Original article

Prevalence of the Alternaria blight of cumin (*Cuminum cyminum* L.) in Bangladesh: Morphology, phylogeny and pathogenic variation of *Alternaria* spp.

Md. Abdul Wadud [a], Sukalpa Das [b], Md. Atiqur Rahman Khokon [c,*]

[a] Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh&Bangladesh Agriculture Research Institute, Bangladesh
[b] Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh&Department of Agricultural Extension, Bangladesh
[c] Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh

ARTICLE INFO

ABSTRACT

Cumin (*Cuminum cyminum* L.) is an important spice crop worldwide and its production is hampered by the infection of Alternaria blight. Cultivation of cumin in Bangladesh is very limited due to the lack of appropriate germplasm and adequate scientific information regarding the prevalence of Alternaria blight. Field trials were conducted with four advanced lines of cumin *viz.* CN026, CN028, CN031 and CN038 in five agro-ecological zones (AEZ) to know the adaptation possibility of these lines against the incidence and severity of Alternaria blight of cumin in Bangladesh. Among all lines, CN026 was found as the best in germination capacity and other yield parameters in all locations. The incidence and severity of the disease was observed as high as 98% and 88%, respectively, however, out of the five locations, the incidence and severity of the disease was the lowest in Bogura for the line CN026. In an attempt to identify the causal organism of the Alternaria blight of cumin by using molecular tools, a total of twenty three isolates were collected from the plants showing Alternaria blight symptoms from different AEZ in Bangladesh. Based on the molecular analysis, the isolates were identified as *Alternaria alternata*, *A. burnsii*, *A. gaisen* and *A. tenuissima*. *A. alternata* was the most prevalent species followed by *A. tenuissima*. The isolates of the identified species were found to have genetic, morphological and pathogenic variation. An isolate of *A. alternata* was observed as the most virulent among the isolates. This is the first report of *A. alternata* causing Alternaria blight disease of cumin in Bangladesh. The findings of this experiment will help in selecting suitable cumin germplasm and designing proper management strategies against Alternaria blight of cumin in Bangladesh.

© 2021 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Cumin (*Cuminum cyminum* L.*)* belongs to the family Apiaceae (Umbelliferae) and is believed to be the native of the mediterranean and near eastern regions of the globe (Nabhan, 2014). It is widely cultivated in Uzbekistan, Tajikistan, Turkey, Morocco, Egypt, India, Syria, Mexico, Pakistan and Chile (Azeez et al., 2008). It grows best on well drained sandy loam to loamy soil with a pH range of 6.8 to 8.3 (Weiss, 2002). Cumin seeds are used as spice in culinary for flavoring soups, sauces, pickles and for seasoning breads and cakes. Cumin is an important ingredient in Bangladeshi cuisine. To meet the internal demand, a huge amount of cumin is imported every year in Bangladesh in expense of costly foreign exchange. In 2019–20, about 27,000 to 28,000 tons of cumin was imported in Bangladesh (The Financial Express, 2020). Therefore, cultivation of cumin in Bangladesh might reduce its dependency on other countries. Despite having congenial climatic and edaphic conditions, commercial cultivation of cumin is not well adopted in Bangladesh due to many socio-economic reasons including the lack of appropriate germplasm and adequate scientific information regarding the infestation of different pathogens.

Production of cumin is seriously affected by the infection of Alternaria blight caused by *Alternaria* spp. The disease appears in devastating form every year in the most cumin-growing areas in

* Corresponding author.
  E-mail address: atiq.ppath@bau.edu.bd (Md. Atiqur Rahman Khokon).

the world and can cause up to 80% of yield loss (Gemawat and Prasad, 1972). Initially, blight affected plants show minute whitish to black isolated necrotic areas on the aerial parts, especially on tips of young leaves leading to the death of the whole plant or the affected parts (Uppal et al., 1938; Sharma, 2010). Diseased seeds are small, de-shaped, shriveled, light weight and black in colour (Gemawat and Prasad, 1972). High humidity during flowering and fruit setting, the most vulnerable stage of the growth of the crop, is conducive for the development of Alternaria blight in cumin (Uppal et al., 1938; Patel et al., 1957; Gemawat and Prasad, 1971). Correct species identification is an imperative for designing successful management approaches against any plant pathogen. Different species and isolates of *Alternaria* vary in radial mycelial growth, conidia structure, colony character, sporulation and pathogenic capability (Ansari et al., 1989; Patni et al., 2005; Kaur et al., 2007). For delimiting the species of fungal pathogens and to study their diversity, molecular tools are being used increasingly including other morphological characters (Benali et al., 2011). To the best of our knowledge, there is no extensive study conducted so far in Bangladesh about the prevalence of the Alternaria blight of cumin and its causal organisms.

The present study was undertaken 1) to know the prevalence of the Alternaria blight of cumin in Bangladesh 2) to study the feasibility of introducing some advanced lines of cumin, better adapted against the Alternaria blight, in Bangladesh 3) to identify and characterize species of fungus associated with the Alternaria blight of cumin in Bangladesh using morphological and molecular features 4) to discriminate the pathogenicity of the isolates of fungus associated with the Alternaria blight of cumin collected from different locations of Bangladesh.

2. Materials and methods

Assessment of germination and yield parameters

The experiment was conducted at five sub-centers under Spices Research Centre (SRC) of Bangladesh Agricultural Research Institute situated in different Agro-ecological Zones (AEZ) in Bangladesh (Tables 1 & 2). The experiment was laid out in a randomized complete block design (RCBD) with four replications during November 2019 to March 2020. Standard procedures of cultivating cumin were followed for the land preparation and subsequent intercultural operations (Verma et al., 2018). Four advanced cumin lines *viz.* CN026, CN028, CN031 and CN038 were used in the experiment. One hundred seeds of each cumin line were sown in each location and percentage of germination was determined by calculating the number of plants grown. For assessing the field performance of four cumin lines, mean of yield parameters of five locations were used (Table 6).

Incidence and Severity of the disease

The incidence and severity of Alternaria blight were recorded for three times starting from the fifty five days after emergence (DAE) of cumin seedlings at the interval of 10 days. For determining disease severity, ten plants were randomly selected from each plot of all locations and percent disease index (PDI) was calculated.

$$\% \text{ Disease incidence (Chester, 1959; Wheeler, 1969)} = \frac{\text{No. of infected plants per unit area} \times 100}{\text{Total no. of plants per unit area}}$$

$$\text{PDI (Chester, 1959; Wheeler, 1969)} = \frac{\text{Sum of all disease rating} \times 100}{\text{Total no. of plants assessed} \times \text{Maximum possible rating}}$$

The disease intensity was calculated with the help of disease rating scale (0–5) where, (0 = Free from disease, 1 = 1–10% area of leaf & umbel blighted, 2 = 11–20% area of leaf, stem & umbel blighted, 3 = 21–35% area of leaf, stem & umbel blighted, 4 = 36–60% area of leaf, stem & umbel blighted and 5 = More than 60% area of leaf, stem & umbel blighted (Jat, 2015).

Isolation, purification and identification of the pathogen

A total of twenty three samples of Alternaria blight of cumin were collected from five locations (Table 1). The diseased parts were cut into small pieces of 3 mm diameter with some healthy part. The cut pieces were washed in tap water and surface sterilized with 10% NaOCl for 30 sec and blotted to dry on sterilized blotting paper. After drying, three pieces were aseptically placed into petri dishes containing Potato Dextrose Agar (PDA) medium. The PDA with small pieces of infected plant parts were incubated at a temperature of 26 ± 1 °C for 7 days (ISTA, 1976). Fungal pathogens were identified on the basis of their morphological, cultural and pathogenic characteristics (Rifai, 1969; Aneja, 2004).

Cultural variability

Purified culture of each isolate was prepared following the hyphae tip culture method (Tutte, 1969). The growth patterns of twenty three isolates of the pathogen were recorded on PDA medium by inoculating with 5 mm discs cut from PDA culture of the isolates. The discs were placed at the center of the Petri plates containing PDA. Three plates of each isolate were incubated at 26 ± 1 °C and inoculated. The growth rate, colony characters, growth habit and sporulation were recorded every 4 days after incubation and continued to 11 days after incubation. Observations on variation in conidial dimension were recorded with the help of a compound microscope.

Measurement of fungal sporulation

To determine the conidial concentration of each isolate, 5 ml of double distilled water (DDW) and 2.5 ml of ethanol was added in each petri plate containing 14 days old pure culture. The surface of the fungal growth on PDA was brushed gently with a toothbrush to disperse the spores. Three ml of suspension and 22 ml of DDW were taken in a test tube and mixed well by shaking. The final suspension (30 μl) was placed in a haemocytometer and the numbers of conidia/ml were counted under a compound microscope and number of conidia/ml was estimated (Nur-E-Nasreen et al., 2017). The procedure was repeated for thrice and a mean number of conidia/ml was estimated.

Table 1
The list of isolates of *Alternaria* spp. collected from different locations of Bangladesh with their corresponding GenBank Accession number.

Serial No.	Location	*AEZ	Isolate Code (GenBank Accession number)
1	Bogura	3	BoCA1 (MN989187)
2	Bogura	3	BoCA4 (MN989188)
3	Bogura	3	BoCA5 (MN989189)
4	Bogura	3	BoCA8 (MN989190)
5	Bogura	3	BoCA11 (MN989191)
6	Bogura	3	BoCA12 (MN989192)
7	Magura	11	MaCA2 (MN989193)
8	Magura	11	MaCA3 (MN989194)
9	Magura	11	MaCA4 (MN989195)
10	Faridpur	12	FaCA3 (MN989196)
11	Lalmonirhat	2	LaCA1 (MN989197)
12	Lalmonirhat	2	LaCA3 (MN989198)
13	Bogura	3	BoCA6 (MN989199)
14	Bogura	3	BoCA3 (MN989200)
15	Bogura	3	BoCA2 (MN989213)
16	Bogura	3	BoCA9 (MN989214)
17	Bogura	3	BoCA10 (MN989215)
18	Bogura	3	BoCA13 (MN989216)
19	Gazipur	8	GaCA1 (MN989217)
20	Gazipur	8	GaCA5 (MN989218)
21	Magura	11	MaCA1 (MN989219)
22	Faridpur	12	FaCA4 (MN989220)
23	Lalmonirhat	2	LaCA2 (MN989221)

* Agro-ecological Zones (AEZ).

Table 2
Weather parameters of experiment sites during November 2019 to March 2020.

Location	*AEZ	Weather parameter	November, 2019	December, 2019	January, 2020	February, 2020	March, 2020	Average weather parameter
Bogura	3	Temperature (^{0}c)	25.32	21.34	19.18	22.11	25.18	22.63
		Rainfall (mm)	0.033	0.00	0.00	0.138	0.90	0.214
		Relative Humidity (%)	87.08	91.44	93.58	86.0	75.97	86.81
Gazipur	8	Temperature (^{0}c)	24.6	19.23	18.24	22.71	24.92	21.94
		Rainfall (mm)	0.70	0.00	0.94	0.00	0.06	0.34
		Relative Humidity (%)	89.55	86.48	80.53	79.31	75.97	82.37
Lalmonirhat	2	Temperature (^{0}c)	18.56	15.68	13.15	17.58	22.45	17.48
		Rainfall (mm)	0.81	0.00	0.11	0.116	0.65	0.337
		Relative Humidity (%)	87.60	88.60	90.12	82.50	74.60	84.68
Faridpur	12	Temperature (^{0}c)	23.14	17.56	16.41	19.26	22.84	19.84
		Rainfall (mm)	32.4	6.2	16.6	0.6	3.8	11.92
		Relative Humidity (%)	86.84	87.18	88.03	79.62	77.16	83.77
Magura	11	Temperature (^{0}c)	23.8	19.70	20.20	24.6	27.85	23.23
		Rainfall (mm)	1.6	1.6	0.00	2.0	0.4	1.12
		Relative Humidity (%)	85.50	86.20	87.03	75.32	74.11	81.63

* Agro-ecological Zones (AEZ).

Extraction of genomic DNA

Fungal DNA was extracted following the modified CTAB method (Doyle and Doyle, 1987). The dried mycelia were ground into powder in liquid nitrogen. The homogenized mycelia were transferred to an eppendorf and CTAB extraction buffer was added. The sample was incubated at 65 °C for 30 min in a hot water bath and centrifuged at 10000 rpm for 10 min. Equal volume of Phenol:Chloroform:Iso-amylalcohol (25:24:1) were mixed and centrifuged at 10000 rpm for 10 min. The supernatant and equal volume of ice-cold isopropanol was added. The sample was incubated at −20 °C for 1.30 h and followed by centrifugation at 13000 rpm for 15 min. The DNA pellet left at the bottom was washed with 70% ethanol, air-dried and dissolved in TE buffer. The DNA stock solution was stored at −20 °C.

PCR amplification

The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) of 23 isolates were amplified using primer set ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATT GATATGC-3′) (White et al., 1990). The amplification reaction for the simplex PCR consisted of a volume of 25 μl PCR mix that was made up by adding 12.5 μl ready to use master mix (Promega, Madison, WI, USA), 9.5 μl nuclease-free water, 1 μl of each 10 μM forward and reverse primer, and 1 μl of the respective isolate's DNA. PCR cycle for ITS sequence amplification consisted of initial denaturation (94 °C for 5 min) followed by 35 cycles of denaturation (94 °C for 30 sec), annealing (56 °C for 30 sec), extension (72 °C for 1.0 min 30 sec) and a final extension of 10 min at 72 °C.

Gel electrophoresis

After DNA amplification, a 10 μl of PCR product from each sample was loaded into 1% agarose gel and stained with Ethidium Bromide (0.5 mg/ml). Electrophoresis was conducted in the Tris-Borate-EDTA (TBE) buffer at 80 V for 45 min. DNA bands were visualized and photographed under UV light by GelView Master (Dynamica Scientific Ltd.). The length of each amplified DNA fragment was compared with a 1 kb DNA ladder (Promega, Madison, WI, USA).

Sequencing

The amplified fragments were sent to the National Institute of Biotechnology (NIB), Dhaka, Bangladesh and were sequenced bi-directionally. The nucleotide sequences were submitted to the National Center for Biotechnology Information (NCBI) GenBank and their accession were obtained (Table1). Primers used for the sequencing reactions were the same as those used in the amplification step.

Phylogenetic analyses

The sequences of *Alternaria* spp. used in the phylogenetic analyses were downloaded from the NCBI Genbank based on the highest match of Nucleotide Basic Local Alignment Search Tool (BLASTN) results against the isolates of *Alternaria* spp. identified in this study. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. A bootstrap analysis using 1000 replications of the sequence data was carried out (Felsenstein, 1985).

Pathogenic variability

Pathogenicity of all the isolates of *Alternaria* spp. was tested on the cumin line CN026 grown on sterilized soil in pots by the spray inoculation technique (Dhingra and Sinclair, 1995). This line was selected for the experiment because it showed better adaptability in the climatic conditions of Bangladesh in terms of germination capacity, yield, incidence and severity of Alternaria blight of cumin. Each pot received 10–15 seeds of the cumin line (Shekhawat et al., 2013). The concentration of the spores was maintained at 1×10^3 ml^{-1} during inoculation. Forty to fifty days old plants were inoculated by spraying the conidial suspension with a hand-held atomizer. The inoculated plants were kept in a humid chamber for 24 h before transferring to a cage house. High humidity was maintained throughout the disease development period by frequent irrigations. Experiments were conducted in Completely Randomized Design (CRD). Each treatment was replicated three times. The disease intensity was calculated with the help of a disease rating scale (***Jat, 2013). Re-isolation was done by collecting the infected plant parts after 10 days of inoculation. The isolated cultures were compared with the original one to confirm the pathogenicity.

2.1. Statistical analysis

Statistical analyses were done by R statistical software (http://www.R-project.org). ANOVA was performed on germination, yield and yield contributing characters of cumin lines, disease incidence and disease severity, radial mycelia growth, length and breadth of conidia, beak length, and number of vertical and horizontal septa by the Agricolae R package and AOV function (R Core Team, 2020).

Table 3
The germination (%) of cumin seeds in different locations.

Location	Germination (%)
SRC, Bogura	80.12 a
SRC, Gazipur	75.06b
SRC, Lalmanirhat	64.06 e
SRC, Magura	72.81c
SRC, Faridpur	69.87 d
Level of significance	**
CV (%)	4.088

Spice Research Centre (SRC); **1% level of probability; Means followed by the same letter in a column did not differ significantly.

Table 4
The germination (%) of different cumin lines.

Cumin line	Germination (%)
CN026	75.20 a
CN028	71.10b
CN031	69.30b
CN038	73.95 a
L.S.	**
CV (%)	4.088

** 1% level of probability; Means followed by the same letter in a column did not differ significantly.

3. Results

Germination percentage and yield parameters of cumin lines: One of the objectives of this study was to introduce some advanced lines of cumin in Bangladesh. To achieve this objective, germination capacity and different yield parameters of four cumin lines were assessed. The overall germination percentage of four lines varied significantly at different locations (Table 3). The highest (80.12%) germination was recorded at SRC, Bogura and the lowest (64.06%) was at SRC, Lalmanirhat. Significant variation in the germination percentage was observed in the four cumin lines (Table 4). The cumin lines CN026 and CN038 had significantly higher germination than the other two lines. The interaction effect of the cumin lines and locations on the germination percentage was significant (Table 5). All of the four cumin lines had higher germination at SRC, Bogura. The highest germination was recorded in the CN026 (85.00%) and CN038 (82.75%) at SRC, Bogura. There was a significant difference among the yield parameters of the cumin lines in all of the locations (Table 6). All of the yields contributing characters were higher for the line CN026. The highest seed (592.85 kg/ha) yield was recorded from the line CN026 and the lowest (279.34 kg/ha) was obtained in the CN031.

Incidence and severity of Alternaria blight disease: The incidence and severity of the Alternaria blight was observed at five locations on four cumin lines at different DAE of seedlings. Significant variation in the incidence and severity of the disease was observed at five locations at different DAE (Table 7). Both incidence and severity of the disease increased with the progress of time dur-

Table 5
The germination (%) of different cumin lines in different locations.

Location	Cumin line	Germination (%)
SRC, Bogura	CN026	85.00 a
	CN028	77.50 bc
	CN031	75.25b-e
	CN038	82.75 a
SRC, Gazipur	CN026	78.00b
	CN028	74.75b-e
	CN031	73.25 d-f
	CN038	74.25b-e
SRC, Lalmanirhat	CN026	65.25 h-j
	CN028	64.75 ij
	CN031	63.25 j
	CN038	63 j
SRC, Magura	CN026	76.25b-d
	CN028	70 fg
	CN031	69 gh
	CN038	76.00b-d
SRC, Faridpur	CN026	71.50 e-g
	CN028	68.50 g-i
	CN031	65.75 h-j
	CN038	73.75c-f
L.S.		**
CV (%)		4.089

Spice Research Centre (SRC); **1% level of probability. Means followed by the same letter in a column did not differ significantly.

Table 6
Yield parameters of different cumin lines.

Cumin line	Plant height (cm)	Primary Branch/plant (no.)	Umbel/plant (No.)	Umbel let/umbel (No.)	Seeds/umbel let (No.)	1000 seed wt. (g)	Yield (kg/ha)
CN026	51.30 a	5.17 a	95.46 a	6.19 a	5.75 a	5.82 a	592.85 a
CN028	44.53 ab	4.83 ab	83.88 ab	5.05 abc	5.03 ab	5.10 ab	450.60 abc
CN031	42.75 bc	68 abc	79.97 a-d	5.23b	4.67 ab	4.83 abc	279.34 cde
CN038	38.98b-e	4.50 a-d	81.44 abc	4.50 bc	4.59 ab	4.33 bc	485.31 ab
L.S.	**	**	**	**	**	**	**
CV (%)	6.48	7.42	13.22	9.41	8.45	9.67	24.42

** 1% level of probability; Means followed by the same letter in a column did not differ significantly.

Table 7
The incidence and severity of Alternaria blight of cumin in different locations.

Location	Disease incidence (%)			Disease severity (%)		
	55 DAE	65 DAE	75 DAE	55 DAE	65 DAE	75 DAE
SRC, Bogura	30.14 e	75.73c	91.69b	48.96c	69.71c	79.90b
SRC, Gazipur	44.56b	77.93 bc	94.20 ab	52.81b	77.12 a	85.80 a
SRC, Lalmanirhat	43.22c	77.82 bc	97.73 a	55.31 a	78.74 a	87.97 a
SRC, Magura	38.05 d	79.34b	97.71 a	53.33 ab	73.27b	85.41 a
SRC, Faridpur	46.03 a	83.89 a	97.77 a	55.01 ab	69.86c	82.28b
L.S.	**	**	*	**	**	**
CV (%)	4.025	4.155	6.297	6.653	4.675	5.039

Spice Research Centre (SRC); Days after Emergence (DAE) **1% level of probability; *5% level of probability; Means followed by the same letter in a column did not differ significantly.

Table 8
The incidence and severity of Alternaria blight of cumin in different lines.

Cumin line	Disease incidence (%)			Disease severity (%)		
	55 DAE	65 DAE	75 DAE	55 DAE	65 DAE	75 DAE
CN026	39.52b	76.09c	95.01	47.96c	70.39b	82.93
CN028	40.18 ab	80.04 ab	95.96	55.04 ab	74.99 a	85.33
CN031	41.05 a	81.56 a	96.69	56.01 a	75.47 a	84.82
CN038	40.85 a	78.09 bc	95.62	53.36b	74.11 a	84.02
L.S.	*	**	NS	**	**	NS
CV (%)	4.0246	4.155	6.297	6.653	4.675	5.039

Days after Emergence (DAE); **1% level of probability; *5% level of probability; Non Significant (NS); Means followed by the same letter in a column did not differ significantly.

ing the experiment period at all locations for all cumin lines and reached the maximum of 98% and 88%, respectively, at 75 DAE. At all intervals of time the highest incidence of the disease was found at SRC, Faridpur whereas the lowest was recorded at SRC, Bogura. However, at all locations except SRC, Bogura, the incidence of the disease was statistically similar at 75 DAE. At 55 DAE, the highest severity of the disease was seen at Faridpur (55%), but at 65 and 75 DAE, Lalmonirhat had the highest severity of 79% and 88%, respectively. The lowest severity of the disease was observed at Bogura at all intervals of time. The incidence and severity of Alternaria blight varied significantly among the four cumin lines at 55 and 65 DAE (Table 8). The incidence and severity of the disease was the highest for the line CN031 and the lowest for the CN026 at all DAE, however, at 75 DAE the difference was non-significant. The interaction effect of locations and cumin lines on the incidence of the disease was found to be significant at 55 DAE and 65 DAE, while it was non-significant at 75 DAE (Table 9). On the other hand, interaction effect on the severity of the disease was significant at all DAE. The cumin line CN026 had the lowest incidence and severity of the disease at Bogura at all intervals of time. The data obtained in this experiment suggested that the cumin line CN026 was a better candidate to be adapted in Bangladesh as it had shown higher germination capacity and better yield parameters with lower incidence and severity of being affected by the Alternaria blight.

Phylogeny of *Alternaria* spp.: A total of twenty three fungal isolates were collected from the infected leaf, stem and twig of cumin having the symptom of the Alternaria blight (Table 1). DNA was successfully extracted from the fungal isolates using CTAB method. The preliminary fingerprinting of the twenty three isolates using the primers ITS1 and ITS4 confirmed these isolates as fungi (Fig. 1). The PCR amplification yielded DNA fragments of approximately 570 bp for all isolates that is the characteristic band size of *Alternaria* spp. The PCR products were sequenced bi-directionally and BLASTN search was conducted. The fungal isolates were found in match with the GenBank accessions of *A. alternata*, *A. tenuissima*, *A. burnsii* and *A. gaisen*. The phylogenetic tree constructed with the sequence data of twenty three isolates and the corresponding accessions of GenBank revealed the isolates as different species of *Alternaria* (Table 1 and Fig. 2). Twelve isolates out of twenty three (isolate no. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) had been found in homology with the GenBank accessions of *A. alternata*, nine (isolate no. 15, 16, 17, 18, 19, 20, 21, 22, 23) with *A. tenuissima*, one (isolate no. 13) with *A. burnsii*, one (isolate no. 14) with *A. gaisen* (Table 1). The findings suggested that *A. alternata* and *A. tenuissima* were the most prevalent species in causing the Alternaria blight in cumin among the sampled locations. *A. alternata* and *A. tenuissima* were detected in all locations whereas *A. gaisen* and *A. burnsii* were detected in Bogura only. The colony characters of the isolates revealed that the isolates molecularly identified as *A. alternata*, *A. tenuissima*, *A. burnsii* and *A. gaisen* had diverse up-side and reverse side color with distinct appearance, texture and margin of the colony (Table 10).

Table 9
The incidence and severity of Alternaria blight of cumin in different lines in different locations.

Location Cumin line		Disease severity (%)			Disease severity (%)		
		55 DAE	65 DAE	75 DAE	55 DAE	65 DAE	75 DAE
SRC, Bogura	CN026	25.58 i	71.21 h	91.22	40.37 h	62.47 h	74.37e
	CN028	31.32 h	79.26 d-f	92.12	51.47 d-g	71.37 d-g	84.37 ab
	CN031	32.16 h	83.61 a-d	92.60	53.82b-f	76.85 a-c	77.75 de
	CN038	31.47 h	77.66 e-g	90.85	50.17 e-g	68.75 fg	83.10 a-d
SRC, Gazipur	CN026	44.75 cd	70.98 h	94.07	48.12 g	73.87b-e	82.72 a-d
	CN028	44.98 cd	78.26 ef	94.40	52.62c-g	76.62 a-c	85.85 ab
	CN031	44.34 cd	80.08b-f	97.10	53.72b-f	78.25 ab	88.25 a
	CN038	44.16 cd	73.61 gh	91.22	56.75 a-c	79.75 a	86.38 ab
SRC, Lalmonirhat	CN026	43.15 d	76.64 fg	94.07	50.00 fg	76.85 a-c	88.10 ab
	CN028	45.63 bc	77.29 fg	94.40	55.10 a-e	81.27 a	87.17 ab
	CN031	43.33 cd	80.22b-f	97.10	59.52 a	80.00 a	88.25 a
	CN038	40.77 e	77.12 fg	98.12	56.62 a-c	76.85 a-c	88.37 a
SRC, Magura	CN026	40.02 ef	82.28 a-e	97.35	51.25 d-g	71.12 d-g	83.87 bc
	CN028	47.60 ab	84.42 ab	98.00	58.00 ab	74.74b-d	86.50 ab
	CN031	47.91 ab	83.95 a-c	97.60	57.25 a-c	72.87c-f	87.25 ab
	CN038	40.24 ef	77.13 fg	98.12	53.75b-f	74.35 b-d	84.00 a-c
SRC, Faridpur	CN026	38.34 fg	79.32c-f	96.86	50.07 fg	67.62 g	82.20b-d
	CN028	37.10 g	80.98 a-f	97.64	58.00 ab	71.35 d-g	82.75 ab
	CN031	36.49 g	79.92b-f	98.23	55.75 a-d	69.37 e-g	85.96 ab
	CN038	48.61 ab	84.94 a	98.12	49.50 fg	70.47 d-g	78.22c-e
L.S.		**	*	NS	*	*	*
CV (%)		4.022	4.155	6.297	6.653	4.675	5.039

Spice Research Centre (SRC); Days after Emergence (DAE) **1% level of probability; * 5% level of probability; NS = Non significant; Means followed by the same letter in a column did not differ significantly.
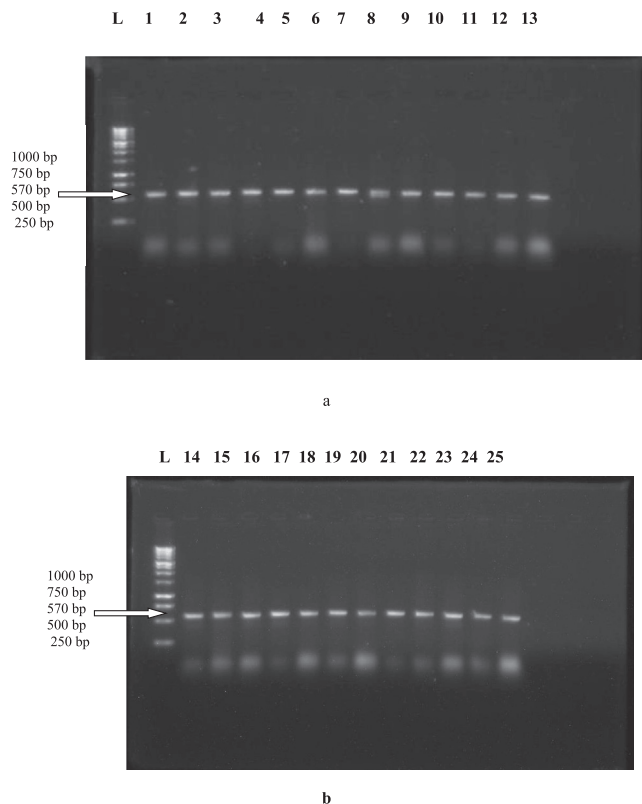
Fig. 1. (a, b) Electrophoresis in agarose gel of polymerase chain reaction products obtained by using the primer ITS1 and ITS4. L: 1 kb ladder (Promega). Lane 1 – 25 are the PCR products of the DNA samples obtained from the different locations of Bangladesh (Table 1). A product length of 570 bp is the characteristic of fungus obtained by ITS1 and ITS4 primers.
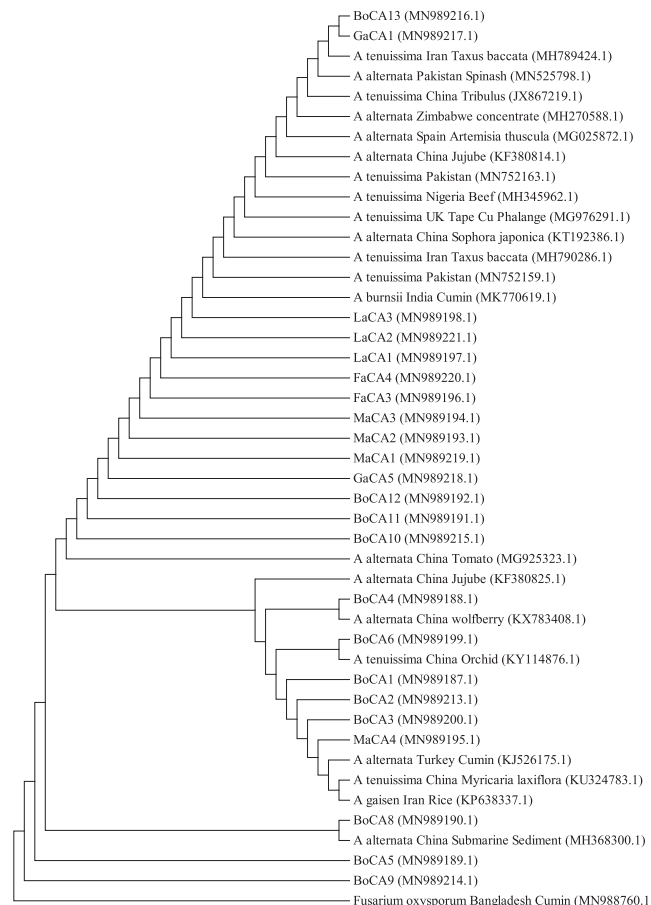

Fig. 2. The evolutionary relationship among the populations was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The sequences used in the phylogenetic analysis were chosen from the best matches in GenBank based on BLASTN against the sequences of four *Alternaria species* recovered from this study. *Fusarium oxysporum* was used as out-group. For isolate codes, please see Table 1.

Morphological variation of *Alternaria* spp.: The isolates significantly varied in radial mycelial growth (Table 11). The radial mycelial growths of the isolates were measured from four to eleven days after inoculation (DAI) on PDA media. Maximum growth (39.03 mm) was recorded in the MaCA3 which was followed by

Table 10
Colony characters of different isolates of *Alternaria* spp. of cumin collected from different locations of Bangladesh.

SL No.	Isolate code	Location	Colony characters			Pathogen isolated
			Appearance, texture and margin	Up side of colony	Reverse side colony	
1	BoCA1	Bogura	Umbronate, velveti, White, irregular margin	Olive green	Dark black	*Alternaria alternata*
2	BoCA4	Bogura	Umbronate, velveti, Whitish, irregular margin	Green	Light green	
3	BoCA5	Bogura	Umbronate, velveti, White, regular margin	Whitish black	Light brown	
4	BoCA8	Bogura	Umbronate, velveti, Olive white, regular margin	Olive white	Brownish white or light brown	
5	BoCA11	Bogura	Whitish, regular margin	Olive green	Dark black	
6	BoCA12	Bogura	Umbronate, cottony and whitish regular margin	Green	Light black	
7	MaCA2	Magura	Umbronate, velveti, Blackish white, irregular margin	Whitish olive green	Brown	
8	MaCA3	Magura	Umbronate, velveti, White, regular margin	Greenish white	Black	
9	MaCA4	Magura	Umbronate, velveti, White, regular margin	Greenish white	Dark black	
10	FaCA3	Faridpur	Rugase, velveti, Whitish olive, irregular margin	Greenish white	Blackish white	
11	LaCA1	Lalmonirhat	Umbronate, velveti, Whitish, regular margin	Blackish white	Light black	
12	LaCA3	Lalmonirhat	Greenish white, irregular margin	Greenish ash	Blackish white	
13	BoCA6	Bogura	Umbronate, cottony, Olive white, regular margin	Olive green	Dark black	*A. burnsii*
14	BoCA3	Bogura	Umbronate, velveti, White, irregular margin	Green	Black	*A. gaisen*
15	BoCA2	Bogura	Brown, irregular margin	Blackish ash	Dark black	*A. tenuissima*
16	BoCA9	Bogura	Velveti, White, irregular margin	Greenish white	Dark black	
17	BoCA10	Bogura	Rugose, velveti, White, regular margin	Greenish white	Light brown	
18	BoCA13	Bogura	Whitish, regular margin	Blackish white	Black	
19	GaCA1	Gazipur	Umbronate, cottony, Greenish white, regular margin	Green	Dark black	
20	GaCA5	Gazipur	Cottony, White, regular margin	Olive green	Black	
21	MaCA1	Magura	Ash, regular margin	Greenish white	Dark black	
22	FaCA4	Faridpur	Rugose, velveti, Greenish white, irregular margin	Dark green	Black	
23	LaCA2	Lalmonirhat	Umbronate, velveti, Blackish white, regular margin	Dark black	Dark black	

Table 11
Radial mycelial growth of different isolates of *Alternaria* spp. on culture medium collected from different locations of Bangladesh.

Isolate code	Radial mycelial growth (mm) at different days after inoculation (DAI)							
	4	5	6	7	8	9	10	11
BoCA1	19.33 hi	22.12f-i	25.33 e-i	29.25 d-h	30.58f-i	32.67 hi	34.83 e-h	37.17 d-i
BoCA4	20.92 a-e	24.25 ab	25.45 d-h	28.56f-i	29.80 i	31.42 jk	35.92 bcd	37.83b-f
BoCA5	20.80b-e	22.50 e-h	25.67c-g	29.42 d-g	31.67 de	34.08c-f	35.77b-e	37.08 d-i
BoCA8	19.47 ghi	22.67 d-g	25.25 e-i	29.50c-f	31.58 def	32.75 hi	34.67f-i	36.42 hij
BoCA11	20.60c-f	23.25b-e	27.05b	30.79 ab	32.92 abc	34.17c-f	35.56c-f	38.80 abc
BoCA12	21.60 ab	23.72 bcd	25.21 e-j	29.25 d-h	32.44 bcd	34.87 bc	36.63b	37.63c-g
MaCA2	21.42 abc	23.25b-e	26.08b-f	29.92b-e	32.33 bcd	33.75 efg	35.42c-g	38.07 a-e
MaCA3	20.27 d-h	23.42b-e	26.58 bc	29.55c-f	33.17 ab	36.00 a	37.76 a	39.03 a
MaCA4	20.75b-f	23.42b-e	26.33 bcd	30.08 bcd	32.08 cde	33.50 fgh	35.50c-f	36.92 e-j
FaCA3	20.62c-f	22.83 d-g	26.13b-f	27.00 L	28.23 j	29.20 L	30.83 k	33.02 k
LaCA1	21.33 abc	23.50b-e	26.63 bc	30.47 bc	32.13b-e	34.67b-e	36.33 bc	37.50 d-h
LaCA3	21.83a	23.33b-e	25.17f-j	28.47 g-j	30.49 ghi	33.90 def	36.03 bc	38.07 a-e
BoCA6	21.17 a-d	25.00 a	28.17 a	31.67 a	33.88 a	35.60 ab	37.67 a	39.25 a
BoCA3	20. 42c-g	21.97 ghi	24.46 ijk	29.02 e-i	33.00 bc	34.17c-f	35.93 bcd	37.25 d-i
BoCA2	19.50 ghi	21.45 hi	23.88 k	27.13 kl	31.25 efg	32.92 ghi	35.83 bcd	38.92 ab
BoCA9	20.84 a-e	23.25b-e	26.08b-f	29.67 cde	31.17 e-h	32.33 ij	33.75 ij	36.13 ij
BoCA10	20.08 e-h	21.92 ghi	24.67 h-k	28.05 ijk	30.52 ghi	32.58 hi	35.67b-e	38.17 a-d
BoCA13	20.42c-g	23.08c-f	25.08 g-j	29.08 d-h	31.83 de	34.08c-f	35.01 d-h	37.57 d-h
GaCA1	20.85 a-e	24.08 abc	26.92b	30.48 bc	33.67 a	34.77 bcd	36.13 bc	36.92 e-j
GaCA5	19.92 e-h	22.83 d-g	25.17f-j	27.48 jkl	29.72 i	30.57 k	33.00 j	35.87 j
MaCA1	19.75f-i	22.58 efg	25.00 g-j	28.33 hij	30.52 ghi	32.17 ij	34.33 hi	36.08 ij
FaCA4	18.83 i	21.17 i	24.25 jk	27.50 jkl	30.18 hi	33.35 fgh	35.02 d-h	36.58 g-j
LaCA2	20.25 d-h	23.33b-e	26.12b-e	28.53f-i	31.10 e-h	32.77 hi	34.51 ghi	36.83f-j
L.S.	**	**	**	**	**	**	**	**
CV (%)	3.05	2.79	2.30	2.13	2.02	1.74	1.67	1.94

**1% level of probability; Means followed by the same letter in a column did not differ significantly.

the BoCA11, MaCA2 and LaCA3 at eleven DAI and the minimum (33.02 mm) was observed in the FaCA3. In addition to these, color, size and shape of the conidia with their spore production capacity were found varied significantly among the stains (Table 12). When cumin plants were inoculated with the spore suspensions of the *Alternaria* isolates, infection started as minute necrotic lesions on all of the above ground plant parts which turned purple at advanced stages and later turned brown to black in 36 to 72 h resulting into blighting of the affected plant. These typical blight symptoms were found at 6 to 7 DAI. The symptoms were not observed in control plants treated with water. The pathogens were re-isolated from the infected plant parts at 10 days after inoculation. The isolated cultures were compared with the original one to confirm the pathogenicity. The pathogenic variation among the isolates was examined by inoculating the line CN026. There were significant differences in the incidence and severity of the disease among the isolates of the four *Alternaria* species (Table 13). The MaCA2 of *A. alternata* was observed as the most pathogenic isolate.

Discussions

Cumin is an important spice widely used in Bangladeshi cuisine. Its cultivation in Bangladesh is limited due to many socio-economic factors including the lack of high yielding and disease resistant variety. Alternaria blight is one of the major diseases of cumin causing huge economic loss in its production worldwide. In the present study, field trials were conducted with four advanced lines of cumin *viz.* CN026, CN028, CN031 and CN038 in five AEZ of Bangladesh to know their performances against the incidence and severity of the Alternaria blight. Further attempts were made to identify the causal organism of the Alternaria blight of cumin in Bangladesh and to study their diversity based on the morphological and molecular characteristics.

Among the five locations, all the cumin lines had the highest germination at Bogura and the lowest at Lalmonirhat. In this experiment, the incidence and severity of the Alternaria blight of four germplasms was observed ranging from 30% to 97% and 48% to 85%, respectively, at different locations. The incidence and sever-

ity of the disease was the least in Bogura and the highest in Farid-pur. The findings of the present investigation are in agreement with Özer and Bayraktar (2015). They reported that the severity of the blight caused by *A. burnsii* and *A. alternata* was ranging from 90% to 98%. Singh et al. (2015) also observed the disease severity of 62% to 68% and 62% to 63%, by inoculating *A. burnsii* and *A. alternata*, respectively. Climatic and edaphic factors might have an impact on the production of cumin and the prevalence of the Alternaria blight. Gemawat and Prasad (1971) has reported that high humidity (90% and above) for about 3 days, temperature between 23 and 28 °C, rainfall, hours of sunlight and wind speed played important roles in the development of the cumin blight caused by *A. burnsii*. Sharma and Pandey (2013) has also noted that the maximum temperature between 29 and 35.5 °C and the minimum temperature between 9.6 and 19.7 °C, average relative humidity of more than 60% in the morning and 28% in the afternoon, wind speed of 2.1–4.8 km/hour and bright hours of sunshine were favor-able for the development of the Alternaria blight of cumin. Singh and Shukla (1986) observed that the infection by *A. alternata* is favored by the temperature of 28.7 °C to 32.2 °C and the relative humidity of 74%. Higher incidences and severity of the Alternaria blight were reported from the higher elevations also (Lenne, 1991).

As Bangladesh has the climatic factors favorable for the development of Alternaria blight, the findings of this experiment outlined the importance of identifying the causal organism, use of the selective cultivars, careful selection of the location and timeliness of undertaking control measures for the safe cultivation of cumin to avoid the disease. In this experiment, four advanced lines of cumin were tested in five different locations to observe their germination capacity, yield performance and their propensity of being infected by Alternaria blight. In all locations, the line CN026 showed the highest germination capacity and yield with the lowest incidence and severity of the Alternaria blight that proved its better adaptability in the climatic conditions of Bangladesh. The primary means for identification of *Alternaria* species are morphological traits that include the appearance, texture, margin, color of the upsides & reverse sides of colonies, properties of coni-

Table 12
Morpho-physiological characters of conidia of different isolates of *Alternaria* spp. of cumin collected from different locations of Bangladesh.

Isolate code	Characteristics of conidia		Mean length of conidia (μm)	Mean breadth of conidia (μm)	Mean beak length of conidia (μm)	Number of vertical septa	Number of horizontal septa	Number of spores/ml (x10^6)
	Shape	Color						
BoCA1	Clavate to obclavate	Brown	52.93 ab	20.37 efg	7.75 hijk	0.94 e-i	1.68 j	2.38 e
BoCA4	Obclavate	Light brown	46.64 ef	22.80 a-d	8.24 ghi	0.77 hi	2.36 d-h	3.62 a
BoCA5	Obclavate to ovoid	Brown	46.18 ef	20.34 efg	6.98 k	0.78 hi	2.44c-g	2.26 ef
BoCA8	Clavate	Dark brown	56.09 a	24.19 a	8.39 fgh	1.18c-h	2.95 ab	1.51 g
BoCA11	Obclavate	Light brown	44.90 fg	17.13 ij	9.86 bcd	0.60 ij	2.40c-h	2.83 d
BoCA12	Obclavate	Brown	42.10 ghi	19.45 fgh	8.32 fghi	0.88f-i	2.48b-g	1.39 gh
MaCA2	Obclavate	Light brown	50.87 bcd	23.32 ab	9.85 bcd	1.20c-h	2.30f-i	3.36 ab
MaCA3	Clavate to obclavate	Dark brown	38.90 ijk	19.97 efg	10.12 abc	1.03 d-i	1.83 ij	1.58 g
MaCA4	Clavate	Dark brown	44.51 fgh	21.50 cde	7.04 jk	1.50 abc	2.33 e-h	0.953 ij
FaCA3	Obclavate	Dark brown	47.46 def	19.32 fgh	10.59 ab	1.30c-f	2.80 a-e	1.33 gh
LaCA1	Obclavate	Dark brown	41.10 hij	21.04 def	9.61 cde	1.93 a	2.70 a-f	2.46 e
LaCA3	Clavate to Obclavate	Brown	44.11 fgh	19.86 e-h	9.24 cdef	1.60 abc	3.10 a	2.31 ef
BoCA6	obovate to obclavate	Light brown	48.45 cde	16.66 ij	10.19 abc	0.67 i	2.88 abc	2.04f
BoCA3	Ovoid	Brown	37.67 jk	20.38 efg	7.41 ijk	0.59 ij	1.79 j	2.24 ef
BoCA2	Obclavate	Light brown	37.20 k	21.51 cde	8.66 e-h	0.82 ghi	1.93 hij	0.86 j
BoCA9	|Obclavate	Light brown	50.42 bcd	22.40 a-d	10.97 a	1.26c-g	2.86 abc	2.89 cd
BoCA10	Obclavate to ovoid	Brown	54.75 a	23.18 abc	7.99 hij	1.36 cde	3.02 a	2.83 d
BoCA13	Obclavate	Brown	47.48 def	23.57 a	8.70 e-h	1.91 ab	2.74 a-f	3.12 bc
GaCA1	Obclavate	Brown	38.01 jk	18.11 hi	9.25 cdef	0.60 ij	2.13 g-j	1.18 hi
GaCA5	Obclavate	Dark brown	50.82 bcd	21.62b-e	10.19 abc	1.47 bcd	3.00 a	2.82 d
MaCA1	Obclavate	Light brown	48.57 cde	23.16 abc	6.92 k	1.18c-h	2.70 a-f	3.21b
FaCA4	Obclavate	Light brown	42.41 gh	15.86 j	9.02 d-g	0.17 j	2.47c-g	1.38 gh
LaCA2	Oblavate	Dark brown	51.23 bc	19.24 gh	10.11 abc	0.93 e-i	2.83 a-d	2.38 e
L.S.			**	**	**	**	**	**
CV (%)			4.59	5.29	6.62	25.73	11.58	7.34

**1% level of probability; Means followed by the same letter in a column did not differ significantly.

dia and pattern of sporulation (Simmons, 2007). However, molecular methods have been suggested by researchers to complement the morphology based approaches (Kang et al., 2001). On the basis of the band size of the PCR products, DNA sequence analysis and phylogenetic study, four species of *Alternaria* were identified in the present work. In the order of prevalence, these were *A. alternata*, *A. tenuissima*, *A. burnsii* and *A. gaisen*. Sharma et al., (2013) found that the band size of PCR products of *Alternaria* spp of cauliflower and mustard was ~550 to 600 bp in India. Zheng et al. (2015) also identified the *Alternaria* spp. of potato in China following the similar molecular methods. The analysis of the observed morphological traits and the molecular characters indicated the diversity of *Alternaria* isolates of cumin in Bangladesh. Uppal et al. (1938) and Shakir et al. (1995) identified *A. burnsii* as the pathogen for causing Alternaria blight of cumin in India and Pakistan, respectively. On the other hand, *A. alternata* was found to be associated with Alternaria blight of cumin in Turkey (Özer and Bayraktar, 2016). However, this is the first report of *A. alternata* causing Alternaria blight of cumin in Bangladesh.

In this experiment, a considerable amount of morphological, cultural and pathogenic variability were recorded among the different isolates of *A. alternata*, *A. burnsii*, *A. gaisen* and *A. tenuissima*.

All *Alternaria* strains obtained from five locations of Bangladesh were found to have separated in different phylogenetic groups. Likewise, pathogenic variability among the isolates was also observed. There are 30 AEZ in Bangladesh based on physiographic, soil, hydrological and agro-climatic characteristics (Bangladesh Agricultural Research Council, 2005). The locations from where samples were collected are situated in different AEZ. Navas et al. (2001) reported that ecological factors or the size of the area of origin of the populations might also be involved in the production of such variation. In this experiment, *A. alternata* was found as the most virulent species. In an experiment, Zheng et al. (2015) had also observed that among the three species of *Alternaria*, *A. alternata* was more virulent than *A. tenuissima* and *A. solani* to cause foliar diseases of potato in China.

To the best of our knowledge, this is the first extensive study regarding the prevalence of the Alternaria blight of cumin in Bangladesh. In this work, a promising line of cumin (CN026) was selected as having the better resilience against the incidence and severity of the Alternaria blight with higher germination and yield potential. The causal organisms of the Alternaria blight were also identified using molecular and morphological tools. All of these findings have formed the foundation of further research that might

Table 13
Pathogenic variation of the different isolates of *Alternaria* spp. on the cumin line CN026.

Isolate code	Pathogen isolated	Disease incidence (%)		Disease severity (%)	
		50 DAS	55 DAS	50 DAS	55 DAS
BoCA1	*Alternaria alternata*	33.16 ef (30.00)	42.12 cd (45.00)	25.57 ef (18.67)	25.08 fg (18)
BoCA4		17.33 g (13.33)	19.33 e-h (16.67)	8.29 g (3.33)	7.55 h (2.67)
BoCA5		34.15 ef (31.67)	29.92 def (25.00)	26.04 ef (19.33)	27.02f (20.67)
BoCA8		12.40 gh (6.67)	12.40 fgh (6.67)	4.67 (10.31 g)	11.06 gh (5.33)
BoCA11		57.98b (71.67)	72.67 ab (86.67)	45.19 bc (50.33)	62.57 bc (71.33)
BoCA12		57.98b (71.67)	70.66 ab (83.33)	55.85b (68.33)	72.67 ab (86.67)
MaCA2		71.56 a (90.00)	89.66 a (100.00)	68.85 a (86.67)	85.05 a (98.00)
MaCA3		60.07 ab (75.00)	73.44 ab (88.33)	51.91b (61.33)	59.85 bcd (74.67)
MaCA4		52.77bcd (63.33)	63.93b (76.67)	48.28 bc (55.33)	67.75b (80.00)
FaCA3		41.16def (43.33)	74.89 ab (90.00)	29.92 def (25.00)	39.21 ef (40.00)
LaCA1		6.37 gh (3.33)	6.37 gh (3.33)	2.93 g (0.67)	2.93 h (0.67)
LaCA3		47.88 bcd (55.00)	57.78 bc (70.00)	38.44 cd (38.67)	46.09 de (52.00)
BoCA6	*A. burnsii*	9.08 gh (6.67)	9.08 fgh (6.67)	7.549 g (4.67)	4.07 h (1.33)
BoCA3	*A. gaisen*	29.92f (25.00)	22.59 d-g (15.00)	22.98f (15.33)	28.18f (22.33)
BoCA2	*A. tenuissima*	12.40 gh (6.67)	12.40 fgh (6.67)	11.28 g (4.00)	8.29 h (3.33)
BoCA9		32.01f (28.33)	42.12 cd (45.00)	27.22 def (21.00)	26.71f (20.33)
BoCA10		30.99f (26.67)	39.15 cde (40.00)	22.98f (15.33)	30.19f (25.33)
BoCA13		56.99 bc (70.00)	59.21 bc7 (3.33)	52.24b (62.33)	70.57b (87.33)
GaCA1		48.84 bcd (56.67)	72.67 ab (86.67)	55.98b (68.33)	53.14 de (64.00)
GaCA5		45.00 cde (50.00)	64.81b (73.33)	48.16 bc (55.33)	53.17 cde (64.00)
MaCA1		51.14 bcd (60.00)	63.93b (80.00)	36.79cde (36.00)	52.35 cde (62.67)
FaCA4		42.12 def (45.00)	59.89 bc (66.67)	31.72 def (29.33)	29.50f (24.33)
LaCA2		35.01 ef (33.33)	42.12 cd (45.00)	25.63 ef (20.00)	30.34f (25.67)
Control		0.3375 h (0.00)	0.3375 h (0.00)	0.3375 g (0.00)	0.3375 h (0.00)
L.S.		**	**	**	**
C.V		20.43	28.80	22.63	23.37

** 1% level of probability; Means followed by the same letter in a column did not differ significantly; Arcsine transformed values are followed by raw data within parenthesis DAS = Days after sowing.

help in designing successful management options against the Alternaria blight of cumin leading to its increased cultivation and production in Bangladesh.

Declaration of Competing Interest

The author declare that there is no conflict of interest.

Acknowledgements

Funding

References

Ansari, N.A., Khan, M.W., Muheet, A., 1989. Effect of some factors on growth and sporulation of Alternaria brassicae causing Alternaria blight of rapeseed and mustard. Acta Botanica Indica 17, 49–53.

Aneja, K.R., 2004. Experiment in microbiology, plant pathology and biotechnology. New International (P) Limited Publisher, India, pp. 121–128.

Azeez, S. V.A., Champakam, B., Zachariah, T.J., 2008. Cumin. In: Parthasarathy eds.: Chemistry of Spices. CABI International, Wallingford, UK. This review article deals with botany, distribution and medicinal properties of cumin, and with the chemistry of cumin oil.

Bangladesh Agricultural Research Council. 2005. Dhaka.

Benali, S., Mohamed, B., Eddine, H.J., Neema, C., 2011. Advances of molecular markers application in plant pathology research. Eur. J. Sci. Res. 50, 110–123.

Chester, K.S., 1959. How sick the plant? In: Plant Pathology an advanced treatise (Eds. J.G. Horshfall and A.E. Diamond). Academic Press, New York. 1, 199-242.

Dhingra, O.B., Sinclair, J.B., 1995. Basic Plant Pathology Methods. 2nd Edition, CRC Press, Boca Raton Florida, USA.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 39,783-791.

Gemawat, P.D., Prasad, N., 1971. Epidemiological studies on Alternaria blight of Cuminum cyminum, Indian. J. Mycol. Pl. Pathol. 2, 65–75.

Gemawat, P.D., Prasad, N., 1972. Epidemiological studies on Alternaria blight of cuminum cyminum, Indian. J. Mycol. Pl. Path. 2 (1), 65–75.

ISTA., International Seed Testing Association. 1976.

Jat, V.D., 2015. Effect of Culture Filtrate of Blight Pathogen [Alternaria alternata (Fr.) Keissler] on Coriander and its Management. M. Sc. thesis, Sri Karan Narendra Agriculture University, Jobner, India.

Kang, J.C., Crous, P.W., Schoch, C.L., 2001. Species concepts in the Cylindrocladium floridanum and Cy. spathiphylli complexes (Hypocreaceae) based on multi-allelic sequence data, sexual compatibility and morphology. Systematic and Applied Microbiology. 24, 206–17.

Kaur, S., G., Singh , Banga ,S.S., 2007. Documenting variation in Alternaria brassicae isolates based on conidial morphology, fungicidal sensitivity and molecular profile. Proceeding of the 12th International Rapeseed Congress, Wuhan, China. 4, 87-89.

Kumar S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.Molecular Biology and Evolution. 33, 1870-1874.

Lenne, J.M., 1991. Diseases and pests of sweetpotatoes: South East Asia, the pacific and East Africa. Nature Research Institute Bulletin, No, p. 46.

Nabhan, G.P., 2014. My library My History Books on Google Play Cumin, Camels, and Caravans: A Spice Odyssey. Univ of California Press. ISBN 978-0-520-26720-6. p. 234.

Nasreen, N.E., Meah, M.B., Tumpa, F.H., Hossain, M.A., 2017. Effect of Media Composition on Vegetative and Reproductive Growth of Alternaria brassicicola and Bipolaris sorokiniana. Curr. Agri. Res. 5(3), 266- 278.

Navas, A., Castagnone-Sereno, P., Blazquez, J., 2001. Genetic structure and diversity within local population of Meloidogyne (Nematoda: Meloidogynidae). Nematology. 3, 243–253.

Özer, G., Bayraktar, H., 2015. Determination of fungal pathogens associated with Cuminum cyminum in Turkey. Plant Protect. Sci. 51, 74–79.

Patel, P.N., Prasad, N., Mathur, R.L., Mathur, B.L., 1957. Fusarium wilt of cumin. Curr. Sci. 26, 181–182.

Patni, C.S., Kolte, S.J., Awasthi, R.P., 2005. Cultural variability of Alternaria brassicae, causing Alternaria blight of mustard. Ann. Plant Physiol. 19, 231–242.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL:http://www.r-project.org/index.html.

Rifai, A., 1969. A revision of the genus Trichoderma. Mycological Papers. 116, 1–56.

Saitou, N., Nei, M., 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Shakir, A.S., Mirza, J.H., Sahi, S.T., Ansar, M., 1995. First report of *Alternaria burnsii*, the causal organism of cumin blight in Pakistan. Pakistan J. Phytopathol. 7 (2), 219.

Sharma, S., 2010. Studies on cumin blight incited by Alternaria burnsii (Uppal Patel and Kamat) and its management. M. Sc. thesis, Anand Agricultural University, Anand, India.

Sharma, S., Pandey, R.N., 2013. Survial, epiderminolgy and management of Alternata blight of cumin in Gujarat. Bioinfolet. 10 (2B), 639–642.

Shekhawat, N., Trivedi, A., Sharma, S.K., Kumar, A., 2013. Cultural, morphological and pathogenic variability in *Alternaria burnsii* causing blight of cumin. J. Mycol. Pl. Path. 43 (1), 80–83.

Simmons, E.G., 2007. Alternaria. An Identification Manual. CBS Biodiversity Series No. 6. CBS Fungal Biodiversity Centre, Utrecht, the Netherlands. pp.775.

Singh, M., Shukla, T.N., 1986. Epidemiology of Alternaria leaf spot and fruit rot of brinjal. Indian Phytopathol. 39, 119–120.

Singh, S., Trivedi, A., Mathur, K., Padamini, R., 2015. Assessment of yield loss of cumin (Cuminum cyminum) caused by Alternaria leaf blight and pathogen recovery from infected seeds. Indian Phytopath. 68 (3), 350–352.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. (USA). 101, 11030–11035.

The Daily Financial Express. 5 May 2020. Dhaka, Bangladesh.

Tutte, J., 1969. Plant pathological methods: Fungi and bacteria. Burgess Publishing Company, U.S.A., p. 229.

Uppal, B.N., Patel, M.K., Kamat, M.K., 1938. Alter naria blight of cumin. Indian J. Agric. Sci. 8, 9–62.

Verma, A.K., Singh, R., Choudhary, S., Lal, G., 2018. Cultivation of dollar earning cumin crop for higher income. Acta Scientific Agriculture. 2 (3), 46–48.

Weiss, E.A., 2002. Spice Crops. CABI International, Wallingford,UK, [Family wise description of spice crops belonging to cruciferae, lauraceae, leguminosae, myristicaceae, myrtaceaeumbelliferae and zingiberaceae. All aspects of cultivation processing and industrial application are discussed]. P. 299.

Wheeler, B.E.J., 1969. An introduction of plant diseases. John Willy & Sons Ltd., London. pp. 301.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego. pp. 315–322.

Zheng, H.H., Zhao, J., Wang, T.Y., Wu, X.H., 2015. Characterization of Alternaria species associated with potato foliar diseases in China. Plant Pathology. 64, 425-433.