INCIDENCE AND BIOLOGY OF *CORYNESPORA CASSIICOLA* (BERK. & CURT.) WEI. DISEASE OF OKRA IN BANGLADESH

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Abstract

Eight commercial okra cultivars were evaluated to determine the magnitude of incidence of *Corynespora cassiicola* (Berk. & Curt.) Wei. Maximum and significant incidence of *C. cassiicola* was 14.86 in the cultivar BARI 1. In rest of the cultivars, the occurrence of this fungus was very poor to nil. *Corynespora cassiicola* appeared to be a minor pathogen for okra. Effects of temperature, pH and culture media on growth and sporulation of the fungus were investigated. Potato dextrose agar medium was found to be the most suitable for optimum growth and sporulation of this fungus at pH 7 and 25ºC. The conidial length significantly varied with the changes of pH. The correlation between pH and conidial length was found negative and highly significant.

Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is a popular and important vegetable crop grown mainly for its tender green fruits in Bangladesh, India and Pakistan. The green fruits are rich in vitamin A and C and minerals like Ca, Mg and Fe. Okra seeds are also good sources of protein and vegetable oil (Yadav and Dhankhar 2001). In Bangladesh, this crop is grown during March to October and suffers from a number of viral and fungal diseases (Ali *et al*. 2000, Fakir 1976). Fernandes *et al*. (1990) carried out a preliminary study on health status of okra seeds, collected from different municipalities of Rio de Jenerio state, Brazil and isolated 15 species of pathogenic fungi. *Corynespora cassiicola* (Berk & Curt.) Wei., was reported for the first time in 1945 as *Helminthosporium vignae* Olive and designated as a pathogen of cowpea (*Vigna sinensis* (L.) Endl. and soybean (*Glycine max* (L.) Merr.) (Olive *et al*. 1945). This fungus has been reported as a pathogen for target spot, leaf spot and leaf blight diseases of a number of dicotyledonous plants including okra with a varied degree of virulence depending on the host species throughout the world (Wei 1950, Castro 1979). Fakir (1977), Rahman (1988) and Shamsi and Naher (2010) reported *C. cassiicola* diseases in *Corchorus capsularis* L., *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. and *Vigna sinensis* L. respectively from Bangladesh.

In okra, *C. cassiicola* was first reported as a seed borne pathogen by Fakir (1976) while Ahmed and Khair (2008) reported this pathogen responsible for stem necrosis. However, the incidence and biology of *C. cassiicola* causing diseases in okra has not yet been reported. Therefore, the present work was undertaken to investigate the disease incidence of *C. cassiicola* in commercial cultivars of okra as well as to study the biology of the fungus.

Materials and Methods

Seven okra cultivars, namely BARI 1, Parvanikranti, Chittagong iron, Parash 2, Sabuj bangla, Pan and Sirajuddoula were collected from commercial seed stores of Siddique bazaar, Dhaka, Bangladesh. In addition, an introduced cultivar, 'red okra', brought from China was acclimatized in the field condition of Bangladesh and included in the present study. Survey on disease incidence of *C. cassiicola* was carried out for three consecutive year (2005 to 2007) in the

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experimental plots at the Botanical garden of Jahangirnagar University following completely randomized block design (RBD). Diseased plants were categorized on the basis of the percentage of diseased surface area (including leaf and stem) according to the scale and formula of Singh (1984).

In order to test the pathogenicity, the causal fungus was first isolated from the transitional zone of healthy and diseased tissue on 2% potato dextrose agar (PDA) medium following Dhingra and Sinclair (1985) and was identified on the basis of microscopic characters and cultural behaviour (Ellis 1971, Subramanian 1971). Stems and fruits of okra, grown under restricted condition, were surface sterilized with 0.5% NaOCl then washed with sterilized distilled water. Pathogenicity tests were performed by pricking surfaces of stems and fruits of okra, up to a depth of 0.5 mm, of each of 25 test plants, on which a mycelium plug (2.0 mm in diameter) cut from the edge of a 7-day-old monosporic fungal cultures was placed. In control plants a sterile PDA plug was placed on each wound. To conduct leaf-pathogenicity test, a conidial suspension (ca. 2×10^{-4} conidia/ml) was prepared in sterile water by harvesting conidia from 10-day-old fungal cultures and the suspension was sprayed on to the 25 leaves of three healthy plants. Inoculated plants were kept in humid condition by repeated spraying with sterile water in a polyethylene chamber for the first 48 hrs and thereafter placed in controlled environment. Symptoms similar to those shown by naturally infected plants developed on the wounded stems, fruits and leaves 7 days after inoculation. *C. cassiicola* was re-isolated successfully from the inoculated plants, confirming Koch's postulates. Control plants remained symptomless.

Potato dextrose agar, corn-meal agar, Czapek's Dox agar, malt extract agar, Richards' medium, Waksman's medium and okra leaf extract agar were tested to select the appropriate growth medium for the fungi. Besides, the test fungus was grown on PDA with varying pH and temperature to assess its biological features. PDA medium was adjusted to different pH levels *viz.,* 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 with 1N NaOH or 1N HCl by using pH meter.

Results and Discussion

Okra plants infected with *C. cassiicola* showed necrotic leaf spot, stem necrosis and fruit rot. Infected leaves consist of lesions with pale yellow to brownish centers having irregular outer rings of gray to dark brown margin (Fig. 1A). Sometime the centre of the lesion was found cracked. The symptom on the infected stem first appeared as a number of very minute, dark, water soaked circular spots that coalesced later and formed a large necrotic subcircular to irregular region having dark gray to black coloured lesion (Fig. 1B). Initial symptom appeared on the surface of the fruits, in the form of water-soaked lesions of elliptical to irregular in shape. Later the lesions coalesced and produced larger irregular necrotic areas (Fig. 1C). It was confirmed by Koch's postulates that *C. cassiicola* was the causal pathogen of the disease symptoms (Fig. 1D, E). Partially affected plants bare fruits, which are few in number and of low quality. Severely affected plant showed wilting and died subsequently. Mukerji and Bhasin (1986) listed the 31 fungal pathogens, which caused okra associated diseases where *C. cassiicola* was absent in the list.

Seven commercial cultivars of okra along with an acclimatized cultivar 'red okra' were surveyed for three consecutive years to assess the level of incidence of *C. cassiicola* causing leaf spot, stem necrosis and fruit rot disease. Disease incidence presented in Table 1 is the sum total of disease incidence on leaf, stem and fruits. The disease occurred usually after the maturity (45-50 DAS) of Okra plants. Maximum and significant (at 0.05% level) disease incidence were 14.86, 12.86 and 9.51 recorded during the year 2005, 2006 and 2007, respectively in the cultivar BARI 1. In rest of the cultivars the occurrence of this fungus was very poor to nil (Table 1). Disease

incidence in the particular cultivars did not change much with the change of cultivating year. Therefore, variation in disease incidence could be attributed to the genotypic variation rather than seasonal.
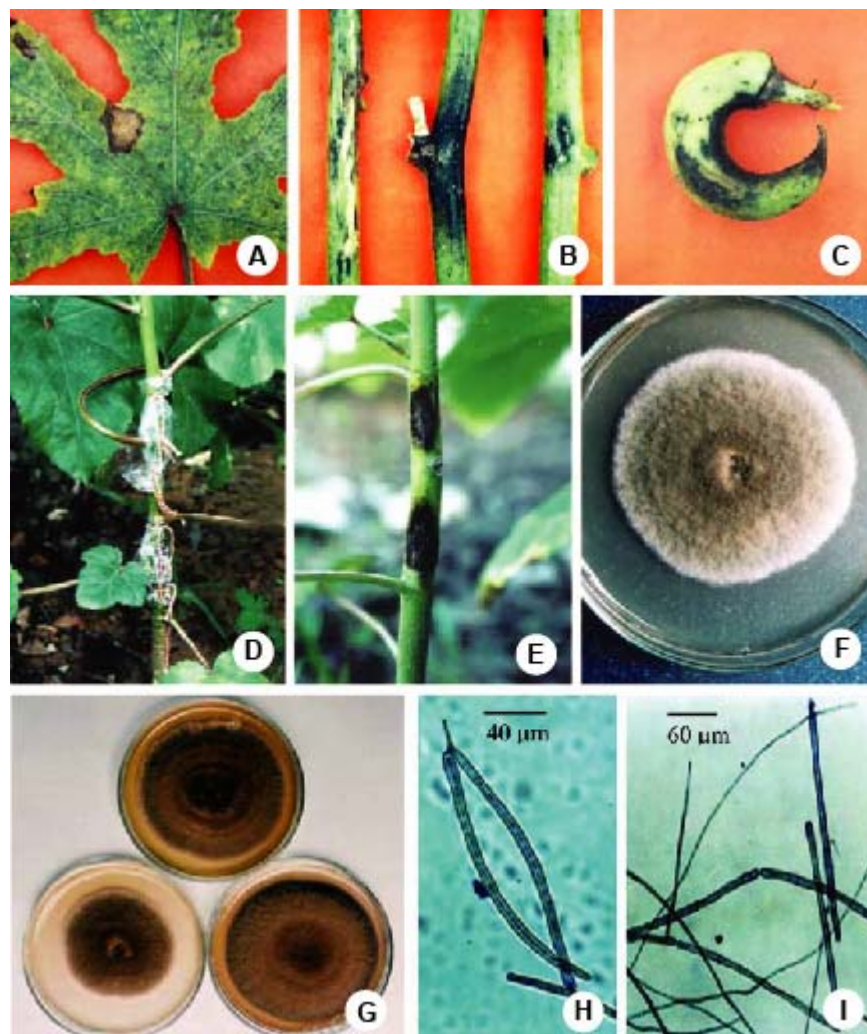

Fig. 1. *Corynespora cassiicola* disease of okra. A. Leaf spot symptom. B. Different magnitude of stem necrosis and C. Fruit rot symptom produced by *C. cassiicola* under natural condition.. D & E. Appearance of stem necrosis symptom after artificial inoculation. F & G. Typical colony of *C. cassiicola* on PDA medium. H. Characteristic conidial feature (× 200)and I. Conidial chain with mycelium (× 100) of *C. cassiicola* viewed under microscope.

Leaves, stems and fruits of eight commercial cultivars of okra were inoculated with *C. cassiicola* to test the magnitude of pathogenicity on each of the plant part (Fig. 2). In each of the three trial, 25 inoculation sites made separately in leaves, stems and fruits under controlled condition where relative humidity was 97±2%. Significant and maximum number of disease symptoms on leaves, stems and fruits were produced in the cultivar BARI 1, followed by

Parvanikranti, Sirajuddoula and Pan cultivars respectively. Sabuj bangla cultivar did not show any disease symptom whereas cultivars Parash 2 and Red okra showed very little response to this pathogen. The diminutive occurrence of *C. cassiicola* in eight cultivars indicates it as a minor pathogen of okra.

Table 1. Incidence of *Corynespora cassiicola* recorded as per cent disease index (PDI) in eight cultivars of okra during summer 2005 to 2007.

Cultivars	Cultivation year		
	2005	2006	2007
Red okra	0.0c	0.0c	0.0c
BARI 1	14.86a	12.86a	9.51a
Parvanikranti	3.33b	0.72bc	2.33b
Chittagong iron	1.07b	0.0a	1.33b
Parash 2	0.72bc	0.24b	0.0c
Sabuj bangla	0.0c	0.0c	0.0c
Pan	1.33bc	0.0c	0.0c
Sirajuddoula	1.91bc	1.32bc	1.76b

Data are average of five replications. In a column means followed by a common letter are not different significantly at 5% level by DMRT.
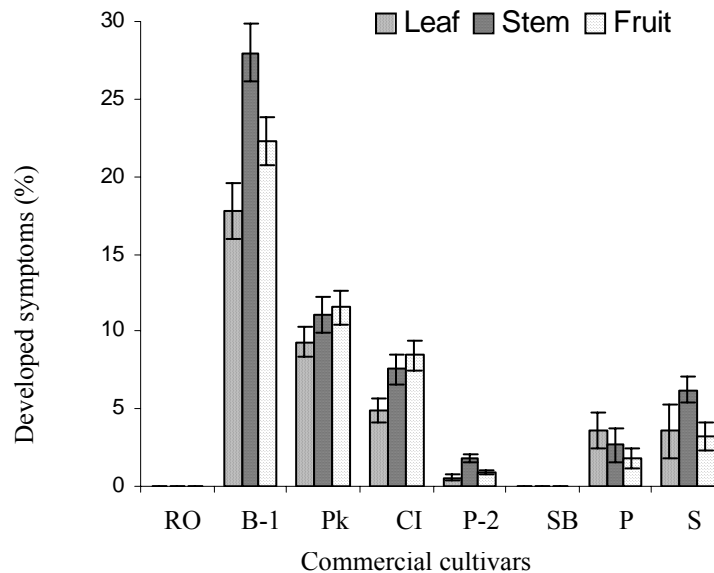

Fig. 2. Percentage of disease symptom (±SE) produced in eight cultivars of okra after artificial inoculation under controlled condition. RO = Red okra, B-1 = BARI-1, Pk = Parvanikranti, CI = Chittagong Iron, P-2 = Parash-2, SB = Sabuj bangla, P = Pan and S = Sirajuddoula

Colony of *C. cassiicola* on PDA medium appeared as effuse, gray to light olivaceous green at immature stage and turned brown to dark blackish brown at maturity, often hairy or velvety (Fig. 1F, G). Conidia formed solitary or in chains of 2 - 6, very variable in shape, obclavate to cylindrical, straight or curved, pale olivaceous brown or brown, smooth, with 4 - 20 pseudosepta and measured 41.2 - 219.7μ long and 9.2 - 21.5μ wide (Fig. 1H, 1I). The potato dextrose agar (PDA) medium at 25ºC temperature was found optimum for the growth and sporulation of the test fungus, which greatly corroborates with the findings of Tsay and Kuo (1991). Effect of temperature on spore germination and mycelial growth was determined by growing the fungus on PDA medium at pH 7 with varying temperature. The fungus grew about 7.87 cm in diameter of colonies at 25ºC in one week. Whereas, the optimum temperature for spore germination and mycelial growth ranged between 25 and 30ºC, which was statistically significant at 5% level by DMRT (Table 2).

Table 2. Effect of temperature on spore germination and mycelial growth of *Corynespora cassiicola*.

Temperature (ºC)	Spore germination (%)*	Diameter of colony (cm)**
10	15.5d	2.22d
15	55.8c	4.13c
20	73.6b	5.66b
25	87.3a	7.87a
30	94.1a	7.61a
35	91.6a	4.46c

*Inoculum on water agar for 3 hrs and 3 replications for each treatment. **Growth on PDA for 7 days and 3 replications for each treatment. In a column means followed by the same letter are not different significantly at 5% level by DMRT.

Vegetative growth of *C. cassiicola* was investigated using some common culture media at pH 7 and 25ºC and has been presented in Table 3. After a week of incubation, maximum mycelial growth was recorded on PDA (8.27 cm) followed by okra leaf extract agar (7.44 cm) and Czapex dox's agar (7.03 cm) respectively. Minimum mycelial growth was recorded in corn-meal agar (1.08 cm) followed by Waksman's medium (2.23 cm). Richard's medium and malt extract agar

Table 3. Effect of different culture media on growth of the *Corynespora cassiicola*.

Culture medium	Mycelial growth (cm) at constant temperature and pH						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Potato dextrose agar	0.63a	1.42a	2.68a	3.67a	5.38a	7.71a	8.27a
Corn-meal agar	0.08c	0.18d	0.33d	0.48d	0.70e	1.00e	1.08f
Czapek's Dox agar	0.54a	1.21b	2.28ab	3.12ab	4.57b	6.55ab	7.03b
Malt extract agar	0.23b	0.53c	0.99c	1.36cd	1.99d	2.85d	3.06d
Richards' medium	0.38ab	0.85bc	1.61b	2.20c	3.23c	4.63e	4.96c
Waksman's medium	0.17bc	0.38d	0.72c	0.99cd	1.45d	2.08d	2.23c
Okra leaf extract agar	0.57a	1.28b	2.41ab	3.30ab	4.84b	6.94ab	7.44b

Note: Data are averages of three replications. In a column means followed by the same letter are not different significantly at 5% level by DMRT.

media were found moderately suitable for *C. cassiicola*. In contrast, Oluma and Amuta (1999) reported faster growth of this fungus on malt-extract agar (MEA) media and slowest growth on potato-dextrose agar (PDA) supplemented with thiamine. However, Fernando *et al*. (2012) reported that PDA was one of the best media for growth and sporulation of *C. cassiicola*.

To determine the optimum pH, the fungus was grown on PDA at 25ºC and varying pH (4-10) for seven days. Initially, the mycelial growth of the fungus in different pH did not vary much but at maturity pH values were found to set profound effect on growth. From day 3 to 5, the fungus showed consistent mycelial growth from pH 6 to 8 but on 6th and 7th day, statistically significant maximum growth was observed at pH 7 and 8, followed by pH 5, 6 and 9 respectively (Table 4). The optimum pH for the growth and sporulation ranged between 5 and 8, which corroborates with the findings of Chunxia *et al*. (2010) as they reported 4 - 8 pH was optimum for growth of this fungus.

Table 4. Effect of pH on the growth of *Corynespora cassiicola*.

pH of PDA medium	Mycelial growth (cm) at 25ºC						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
4	0.33cd	0.83b	2.07b	2.67d	3.33c	3.62d	4.60d
5	0.45bc	1.01ab	2.56a	3.33c	4.37b	5.63b	6.65b
6	0.62a	1.22a	2.87a	3.92ab	5.03a	5.70b	6.87b
7	0.55ab	1.13ab	2.61a	3.87ab	4.94a	6.03a	7.49a
8	0.41c	0.93b	2.73a	4.03ab	5.14a	5.91a	7.36a
9	0.32cd	0.8bc	2.62a	3.73bc	4.81a	5.35bc	6.12bc
10	0.21d	0.53c	2.26b	4.33a	5.13a	5.15c	5.51c

*Mean of three replications. In a column means followed by a common letter are not different significantly at the 5% level of significance by DMRT.

pH of the medium and corresponding frequency of average spore length have been presented in Table 5. The maximum frequency of spore-length ranged between 25 and 74.9 μ and pH 5 to 10. At pH 4, most of the spore lengths were between 75 and 99.9 μ. Maximum spore length ranged between 150 and 174.9 μ, observed at pH 4 to 6. Though spores with minimum length (1 - 24.9 μ) were common in different pH but the frequency was relatively higher at pH 4 and 10 (Table 5).

Table 5. Effect of pH on spore length of *Corynespora cassiicola*.

Spore length (µm)	pH						
	4	5	6	7	8	9	10
1 - 24.9	8.33b	3.33c	1.33c	4.00c	1.67c	5.00c	7.33b
25 - 49.9	13.00ab	19.00a	25.33a	23.67a	29.67a	24.33a	17.67a
50 - 74.9	14.67ab	15.33a	7.00b	14.67b	11.67b	11.67b	18.67a
75 - 99.9	18.33a	9.67bc	9.67b	10.33b	8.00bc	7.33c	5.00bc
100 - 124.9	3.33c	6.00bc	7.00b	1.33c	1.33c	3.67c	2.67bc
125 - 149.9	2.33c	3.33c	3.00c	2.33c	1.67c	0.00d	0.00d
150 - 174.9	1.33c	2.33c	2.67c	0.00c	0.00c	0.00d	0.00d

Frequency of spores expressed as per cent (%) values. Data are average of three observations. In a column means followed by a common letter are not different significantly at the 5% level of significance by DMRT.

Karl Pearson's co-efficient of rank correlation between pH and spore length was –0.297; which indicates the spore length decreases with the increase of pH values. t-test was performed to assess the level of significance. At the 99% confidence level the calculated value of 't' was 3.86, greater than the tabulated value of $t_{(\alpha=0.01)} = 2.57$.

The correlation between pH and spore length was little and negative, but statistically highly significant. Alternately it may be inferred that on PDA medium at 25°C temperature, lower pH values favours increase in spore length of *C. cassiicola* but reduces spore frequency at per unit area. The fungus showed variable conidial length with pH. *C. cassiicola* is regarded as a very variable species within the genus. A comparison of *C. cassiicola* collected on 20 different host plants from various parts of the world has shown a great variation of conidial size for this species (Onesirosan *et al*. 1974). Another study, involving the comparison of genetic differences among different isolates of *C. cassiicola*, using PCR-based techniques, has shown that there were no detectable genetic differences among such isolates (Silva *et al*. 1998). Thus the phenomenon of producing variable conidial length remained unexplained.

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