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# Research Article Morphological Characterization of *Colletotrichum gloeosporioiedes* Identified from Anthracnose of *Mangifera indica* L.

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# Abstract

**Background and Objective:** *Mangifera indica* L. (mango) is affected by various diseases at different developmental stages. One of the most common diseases of mango is anthracnose caused by *Colletotrichum gloeosporioides* in the world. Proper identification of causal organism is difficult because of the morphological variation. The objective of this study was to characterize the morphological variation of *C. gloeosporioides*. **Materials and Methods:** One hundred and forty isolates of *C. gloeosporioides* were taken from anthracnose lesions on fruits (51), leaves (59), flower clusters (17) and on twigs (13) of mango from geographic nine regions of Bangladesh. Cultural methods (mycelial growth rate, color, texture, acervuli, conidial size and setae) and microscopic measurements (ocular micrometer and stage micrometer) were used to characterize the isolates. The experiment was conducted by following Completely Randomized Design (CRD) with five replicates. One way analysis of variance was done to check the significant (p<0.05) differences. **Results:** All of the isolates varied significantly (p<0.05) among different plant parts and also among the origins. They followed the order of F<FLC<T<L and S2<S4<S9<S1<S8<S3<S6<S5<S7, respectively. Mycelial growth, size of conidia, acervuli (No. cm<sup>-2</sup>) ranged from 9.5-10.6 mm day<sup>-1</sup>, 17.82-30.26 and 1.00-5.40 µm, respectively. Mycelial color (5), texture (6) and setae were present. Isolates were clustered into four distinct groups. **Conclusion:** It is concluded that morphological variations of *C. gloeosporioides* among different plant parts, conidial size, acervuli production, mycelial color, texture and setae.

Key words: Mangifera indica L., C. gloeosporioides, acervuli, conidia, growth rate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

*Mangifera indica* L. (Mango) belongs to the family-Anacardiaceae. It grows in 87 countries in the world<sup>1</sup>. In terms of total area and fruit production, mango heads the list in area and third in production. It occupies 79066 ha of land. Total production is 1047849 t annum<sup>-1</sup> with an average yield of 13.25 t ha<sup>-1</sup> in Bangladesh<sup>2</sup>. However, this quantity is very low compared to those in India, Pakistan and many other mango-producing countries in the world<sup>3</sup>. India is the leading country for mango production (70%) followed by China and Thailand<sup>4</sup>.

Mango is affected by a large number of diseases at different developmental stages<sup>1</sup>. The most common diseases are anthracnose, stem-end rot, powdery mildew and mango malformation<sup>5</sup>. Anthracnose caused by *C. gloeosporioiedes* is the most widespread and serious pre-and postharvest disease in Bangladesh<sup>3</sup>. Approximately 25-30% loss of total mango production in Bangladesh<sup>3</sup>, 15-20% in India and 30-60% in the world<sup>5</sup> is due to anthracnose and stem end rot diseases. *Colletotrichum* species cause anthracnose, which can cause considerable damage in a large number of crops, such as cereals, coffee and legumes<sup>6,7</sup>. Even greater economic loss is due to post harvest anthracnose disease of tropical and subtropical fruits such as avocado, banana and mango<sup>8,9</sup>.

Anthracnose disease symptoms in mango are characterized by necrosis or blight on leaves, fruits, flower clusters and twigs. Anthracnose pathogen invades inflorescences, fruits, leaves and stems of mango plant. It appears to be irregular-shape black necrotic spots on both surfaces of the mango leaf. Acervuli (fruiting structure) on black sunken lesion was developed in the necrotic tissue. Acervulus produces mass of conidia. Then conidia were spread and invade upon young tissue under favorable conditions<sup>5</sup>. Phytopathogens secrete cell wall degrading enzymes and then utilize the components of the host cells as nutrients<sup>10</sup>. It exhibits morphological variations, which makes it difficult to classify<sup>11</sup>. Due to variability in pathogenic isolates, expanded

active disease cycle is very complicated to manage<sup>12</sup>. However, identification of causal organism is crucial for taking effective control measure since *C. gloeosporioides* is sensitive to fungicide. The genus *Colletotrichum* possesses many morphologically similar taxa comprising endophytic, saprobic and plant pathogenic fungi<sup>13</sup>.

Conventionally, Colletotrichum species identification was done by a variety of cultural and morphological characteristics growth rate, size of conidia, presence or absence of setae, sensitivity of fungicide, colony color etc<sup>6,8,13,14</sup>. This pathogen may perform as an excellent model for studying pathogenicity, from saprophyte to pathogen<sup>15</sup>. Several researchers noticed conidial morphology of Colletotrichum spp. in different countries<sup>13,15,16</sup>. Unfortunately, there is no such type of measurement in Bangladesh of this species. Identification of the causal agent (Colletotrichum spp.) of anthracnose in mango is not specific since it may differ from conidial dimension and growth rate. Thus, it is necessary to determine the criteria for identifying the complexity of C. gloeosporioides. Therefore, the objective of this study was to verify the morphological variation of C. gloeosporioides for accurate identification and its managements. It will help the researcher to easy determination of the aggressiveness of the isolate types and the highest variation zone in Bangladesh.

#### MATERIALS AND METHODS

#### Collection and isolation of Colletotrichum gloeosporioiedes.

One hundred and forty isolates of *C. gloeosporioides* were collected from anthracnose lesions on fruits (51), leaves (59), flower clusters (17) and twigs (13) of *Mangifera indica* L. (mango) in different (Khulna, Satkhira, Jessore, Bagerhat, Jhenaidah, Rajshahi, Chapainababgonj, Rangpur and Dinajpur) regions of Bangladesh in 2015 (Table 1, Fig. 1a-d). *Colletotrichum* enter into the host tissues directly through a penetration peg that emerges from dome-shaped appressoria. Which are darkly pigmented with melanin<sup>17</sup>. The infected plant parts were cut into  $5 \times 5$  mm<sup>18</sup> (Fig. 1e).

Table 1: Origin and number of the *C. gloeosporioides* isolates obtained from anthracnose affected mangoes

Table 1. Origin and number of the c. glocosponolices isolates obtained non-antihachose anected mangoes						
Origins	Latitude	Longitude	Anthracnose affected plant parts	No. of isolates		
S1	22°71′	89°07′	L (15), F (10), FLC (5), T (4)	34		
S2	22°84′	89°54′	L (4), F (4), FLC (1), T (1)	10		
S3	23°17′	89°18′	L (5), F (5), FLC (2), T (1)	13		
S4	22°33′	89°77′	L (5), F (4), FLC (1), T (1)	11		
S5	23°55′	89°17′	L (4), F (3), FLC (1), T (1)	9		
S6	24°36′	88°62′	L (9), F (6), FLC (1), T (1)	17		
S7	24°57′	88°27′	L (8), F (10), FLC (4), T (2)	24		
S8	25°62′	88°63′	L (5), F (5), FLC (1), T (1)	12		
S9	25°74′	88°27′	L (4), F (4), FLC (1), T (1)	10		

S1: Satkhira, S2: Khulna, S3: Jessore, S4: Bagherhat, S5: Jhenaidah, S6: Rajshahi, S7: Chapai, S8: Dinajpur, S9: Rangpur and L: Leaves, F: Fruits, FLC: Flower clusters, T: Twigs



Fig. 1(a-i): Collection and isolation of *C. gloeosporioides* from anthracnose affected mangoes on Oat Meal Agar (OMA) media,
(a) Fruits, (b) Leaves, (c) Flower clusters, (d) Twigs, (e) 5×5 mm cut tissue, (f) Prepared sample on petri-plate, (g) Pure culture of acervulus and (h, i) Stored at 4°C into OMA slants

Surface of the specimens were sterilized with 1% sodium hypochlorite (NaOCI) for 1 min<sup>19</sup> and washed with sterile water and dried with sterilized filter paper (Whatman 1). The prepared samples were placed on Oat Meal Agar (OMA)<sup>20</sup> contained petri-plates (Fig. 1f). The plates were incubated at room temperature (25°C) and observed for the fungal growth<sup>21</sup> (Fig. 1g). The pathogen was identified at the species level depending upon their cultural and morphological characters<sup>22</sup>. The prepared the glass slides were placed under compound microscope with 10 times (10x) and four hundred times (400x) magnifications to observe images for the presence of conidia and setae. The growing edges of fungal mycelia were then transferred aseptically to OMA slants. Isolates were identified following sporulation and pure cultures (single acervulus culture) were stored at 4°C on OMA slants (Fig. 1h, 1i, Fig. 2a-c).

Pathogenicity The identified isolates of test: C. gloeosporioides were confirmed by Koch's Postulates methods. Various plant parts were used for confirmation. However, only pathogenicity on leaves was discussed for this study. Healthy excised mango leaves, 8 cm in middle portion were placed in petri-plates with five replicates. The conidial suspension  $5 \times 10^6$  spore mL<sup>-1</sup> (supplemented with 0.01%) tween 80 in a ratio of 1:1 v/v) was prepared<sup>23</sup> from the conidia of 18 day old culture. This suspension was tested by the Drop Inoculation Method on the excised mango leaves. The leaves were slightly injured by Pin Prick Method<sup>24</sup> and then inoculation by dropper (pre sterilized by 70% ethanol)<sup>25</sup>. The excised leaf inoculated with only sterile distilled water was considered as control. Small brown spots (initiated symptoms 8 days after inoculation) on leaves gradually enlarged and center of the lesions turned into dark brown color. After



Fig. 2(a-i): Identification and pathogenecity test of *C. gloeosporioides*, (a-c) Conidia and setae, (d-f) After day 7 and 12 acervuli with sticky masses of spores on inoculated excised healthy mango leaves, (g) 18 days old culture and (h, i) Pure culture of acervulus after 18 and 28 days on OMA containing petri-plates

12 days numerous acervuli with masses of spores were produced on inoculated excised mango leaves (Fig. 2d-f). Pure culture of *C. gloeosporioides* on OMA containing petri-plates was kept under laboratory condition. After 18 and 28 days numerous acervuli with sticky masses of spores were produced (Fig. 2g-i). The presence of conidia was similar to the original isolates.

**Morphological characterization:** Morphological characterization was done through observation of six parameters, such as Mycelia Growth Rate (MGR) (mm day<sup>-1</sup>), size of conidia (CS) (µm), number of acervuli production (NOA) (cm<sup>-2</sup>), texture (MT), (fluffy/submissive), presence or absence of setae (S) and cultural characterization through color of the upper surface and reverse side (MC). After 8 days, growth rate was recorded. Mycelial color

and appearance of 18 days old growth culture were recorded. Colony diameter of every culture was recorded daily until the mycelium touches the petri-dishes (for 8 days). Growth rate was calculated as the 8 day average of mean daily growth (mm day<sup>-1</sup>). Average Linear Growth Rate (ALGR) was measured according to Jahan *et al.*<sup>26</sup>.

$$ALGR (mm day^{-1}) = \frac{C8-C0}{8}$$

where, C8 is colony diameter after 8 days of inoculation and C0 is Initial Colony diameter of inoculation.

Fifty spores of each isolate were selected randomly for measurement of width and length, using a calibrated ocular micrometer and stage micrometer<sup>27</sup>.

**Statistics analysis:** Descriptive statistics (mean and standard deviation), box and mean plots of different parameters were developed by using STATISTICA software (version 10). One way analysis of variance was carried out to examine the significant (p<0.05) difference of all the parameters. Tukey *post hoc* test was carried out to compare the mean value of different morphological characters. A dendrogram (cluster analysis) was made for all the isolates by using STAR software.

#### RESULTS

Morphological variation of Colletotrichum gloeosporioides among different plant parts: Both cultural and morphological variations of C. gloeosporioides grown on Oat Meal Agar (OMA) after 18 days of incubation at 25°C were observed among different plant parts of mango. All the six characters topography (White fluffy, dense, light grey and dark olive) and setae (present or not) sometimes were showed variation in some isolates, whereas other 4 characters were same in presence of acervuli with masses of cylindrical hyaline conidia. Four isolate types varied significantly (p<0.05) among themselves and followed the order of F<FLC<T<L. These isolate types also varied among themselves in terms of mycelial growth rate and conidial size. However, they didn't vary in case of number of acervuli per square cm, mycelia color, mycelial texture and setae production. The MGR was the highest 9.9-10.21 mm day<sup>-1</sup> in fruits and lowest in leaves; CS was the highest 22.09-27.54 µm in fruits and lowest in twigs; NOA was the highest 2.11-2.57 cm<sup>-2</sup> in fruits and lowest in flower cluster; MC was the highest 2.12-2.77 in twigs and

lowest in leaves; MT was the highest 2.90-3.12 in flower cluster and lowest in fruits; S was the highest 1.69-1.81 in twigs and lowest in leaves. Box plots of six parameters showed differences within the isolate types (Table 2, 3, Fig. 3).

Morphological variation of Colletotrichum gloeosporioides among isolates origin: Morphological variation of C. gloeosporioides grown on OMA after 18 days of incubation at 25°C were observed among isolates origin differences. Nine sources of isolates origin varied significantly (p < 0.05) among themselves and followed the order of S2<S4<S9<S1<S8<S3<S6<S5<S7. These isolates origin also varied in terms of number of acervuli production (per square centimeter). However, they didn't vary in case of mycelial growth rate, conidial size, mycelia color, mycelial texture and setae production. The MGR was the highest 9.98-10.20 mm day<sup>-1</sup> in S2 and lowest in S4; CS was the highest 23.52-25.98 µm in S4 and lowest in S7; NOA was the highest 1.94-3.06 cm<sup>-2</sup> in S2 and lowest in S7; MC was the highest 1.78-2.71 in S7 and lowest in S5; MT was the highest 2.65-3.58 in S8 and lowest in S1; S was the highest 1.67-1.92 in S8 and lowest in S5. Box plots of six parameters showed differences within the isolates origin (Table 4, Fig. 4).

**Morphological grouping of** *Colletotrichum gloeosporioides* **among 140 isolates:** Morphological group of *C. gloeosporioides* were observed by cluster analysis produced dendrogram which clustered 140 isolates into four distinct groups such as group 1 (G1), group 2 (G2), group 3 (G3) and group 4 (G4), (Table 5, Fig. 5).

Table 2: Cultural variation of C. gloeosporioides among different plant parts of mango grown on Oat Meal Agar (OMA) media after 18 days of incubation at 25°C

Type of isolates	Cultural characters							
	TGP	CC	S	A	SC	СМ		
F	White fluffy	Hyaline	Present	Present	Cylindrical	Present		
L	White dense	Hyaline	Present	Present	Cylindrical	Present		
FLC	Light grey	Hyaline	Present	Present	Cylindrical	Present		
T	Dark olive	Hyaline	Present	Present	Cylindrical	Present		

TGP: Topography, CC: Conidial color, S: Setae, A: Acervuli, SC: Shape of conidia, CM: Conidial masses

Table 3: Morphological variation of C. gloeosporioides among different plant parts of mango grown on OMA after 18 days of incubation at 25°C

Type of isolates	Parameters (Mean±SD)						
	 MGR	CS	NOA	MC	MT	 S	
F	10.21±0.20ª	27.54±2.13ª	2.57±1.28ª	2.33±1.49ª	2.90±1.77ª	1.80±0.40ª	
L	9.91±0.21°	23.42±2.60 <sup>b</sup>	2.36±1.06ª	2.12±1.40ª	2.97±1.78ª	$1.81 \pm 0.39^{\circ}$	
FLC	10.04±0.27 <sup>bc</sup>	22.31±1.47 <sup>b</sup>	2.11±0.88ª	2.41±1.58ª	3.12±1.83ª	1.76±0.44ª	
Т	10.08±0.31 <sup>b</sup>	22.09±3.90 <sup>b</sup>	2.37±1.15ª	2.77±1.69ª	3.00±1.73ª	1.69±0.48ª	

OMA: Oat meal agar, DMRT was represented in small letters. Same letters of each column are not significantly different (p<0.05) and MGR: Mycelial growth rate (mm day<sup>-1</sup>), CS: Conidial size ( $\mu$ m), NOA: No. of acervuli (cm<sup>-2</sup>), MC: Mycelial color, MT: Mycelial texture and S: Setae



Fig. 3(a-f): Box plot of six parameters (Growth rate of mycelium, size of conidia, number of acervuli, setae, mycelial color and texture) showing value differences within isolate types

Open boxes indicate inter-quartile range (25th-75th percentile), vertical bars show minimum and maximum value and markers in each box represent median

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Parameters (Mean±SD)						
MGR	CS	NOA	MC	MT	S	
10.09±0.29ª	25.86±3.55ª	2.59±1.14 <sup>ab</sup>	2.00±1.46 <sup>a</sup>	2.65±1.67ª	1.79±0.41ª	
10.20±0.33ª	23.97±4.41ª	3.06±1.05ª	2.40±1.71ª	2.90±1.91ª	$1.70 \pm 0.48^{a}$	
10.01±0.30ª	23.55±4.18ª	$2.42 \pm 1.34^{ab}$	2.54±1.56ª	2.92±1.89ª	1.77±0.44ª	
9.98±0.17ª	25.98±3.16ª	2.65±0.96 <sup>ab</sup>	2.09±1.38ª	3.18±1.99ª	1.73±0.47ª	
10.08±0.28ª	25.50±2.85ª	2.09±0.95 <sup>ab</sup>	1.78±1.30ª	$3.00 \pm 2.00^{a}$	1.67±0.50ª	
9.99±0.16ª	23.67±2.46ª	2.11±0.99 <sup>ab</sup>	$2.00 \pm 0.94^{a}$	2.76±1.68ª	1.82±0.39ª	
9.99±0.22ª	23.52±1.70ª	1.94±0.92 <sup>b</sup>	2.71±1.63ª	3.25±1.87ª	1.79±0.41ª	
10.12±0.31ª	24.38±2.85ª	2.53±1.45 <sup>ab</sup>	2.58±1.78ª	3.58±1.73ª	1.92±0.29ª	
10.05±0.27ª	25.30±4.25ª	2.62±1.33 <sup>ab</sup>	2.70±1.57ª	2.80±1.55ª	1.90±0.32ª	
	Parameters (Mean: MGR 10.09±0.29 <sup>a</sup> 10.20±0.33 <sup>a</sup> 10.01±0.30 <sup>a</sup> 9.98±0.17 <sup>a</sup> 10.08±0.28 <sup>a</sup> 9.99±0.16 <sup>a</sup> 9.99±0.22 <sup>a</sup> 10.12±0.31 <sup>a</sup> 10.05±0.27 <sup>a</sup>	Parameters (Mean±SD)           MGR         CS           10.09±0.29 <sup>a</sup> 25.86±3.55 <sup>a</sup> 10.20±0.33 <sup>a</sup> 23.97±4.41 <sup>a</sup> 10.01±0.30 <sup>a</sup> 23.55±4.18 <sup>a</sup> 9.98±0.17 <sup>a</sup> 25.98±3.16 <sup>a</sup> 10.08±0.28 <sup>a</sup> 25.50±2.85 <sup>a</sup> 9.99±0.16 <sup>a</sup> 23.67±2.46 <sup>a</sup> 9.99±0.22 <sup>a</sup> 23.52±1.70 <sup>a</sup> 10.12±0.31 <sup>a</sup> 24.38±2.85 <sup>a</sup> 10.05±0.27 <sup>a</sup> 25.30±4.25 <sup>a</sup>	Parameters (Mean±SD)MGRCSNOA $10.09 \pm 0.29^{a}$ $25.86 \pm 3.55^{a}$ $2.59 \pm 1.14^{ab}$ $10.20 \pm 0.33^{a}$ $23.97 \pm 4.41^{a}$ $3.06 \pm 1.05^{a}$ $10.01 \pm 0.30^{a}$ $23.55 \pm 4.18^{a}$ $2.42 \pm 1.34^{ab}$ $9.98 \pm 0.17^{a}$ $25.98 \pm 3.16^{a}$ $2.65 \pm 0.96^{ab}$ $10.08 \pm 0.28^{a}$ $25.50 \pm 2.85^{a}$ $2.09 \pm 0.95^{ab}$ $9.99 \pm 0.16^{a}$ $23.67 \pm 2.46^{a}$ $2.11 \pm 0.99^{ab}$ $9.99 \pm 0.22^{a}$ $23.52 \pm 1.70^{a}$ $1.94 \pm 0.92^{b}$ $10.12 \pm 0.31^{a}$ $24.38 \pm 2.85^{a}$ $2.53 \pm 1.45^{ab}$ $10.05 \pm 0.27^{a}$ $25.30 \pm 4.25^{a}$ $2.62 \pm 1.33^{ab}$	Parameters (Mean±SD)MGRCSNOAMC $10.09 \pm 0.29^{a}$ $25.86 \pm 3.55^{a}$ $2.59 \pm 1.14^{ab}$ $2.00 \pm 1.46^{a}$ $10.20 \pm 0.33^{a}$ $23.97 \pm 4.41^{a}$ $3.06 \pm 1.05^{a}$ $2.40 \pm 1.71^{a}$ $10.01 \pm 0.30^{a}$ $23.55 \pm 4.18^{a}$ $2.42 \pm 1.34^{ab}$ $2.54 \pm 1.56^{a}$ $9.98 \pm 0.17^{a}$ $25.98 \pm 3.16^{a}$ $2.65 \pm 0.96^{ab}$ $2.09 \pm 1.38^{a}$ $10.08 \pm 0.28^{a}$ $25.50 \pm 2.85^{a}$ $2.09 \pm 0.95^{ab}$ $1.78 \pm 1.30^{a}$ $9.99 \pm 0.16^{a}$ $23.67 \pm 2.46^{a}$ $2.11 \pm 0.99^{ab}$ $2.00 \pm 0.94^{a}$ $9.99 \pm 0.22^{a}$ $23.52 \pm 1.70^{a}$ $1.94 \pm 0.92^{b}$ $2.71 \pm 1.63^{a}$ $10.12 \pm 0.31^{a}$ $24.38 \pm 2.85^{a}$ $2.53 \pm 1.45^{ab}$ $2.58 \pm 1.78^{a}$ $10.05 \pm 0.27^{a}$ $25.30 \pm 4.25^{a}$ $2.62 \pm 1.33^{ab}$ $2.70 \pm 1.57^{a}$	Parameters (Mean±SD)MGRCSNOAMCMT $10.09 \pm 0.29^{a}$ $25.86 \pm 3.55^{a}$ $2.59 \pm 1.14^{ab}$ $2.00 \pm 1.46^{a}$ $2.65 \pm 1.67^{a}$ $10.20 \pm 0.33^{a}$ $23.97 \pm 4.41^{a}$ $3.06 \pm 1.05^{a}$ $2.40 \pm 1.71^{a}$ $2.90 \pm 1.91^{a}$ $10.01 \pm 0.30^{a}$ $23.55 \pm 4.18^{a}$ $2.42 \pm 1.34^{ab}$ $2.54 \pm 1.56^{a}$ $2.92 \pm 1.89^{a}$ $9.98 \pm 0.17^{a}$ $25.98 \pm 3.16^{a}$ $2.09 \pm 0.96^{ab}$ $2.09 \pm 1.38^{a}$ $3.18 \pm 1.99^{a}$ $10.08 \pm 0.28^{a}$ $25.50 \pm 2.85^{a}$ $2.09 \pm 0.95^{ab}$ $1.78 \pm 1.30^{a}$ $3.00 \pm 2.00^{a}$ $9.99 \pm 0.16^{a}$ $23.67 \pm 2.46^{a}$ $2.11 \pm 0.99^{ab}$ $2.00 \pm 0.94^{a}$ $2.76 \pm 1.68^{a}$ $9.99 \pm 0.22^{a}$ $23.52 \pm 1.70^{a}$ $1.94 \pm 0.92^{b}$ $2.71 \pm 1.63^{a}$ $3.25 \pm 1.87^{a}$ $10.12 \pm 0.31^{a}$ $24.38 \pm 2.85^{a}$ $2.53 \pm 1.45^{ab}$ $2.58 \pm 1.78^{a}$ $3.58 \pm 1.73^{a}$ $10.05 \pm 0.27^{a}$ $25.30 \pm 4.25^{a}$ $2.62 \pm 1.33^{ab}$ $2.70 \pm 1.57^{a}$ $2.80 \pm 1.55^{a}$	

#### Table 4: Morphological variation of *C. gloeosporioides* among 9 different districts origins

DMRT was represented in small letters. Same letters of each column are not significantly different (p<0.05), MGR: Mycelial growth rate (mm day<sup>-1</sup>), CS: Conidial size ( $\mu$ m), NOA: No. of acervuli (cm<sup>-2</sup>), MC: Mycelial color, MT: Mycelial texture and S: Setae

Table 5: Cluster analysis of 140 C. gloeosporioides isolates

Groups	Isolates	Member of clusters
G1	22	F1 F2 F5 F9 F11 F12 F13 F14 F15 F18 F20 F21 F42 F45 F46 F47 F48 F51 L1 L2 L29 T8
G2	40	F3 F4 F6 F7 F8 F10 F28 F29 F31 F32 F34 F38 F40 F49 F50 L5 L9 L12 L15 L16 L19 L20 L21 L25 L30 L32 L33 L34 L41 L43 L48 L51
		L55 L59 FLC1 FLC11 FLC12 FLC15 T3 T9
G3	57	F16 F17 F19 F22 F23 F25 F26 F30 F33 F35 F37 F41 F43 F44 L4 L7 L8 L10 L14 L22 L24 L26 L27 L28 L31 L35 L36 L37 L38 L42
		L44 L45 L46 L47 L49 L52 L53 L54 L56 L57 L58 FLC2 FLC4 FLC5 FLC6 FLC7 FLC9 FLC13 FLC14 FLC16 T1 T2 T5 T7 T10 T12 T13
G4	21	F24 F27 F36 F39 L3 L6 L11 L13 L17 L18 L23 L39 L40 L50 FLC3 FLC8 FLC10 FLC17 T4 T6 T11

G1: Group 1, G2: Group 2, G3: Group 3 and G4: Group 4

#### Table 6: Morphological grouping of C. gloeosporioides among 140 isolates

Groups	Parameters (Mean±SD)						
	MGR	CS	NOA	MC	MT	S	
G1	10.38±0.20ª	28.72±1.53ª	3.83±0.85ª	2.23±1.41 <sup>ab</sup>	2.05±1.36 <sup>bc</sup>	1.64±0.49 <sup>b</sup>	
G2	9.97±0.18 <sup>b</sup>	24.96±3.24 <sup>b</sup>	2.04±0.85 <sup>b</sup>	1.57±0.98 <sup>b</sup>	1.60±0.71°	$2.00 \pm 0.00^{a}$	
G3	9.98±0.24 <sup>b</sup>	23.64±2.76 <sup>bc</sup>	2.29±1.04 <sup>b</sup>	2.74±1.61ª	4.39±1.42ª	$2.00 \pm 0.00^{a}$	
G4	10.04±0.24 <sup>b</sup>	22.63±2.55°	1.93±1.01 <sup>b</sup>	2.52±1.54ª	2.67±1.56 <sup>b</sup>	1.00±0.00°	

DMRT was represented in small letters. Same letters of each column are not significantly different (p<0.05), MGR: Mycelial growth rate (mm day<sup>-1</sup>), CS: Conidial size ( $\mu$ m), NOA: No. of acervuli (cm<sup>-2</sup>), MC: Mycelial color, MT: Mycelial texture and S: Setae

Four groups of isolates varied significantly (p<0.05) among themselves. The G1 was varied significantly based on MGR, CS and NOA and G3 was MC, MT and S. CS was highest varied in G2 and lowest in G1. The NOA was highest varied in G3 and lowest in both G1 and G2. The MGR was the highest 9.97-10.38 mm day<sup>-1</sup> in G1 and lowest in G2, CS was the highest 22.63-28.72  $\mu$ m in G2 and lowest in G4, NOA was the highest 1.93-3.83 cm<sup>-2</sup> in G1 and lowest in G4, MC was the highest 1.57-2.74 in G3 and lowest in G2, MT was the highest 1.60-4.39 in G3 and lowest in G4, Table 6).

Mean plot of six parameters in each group of *C. gloeosporioides* were varied themselves. The G1 was showed the highest variability in terms of all characters. The G2, G3 and G4 were highly varied except setae. Setae were always presence in these 3 groups. All the parameters highest variability in conidial size and the lowest in setae and followed the order of CS<MT<MC<NOA<MGR<S (Fig. 6).

Morphology of *Colletotrichum* colonies varies within and among groups, depending on culture medium, substrate and temperature, among other factors. Different appearances of mycelial growth were observed among 140 isolates of *C. gloeosporioides* (4 clustered) grown on OMA containing petri-dishes after 18 and 28 Days After Inoculation (DAI) was observed (Fig. 7). Variation in mycelial growth occurred between isolates. Mycelial growth was very profuse, moderate and least. For all isolates, number of acervuli and masses of conidia production was extensive on OMA media.

#### DISCUSSION

Present results verified the identity of *C. gloeosporioides* population associated with anthracnose of mango fruits, leaves, flower clusters and twigs in Bangladesh. The study was found variability in all the places with various stages of the same plant. Many researchers were characterized variation in



Fig. 4(a-f): Box plots of six parameters (Growth rate of mycelium, size of conidia, number of acervuli, setae, mycelial color and texture) showing differences within isolates origins

Open boxes indicate inter-quartile range (25th-75th percentile); vertical bars show minimum and maximum value and markers in each box represent median, S1: Satkhira, S2: Khulna, S3: Jessore, S4: Bagherhat, S5: Jhenaidah, S6: Rajshahi, S7: Chapai, S8: Dinajpur and S9: Rangpur districts

EZ Group 1 Isolates 22 Isolates Group 4 21 101 179 173 174 1710 1710 Ē OL LE 6† 857 977 -C 하셔Ⴆ 유지 비미마 Group 3 Isolates 57 Ē --T -E Isolates Group 2 Æ 40 È Æ 100 80 20 0 60 40 Simiilarity distances

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Fig. 5: Dendrogram showing group variation among 140 isolates of *C. gloeosporioides* from mango All bootstrapped numbers are reported for clusters that are differentiated to other isolates or clusters

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Fig. 6(a-d): Continue



Fig. 6(a-d): Continue

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Fig. 6(a-d): Continue



Fig. 6(a-d): Mean plot of six parameters (Growth rate of mycelium, size of conidia, number of acervuli, setae, mycelial color and texture) in each group of *C. gloeosporioides* isolates from anthracnose of mango, (a) Group 1 composed of 22 isolates, (b) Group 2 composed of 40 isolates, (c) Group 3 composed of 57 isolates and (d) Group 4 composed of 21 isolates



Fig. 7(a-h): Mycelial growth of *C. gloeosporioides* after 18 [(a) White Cottony, (b) Dense white, (c) Grey, (d) Pale grey] and 28 Days after Inoculation (DAI) [(e) Olive green, (f) Black, (g) Dark olive, (h) Light olive] on OMA containing petri-dishes

different places and countries are different due to high host range<sup>11</sup>. The basic criteria for identifying *C. gloeosporioides* are CS and NOA. All of the isolates were categorized into four morphological groups. The size of conidia of G2 were highest than that of other groups. The G1 isolates grew faster and highly produced number of acervuli. The similar grouping was done by where 38 isolates were divided based on conidial morphology into three groups<sup>28</sup>.

The findings reported that, observations and measurements of MGR, CS, NOA, MC, MT and S have usually been made within the species compared that fruits isolates were significantly (p<0.05) fast growing, enlarged conidial size and highly produced acervuli, whereas leaves isolates were slow grower, less setae and mycelial color variation. In related studies, according to Abera *et al.*<sup>13</sup>, *C. gloeosporioides* produced symptoms and the shape or size of conidia (6.0-10×2.0-2.5 µm) was slightly different from those found on white fleshed species in Okinawa Prefecture.

In the study, the highly variable isolates were S2 based on high growth rate with large conidia but number of acervuli production in S8. Similarly, the lowest variability in S7 due to both small conidia and low acervuli production and S6 was the slow grow at p<0.05 level of significance. This result is in agreement with a previous study by Sangdee *et al.*<sup>29</sup> who found a morphological variation of conidial size among *Colletotrichum* species. However, similarity in disease symptoms caused by the target pathogen implied that it would not be easy to determine whether the morphological features had been effective in disease incidence of the mango anthracnose until a proper assessment is made. This study also has shown the variation in the virulence of mango anthracnose pathogen isolates, which has suggestions for both disease control and the host adaptability of pathogen populations.

Fruits are the edible, most frequently utilizable and commercially valuable part of mango tree. Similarly, green leaves are photosynthetically active part. In this way, different parts of a mango tree bear their significance. Therefore, growth and infestation variability of *C. gloeosporioides* on different plant parts were studied. According to Vithanage *et al.*<sup>28</sup> anthracnose affected lesions of ripe mangoes and De Souza Serra *et al.*<sup>30</sup> leaves were used to characterize the morphological features. It can be beneficial for accurate identification of many researchers particularly, bio-security, plant breeding and integrated disease management. Thus, a new concept on this variability of *C. gloeosporioides* in Bangladesh and possibly to prevent yield losses through effective control measure.

### CONCLUSION

The present study revealed that morphological variation of *C. gloeosporioides* existed among different parts of plant as well as among different source of origins of mango cultivers in respect of mycelial growth rate, conidial size, number of acervuli, mycelial color, texture and setae.

#### SIGNIFICANCE STATEMENTS

Morphological characterization of *Colletotrichum gloeosporioides* are needed to improve the scope of our knowledge of the anthracnose causing populations in different developmental stages and origins of same plants. Proper identification is important in the development of management perspective.

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