease on Growth of Different Chilli Cultivars

Percent reduction of plant height and canopy diameter of chilli due to anthracnose diease were presented in the Table 1. Highest percent reduction of plant height and canopy diameter were found in V_1/BARI Marich-1 (36.39%) and (35.74%) respectively. On the other hand the cultivar V_7/pusajawla (36.17%), V_6/chandpur (34.95%), V_4/jamalpur (30.23%) and V_9/kustia (30.15%) have been recorded statistical identical percent reduction of plant height with V_1/BARI Marich-1 (36.39%) but lowest percent reduction of canopy diameter as compared to other. The plant height reduction as well as canopy diameter reduction both the cases the cultivar V_8/comilla-2 showed resistant reaction against the disease. The results suggested that the cultivar V_8/comilla-2 have been showed some sorts of field resistant against the anthracnose disease.

Table 1 Percent reduction of plant height and canopy diameter of eleven chilli cultivars due to anthracnose disease.

Variety	Plant height		% Reduction	Canopy diameter		% Reduction
	Healthy	Infected		Healthy	Infected	
BARI-1(V_1)	41.66 f	26.45 h	36.39 a (37.05)	53.74 ab	34.50 f	35.74 a (36.70)
Chittagong (V_2)	71.53 b	60.38 b	15.56 d (23.22)	48.22 c	37.69def	21.90 b (27.82)
Comilla -1(V_3)	69.03 bc	51.00 c	26.04 bc (30.64)	49.53 bc	36.89 def	25.40 b (30.18)
Jamalpur (V_4)	49.10 e	34.13 g	30.23 ab (33.29)	48.68 c	35.60 ef	26.59 b (30.93)
Gazipur (V_5)	63.46 d	46.85 d	26.07 bc (30.69)	54.50 a	41.13 e	24.30 b (29.28)
Chandpur (V_6)	65.00 cd	42.31 ef	34.95 a (36.24)	55.30 a	44.68 b	18.95 b (25.50)
Pusa jawla (V_7)	63.78 d	40.64 f	36.17 a (36.93)	54.44 a	45.11 b	18.82 b (25.40)
Comilla-2 (V_8)	80.59 a	80.59 a	0.00 e (0.57)	50.81 abc	50.81 a	0.00 c (0.57)
Kustia (V_9)	47.90 ef	33.34 g	30.15 ab (33.29)	47.59 c	37.40 def	21.36 b (27.51)
Bogra (V_{10})	72.50 b	57.23 b	20.97 c (27.21)	47.64 c	38.15 cde	19.75 b (26.33)
Balujhuri (V_{11})	61.57 d	45.00 de	26.95 bc (31.18)	50.87 abc	39.71 cd	21.90 b (27.84)
CV%	5.25	5.62	8.83	5.75	5.07	13.46

Means followed by same letters in row and column are not significantly different at 5% level by DMRT. (Data on the parenthesis are arcsine transformed value)

Effect of Anthracnose Disease on Yield of Different Chilli Varieties

Among the eleven chilli cultivars were tested in this study the number of fruit per plant were recorded very low in infected plants as compared to healthy plants (Table 2). The percent reduction of fruit per plant due to anthracnose disease caused by *Colletotruchum capsici* were observed in all the tested cultivars except V_8/comilla-2 which did not develop any symptom of anthracnose disease in field condition. The highest reduction of fruits/plant were recorded in V_5/gazipur (58.80%) which was statistically identical with V_7/pusjawla (58.01%), V_{11}/balujhuri (53.32%), V_2/chittagong (51.80%), V_{10}/bogra (51.03%) and V_6/chandpur (50.23%). However, the variety V_1/BARI Marich-1 have been shown lowest percent reduction of fruits/plant as compared to other cultivars but in earlier it was shown highest reduction of plant height and canopy diameter. In case of the rest cultivars the percent reduction of fruits/plant were ranged from 38.00-46.92% (Table 2). Due to incidence of *Colletotruchum capsici* the leaves, flowers and young fruits were dropped from the infected plants. (Kannan et al., 1998) described the similar symptoms in case of *Colletotruchum capsici* infection in chilli.

Among the eleven varieties V_8/comilla-2 have been showed the better performance in respect of total yield (12.52 t ha^{-1}) which was statistically identical with V_{10}/bogra (12.22 t ha^{-1}), V_7/pusajawla (11.51t/ha), V_{11}/balujhuri (11.48 t ha^{-1}) and V_6/chandpur (11.40 t ha^{-1}). The lowest yield was recorded in V_9/kustia (5.45 t ha^{-1}) which was statistically not identical with rest of the cultivars tested in this study. Marketable yield was reduced due to fruit infection by *C. capsici*. Among the eleven cultivars the highest fruit infection was found in V_{11}/balujhuri (11.57%) which was statistically identical with V_7/puajawla (11.19%), V_{11}/balujhuri (11.16%), V_9/kustia (10.42%) and V_4/Jamalpur (10.18%). However, the lowest fruit infection was found in V_8/comilla-2 (2.53%). With the increase of fruit infection marketable yield was decreased at similar rate. Highest marketable yield was found in V_8/comilla-2 (12.52 t ha^{-1}) which was statistically identical with V_{10}/Bogra (11.77 t ha^{-1}) where the fruit infection were (2.53%) and (3.76%), respectively (Table 3). Almost similar pattern of resistance in chillies against ripe fruit rot pathogen has also been reported by many workers from different part of the world. (Ullasa et al., 1981) evaluated 298 entries of *capsicum spp.*

Against chilli anthracnose and recorded resistance only 22 lines. In this study we would like to report that the cultivar V_8/comilla -2 having some sorts of capability of filed resistance which might be use as a breeding material.

Table 2 Effect of anthracnose disease on chilli in respect of percent reduction of number of fruit per plant.

Variety	Number of fruit per plant		%Reduction
	Healthy	Infected	
BARI Marich-1(V_1)	169.3 cd	92.3 c	45.46 bc (42.39)
Chittagong (V_2)	176.3 cd	84.8 cde	51.80 ab (46.04)
Comilla -1(V_3)	149.0 de	89.0 cd	38.30 c (37.96)
Jamalpur (V_4)	133.0 e	72.3 f	45.18 bc (42.23)
Gazipur (V_5)	216.3 b	88.8 cd	58.80 a (50.18)
Chandpur (V_6)	182.5 c	90.0 cd	50.23 ab (45.13)
Pusa jawla (V_7)	179.5 c	74.3 ef	58.01 a (49.66)
Comilla-2 (V_8)	266.0 a	266.0 a	0.00 d (0.57)
Kustia (V_9)	106.0 f	56.5 g	46.92 bc (43.23)
Bogra (V_{10})	230.0 b	112.5 b	51.03 ab (45.59)
Balujhuri (V_{11})	170.8 cd	79.0 def	53.32 ab (47.02)
CV%	10.15	7.34	8.66

Means followed by same letters in row and column are not significantly different at 5% level by DMRT. (Data on the parenthesis are arcsine transformed value)

Table 3 Effect of anthracnose of chilli on marketable yield loss in eleven chilli cultivars.

Variety	Total yield (t ha^{-1})	% Fruit infection	Marketable yield (t ha^{-1})
BARI Marich-1(V_1)	7.56 c	8.86 cd (17.27)	6.89d
Chittagong (V_2)	7.92 bc	8.08 d (16.50)	7.29 cd
Comilla -1(V_3)	7.95 bc	6.60 e (14.87)	7.39 cd
Jamalpur (V_4)	6.93 c	10.18 abc (18.60)	6.23 d
Gazipur (V_5)	9.18 b	9.13 bcd (17.54)	8.31 c
Chandpur (V_6)	11.40 a	11.57 a (19.86)	10.12 b
Pusa jawla (V_7)	11.51 a	11.19 a (19.52)	10.18 b
Comilla-2 (V_8)	12.52 a	2.53 g (9.09)	12.22 a
Kustia (V_9)	5.45 d	10.42 ab (18.80)	4.75 e
Bogra (V_{10})	12.22 a	3.76 f (11.15)	11.77 a
Balujhuri (V_{11})	11.48 a	11.16 a (19.49)	10.22 b
CV%	10.77	5.45	9.85

Means followed by same letters in row and column are not significantly different at 5% level by DMRT. (Data on the parenthesis are arcsine transformed value)

Acknowledgments

The authors humbly express their gratitude to the Director General & Director Training and Communication, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur for their support during the research works. The authors also express their gratitude to the Project Director (Spices Research Centre) for financial support.

References

Agostini, J.P. and L.W. Timmer. 1992. Selective isolation procedure for differentiation of two strains of *Colletotrichum gloeosporioides* from citrus. Plant Dis.76: 1176-78.

Anonymous. 2007. Yearbook of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics, Statistical Division, Ministry of Planning. Government of Bangladesh.

Azad, A.K.M., M.M. Kamal, S.H. Howlader, M. Hossain, and A.M. Akanda. 2005. Morphology of six isolates of Colletotrichum species and their host range. Bangladesh J. Plant Pathol. 21: 71-76.

Dasgupta, B. 1981. Sporulation and relative virulence among isolates of *Colletotrichum capsici* causing anthracnose of betel vine. India Phytopath 32: 196-99.

Daykin, M.E. and R.D. Millholland. 1984. Ripe fruit rot of muscadine grape cause by *Colletotrichum gloeo-sporioides* and its control. Phytopathology 74: 770-774.

Eastburn, D.M. and W.D. Gubler. 1990. Strawberry anthracnose: Detection and Survival of *Colletotrichum acutatum* in soil. Plant Dis.74: 161-63.

FAOSTAT. 2011. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor.

Gomez, K.A. and A.A. Gomez. 1984. Statististical Procedures for Agricultural Research. 2 edn. John. Wiley and Sons. Inc. New York.

Howard, L.R. 2000. Changes on phytochemical antioxidant activity of selected pepper species as influenced by maturity. J. Agric. Food Chem. 48: 1713-20.

Kannan, R., M. Ananthan and P. Blalsubramani. 1998. Anthracnose a Menace in Chilli Cultivation. Spice India 11:2-3. Kew, Surry, England.

Mian, I.H. 1995. Methods in Plant Pathology. IPSA-JICA Project.

Mordue, J.E.M. 1971. Description of Pathogenic Fungi and Bacteria No. 315. Kew, Surrey, England.

Sutton, B.C. 1980. The Coelomycetes, Fungi Imperfecti with Pycnidia, Acervuli and Stromata. Commonwealth Mycological Institute, Kew, Surrey, UK.

Ullasa, B.A., R.D. Rawal, H.S. Soshi, D.P. Singh and M.C. Joshi. 1981. Reaction to sweet pepper genotype to anthracnose, Cercospora leaf spot and powdery mildew. Plant Dis. 65: 600-601.

Winch, J.E., G.V.H. Jackson and I.S. Cole. 1984. Studies of *Colletotrichum gloeosporioides* diseases on yam, *Dioscorea alata* in Solmon Island. Plant Pathol. 33: 467-77.

Manuscript received 28 August 2011, accepted 20 October 2011