Original article

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

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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 a devastating form every year in the most cumin-growing areas in Bangladesh.
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.

\[
\text{PDI} = \left( \frac{\text{Sum of all disease rating}}{\text{Total no. of plants per unit area}} \right) \times 100
\]

| Serial No. | Location | *AEZ | Isolate Code | GenBank Accession number |
|-----------|----------|------|--------------|--------------------------|
| 1         | Bogura   | 3    | BoCA1 (MN989187) |                          |
| 2         | Bogura   | 3    | BoCA4 (MN909188) |                          |
| 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   | MaCA6 (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    | BoCA3 (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).
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:isoamylalcohol (25:24:1) were mixed and centrifuged at 10000 rpm for 10 min. Equal volume of Phenol:Chloroform:isoamylalcohol 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. 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′-TCCGATATGGAACTTCGCCC-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).

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 × 10⁷ 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).
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.

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         |
| CV (%)         | 4.088           |

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           |

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 gb           |
|                | 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       |

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        |

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 | 91.60b | 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.
The incidence and severity of Alternaria blight of cumin in different lines in different locations. The incidence and severity of Alternaria blight of cumin in different lines. Parameters with lower incidence and severity of being affected by desh as it had shown higher germination capacity and better yield. Cumin line CN026 was a better candidate to be adapted in Bangladesh. The data obtained in this experiment suggested that the incidence and severity of the disease at Bogura at all intervals of time was significant at all DAE. The cumin line CN026 had the lowest incidence and severity at 55 and 65 DAE (Table 8). The incidence and severity 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 severity of the disease was significant. The interaction effect of locations and cumin lines on the severity of the disease was statistically similar at 75 DAE. At 55 DAE, the difference was non-significant at 75 DAE (Table 9).

### Table 8

| 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 |
| LS         | *       | NS     | NS    | *       | *       | NS    |
| CV (%)     | 4.0246 | 4.155  | 6.297 | 6.653   | 4.675   | 5.039 |

### Table 9

| 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-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 | 52.62 a-c | 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 a |
|                     | CN028 | 45.63 bc | 77.29 g | 94.40 | 55.10 a-c | 81.27 a | 87.17 ab |
|                     | CN031 | 43.33 cd | 80.22b-f | 97.10 | 59.52 a | 80.00 a-b | 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 | 53.73b-f | 74.35b-d | 84.00 a-c |
| SRC, Faridpur       | CN026 | 38.34 fg | 79.32c-f | 96.86 | 50.07 fg | 67.62 g | 82.20 b-d |
|                     | CN028 | 37.10 g | 80.98 a-f | 97.64 | 58.00 ab | 71.35 d-g | 82.75 a-d |
|                     | 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 |
| LS                  | *      | *      | NS    | *      | *      | NS    |
| CV (%)              | 4.022  | 4.155  | 6.297 | 6.653   | 4.675   | 5.039 |

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.

RNA and DNA were extracted from the fungal isolates using the 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. burnsi 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 (Fig. 1 and Table 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. burnsi, 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. burnsi were detected in Bogura only. The colony characters of the isolates revealed that the isolates molecularly identified as A. alternata, A. tenuissima, A. burnsi and A. gaisen had diverse up-side and reverse side color with distinct appearance, texture and margin of the colony (Table 10).
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**

| SL No. | Isolate code | Location | Colony characters | Up side of colony | Reverse side colony | Pathogen isolated |
|-------|--------------|----------|-------------------|-------------------|---------------------|-------------------|
| 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               | *A. burnsii*      |
| 9     | MaCA4        | Magura   | Umbronate, velveti, White, regular margin | Greenish white    | Dark black          | *A. gaisen*      |
| 10    | FaCA3        | Faridpur | Rugose, velveti, Whitish olive, irregular margin | Greenish white    | Blackish white      |                   |
| 11    | LaCA1        | Lalmonirhat | Umbronate, velveti, Whitish, regular margin | Blackish white    | Light black         | *A. tenuissima*  |
| 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. tenuissima*  |
| 14    | BoCA3        | Bogura   | Umbronate, velveti, White, irregular margin | Green             | Black               |                   |
| 15    | BoCA2        | Bogura   | Brown, irregular margin | Blackish ash      | Dark black          | *A. tenissima*    |
| 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        | Black               |                   |
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               | 22.12 i                | 25.33 e-i              | 29.25 d-h              | 30.58 i-f              | 32.67 hi               | 34.83 e-h              | 37.17 d-i              |
| BoCA4        | 20.92 a-e              | 24.25 ab               | 25.45 d-h              | 28.56 i-f              | 29.80 i                | 31.42 jk               | 35.92 bcd              | 37.83 b-f              |
| BoCA5        | 20.80 b-e              | 22.50 e-h              | 25.67-g                | 29.42 d-g              | 31.67 de               | 34.08 c-f              | 35.77 b-e              | 37.08 d-i              |
| BoCA8        | 19.47 ghi              | 22.67 d-g              | 25.25 e-i              | 29.50 c-f              | 31.58 def              | 32.75 hi               | 34.67 f-i              | 36.42 h-j              |
| BoCA11       | 20.60 f-c              | 23.25 b-e              | 27.05 b                | 30.79 ab               | 32.92 abc              | 34.17 c-f              | 35.56 c-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               | 36.63 b                | 37.63 b-g              |
| MaCA2        | 21.42 abc              | 23.25 b-e              | 26.06 b-f              | 29.50 b-e              | 32.33 bcd              | 33.75 efg              | 35.42 d-f              | 38.07 c-e              |
| MaCA3        | 20.27 d-h              | 23.42 b-e              | 26.58 bc               | 29.55 c-f              | 33.17 ab               | 36.00 a                | 37.76 a                | 39.03 a                |
| MaCA4        | 20.75 b-f              | 23.42 b-e              | 26.33 bcd              | 30.08 bcd              | 32.08 cde              | 33.50 fgh              | 35.50 c-f              | 36.92 e-j              |
| FaCA3        | 20.62 f-c              | 22.83 d-g              | 26.13 b-f              | 29.00 L                | 28.23 j                | 29.20 L                | 30.83 k                | 33.02 k                |
| LaCA1        | 21.33 abc              | 23.50 b-e              | 26.63 bc               | 30.47 bc               | 32.13 b-e              | 34.67 b-e              | 36.33 bc               | 37.50 d-h              |
| LaCA3        | 21.83a                 | 23.39 b-e              | 25.17 f-j              | 28.47 g-j              | 30.49 ghi              | 33.90 def              | 36.03 bcd              | 38.07 a-e              |
| BoCA6        | 21.17 a-d              | 25.00 a                | 28.17 a                | 31.67 e                | 33.88 a                | 35.60 ab               | 37.67 a                | 39.25 a                |
| BoCA3        | 20.42 c-g              | 21.97 ghi              | 24.46 ijk              | 29.02 e-i              | 33.00 bc               | 34.17 c-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 abj              |
| BoCA9        | 20.84 a-e              | 23.25 b-e              | 26.08 b-f              | 29.67 cde              | 31.17 e-h              | 33.88 a                | 35.60 ab               | 37.67 a                |
| BoCA10       | 20.08 e-h              | 21.92 ghi              | 24.67 h-k              | 28.05 ijk              | 30.52 ghi              | 32.58 hi               | 35.67 b-e              | 38.17 d-f              |
| BoCA13       | 20.42 g-c              | 23.08 f-e              | 25.08 g-j              | 29.08 d-h              | 31.83 de               | 34.08 c-f              | 35.01 d-h              | 37.57 d-h              |
| GaCA1        | 20.85 a-e              | 24.08 abc              | 26.92 b                | 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              | 25.17 f-j              | 27.48 jkl              | 29.72 i                | 30.57 k                | 33.06 o                | 35.87 j                |
| MaCA4        | 19.75 f-c              | 22.58 efg              | 25.00 e-j              | 28.33 h                 | 30.52 ghi              | 32.17 i                | 34.33 h                | 36.08 i                |
| FaCA4        | 18.83 i                | 21.17 l                | 24.25 jk               | 27.50 jkl              | 30.18 h                | 33.35 fgh              | 35.02 d-h              | 36.58 g-j              |
| LaCA2        | 20.25 d-h              | 23.39 b-e              | 26.12 b-e              | 28.53 f-i              | 31.10 e-h              | 32.77 hi               | 34.51 ghi              | 36.83 f-i              |
| LS:          | **                     | **                     | **                     | **                     | **                     | **                     | **                     | **                     |
| 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 the advanced lines of cumin plants were inoculated with the spore suspensions 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 favorable 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-
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 el 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

| 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) |
|--------------|---------------------------|-----------------------------|-------------------------------|-------------------------------|--------------------------|---------------------------|-----------------------------|
| 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 ghij                 | 0.77 hi                   | 2.36 d                      | 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 ab                      | 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 bc                      | 23.32 ab                      | 9.85 bcd                  | 1.20c-h                   | 2.30f-i                     | 3.36 ab                     |
| MaCA3        | Obclavate to clavate       | Dark brown                 | 38.90 jk                      | 19.97 efg                     | 10.12 abc                 | 1.03 d-i                  | 1.83 ij                      | 1.58 g                      |
| MaCA4        | Obclavate                  | 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 hj                      | 21.04 def                     | 9.61 cde                  | 1.93 a                    | 2.70 a-f                     | 2.46 e                      |
| LaCA3        | Obclavate to obclavate     | Dark brown                 | 44.11 fgh                     | 19.86 e-h                     | 9.24 cdef                 | 1.60 abc                  | 3.10 a                      | 2.31 ef                     |
| BoCA6        | Obclavate to obclavate     | Light brown                | 48.45 cde                     | 16.66 ij                      | 10.19 abc                 | 0.67 i                    | 2.88 abc                     | 2.04f                      |
| BoCA3        | Ovoid                      | Light brown                | 37.67 jk                      | 20.38 efg                     | 7.41 ikj                  | 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 ghij                 | 1.93 hjij                    | 0.86 j                      |
| BoCA9        | Obclavate to ovoid         | 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         | Light 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                     |
| GaCA5        | Obclavate                  | Brown                       | 38.01 jk                      | 18.11 hi                     | 9.25 cdef                 | 0.60 ij                   | 2.13 g-j                     | 1.18 hi                     |
| 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.**: 4.59 5.29 6.62 25.73 11.58 7.34

**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.**
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.

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