

Morphological and physiological variation among different isolates of *Alternaria spp.* from Rapeseed-Mustard

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Abstract— To find out the Morphological variation on growth and sporulation of *Alternaria* species of *Alternaria* leaf blight of mustard from 10 representative geographical locations of Bangladesh, this experiment was conducted at Plant Pathology Laboratory, Oilseed Research center, Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur, Bangladesh. All the isolates showed high level of variability in in-vitro in respect of radial mycelial growth, colony colour, sub surface colour, colony shape, colony texture, zonation (surface and sub surface), length and width of conidia, beak length and number of septa. The maximum and minimum radial mycelial growth was recorded 90 mm in isolate NAT_{Ab} and 83.67 mm in isolate GAZ_{Ab}, respectively at 14 days after incubation. Significant variation in conidial length, width, beak and no. of conidia observed in all isolates. The length of conidia ranged from 41.56 to 117.54 μm with 3 to 11 transverse and 0 to 3 vertical septa. The width and beak length varied from 10.34 to 23.12 μm and 16.78 to 72.65 μm, respectively. Surface colour were olivaceous green to black and circular shaped colonies were observed in all isolates on PDA medium. Colony texture were cottony to velvety. Subsurface colour varied from light brown to black and pinkish. Zonation found in some isolates and some did not produce on both surface and subsurface. All conidia were muriform and light brown to deep brown in colour. Potato Carrot Dextrose Agar medium (PCDA) and 25 ° C temperature were found optimum for different isolates for mycelial growth and sporulation.

Keywords—*Alternaria brassicae*, mustard, morphology, physiology, culture media, variability.

I. INTRODUCTION

Rapeseed-Mustard (*Brassica spp.*) is the principal oil-producing crop of Bangladesh yielding 77.51% [4] of total

oilseed production from 60.3% of the total area coverage. This crop is cultivated, at present, in about 802882 acres. The production is about 359452 lac metric tons oil [4]. The average yield of mustard is 447 Kg/ha. Total production and per hectare seed yield of this crop may be increased by using high yielding variety (HYV) and improved production technologies.

Rapeseed-mustard is cultivated almost all over the world. It is grown in tropical as well as temperate agro climatic zones and are the best adapted to areas having a relatively cool, moist climate during the growing season. [18]. *Alternaria* leaf blight caused by *Alternaria brassicae* is one of the major diseases of mustard [21,29,15,1, 10,37, 7]. This disease reduces mustard yield up to 47% [30] in India. It is a prominent disease in India, Australia, Canada, Africa, England, Germany, France, Sri Lanka, Spain and Sweden, all most all around the world [12].

Around the initial site of host leaf *Alternaria* morphologically produces a series of concentric rings [2]. *A. brassicae* is a necrotrophic pathogen produce lesion on leaves, stem and siliquae which affect seed quantity, quality by reducing oil content, seed size and seed colour [8]. This disease may cause significant losses in both temperate and tropical Brassica crops [20].

The major aspects of biology of an organism are the morphological and physiological characters of an individual within a species. Although, it is not frequent in asexually produced individual of the progeny. Variability studies are important to document the changes occurring in populations and individuals as variability in morphological and physiological traits indicate the existence of different pathotypes [21]. Anamorph form of this pathogen shows great variability in morphology, physiology and pathogenicity. Several researchers have reported existence

of variability based on morphology, sporulation, growth and cultural characteristics.

We know, every pathogen species has numerous biotypes, races or pathotype with specific genes in the respective host plants [36]. Proper understanding in the variation of pathogen population is highly crucial in the process of breeding for resistance against a particular disease.

Considering the above fact this research was undertaken to Find out the morphological and physiological variation among different isolates of *Alternaria spp.*

II. MATERIALS AND METHODS

The experiment was conducted in the Plant Pathology Laboratory, Oil Seed Research Center, Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur, Bangladesh during the period from July 2015 to March 2016.

2.1 Collection of leaf sample

Mustard leaves having typical symptoms were collected from 10 mustard growing districts of Bangladesh namely Dhaka, Rajshahi, Natore, Naogaon, Bogra, Lalmonirhat, Gazipur, Rangpur, Pabna and Mymensingh.

2.2 Designation of collected isolates

The collected isolates were designed as DHA_{Ab}, GAZ_{Ab}, MYM_{Ab} based on their collected location. For example an isolate collected from Dhaka and recognized as first three letters of the area and Ab indicate *Alternaria brassicae* (Table 1).

Table.1: Designation of collected isolates of *Alternaria Brassicae*

District/Thana	Isolates designation	Village/Place
Dhaka (SAU)	DHA _{Ab}	Agronomy field
Gazipur (BARI)	GAZ _{Ab}	Oil Research field
Mymensingh (BAU)	MYM _{Ab}	Horticulture field
Pabna	PAB _{Ab}	Bhabarhat
Rangpur	RAN _{Ab}	Tillalpara
Natore	NAT _{Ab}	Dayarampur
Naogaon	NAO _{Ab}	Kamalpara
Lalmonirhat	LAL _{Ab}	Benupara
Bogra	BOG _{Ab}	Munail
Rajshahi	RAJ _{Ab}	Khorkhori

SAU = Sher-e- Bangla Agricultural University

BAU = Bangladesh Agricultural University

BARI = Bangladesh Agricultural Research Institute

2.3 Preparation of PDA medium

Potato dextrose agar (PDA) were prepared by 200gm potato extract, 1000ml distilled water, 17 gm agar, 20gm dextrose in a conical flask and autoclaved at 121 c under 15 psi for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile Petri plates.

2.4 Preparation of CDA

Carrot dextrose agar were prepared by 200gm carrot extract, 1000ml distilled water, 17 gm agar, 20gm dextrose in a conical flask and autoclaved at 121 c under 15 psi for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile Petri plates.

2.5 Preparation of Potato-Carrot Dextrose Agar (PCDA)

The combination of Potato-Carrot dextrose agar prepared by 100ml potato+100ml carrot extract, 1000ml distilled water, 17 gm agar, 20 gm dextrose in a conical flask and autoclaved at 121 c under 15 psi for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile petriplates.

2.6 Isolation and Identification of *Alternaria spp.*

The pathogen was isolated by tissue planting method and incubated at 25±1° C for 7 days. After incubation the fungus mycelia were examined under stereomicroscope (Model: Motic, SMZ-168) & compound microscope (Model: Omano, OMTM-85) for identification of the pathogen. The fungus was identified following the keys of Eills[9] .

2.7 Purification and preservation of the pathogen

The pure culture of *A. brassicae* from the PDA was transferred to PDA slants and allowed to grow at 25± 1°C for 7 days. After incubation PDA slants were preserved in refrigerator at 4°C for further study.

2.8 Colony characters of *Alternaria spp.*

Colony characters in terms of surface colour, colony shape, colony texture, zonation (surface and subsurface) and subsurface colour were studied.

2.9 Morphological variability of *Alternaria spp.*

All the isolates were studied for morphological variations. In terms of conidia color, shape, size, septation, was observed on PDA medium.

2.10 Effect of culture media on growth, spore production and time of sporulation

Mycelial discs of 7 days old culture of *Alternaria* spp. isolates were transferred to the center of PDA, CDA and PCDA and incubated at 25°C and 22±1°C and data were recorded on growth, spore production and time of sporulation. 3 replications were maintained for each isolates in a completely randomized design. The colony diameter was recorded on 2, 4, 6, 8, 10, 12, 14 days after inoculation.

2.11 Data Analysis

For cultural, morphological and the treatment means the data were statistically analyzed by Duncan's Multiple Range test (DMRT) with significance level at 5% [13]. The package used for analysis was MSTAT-C version -88, developed by Michigan State University, Agricultural University of Norway [11].

III. RESULTS

3.1 Colony characters of isolates of *Alternaria* spp. on PDA

Variation was observed in colony characters of 10 isolates of *A. brassicae* like surface colour, shape, texture, zonation and subsurface colour are presented Table 2 and Figure 1.

3.2 Morphological variation of conidia of different isolates of *Alternaria* spp.

3.2.1 Size of conidia of *Alternaria* spp. on PDA

Remarkable variation was observed in length, breadth and beak size of conidia of different isolates of *A. brassicae* on PDA (Table 3). The length of conidia of different isolates

varied from 41.56µm to 117.54µm. The maximum mean length was recorded at MYM_{Ab} 113.1µm. The minimum length was recorded at isolates GAZ_{Ab} 63.63µm.

The breadth of conidia of different isolates varied from 10.34 µm to 23.12 µm. The maximum mean breadth was recorded at PAB_{Ab} 17.36 µm. The minimum mean breadth was recorded at LAL_{Ab} 20.29 µm. The beak of conidia of different isolates varied from 16.78 µm to 72.65 µm. The maximum mean beak was recorded at PAB_{Ab} 43.26 µm. The minimum mean beak was recorded at GAZ_{Ab} 24.84 µm.

3.2.2 Conidial characteristics of *Alternaria* spp. on PDA

All isolates were muriform. Colour of isolates of *A. brassicae* varied from light brown to deep brown (Table 4). Isolates NAT_{Ab} and GAZ_{Ab} show light brown colour, DHA_{Ab}, MYM_{Ab}, LAL_{Ab}, BOG_{Ab} and RAJ_{Ab} show brown

Table.3: Size of conidia of different isolates of *Alternaria* spp. on PDA

Isolate	Length(µm) ¹	Breadth(µm) ¹	Beak(µm) ¹
DHA _{Ab}	88.55 d	18.12 b	28.09 e
GAZ _{Ab}	63.63 e	18.09 b	24.84 e
MYM _{Ab}	113.1 a	18.56 ab	38.76 bc
PAB _{Ab}	103.4 bc	17.36 b	43.26 a
RAN _{Ab}	99.33 bc	17.90 b	37.19 c
NAT _{Ab}	90.03 d	17.87 b	37.93 c
NAO _{Ab}	87.73 d	20.17 a	25.98 e
LAL _{Ab}	101.5 bc	20.29 a	38.95 bc
BOG _{Ab}	97.61 c	18.23 b	41.89 ab
RAJ _{Ab}	104.7 b	19.12 ab	33.23 d
LSD	6.82	1.82	3.47
(0.05)			
CV (%)	4.22	5.75	5.82

¹Mean of 15 replications for each isolates

Table.2: Colony Characters of different isolates of *Alternaria* spp. on PDA

Isolates	Colour		Texture	Colony shape	Zonation	
	Surface	Subsurface			Surface	Subsurface
DHA _{Ab}	Olivaceous green	Black center with white surroundings	Cottony	Circular	No zonation	Zonation
GAZ _{Ab}	Olivaceous green	Black center with pinkish surroundings	Cottony	Circular	Zonation	Zonation
MYM _{Ab}	Black	Brownish	Velvety	Circular	No zonation	Zonation
PAB _{Ab}	Olivaceous green	Black center with pinkish surroundings	Cottony	Circular	No zonation	Zonation
RAN _{Ab}	Black	Black	Cottony	Circular	No zonation	No zonation
NAT _{Ab}	Black	Black center with	Cottony	Circular	Zonation	No zonation

white surroundings						
NAO _{Ab}	Olivaceous green	Greenish black	Velvety	Circular	Zonation	No zonation
LAL _{Ab}	Olivaceous green	Light brown	Velvety	Circular	No zonation	Zonation
BOG _{Ab}	Black	Black	Cottony	Circular	No zonation	No zonation
RAJ _{Ab}	Olivaceous green	Brownish green with pinkish surroundings	Cottony	Circular	Zonation	Zonation

Table.4: Conidial characteristics of *Alternaria* spp.

Isolates	From	Colour	Septation (Range)	
			Horizontal	Vertical
DHA _{Ab}	Muriform	Brown	5-9	0-1
GAZ _{Ab}	Muriform	Light brown	3-7	0-2
MYM _{Ab}	Muriform	Brown	5-7	2-3
PAB _{Ab}	Muriform	Deep Brown	5-7	1-2
RAN _{Ab}	Muriform	Deep Brown	5-7	2-3
NAT _{Ab}	Muriform	Light brown	7-11	2-3
NAO _{Ab}	Muriform	Deep Brown	5-8	1-3
LAL _{Ab}	Muriform	Brown	7-9	1-3
BOG _{Ab}	Muriform	Brown	7-11	0-3
RAJ _{Ab}	Muriform	Brown	5-9	1-2

colour. PAB_{Ab}, RAN_{Ab} and NAO_{Ab} show deep brown in colour. (Figure 2)

Variation in septation observed in isolates of *A.brassicacae*. The horizontal septation varied from 3-7 to 7-11. The vertical septation varied from 0-1 to 2-3. The maximum horizontal septation observed isolates BOG_{Ab} (7-11) and minimum septation observed in isolates GAZ_{Ab}. The maximum vertical (2-3) septation observed in isolates MYM_{Ab}, RAN_{Ab} and NAT_{Ab}. The minimum vertical (0-1) septation observed in isolate DHA_{Ab}.

3.3 Cultural variability of *Alternaria brassicae*

3.3.1 Radial mycelial growth of 10 isolates of *Alternaria* spp. on PDA

Radial mycelial growth of different isolates of *Alternaria* spp. significantly varied on PDA (Table 5 and Plate 1). After 2 days of inoculation the maximum radial mycelial growth of *A. brassicae* (30.50 mm) was observed in DHA_{Ab}, followed by PAB_{Ab} (27.00 mm). The minimum radial mycelial growth (14.67 mm) was recorded in NAO_{Ab} which was statistically similar to GAZ_{Ab} (17.33 mm).

After 4th day, 6th day, 8th day, 10th day and 12th day of inoculation the maximum radial mycelial growth of *Alternaria* spp. were recorded in DHA_{Ab} which were 48.33 mm, 64.00 mm, 79.93 mm, 89.33 mm and 90.00 mm,

respectively and the minimum radial mycelial growth were recorded in NAO_{Ab} which was 33.50 mm.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. was measured in DHA_{Ab} which was (90.00 mm), followed by PAB_{Ab} (88.33 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} which was (76.67 mm) which was statistically similar to MYM_{Ab} (79.67 mm).

3.3.2 Radial mycelial growth of 10 isolates of *Alternaria* spp. on CDA

After 2 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. 29.67 mm was measured in NAT_{Ab}, followed by RAJ_{Ab} (28.67 mm). The minimum radial mycelial growth was recorded in GAZ_{Ab} (17.67 mm) which was statistically similar to MYM_{Ab} (19.17 mm).

After 4th days, 6th days, 8th days, 10th day and 12th days of inoculation the maximum radial mycelial growth of *A. brassicae* were measured in NAT_{Ab} which was 49.17 mm, 65.33 mm, 82.33 mm, 87.00 mm and 90.00 mm respectively and the minimum radial mycelial growth were recorded in GAZ_{Ab} 26.33 mm, 42.33 mm, 54.33 mm, 73.33 mm and 77.67 mm respectively.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. was measured in NAT_{Ab} (90.00 mm), which was statistically similar to RAJ_{Ab} which was 89.33 mm. The minimum radial mycelial growth was recorded in GAZ_{Ab} (83.67mm) which was statistically similar to MYM_{Ab} (85.33 mm).

3.3.3 Radial mycelial growth of 10 isolates of *Alternaria* spp. on PCDA

After 2 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. was measured in RAJ_{Ab} which was (33.17 mm) which was statically similar to RAN_{Ab} (31.83 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} which was (20.33 mm) which was statistically similar to MYM_{Ab} (23.33 mm).

Table.5: Radial mycelial growth of different isolates of *Alternaria* spp. at different days after incubation on PDA

Isolate	2 th Day	4 th Day	6 th Day	8 th Day	10 th Day	12 th Day	14 th Day
DHA _{Ab}	30.50 a	48.33 a	64.00 a	79.93 a	89.33 a	90.00 a	90.00 a
GAZ _{Ab}	17.33 f	38.00 d	51.67 def	69.00 bc	76.33 cd	79.33 bcd	80.33 cd
MYM _{Ab}	20.33 e	41.83 bcd	50.50 ef	67.67 c	73.00 de	77.00 cd	79.67 cd
PAB _{Ab}	27.00 b	42.33 bc	59.37 abc	73.33 abc	81.33 bc	82.67 abc	83.00 abcd
RAN _{Ab}	23.33 d	45.67 ab	55.67 cd	70.57 bc	75.50 cd	78.33 bcd	82.67 bcd
NAT _{Ab}	17.67 f	40.67 cd	56.33 bcd	73.67 abc	79.13 bcd	84.67 abc	85.00 abc
NAO _{Ab}	14.67 g	33.50 e	47.33 f	58.33 d	67.00 e	73.33 d	76.67 d
LAL _{Ab}	21.00 e	41.67 cd	54.67 cde	76.67 ab	84.67 ab	82.67 abc	83.00 abcd
BOG _{Ab}	25.67 bc	43.50 bc	58.50 bc	75.83 ab	80.33 bcd	83.33 abc	85.00 abc
RAJ _{Ab}	24.33 cd	40.83 cd	61.00 ab	74.93 abc	81.00 bc	86.33 ab	88.33 ab
LSD (0.05)	1.97	3.87	5.17	7.77	7.93	8.54	7.10
CV (%)	5.21	5.46	5.42	6.34	5.91	6.14	5.00

Table.6: Radial mycelial growth of different isolates of *Alternaria* spp. at different days after incubation on CDA

Isolate	2 th Day	4 th Day	6 th Day	8 th Day	10 th Day	12 th Day	14 th Day
DHA _{Ab}	25.83 c	42.67 b-d	53.67 cd	64.83 ef	77.67 bc	80.00 de	86.67 ab
GAZ _{Ab}	17.67 e	26.33 f	42.33 f	54.33 g	73.33 c	77.67 e	83.67 b
MYM _{Ab}	19.17 e	33.17 e	48.00 e	61.50 f	82.00 ab	83.67 b-d	85.33 ab
PAB _{Ab}	26.67 bc	43.00 b-d	58.67 b-d	72.67 b-d	81.67 ab	85.33 a-d	87.33 ab
RAN _{Ab}	22.00 d	38.83 d	53.17 de	69.77 de	81.67 ab	82.67 b-e	88.33 ab
NAT _{Ab}	29.67 a	49.17 a	65.33 a	82.33 a	87.00 a	90.00 a	90.00 a
NAO _{Ab}	26.50 bc	42.67 b-d	58.83 bc	70.67 c-e	75.67 bc	85.33 a-d	87.67 ab
LAL _{Ab}	26.00 c	42.00 cd	60.67 ab	75.33 b-d	76.67 bc	81.33 c-e	86.33 ab
BOG _{Ab}	27.33 a-c	46.50 ab	61.67 ab	76.33 a-c	82.33 ab	86.67 a-c	88.67 ab
RAJ _{Ab}	28.67 ab	44.33 bc	63.33 ab	78.33 ab	85.33 a	88.33 ab	89.33 ab
LSD (0.05)	2.36	4.35	5.55	6.49	7.63	5.82	6.28
CV (%)	5.5	6.24	5.76	5.4	5.58	4.06	4.22

Table.7: Radial mycelial growth of different isolates of *Alternaria* spp. at different days after incubation on PCDA

Isolate	2 th Day	4 th Day	6 th Day	8 th Day	10 th Day	12 th Day	14 th Day
DHA _{Ab}	26.67 bc	45.20 cd	61.33 b	75.67 ab	85.00 ab	88.00 a-c	89.00 ab
GAZ _{Ab}	26.67 bc	45.20 cd	62.30 ab	78.27 a	84.20 ab	87.67 a-c	88.00 ab
MYM _{Ab}	23.33 cd	42.33 de	52.33 c	69.33 bc	77.00 bc	81.67 c	85.33 c

PABAb	29.67 ab	50.00 a-c	63.50 ab	78.33 a	85.33 ab	88.67 ab	89.33 ab
RANAb	31.83 a	46.33 b-d	63.00 ab	76.00 a	83.33 bc	86.33 a	89.67 ab
NATAb	24.33 c	39.67 e	64.83 ab	76.10 a	84.83 ab	89.67 ab	90.00 a
NAOAb	20.33 d	33.83 f	48.33 c	64.67 c	70.67 c	74.67 d	79.00 d
LALAb	31.50 a	46.33 b-d	65.33 ab	78.67 a	83.00 ab	85.33 a-c	87.33 bc
BOGAb	31.00 a	50.67 ab	66.33 ab	79.00 a	84.33 ab	88.67 ab	90.00 a
RAJAb	33.17 a	54.83 a	68.17 a	80.27 a	88.33 a	90.00 a	90.00 a
LSD (0.05)	3.62	5.19	6.05	6.64	8.86	6.41	2.66
CV (%)	7.65	6.71	5.77	5.16	6.27	4.39	1.78

After 4th days, 6th days, 8th days, 10th days and 12th days of inoculation the maximum radial mycelial growth of *A. brassicae* 54.83 mm, 68.17 mm, 80.27 mm, 88.33 mm and 90.00 mm were measured in RAJ_{Ab} and the minimum radial mycelial growth were recorded in NAO_{Ab} 33.83 mm, 48.33 mm, 64.67mm, 70.67 mm and 74.67 mm.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. was measured in RAJ_{Ab} 90.00 mm, which was statistically similar to BOG_{Ab} and NAT_{Ab} (90.00 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} (79.00 mm) proceeded by MYM_{Ab} (85.33 mm).

IV. DISCUSSION

A laboratory examination was carried out at Plant Pathology Laboratory of Oil seed Research Center, BARI, Joydevpur, Gazipur to find out morphological and physiological variation among ten different isolates of *Alternaria* spp. isolated from mustard leaf having typical symptoms of *Alternaria* blight.

Leaves of mustard having typical symptoms were collected from ten different location of Bangladesh and causal organisms were isolated on PDA medium. All the isolates produced light brown to deep brown muriform conidia with beak. This finding was supported by previous findings [18]. They were also found muriform conidia which were brownish black. Some researcher worked with *Alternaria* spp. and found muriform, obclavate conidia with brownish black [28].

All 10 isolates showed variations in respect of their cultural and morphological characteristics on different media. In respect of cultural characteristics, the isolates of *Alternaria* spp. showed variation in mycelial growth, colony color, shape, textures, subsurface color, zonation conidia production and sporulation time.

Remarkable effect of different culture media on radial mycelia growth was observed in *Alternaria* spp.. Significant

variation was found in colony color of *A. brassicae* on PDA medium. Most of the colony color of the isolates were olivaceous green to black. The results are partially agreement with [33] who found that the colony color of *A. mali* isolated from apple was light to dark olivaceous with greenish or brownish tinge. In case of *A. alternata* isolated from ribben plants colony colour black to olivaceous-black or grayish colour on PDA medium was found [24]. Thirty two isolates of *A. brassicicola* for colony color and radial growth were observed by [6]. Colony colour of *A. brassicicola* varied from olive green to dark olivaceous black on PDA.

All the isolates of *Alternaria* spp. colony had circular shaped. The results are in agreement with [38] were identified its morphological and cultural characters of *A. brassicae* isolates from four different locations, colonies of all the isolates were circular in shape. The colony shape of *A. solani* isolated from tomato plants were found circular margin with smooth surfaced colony [31]. The entire isolates colony had cottony and velvety texture on PDA medium. The results are in agreement with [3] examined 308 isolates of *Alternaria* spp. colonies generally had a cottony texture on group 4. *Alternaria* blotch, causal organism *A. mali*, colonies varied in their cultural behavior ranging from velvety to cottony [33]. Remarkable variation was observed on spore production and sporulation time on different media and temperature. Potato Carrot Media are found suitable for spore production and sporulation time for maximum isolate followed by CDA and PDA. This result was supported by the [23] found potato carrot broth are suitable for sporulation and spore production *A. brassicae*. Variation were found in mycelial growth, sporulation in different nutrient media like Potato Dextrose Agar, Cauliflower Agar medium and Carrot Potato Agar good for 32 isolates of *A. brassicae* [33].

Variations were observed in accordance with length, breadth and beak on different isolates of *A. brassicae* on PDA media. The length of conidia of different isolates

varied from 41.56µm to 117.54µm. The breadth of conidia of different isolates varied from 10.34 µm to 23.12 µm. The beak of conidia of different isolates varied from 16.78 µm to 72.65 µm. The horizontal septation varied from 3-7 to 7-11. The vertical station varied from 0-1 to 2-3. This result are partially supported with [28] define *A. brassicae* length of conidia varied from 96 µm -114 µm, breadth varied from 17 µm -24 µm and beak length varied from 45 µm-65 µm and transverse and longitudinal septation varied from 10-11 and 0-6 respectively. 322 isolates of *A. brassicae* variation was recorded among conidial length, breadth and beak length which range of 51.4-481.2 µm, 6.9-36.0 µm and 16.3 - 266.9 µm respectively [16]. Average numbers of horizontal septa were 9.7, vertical septa were 0.8. The horizontal septation of 5 different isolates of *A. brassicae* varied from 4-13 and vertical from 0-6[33]. 23 isolates of *A. brassicae* were collected and found maximum length of conidia ranged from 150 - 122 µm with 8 - 9 transverse and 2 vertical septation [27] Eight isolates of *A. solani* were examined [25] and found average conidial size (L×B) was 42.18×15.18 µm and beak size was 13.10µm. In ten isolates of *A. macrospora* size of conidia ranged from 20.81-56.23 x 9.2- 27.10 µm with 1 - 6 transverse and 0 - 4 longitudinal septa were found [14]. ten isolates collected by[26] of *A. alternata* the length and width of conidia were varied from 30.99 -42.47 µm and 11.90-17.37 µm respectively. All isolates produced both beaked and unbeaked conidia. The beak length of conidia varied from 18.7-23.81 µm. *Alternaria blotch*, causal organism *A. mali* 21 isolates of *A. mali* were collected from different locations. Average conidial size ranged from 21.36 to 31.74 x 8.34 to 14.48 µm. Among the isolates of *A. mali* size of conidia 19–50 µm ×5–9 µm in nature and 20–59 µm ×8–13 µm in culture, with 3–8 transverse septa and usually no longitudinal septa or only 1 longitudinal septa were found [33].

V. CONCLUSION

Rapeseed Mustard (*Brassica spp.*) is the principal oil-producing crop of Bangladesh and *Alternaria* leaf blight caused by *Alternaria brassicae*, is one of the major disease of rapeseed mustard. This research was conducted to find out existence of physiological races of *Alternaria* spp. causing *Alternaria* leaf blight of mustard on the basis of cultural and morphological aspects. The experiment was laid out in the completely randomized design with three replications. Ten isolates of *Alternaria* spp. were collected from ten different mustard growing districts of Bangladesh. Three different media and two different temperatures were

used to measure growth and development of *Alternaria* spp..

All the 10 isolates showed variation in the terms of cultural and morphological characteristics. Among three different culture media, potato carrot agar medium at 25°C showed the best performance in the terms of radial mycelial growth. Colour of the colonies of *Alternaria* spp. showed variation among ten isolates. Olivaceous green to black color colony developed on PDA medium. All the isolates produced Circular colony and the texture were cottony to velvety. All isolates showed compact type compactness. Variation also observed between surface and sub surface colour. Surface colour varied from light brown to deep brown. Subsurface colour varied from light brown to black and pinkish. Zonation was present both surface and subsurface in some isolates and some isolates showed no zonation on both side. Effect of media on sporulation significantly differed among the isolates. The highest number of conidia production was recorded 48.17 to 59.79 × 10⁶/ml was counted RAJ_{Ab} on Potato Carrot media at 25°C temperature. Of all the isolates of *Alternaria* spp. with maximum in isolate RAJ_{Ab} and minimum in NAO_{Ab}. Temperature showed an influence on sporulation. (Data not shown).

Effect of media on sporulation time differed significantly among the isolates. The minimum days (4 days) required for sporulation in PCDA followed by CDA.

Remarkable variation among different *Alternaria* spp. isolates were observed in length, breadth and beak size of the conidia. The conidial length varied within a range of 41.56µm to 117.54µm and the breadths were varied from 10.34 µm to 23.12 µm. All isolates were muriform and deep brown to light brown in colour with a beak length of 16.78 µm to 72.65 µm.

On the basis of the above results and discussion it can be summarized that- variability exists in the pathogen of *alternaria* leaf blight caused by *Alternaria* spp. prevailing in the rapeseed mustard growing areas of Bangladesh. Potato Carrot agar medium and 25°C temperature were appeared to be the best medium and temperature respectively for the mycelial growth and sporulation of this fungal pathogen. More research should be conducted on molecular characterization of this isolates to find out the phylogenetic relationship.

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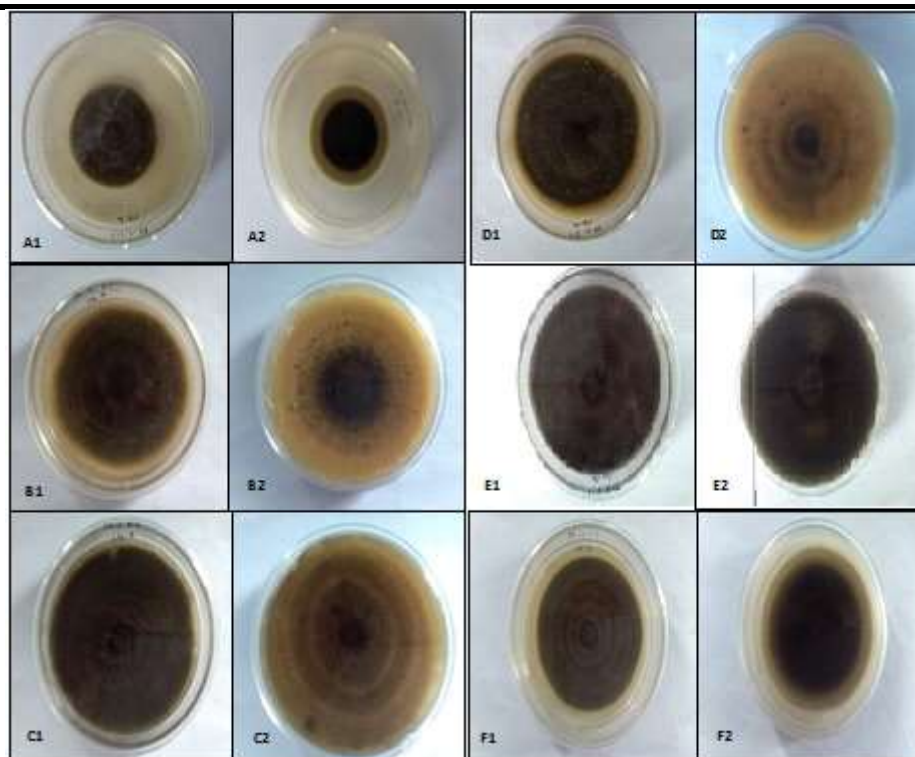


Plate.1: Colony charecters of different isolates of A. Brassicae on PDA media

1. Surface 2. Sub-surface

A. DHA_{Ab} B. GAZ_{Ab} C. MYM_{Ab} D. PAB_{Ab} E. RAN_{Ab} F. NAT_{Ab}

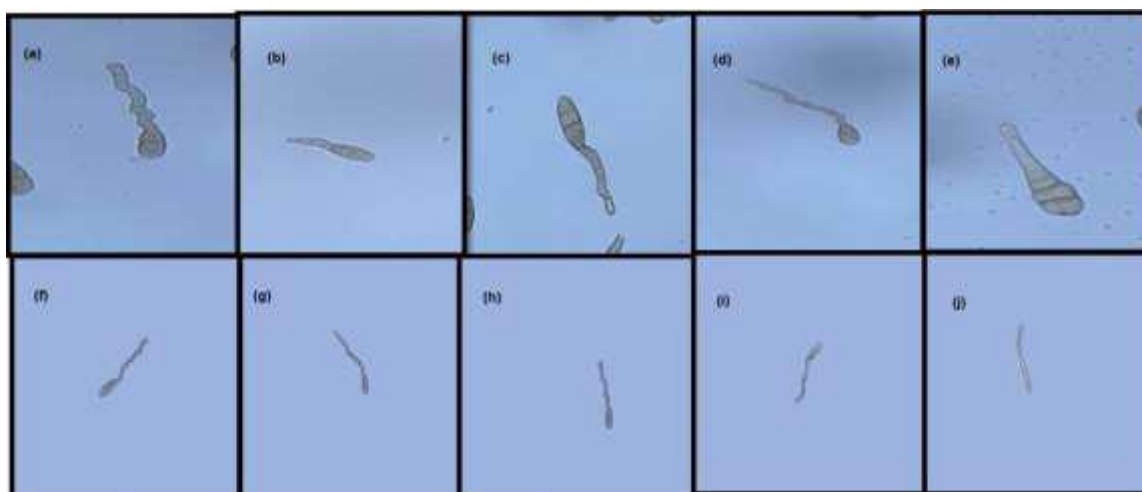


Plate.2: Conidial characteristics of A.brassicae A. DHA_{Ab} B. GAZ_{Ab} C. MYM_{Ab}

D. PAB_{Ab} E. RAN_{Ab} F. NAT_{Ab} G. NAO_{Ab} H. LAL_{Ab} I. BOG_{Ab} J. RAJ_{Ab}