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Full Length Research Paper

Aggressiveness, diversity and distribution of *Alternaria brassicae* isolates infecting oilseed *Brassica* in India

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***Alternaria brassicae* (Berk.) Sacc., a necrotrophic fungus devastating oilseed *Brassica* crops in India, causes up to 47% reduction in seed yield. Morphological characteristics of different isolates revealed variation in growth, shape and pigmentation of colony, conidial measurements and number of septa. Conidial length varied from 106.7 to 285.9 µm, width 33.5 to 57.0 µm and beak length 41.4 to 180.0 µm. Number of horizontal septa varied from 3.2 to 8.0 and vertical 0.3 to 1.4. Different synthetic media showed profound variation in mycelial growth of *A. brassicae* isolates and the poor sporulation indicated that the fungus requires some organic sources of nutrition for better growth and sporulation. The degree of sporulation of *A. brassicae* isolates is a function of nutrition proved for the first time. Per cent inhibition of mycelial growth showed diverge among *A. brassicae* isolates, which may be due to the variation towards fungicidal sensitivity among isolates. Pathogen aggressiveness study demonstrated the existence of considerable variation in tolerance of *Brassica* species to *A. brassicae*, which is proved with the location specific disease severity.**

Key words: Variability, *Alternaria brassicae*, fungicides sensitivity, aggressiveness, *Brassica*.

INTRODUCTION

Indian mustard [*Brassica juncea* (L.) Czern and Coss.] alone contributes about 80% of the total rapeseed-mustard which is one of the major oilseed crops cultivated in India (AICRP-RM, 2011). *Alternaria* blight disease caused by *Alternaria brassicae* (Berk.) Sacc. has been reported from all the continents of the world and is one among the important diseases of Indian mustard causing up to 47% yield losses (Meena et al., 2010a) with no proven source of resistance against the disease reported till date in any of the hosts (Meena et al., 2010b). Little work on variability has been reported on genetic structure of *A. brassicicola* population suggests the occurrence of sexual recombination (Bock et al., 2005). Information on trends in variability of *A. brassicae* population in India is lacking. In view of the economic importance of rapeseed-mustard crops to India, it seemed desirable to learn more of the biology of the *A. brassicae* that attack these crops.

A comparative knowledge of the nutritional patterns and factors influencing its growth are prerequisite to any study leading to the understanding of host-pathogen relationship and specificity. *Alternaria* blight severity on rapeseed-mustard differs among seasons and regions as also between individual crops within a region. This may be due to existence of variability among isolates of *Alternaria* species. Some reports on the existence of morphological variability within the isolates of other *Alternaria* species have been reported by earlier workers (Meena et al., 2005; Varma et al., 2006). Special attention was focused on variability in pathogen diversity and aggressiveness of 30 *A. brassicae* isolates collected from different geographical locations from different *Brassica* hosts.

MATERIALS AND METHODS

Pathosystem

The current study is part of a project on the population of the *Brassica–Alternaria* system. The study area includes three

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Table 1. *Alternaria brassicae* isolates infecting *Brassica* species at different geographical locations.

A. brassicae isolate	Host	Date of collection	Location	Latitude and longitude	Plant part
BAB-02	*B. napus*	15-Feb-05	Jammu, J & K	32° 44' N, 74° 54'E	leaf
BAB-19	*B. juncea*	22-Jun-09	Bharatpur, Raj	27° 15' N, 77° 30'E	seed
BAB-20	*B. juncea*	28-Feb-05	Alwar, Raj	27° 34' N, 76° 36'E	pod
BAB-23	*B. carinata*	23-Feb-05	Behrampore, WB	24° 6' N, 88° 15'E	leaf
BAB-30	*B. rapa* spp *ys*	24-Jan-06	Berhampore, WB	24^{o}6' N, 88^{o}19' E	leaf
BAB-08	*B. juncea*	8-Mar-06	Dhamsya, Jaipur, Raj	26°88' N,76°15'E	leaf
BAB-39	*B. carinata*	1-Feb-10	Kangra, HP	32° 05' N, 76° 18'E	leaf
BAB-40	*B. juncea*	1-Feb-10	Kangra, HP	32° 05' N, 76° 18'	leaf
BAB-41	*B. napus*	3-Mar-10	Kangra, HP	32° 05' N, 76° 18'E	leaf
BAB-42	*B. juncea*	10-Feb-10	Parwai, Jhansi, UP	25° 27' N, 78° 37'E	leaf
BAB-43	*B. juncea*	21-Jan-10	Hazaribag, Jharkhand	23º 59' N, 85º 25'E	leaf
BAB-44	*B. juncea*	30-Jan-10	Nagina, Bijnor, UP	29° 27´N, 78° 29´E	leaf
BAB-45	*B. juncea*	26-Jan-10	Mandore, Jodhpur, Raj	26° 18' N, 73° 04'E	leaf
BAB-47	*B. juncea*	Feb-10	Tonk, Raj	26° 11' N, 75° 50'E	leaf
BAB-48	*B. juncea*	4-Feb-10	Shivrajpur, Kanpur, UP	26° 28'N 80° 21'E	leaf
BAB-49	*B. juncea*	25-Jan-10	Jobner, Jaipur, Raj	26° 95' N,75° 34'E	leaf
BAB-50	*B. juncea*	10-Feb-10	Jhansi, UP	25° 27' N, 78° 37'E	leaf
BAB-04	*B. rapa* spp *toria*	2-Mar-05	Kamrup, Assam	25°74’ N,93° 85’E	pod
BAB-06	*B. juncea*	3-Mar-05	Golaghat, Assam	22° 7' N, 92° 6'E	leaf
BAB-18	*B. juncea*	18-Jun-08	Pantnagar, Uttarakhand	29°03' N, 79°31'E	leaf
BAB-28	*B. juncea*	2-Mar-05	Ri-Bhoi, Meghalaya	25° 50’ N, 90°55’E	leaf
BAB-29	*B. juncea*	5-Mar-05	Dimapur, Nagaland	25° 55' N, 93° 44'E	leaf

BAB: *Brassica Alternaria brassicae.*

sub-regions from the north western to north eastern between Rajasthan (27° 00' N, 74° 00' E) and north east states (Latitude 21° 58' and 24° 35' N, Longitude 92° 15' and 93° 29' E), and comprises a total of 30 populations of *A. brassicae,* which is a common necrotrophic pathogen of *Brassica* species in this region, producing black lesions on leaves, stems and developing pods (Meena et al., 2010). The common occurrence of infected seeds and stubbles in soil indicates the potential for vertical transmission (parent to offspring) to play a role in the epidemiology of the interaction. A survey was conducted to observe the disease pressure under AICRP-RM throughout monitoring for these regions.

Collection of *A. brassicae* isolates

Plant material infected with *A. brassicae* was sampled randomly from different geographical locations on *Brassica* species cultivated in India was collected and designated as BAB stands for *Brassica Alternaria brassicae* (Table 1). These selected infected spots were washed 3-4 times in sterilized distilled water and then surface sterilized by dipping in 4% NaOCl solution for 1 min, followed by washing with sterilized water 3-4 times. Surface sterilized leaf spot pieces were then aseptically transferred into 9 cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25±2°C for seven days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot pieces on the medium were aseptically transferred into another Petri plate containing PDA medium, where it was grown for 15 days at 23±2°C in the BOD incubator. On the basis of their conidiophore and conidial morphology as described by Simmons (2007), the pathogen was identified as *Alternaria brassicae* (Berk.) Sacc. and purified by single spore isolation method. The isolated fungal pathogen cultures were maintained on PDA slants at 4°C. The 22 isolates (NAIMCC F 02599-02620) have been submitted to the National Bureau for Agriculturally Important Microorganisms (ICAR), Mau Nath Bhanjan (Uttar Pradesh, India) to develop the National Repository on *Alternaria* spp.

Single-spore colonies were prepared with the help of stereo microscope by grown on potato dextrose agar (PDA). Sub-culturing was done after three months on PDA. Brassica leaf broth (BLB) medium was used at fourth subculturing to maintain the aggressiveness of the isolates every year. BLB medium was prepared using 250 g fresh leaf of *Brassica juncea* cultivar Varuna in 1 L distilled water supplemented with 15 g sucrose. Culture slants of isolates were stored on PDA in the refrigerator. A total of 22 *A. brassicae* isolates were collected and maintained (Table 1).

Morphological variability

Ocular micrometer was calibrated and by use of micrometry (Meena et al., 2005), morphological variability among the 22 isolates of *A. brassicae* was studied in 2010-11. Total fifty conidia from each slide were examined at 40X magnification of light microscope and measured using ocular and stage micrometer. The average was used to calculate the conidial length, width, beak length and number of horizontal and vertical septa.

Effect of different temperature and relative humidity on *A. brassicae* isolates

Effect of temperature on radial growth of only limited isolates of *A. brassicae* (from Bharatpur, Pantnagar, Alwar, Kanpur, Hazaribag,

Table 2. Sporulation index.

Sign	Index	No of spores per microscopic fields
-	Absent	Nil
+	Trace	1-10
++	Mild	11-30
+++	Moderate	31-50
++++	Abundant	More than 50

Berhampur) was studied. Petri plates containing PDA medium were inoculated with agar blocks taken from fresh actively growing culture plates. Plates were incubated for 10 days at 15, 20, 25, 30, 35 and 40°C temperature in B.O.D. incubator at a constant 100% relative humidity (RH) maintained in separate lower lid of the plate which was fixed with sticky tape on the other lower lid of fungal culture plates by mixing different quantities of KOH and distilled water (Chatopadhyay and Appaji, 2000). Similarly, *A. brassicae* isolates were incubated at 25°C in B.O.D. incubator for 10 days at 100, 95, 90, 85, 80, 75, 70, 60 and 50% RH. Radial growth was measured after 10 days of inoculation for all isolates.

Cultural characteristics

To observe the variation among the isolates in cultural characteristics, that is, colony colour, shape, margin and texture a separate experiment was conducted on PDA and incubated in B.O.B. incubator at 25°C temperature and 100% relative humidity.

Effect of culture media on mycelia growth and sporulation

To study the effect of culture media on mycelia growth and sporulation, five different culture media, viz Asthana and Hawker's, Brown's, Czapek's, Elliot's and Richard's media were prepared in 250 ml conical flask. Each treatment was replicated four times. A 2.0 mm diameter mycelium disc from 10 day old culture grown on PDA was transferred into each 250 ml conical flask containing 25 ml test media were incubated in B.O.D. incubator at 25°C for 20 days. To observe the sporulation on 21 day old culture the filtrate was diluted thousand times and spores per microscopic field were counted with the help of Heamocytometer for sporulation index and results obtained are presented in the table. The fungal growth of each flask was separated on oven dried whatman filter paper No. 42 and subsequently oven dried for 48 h at 50°C to obtain the true weight of the mycelial mat. The dry weight of the fungal growth was obtained by subtracting the weight of filter paper with four replications. The fungal growth of each isolate by weight of oven dried mycelial mat was obtained.

Sporulation index

This is shown is Table 2.

Fungicide sensitivity among isolates

Effect of four fungicides *viz*, Sure (carbendazim 12% + mancozeb 63% WP), Mancozeb, Ridomil-MZ 72 WP (metalaxyl 8% + mancozeb 64% WP) and Kvistin (carbendazim 50% WP) on mycelial growth of *A. brassicae* isolates was studied *in vitro* at 200 and 500 ppm concentrations. Stock solutions (10, 000 mg/l) were prepared for each active ingredient in distilled water. The solvent concentration in both controls and assays never exceeded 1% (v/v). Aliquots of stock solutions were incorporated to autoclaved PDA medium at 45-50°C to get desired concentrations of 200 and 500 ppm by mixed thoroughly before plating using poisoned food technique (Nene and Thapaliyal, 1993). Amended medium with the fungicides was then poured into each Petri plate and inoculated with 2 mm mycelial disc of each isolate separately and incubated at 25±2°C. Medium without fungicide served as control. Thus, isolates were classified into three major groups' *viz*., highly resistant (HR), moderately resistant (MR) and sensitive (S). The radial growth of *A. brassicae* colonies in diameter of tested isolates on PDA medium was measured after 10 days and per cent inhibition was calculated by the following formula.

Percentage inhibition = Growth of the pathogen in control – the presence of antagonist × 100/ Growth of pathogen in control plate.

Pathogen aggressiveness

In this experiment, two leaves (3rd/4th true leaves) were collected from 45 day-old plants having approximately six leaves to determined aggressiveness of *A. brassicae* isolates of 8 different *Brassica* species. Five cultivar/ genotypes of *B. juncea* including PHR-2, PAB-9511 and EC-399299 as tolerant and Varuna and Rohini as susceptible, *Sinapis alba, Eruca sativa, B. oleracea, B. carinata* (Kiran)*, B. rapa* spp *toria* (PT-303)*, B. rapa* spp. *brown sarson* (BSH-1) and *B. napus* (GSL-1) were screened for reaction against 30 isolates of *A. brassicae.*

A replicate for the detached leaf test consisted of three randomly selected leaves from a set of the $3^{rd}/4^{th}$ true leaves collected from several plants that were pooled. The turgidity of detached leaves was maintained by plugging the petioles with moist cotton in 200 mm size Petri plates having fourfold moist sterilized filter paper in bottom. Conidia of a 10 day-old culture were washed off with distilled water and filtered through cheesecloth of 0.1 mm diameter mesh size. The conidial suspension was then shaken and supplemented with 10 µl Tween-20 l^{-1} of suspension. The concentration of the conidial suspension was determined at least three times using a haematocytometer and adjusted to $5x10^4$ conidia ml^{-1} then incubated at 25±2°C for 3 days in the dark controlled-temperature room. Isolate aggressiveness was also evaluated with regard to the growth rate of individual lesions. Lesion size was measured after 10 days of inoculation.

RESULTS AND DISCUSSION

The pathosystem

Severity of *Alternaria* blight on oilseed Brassicas differ from seasons to season and among regions as also between individual crops within a region. This may be due to existence of variability among isolates of *Alternaria* species. Mean maximum Alternaria blight disease severity both on leaves and *pods* was observed during 2009 at Pantnagar followed by Faizabad, Dholi, Kangra, Kanpur, Morena and Hisar where the weather conditions were conducive for development of the pathogen. Disease pressure was observed mild at Bharatpur, Sriganganagar and Jaipur districts of Rajasthan (Table 3). This reflected the adaptation of the respective isolates to the ambient conditions in the different cropping areas, where the disease occurs in varied proportions in different years. Disease dynamics in the *Brassica*-

Table 3. Percent *Alternaria* blight disease severity on *Brassica* spp. during 2009.

Genotypes	JAG	DOL	HSR	PNT	MOR	KNG	NAV	BHP
PHR 2 (*Bj*)	29.9 (25.0)	43.6 (47.5)	31.6 (27.5)	39.0 (39.6)	46.5 (52.7)	41.7 (44.3)	20.1 (11.7)	15.2 (27.1)
PBC 9221 (*Bc*)	15.7 (7.5)	43.6 (47.5)	18.4 (10.0)	39.0 (39.6)	42.9 (46.3)	36.3 (35.0)	7.9 (1.9)	7.7 (16.1)
GSL 1 (*Bn*)	24.7 (17.5)	50.8 (60.0)	15.9 (7.5)	48.6 (56.3)	49.4 (57.7)	39.7 (40.9)	15.2 (6.9)	8.4 (16.8)
VARUNA (*Bj*)	42.1 (45.0)	49.3 (57.5)	38.2 (38.3)	51.0 (60.4)	50.2 (59.0)	53.3 (64.3)	17.4 (8.9)	25.3 (33.0)
C.D. (P < 0.05)	6.5	2.8	3.1	3.7	10.9	4.8	1.5	1.1

Figures in parenthesis are arc sin transformation and others are original values.

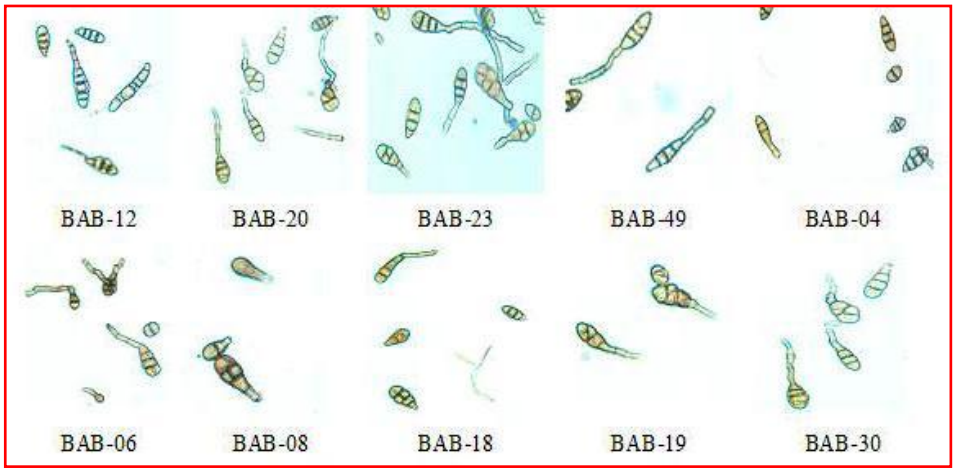

Figure 1. Conidia of different *A. brassicae* isolates.

Alternaria system are highly epidemic, and follow a clear 'boom-and-bust' pattern, with prevalence in local populations often reaching 100% by the end of February during the growing season (Kolte, 1985; Chattopadhyay et al., 2005).

Morphological variability among isolates

The 22 single-spore isolates of *A. brassicae* showed significant (*P*<0.05) morphological variability (Figure 1) in respect of conidia length, conidia width, beak length and number of septa (Table 4). Mean conidia size was 182.8 μm (106.7 - 285.9 μm) x 41.0 μm (33.5 - 57.0 μm) with a beak size of 95.0 μm (41.4 – 180.0 μm). Mean transverse and longitudinal septa number were 4.4 (3.2 – 8.0) and 0.7 (0.1 – 1.4), respectively (Table 4). Finally it was revealed that the smallest size of conidia and lowest number of septa was in Ri-Bhoi, Meghalaya isolate (BAB 28) while maximum size was in Pantnagar, Uttarakhand isolate (BAB 18). Microscopic examination of conidia at 40X magnification revealed variability in conidia size and could be categorized into four groups, that is, Group I: 100-150 μm (BAB-08, BAB-19, BAB-28, BAB-39, BAB-43, BAB-44 and BAB-45), Group II: 150-200 (BAB-02, BAB-04, BAB-20, BAB-29, BAB-30, BAB-40, BAB-42, BAB-47 and BAB-48), Group III: 200-250 μm (BAB-23, BAB-41 and BAB-49) and Group IV: more than 250 μm (BAB-06, BAB-18 and BAB-50). These results are in agreement with earlier workers (Awasthi and Kolte, 1989; Varma et al., 2006; Meena et al., 2005; Kaur et al., 2007; Singh et al., 2007), who observed morphological variability in different geographical isolates within an *Alternaria* species.

Effect of temperature and relative humidity on growth and sporulation

Mycelial growth and sporulation varied among the different isolates at different temperatures. However, the highest radial growth of *A. brassicae* mycelia was at 25°C *in vitro* (Table 5) for all the geographical isolates of the pathogen tested with abundant sporulation being highest in the same. Mycelial growth was most favoured by 100% relative humidity with a gradual reduction in growth, sporulation till 70% RH and a decrease in growth and sporulation at 60 and 50% RH. Variability of several *Alternaria* species in respect of mycelial growth and sporulation at different temperatures, RH ranges has

Table 4. Conidial size of different geographical isolates of *A. brassicae**.

A. brassicae isolates	Length (µm)	Width (µm)	Beak length (µm)	No. of Septa	
				Horizontal	Vertical
BAB-02	152.3	47.5	47.3	3.8	1.3
BAB-04	185.1	47.3	95.2	3.5	0.9
BAB-06	252.1	33.9	178.0	6.8	0.1
BAB-08	106.7	33.5	48.3	3.8	0.3
BAB-18	285.9	47.5	180.0	6.3	0.5
BAB-19	140.6	44.2	61.4	3.2	0.9
BAB-20	189.7	35.6	111.5	4.2	0.3
BAB-23	211.7	41.8	125.9	3.7	0.7
BAB-28	122.2	34.7	44.7	3.4	0.4
BAB-29	198.4	43.4	104.7	3.8	0.9
BAB-30	193.6	57.0	87.3	3.2	0.9
BAB-39	144.5	37.2	70.9	3.2	0.5
BAB-40	198.2	41.6	100.4	5.6	1.1
BAB-41	206.5	44.2	116.8	4.0	0.8
BAB-42	196.6	33.7	113.9	5.5	0.1
BAB-43	147.5	36.6	66.7	3.3	0.5
BAB-44	140.6	35.4	67.9	3.5	0.4
BAB-45	144.1	48.3	41.4	3.4	1.4
BAB-47	168.1	40.4	76.2	4.1	0.9
BAB-48	151.1	33.9	73.9	4.6	0.4
BAB-49	201.2	44.6	105.9	6.1	1.1
BAB-50	284.3	40.0	172.7	8.0	0.6
Mean	182.8	41.0	95.0	4.4	0.7
CV%	26.07	15.25	43.82	30.82	53.41

*Average of 50 conidia in each isolates.

Table 5. Mycelial growth of *A. brassicae* under different temperatures and relative humidity conditions.

A. brassicae isolate	Radial growth (mm)*														
	Temperatures (°C)[1]						Relative Humidity (%)[2]								
	15	20	25	30	35	40	50	60	70	75	80	85	90	95	100
BAB-18	16.7	16.0	24.7	24.7	16.3	12.3	10.0	13.3	20.0	22.3	24.3	26.3	24.7	29.3	31.3
BAB-19	11.0	16.3	27.0	25.3	16.0	12.7	10.5	13.5	20.5	21.7	23.5	25.7	27.0	29.0	30.0
BAB-20	15.3	19.0	30.0	27.3	18.0	12.7	10.3	13.0	20.3	21.3	24.0	26.7	30.0	28.7	29.5
BAB-23	11.0	15.3	24.0	21.0	15.0	13.0	11.7	14.0	21.0	23.0	25.0	27.7	24.0	30.7	31.0
BAB-30	16.7	16.7	28.0	23.3	16.3	12.3	9.7	12.7	19.5	21.5	23.7	26.7	28.0	29.3	30.0
BAB-43	14.3	15.0	29.3	29.0	16.0	10.0	10.7	13.0	20.3	22.0	24.7	27.0	29.3	29.0	30.0
BAB-48	15.7	16.3	24.3	27.0	16.0	14.0	11.0	13.7	19.7	22.7	24.5	27.3	24.3	29.3	30.5
L.S.D.	Temperature: 2.2; Isolate: 1.8						Relative Humidity: 3.2; isolate: 2.1								
($P < 0.05$)	Temperature x isolate: 2.1						Relative humidity x isolate: 2.2								

*mean of three replications, [1]Relative Humidity 100%, [2]Temperature 25°C.

been reported earlier by many workers (Sharma and Tewari, 1998, Meena et al., 2005; Singh et al., 2007). Most favourable optimal temperature 23-25°C for sporulation has been reported (Kadian and Saharan, 1984; Ansari et al., 1989). In the present study, different temperatures were found optimum for mycelial growth and sporulation of different isolates of *A. brassicae*, which showed cultural variability among them. This temperature

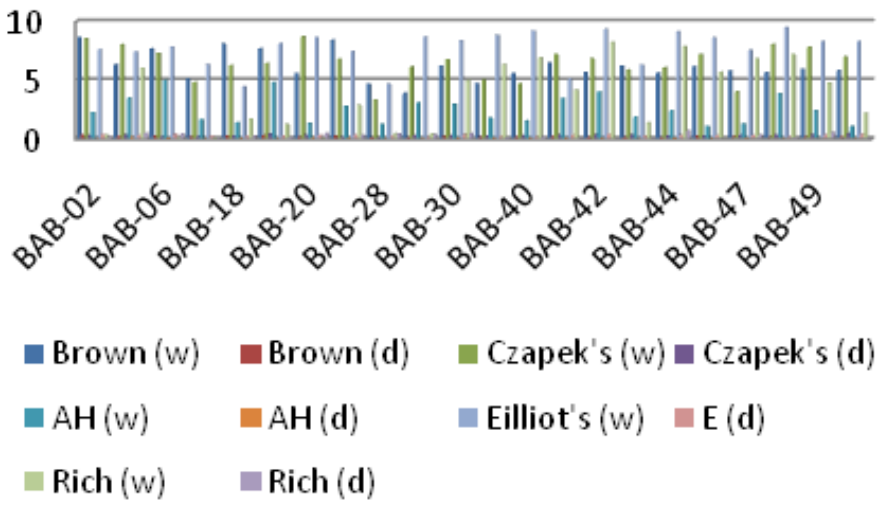

Figure 2. Variability in wet and dry mycelial weight of *A. brassicae* isolates on different media.

ranged from 25 to 30°C and 15 to 35°C for mycelia growth and sporulation, respectively. The enormous disparity available among only twenty two isolates of *A. brassicae* also indicates their ability to adapt to varied climatic situations. These findings are supported by Singh et al. (2007), who also found variability among different *A. brassicae* isolates of different geographical origin for temperature requirement. Further, the higher temperature and RH being favourable for Berhampore isolate could be related with climatic condition of the West Bengal state, where temperature and RH during rapeseed-mustard crop season is generally higher than other geographical regions in India (Table 1).

Cultural characteristic of *A. brassicae* isolates

Isolates of *A. brassicae* showed variable cultural characteristics varied from regular to irregular, cottony white, dark green to light brown mycelial growth and based on characteristics, all *A. brassicae* isolates could be grouped into four colony types. Group 1 isolates produced white to pale gray or apricot orange colonies with a cottony texture. Tufts of sterile white hyphae were present at the center of the colony. Group 2 isolates produced dark olive gray to iron gray colonies with wavy or torn margin and fluffy to woolly colony texture. Group 3 isolates produced pale olive gray to olive gray colonies, often with a very thin (1 to 2 mm) white margin with woolly to cottony colony texture. Diffusible pigments absent, although all isolates produced whitish crystals in agar medium underneath the mycelial mat and some produced crystals in great abundance. Group 4 consisted of colonies showed lettuce green to olive green and usually had a prominent (2 to 5 mm) white margin. Colony texture was fulfy to woolly. This group did not produce diffusible pigments, but about half of the isolates produced whitish crystals in the agar medium underneath the mycelial mat.

Effect of culture media

Different test synthetic broth media showed profound variation in wet biomass of mycelium of *A. brassicae* isolates infecting rapeseed-mustard. Maximum wet mycelial biomass of *A. brassicae* isolates (BAB 48, BAB 42, BAB, 40 and BAB 44) was recorded in Elliot's medium, followed by Brown's medium, Czapeck's medium. However, least mycelial wet biomass was observed for all isolates in Asthana and Howker's medium. Maximum dry mycelial biomass of *A. brassicae* isolates (BAB 50, BAB 19, BAB, 49 and BAB 42) was recorded in Czapeck's medium followed by Elliot's medium. However, least mycelial dry weight was observed for all isolates in Brown's medium and Asthana and Hawker's medium (Figure 2).

Maximum sporulation was observed in BAB 28 followed by BAB-18 isolate in Eilliot's, Richard's and Asthana and Howker's medium. Brown's medium showed no sporulation in all isolates. However, Asthana and Howker's medium showed good sporulation in almost all the isolates (Table 6). Poor sporulation of *A. brassicae* in different synthetic media indicated that the fungus require some organic sources of nutrition for better growth and sporulation.

Fungicide sensitivity among isolates

Maximum per cent mycelial growth inhibition over control at 200 ppm concentration was observed in metalaxyl +

Table 6. Sporulation of *Alternaria brassicae* isolates on different culture media.

Isolates	Brown's	Eilliot's	Asthana and Howker's	Czapek's	Richard's
BAB-02	-	+	++	+	-
BAB-04	-	++	+	++	+++
BAB-06	-	+	+	+	-
BAB-08	-	+	++	+	-
BAB-18	-	++++	++	++	++
BAB-19	-	-	+	-	+
BAB-20	-	-	++++	+	++
BAB-23	-	-	++	+	+
BAB-28	-	++++	++++	++++	+++
BAB-29	-	+	+	+	-
BAB-30	-	-	++	+	++
BAB-39	-	-	++	-	-
BAB-40	-	-	++	+	+
BAB-41	-	++	+	+	-
BAB-42	-	-	+	+	+++
BAB-43	-	+	-	-	+
BAB-44	-	+	++	+	+
BAB-45	-	+	-	-	+
BAB-47	-	+	+	+	+
BAB-48	-	+	+	+	-
BAB-49	-	-	+++	+	-
BAB-50	-	++	+	++	+++

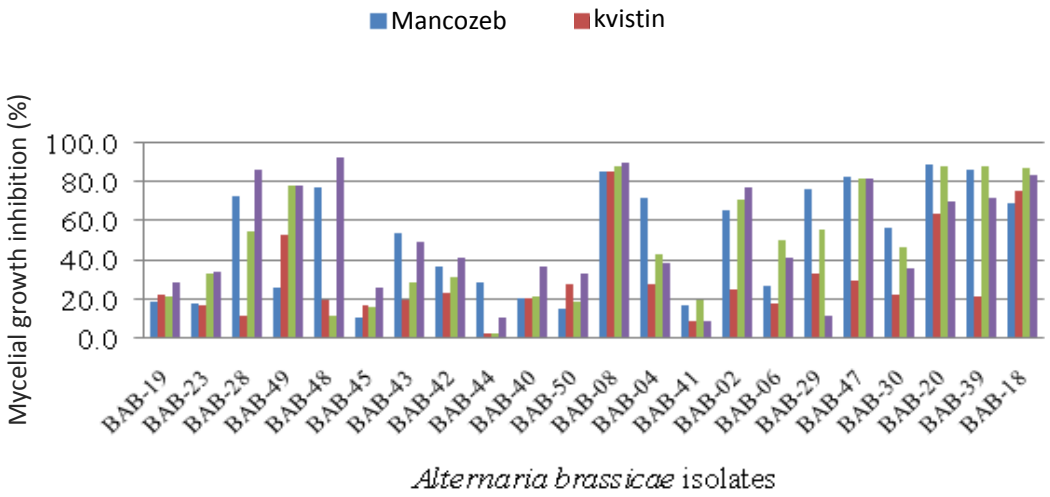

Figure 3. Fungicide sensitivity of *A. brassicae* isolates at 200 ppm concentration.

mancozeb fungicide with a wide variation ranged from 8.9 percent (BAB- 41) to 92.5% (BAB-48). While at 500 ppm, growth inhibition was in carbendazim fungicide with a wide variation ranged from 8.9% (BAB- 41) to 92.5% (BAB-48). However, fungicide carbendazim provided least mycelial growth inhibition over control ranged from 2.6 (BAB-44) to 86.1% (BAB-08). Isolates, BAB-44, BAB-45, BAB-19, BAB-41, BAB-40, BAB-50 and BAB-23 were found sensitive to fungicides which showed similar response against all four test fungicides in inhibiting mycelial growth. Isolates viz., BAB-08, BAB-18, BAB-47, BAB-20, BAB-39 and BAB-48 were found highly resistant to test fungicides, seems to be highly virulent (Figure 3), Based on the mycelial growth in fungicide assay at 500

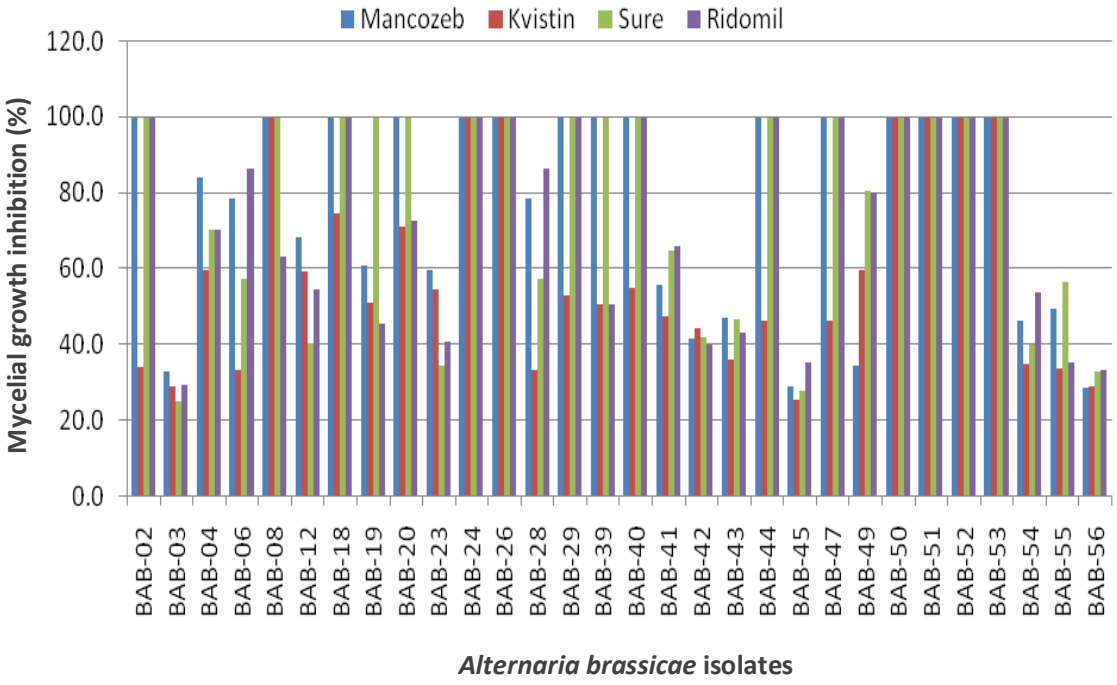

Figure 4. Fungicide sensitivity of *A. brassicae* isolates at 500 ppm concentration.

ppm concentration (Figure 4), *A. brassicae* isolates were categorized into three major group's viz., highly resistant (BAB-02, BAB-18, BAB-24, BAB-26, BAB-29, BAB-40, BAB-44, BAB-47, BAB-50, BAB-51, BAB-52, BAB-53), moderately resistant (BAB-06, BAB-08, BAB-12, BAB-19BAB-23, BAB-39, BAB-41, BAB-43, BAB-54) and sensitive (BAB-03, BAB-04, BAB-20, BAB-28, BAB-42, BAB-45, BAB-49, BAB-55, BAB-56). Similar observations recorded when studying the effect of difenoconazole on *A. alternata* (Reuveni and Sheglov, 2002).

Results indicated that all test fungicides showed inhibition over control (Figure 2) but differ in percent inhibition of mycelial growth which may be due to the variation towards sensitivity among *A. brassicae* isolates. The present findings are in line with Meena et al. (2004) in controlling *A. brassicae* with mancozeb, but differ in percent inhibition of mycelial growth. Our results indicated that mancozeb and sure caused significant reductions of mycelial growth of *A. Brassicae.* However, iprodione was efficient in reducing germ tube length with EC_{50} below 10 mg/l observed for *A. brassicicola* isolates (Huang and Levy, 1995) but these authors also found that this fungicide was able to inhibit germination at concentrations as low as 5 mg/l. During the course of this study, four *A. brassiciae* isolates highly resistant to mancozeb were identified. Resistance of *A. brassicicola* to iprodione has already been documented by Huang and Levy (1995). Our results proved with the earlier study of thirteen isolates of *A. brassicae* tested on oilseed rape differed in their virulence (Mirdha, 1983). Based on radial mycelial growth, *A. brassicae* isolates could be categorized into three major groups *viz.,* highly resistant, moderately resistant and sensitive (Table 7).

Pathogen aggressiveness

Different isolates of *A. brassicae* showed variable response on host differentials of *Brassica* species. Significant tolerance with minimum lesion size was observed in *Brassica alba,* EC-399299, *B. juncea* (PAB 9511), *Eruca sativa* and *B. carinata, B. napus*. Variation in tolerance and susceptibility on same host depending on aggressiveness of isolates revealed that the variability exist among *A. brassicae* isolates (Table 7). Different *Brassica* species showed variable reaction against same isolate, which reflect the variation among the geographical isolates that may be used as host differentials against *A. brassicae*. Highest mean susceptibility for *A. brassicae* isolates was observed on *B. juncea* cultivars Varuna and Rohini while tolerance was recorded on *B. alba* and *B. napus*. Penetration by pathogen through wounds of host plants has been a significant component of infection process for *Alternaria* spp. (Rotem, 1994).

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Table 7. Reaction of different genotypes/ species of *Brassica* against *Alternaria brassicae* isolates.

Isolates	*S. alba*	PHR-2(*B.j*)	(*B.j*) Varuna	(*B.j*) PAB-9511	(*B.j*) Rohini	(*B.j*) EC399299	*B. rapa* (*BSH-1*)	*E. sativa*	*B. rapa* ssp toria	*B. carinata*	*B. napus* (*GSL-1*)	*B. oleracea*
BAB-01	0.0	6.5	2.0	5.5	5.0	3.5	3.9	8.7	6.0	6.8	4.3	6.3
BAB-02	10.3	4.6	9.0	6.6	6.6	4.8	7.5	7.3	5.1	6.0	1.5	7.3
BAB-03	8.5	0.0	4.1	6.8	6.7	11.0	6.0	8.1	5.8	9.3	5.1	8.3
BAB-04	7.4	10.8	8.5	7.4	10.0	9.8	6.6	9.0	8.4	2.8	5.8	9.4
BAB-05	7.5	8.2	15.5	4.6	5.7	6.6	4.6	9.7	8.6	8.3	3.1	8.5
BAB-06	0.0	7.5	17.8	4.4	5.8	6.8	6.0	8.4	10.3	10.3	9.5	8.1
BAB-07	0.0	9.0	4.8	5.8	6.3	10.7	5.8	7.9	5.8	1.5	5.2	9.7
BAB-08	7.0	7.8	17.3	7.1	18.9	7.4	6.2	8.5	8.0	11.1	6.5	10.2
BAB-09	9.8	3.6	6.6	7.0	7.1	8.4	9.6	7.9	12.2	7.1	6.8	7.1
BAB-10	7.9	7.8	17.8	0.0	20.0	8.0	5.1	9.2	7.0	5.0	7.1	9.6
BAB-11	9.3	6.3	6.0	5.4	8.3	9.1	3.8	2.4	8.0	3.3	8.8	8.0
BAB-12	7.1	5.9	8.0	3.8	6.6	9.6	6.3	6.2	7.7	5.0	6.1	9.3
BAB-13	8.2	12.2	18.7	8.5	18.0	8.2	8.9	5.8	8.4	3.4	3.5	8.0
BAB-14	4.0	8.7	9.4	6.8	7.0	6.3	7.8	10.5	9.8	6.3	3.3	9.2
BAB-15	3.5	7.9	7.5	7.8	8.1	6.9	3.6	7.9	6.2	11.6	5.3	5.9
BAB-16	0.0	6.8	4.0	5.3	8.0	6.0	8.0	7.1	6.3	10.5	7.4	8.8
BAB-17	5.9	11.0	7.1	9.0	16.8	7.9	6.6	7.1	10.8	11.0	7.4	8.0
BAB-18	8.3	9.9	17.2	7.6	15.2	5.3	7.3	8.3	6.1	9.0	10.7	6.4
BAB-19	9.6	3.3	5.0	10.5	7.5	1.9	8.0	11.2	7.1	9.3	12.3	8.7
BAB-20	6.3	7.8	11.9	7.5	6.6	0.0	8.1	8.2	8.0	8.3	11.0	11.0
BAB-21	5.8	5.8	9.8	9.2	6.8	6.9	8.6	8.1	7.0	0.0	4.9	9.1
BAB-22	8.8	9.8	5.9	7.1	6.4	8.0	6.0	6.3	7.0	6.7	9.3	10.5
BAB-23	0.0	10.5	7.3	10.1	10.0	7.1	7.7	7.5	5.7	4.9	11.7	9.0
BAB-24	6.3	4.9	17.6	13.0	14.0	4.7	8.6	7.4	7.6	9.3	9.5	10.0
BAB-25	10.1	9.1	7.5	3.9	16.0	7.3	7.7	11.9	6.4	0.0	4.8	10.1
BAB-26	10.1	9.6	7.6	5.0	8.8	5.8	5.8	6.9	3.5	7.3	2.5	10.3
BAB-27	10.8	4.6	17.6	0.0	15.0	5.0	5.1	12.1	9.0	10.5	7.3	13.2
BAB-28	9.3	8.7	8.2	2.9	10.3	9.7	6.0	9.0	5.0	6.8	10.2	8.3
BAB-29	0.0	9.0	6.8	7.0	13.0	8.8	4.5	9.8	8.0	6.0	10.0	9.4
BAB-30	7.8	9.4	8.8	8.3	11.2	9.3	6.9	7.9	7.6	9.6	11.1	10.8
CD at 1%	3.2	3.9	3.0	4.2	3.2	3.4	4.0	2.4	2.2	2.5	2.6	3.8

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