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Studies on the cultural and morphological variability among different isolates of *Alternaria brassicae* inciting Blight disease of cauliflower in Uttar Pradesh, India

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Blight of cauliflower caused by *Alternaria brassicae* is one of the important diseases of cauliflower causing serious yield and quality loss in production due to the seed borne nature of the pathogen. Variation in morphology and cultural characteristics among twenty three isolates of *Alternaria brassicae* from Uttar Pradesh was studied. All the isolates showed high level of variability *in vitro* in respect of their growth at different pH, temperature, dry mycelial weight and sporulation. Though, all the isolates were pathogenic in nature, but pathogenicity was not directly related to the cultural and the morphological characteristics. Sixteen isolates preferred an acidic pH while seven thrived well in a slightly basic pH medium. Both mycelial growth and sporulation were supported by cauliflower leaf extract agar medium while a temperature of 25-30^{0}C was found optimum for growth of all the isolates.

Key words: Cauliflower, *Alternaria brassicae*; variability

INTRODUCTION

Alternaria Blight or Alternaria Leaf blight disease of cauliflower caused by *Alternaria brassicae* (Berk). Sacc. is one of the most destructive fungal diseases and causes significant qualitative and quantitative losses in crop production. Although the occurrence of the disease is global, it is more prevalent in subtropical and temperate countries. The disease causes heavy losses at vegetable curd stage, as well as during seed setting stage. The symptoms are restricted to lower leaves and dark brown to black circular spots cover the leaves. Later on they become large, covered with black spores in concentric zonation and give blighted appearance in cauliflower. Inflorescence, pedicel and siliqua are severely affected resulting in internally seed borne infection of pathogen (Pandey *et al,* 2003). In India it was reported that the 5 – 30% loss caused by *A. brassicae* alone (Pandey *et al,* 2002). The disease severity and average disease incidence in Uttar Pradesh was recorded 10 – 40% and 26% respectively (Pandey *et al,* 2002). *Alternaria brassicae* have different cultural characters after isolation and it is arduous to differentiate it from other *Alternaria* spp., mainly due to analogy in colony characters. Identification and characterization of the pathogen for their variability can be done by several methods. The present study is conducted to characterize the variability among *A. brassicae* isolates so that the first line of information generated thereof will be successfully utilized in resistant breeding programs.

MATERIALS AND METHODS

Isolation and maintenance of A. brassicae isolates

Cauliflower leaves showing leaf blight symptoms were sampled randomly from different cultivars at

different farmers' fields in different geographical locations of Uttar Pradesh (Lat 26.85^0 N; Long 80.91^{0}E) state of India. The isolates of *A. brassicae* were designated based on their place of collection (Table 1). The selected infected spots were washed 3 to 4 times in sterilized distilled water and then surface sterilized by dipping in 4% sodium hypochlorite (NaOCl) solution for 1 min, followed by washing with sterilized water for 4 to 5 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, the pure culture of *A. brassicae* was isolated by selecting growing mycelia tip on PDA and 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 the pathogen was identified as *A. brassicae* (Berk.) Sacc. [Simmons, 1967] and purified by single spore isolation method. The isolated fungal pathogen cultures were maintained on PDA slants at 4°C.

Pathogenicity test in vivo: For this purpose, mycelial mat of 10 days old cultures of the isolates on PDA was taken, blended with sterilized distilled water and filtered through cheesecloth. The spore suspensions (2-3x10^3/ml) were taken in a 100 ml atomizer and sprayed on leaves. Leaves sprayed with sterilized distilled water were kept as control. The plants were kept in fabricated moist chamber for 3 days, and subsequently removed from the chamber and observed under normal conditions in the glasshouse at 28 + 4^{0}C for development of the symptom. The symptoms expressed were studied and re-isolation was made from the infected leaves and the pathogenicity test as above, was repeated twice to confirm the results. Based on the symptom expression and per cent disease incidence, the isolates were categorized as least virulent (>40%), moderately virulent (40-50%), virulent (60- 70%) and highly virulent (<70%) groups.

Effect of different pH on radial growth

Nine pH values ranging from 4.5 to 8.5 i.e. 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, were adjusted through pH meter by adding few drops of hydrochloric acid (HCl) or sodium hydroxide (NaOH) solution in PDA medium. The medium was poured in sterilized Petri plates, cooled and solidified. Ten days old fungal culture discs were cut out by sterilized steel cork borer (5 mm diam.) and transferred to the Petri plates under aseptic conditions in such a position where the mycelium of the culture touches the medium. Five replicates were maintained for each pH and incubated at 25±1°C. The data of radial growth was recorded 10 days after inoculation (DAI). The isolates were categorized in to two groups viz. acidic and alkaline on the basis of their maximum radial growth on pH 6.5 and 7.0 respectively.

Effect of different temperature on radial growth

All twenty three isolates of the pathogen were inoculated on PDA plates. For each temperature range five replication were maintained. The Petri plates were kept at five different temperatures in BOD i.e. 10±1°C, 15±1°C, 30±1°C, 35±1°C, 40±1°C, 50±1°C. The data of radial growth was recorded at 10 DAI.

Effect of different media on mycelial dry weight and sporulation of *A. brassicae* isolates

Two natural media i.e., CLA (Cauliflower Leaf Agar Medium) and ASM (Alternaria Sporulation Medium), four semi-synthetic media viz., PDA, MEA (Malt Extract Agar), CMA (Cornmeal agar), and OMA (Oat meal Agar)) and five synthetic media viz. GPA (Glucose Peptone Agar)), CZPA (Czapek's Dox Agar), RA (Richard's Agar), WFPA (Wet Filter Paper Agar) and YEDA (Yeast Extract Dextrose Agar) were used in the study but in the liquid form (i.e. without Agar). Mycelial discs of 5 mm diameter were cut out from the margins of the colonies of ten days old culture by a sterilized (5 mm diam.) metal corkborer. A single such disc was transferred to each flask by using a sterilized inoculation needle. The inoculated flasks were incubated for 15 days at 24 + 2^{0}C. The growth of different isolates, obtained on liquid media was measured by dry weight. For this purpose, Whatman filter paper number 42 was used. The Whatman filter paper was kept in an oven at 60^{0}C for 48h and then weighed on an electronic balance. Then the mycelial growth of the *Alternaria* isolates was poured on the filter paper kept in funnel and was left for filtration of one hour. The mycelial mat was retained on the filter paper. The Whatman filter paper along with the mycelial growth of different *Alternaria* isolates was kept in a sterilized Petri plate and subjected to drying at 60^{0}C to get constant weight. The filter paper along with dried mycelium was again

weighed and the difference between the final and initial weight of the filter paper denoted the dry weight of the *A*lternaria isolates. Three replications of each treatment were maintained.

To determine conidial concentration of each isolates, cultures grown in the seven (PDA, CZPA, WFPA, YEA, RA, CLEA and GPA) different media plates were considered. Seven days after inoculation, ten milliliter (10 ml) of sterile distilled water was added to culture plate and using a sterile glass slide, the culture surface was gently scrapped to make a conidial suspension. Conidial concentration was determined using a haemocytometer.

RESULTS AND DISCUSSION

Pathogenicity test and categorization of isolates as per virulence

Out of twenty three isolates eight (Gpr- C- A, Brh -C-A, Bas-C-A, Kha-C-A, Mau-C-A, Mah-C-A, Son -C-A, Sid-C-A) were observed as highly virulent ones with a percent disease incidence ranging from 72.00 to 80.66 at 25 DAI. Six (Azm-C-A, Bar-C-A, Deo-C-A, Gon-C-A, Luk-C-A, Vns-C-A) registered as virulent ones with a percent disease incidence ranging from 51.33 to 70.00 at 25 DAI while three(Bl-C-A, Bar-C-A, Sul-C-A,) were recorded as least virulent (Table 2). It should be noted that the disease incidence gradually increased with time in case of all the isolates considered for the study. Similar type of experiment for study of pathogenic variability of different isolates was conducted by Castro *et al,* (2000) in tomato by inoculating spore suspension concentration of 1.25 x 10^3 conidia/ml of *A. solani.* The existence of pathogenic variability among the isolates of *A. brassicae* was also studied and reported by Meena *et al,* (2012).

Table.1. List of Alternaria blight isolates in cauliflower collected during survey (2012 – 13)

Place	District	Isolate code
Shibali campus	Azamgargh	Azm-C-1
Rasara	Ballia	Bl-C-1
Jaraval Road	Baharaich	Brh-C-1
Faizabad Road	Barabanki	Bar-C-1
Manikapur	Basti	Bas-C-1
Jaddupur	Bhadohi	Bh-C-1
Bhatpar Rani	Deoria	Deo-C-1
Akabarpur	Faizabad	Fai-C-1
Gonda Katchahari	Gonda	Gon-C-1
Chauri Chaura	Gorakhpur	Gpr-C-1
Mohamadabad	Ghazipur	Gha-C-1
Badalapur	Jaunpur	Jau-C-1
Pipra Chouraha	Khalilabad	Kha-C-1
Sapaha Village	Kushinagar	Kus-C-1
Bakshi ka talab	Lucknow	Luk-C-1
Haldharpur	Mau	Mau-C-1
Chunar	Mirzapur	Mir-C-1
Laxmiganj	Maharajganj	Mah-C-1
Raibareili	Raibarelli	Rai-C-1
Dudhi	Sonbhadra	Son-C-1
Sidhartha Nagar	S.Nagar	Sid-C-1
Amethi	Sultanpur	Sul-C-1
IIVR	Varanasi	Vns-C-1

Table 2 : *In vivo* pathogenicity of *Alternaria brassicae* isolates

Isolates	Per Cent Disease Incidence		
	10DAI	17 DAI	25 DAI
Azm – C - A	29.833	53.000	70.000
Bl – C - A	19.333	27.333	40.500
Brh – C - A	34.00	57.167	73.000
Bar – C - A	27.10	44.667	61.333
Bas – C - A	36.00	58.33	74.00
Bh – C - A	10.000	26.333	35.000
Deo – C - A	21.460	34.333	51.333
Fai – C - A	20.000	33.667	45.000
Gon – C - A	29.000	45.333	60.333
Gpr – C - A	46.23	62.33	79.67
Gha – C - A	5.000	6.667	8.000
Jau – C - A	17.833	25.000	28.000
Kha – C - A	45.00	61.66	79.33
Kus – C - A	11.333	17.500	20.000
Luk – C - A	35.333	50.167	60.000
Mau – C - A	37.66	60.00	78.00
Mir – C - A	15.000	22.000	25.500
Mah – C - A	49.000	64.000	80.66
Rai – C - A	10.333	17.167	21.500
Son – C - A	45.000	60.000	72.000
Sid – C - A	41.00	61.00	78.33
Sul – C - A	32.000	44.000	50.333
Vns – C - A	31.333	48.833	66.667
CD at 5%	1.53	1.437	2.55
SE (d)	0.75	0.70	1.25
SE (m)	0.53	0.50	0.89
CV	3.72	2.25	3.19

DAI=Days after Inoculation

Table 3 : Effect of pH on growth of *Alternaria brassicae* isolates

Isolates	Radial Growth (mm) on different pH 10 days after Inoculation								
	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
Azm – C - A	19.33	21.33	49.00	55.67	47.33	30.00	15.67	00.00	00.00
Bl – C - A	24.00	25.33	44.33	60.00	64.00	27.33	00.00	00.00	00.00
Brh – C - A	32.97	37.67	49.33	59.33	68.77	69.00	34.00	14.33	9.00
Bar – C - A	22.67	37.70	50.63	59.87	68.83	46.67	34.67	13.66	9.33
Bas – C - A	36.66	40.66	51.33	64.67	71.67	72.33	37.00	17.33	10.66
Bh – C - A	33.00	38.00	50.33	62.67	71.33	68.00	16.66	00.00	00.00
Deo – C - A	30.00	31.33	37.07	31.23	51.75	57.00	30.96	00.00	00.00
Fai – C - A	29.00	33.00	41.87	44.33	52.67	18.00	00.00	00.00	00.00
Gon – C - A	29.43	31.70	45.50	44.67	56.00	22.57	22.33	00.00	00.00
Gpr – C - A	38.00	42.33	53.71	67.11	73.11	75.00	39.30	19.00	13.00
Gha – C - A	28.57	30.00	41.00	55.83	70.00	31.83	16.68	00.00	00.00
Jau – C - A	26.33	28.96	41.13	47.67	64.80	21.00	00.00	09.67	00.00
Kha – C - A	37.66	42.00	53.11	67.00	73.00	74.00	38.66	18.33	12.11
Kus – C - A	33.67	39.00	46.33	59.00	56.67	56.00	28.33	15.30	00.00
Luk – C - A	34.33	38.34	49.33	56.00	67.67	43.00	36.33	15.33	00.00
Mau – C - A	37.11	41.11	52.00	66.00	73.11	72.00	37.33	17.50	11.00
Mir – C - A	34.33	36.33	50.17	60.00	71.00	46.67	35.67	14.00	00.00
Mah – C - A	38.33	42.47	54.00	67.66	75.00	75.66	39.66	19.67	13.66
Rai – C - A	35.57	37.67	51.00	57.57	71.00	47.00	29.00	00.00	00.00
Son – C - A	35.00	40.00	49.76	59.66	71.33	37.97	14.00	00.00	00.00
Sid – C - A	37.33	41.70	52.66	66.33	72.66	73.33	38.00	18.11	11.67
Sul – C - A	22.67	25.33	38.50	39.33	71.33	23.00	00.00	00.00	00.00
Vns – C - A	28.00	30.33	48.27	59.67	69.50	71.00	00.00	00.00	00.00
CD at 5%	3.24	2.83	3.45	3.29	2.75	2.87	2.56	1.07	1.051
SE (d)	1.60	1.39	1.70	1.62	1.25	1.41	1.06	0.77	0.468
SE (m)	1.13	0.98	1.20	1.14	1.08	1.00	1.03	0.51	0.301
CV	6.42	5.07	4.40	3.64	3.07	4.07	6.15	14.43	3.106

Radial growth of different isolates of *A. brassicae* in different pH

Fourteen isolates namely Bl-C-A, Bar-C-A, Bh-C – A, Fai-C-A, Gon - C-A, Gha-C-A, Jau-C-A, Kus-C - A, Luk-C-A, Mau-C-A, Mir-C-A, Rai-C-A, Son-C – A and Sul-C-A were acidic inhabitants having their maximum radial growth at pH 6.5. The range of radial growth observed was 52.67 to 73.11 mm. (Table 3). It is to be noted that when the pH was subsequently increased to 7.0, in all the aforementioned isolates, the radial growth dropped down significantly. Eight isolates among the twenty three used in the study i.e. Brh-C-A, Bas-C-A, Deo-C- A, Gpr-C-A, Kha - C-A, Mah-C -A, Sid-C-A, Vns-C- A, were alkaline inhabitants recording their maximum radial growth on pH 7.0, ranging from 57.0 to 75.66 mm. Similarly an increase in pH to 7.5 resulted in a decrease in radial growth of these isolates as well. It was interesting to note that most of the alkaline inhabitant isolates belonged to similar geographical area. Only one isolate Azm-C- A showed a higher acidic tolerance and its maximum radial growth of 55.67 mm was recorded at pH 6.0. However, the isolates Bl-C-A, Fai-C-A, Jau-C-A, Sul – C-A and Vns-C-A recorded no radial growth beyond 7.0 pH A pH range of 5-10 have been reported congenial for both mycelial growth and sporulation of A. brassicae isolates (Lapis and Ricaforte, 1974; Goyal *et al,* 2011) which is in accordance with the present findings. However, optimum pH level was different for different isolates, which hints at the existence of variability among them.

Table 4: Effect of temperature (^{0}C) on growth of *A. brassicae* isolates

Isolates	Radial Growth (mm) at different temperature (^{0}C) 10 DAI					
	10^{0}C	15^{0}C	25^{0}C	30^{0}C	40^{0}C	50^{0}C
Azm – C - A	7.17	29.67	47.00	41.60	19.33	12.00
Bl – C - A	13.00	18.84	52.00	52.00	14.00	00.00
Brh – C - A	8.83	13.43	49.50	50.80	12.33	9.00
Bar – C - A	6.33	16.37	48.00	40.00	21.33	9.13
Bas – C - A	14.66	56.39	57.00	53.66	36.00	14.00
Bh – C - A	12.33	34.70	51.00	45.83	13.33	00.00
Deo – C - A	8.00	31.97	50.40	44.87	13.67	6.50
Fai – C - A	11.67	15.33	49.67	48.33	16.00	5.37
Gon – C - A	5.67	20.96	48.00	30.67	20.33	5.67
Gpr – C - A	17.11	58.66	60.50	55.67	39.66	16.00
Gha – C - A	6.43	21.40	49.00	46.66	50.00	13.50
Jau – C - A	6.50	53.67	40.00	51.33	12.00	5.97
Kha – C - A	16.80	58.00	59.00	55.11	38.11	15.50
Kus – C - A	6.83	44.33	52.00	54.33	16.00	7.67
Luk – C - A	6.87	45.00	49.87	48.67	8.67	00.00
Mau – C - A	15.70	56.70	57.70	54.00	37.33	14.66
Mir – C - A	6.37	54.43	53.50	52.73	10.17	00.00
Mah – C - A	17.66	60.00	60.66	57.00	41.50	17.33
Rai – C - A	6.94	43.53	49.10	45.36	11.10	00.00
Son – C - A	8.00	42.67	51.73	49.77	10.87	00.00
Sid – C - A	16.00	57.00	59.00	54.00	38.00	15.33
Sul – C - A	6.77	45.97	51.50	52.73	10.67	00.00
Vns – C - A	6.33	49.33	49.66	51.9	7.67	6.17
CD at 5%	1.40	3.83	3.50	5.61	3.50	1.82
SE (d)	0.69	1.88	1.72	2.76	1.73	0.89
SE (m)	0.49	1.33	1.22	1.95	1.22	0.64
CV	10.42	6.70	4.38	6.52	4.38	21.65

DAI=Days afterInoculation

Radial growth of different isolates of A. brassicae at different temperature (^{0}C)

Radial mycelial growth of *A. brassicae* varied among different isolates at different temperatures. Seventeen isolates viz. Azm -C-A, Bar-C-A, Bas-C-A, Bh-C-A, Deo-C-A, Fai-C-A, Gon-C-A, Gpr- C -A, Gha-C-A, Kha-C-A, Luk-C-A, Mau-C-A, Mir- C -A, Mah-C-A, Rai -C-A, Son-C-A, and Sid-C-A recorded maximum radial growth at 25^{0}C temperature. Among them the Mah-C-A and Azm-C-A exhibited highest and lowest radial growth of 60.66 mm and 47.00 mm respectively. On further increase of the temperature, the radial growth of each of these isolates decreased, clearly signifying that a temperature of 25^{0}C was optimum for these isolates. Rest of the six isolates i.e. Bl -C-A, Brh-C-A, Jau-C-A, Kus-C-A, Sul - C-A, and Vns-C -A, recorded maximum radial growth at 30^{0}C temperature and among them Kus – C – A recorded highest radial growth (54.33 mm) while Brh- C -A showed lowest radial growth (50.80 mm) (Table 4). Any further increase in temperature beyond 30^{0}C resulted in the reduced radial growth of these isolates. The variability in mycelial growth of several Alternaria species at different temperature has

Table 5 : Mycelial dry weight of *Alternaria brassicae* isolates on different culture media

Isolates	Mycelial dry weight (mg) 15DAI						
	PDA	CZPA	WFPA	YEA	RA	CLA	GPA
Azm – C - A	126.67	174.17	167.00	118.33	149.00	192.00	119.84
Bl – C - A	130.66	154.00	110.00	131.83	135.00	182.00	90.33
Brh – C - A	134.00	160.00	152.00	146.50	135.00	180.00	108.50
Bar – C - A	132.00	140.00	138.00	118.67	160.00	162.00	109.00
Bas – C - A	145.00	160.67	140.00	130.90	140.00	170.00	121.00
Bh – C - A	140.33	150.50	132.84	129.33	129.00	156.50	111.00
Deo – C - A	130.68	143.17	124.00	121.33	128.67	149.67	121.00
Fai – C - A	134.00	141.67	132.00	122.00	138.00	157.33	112.00
Gon – C - A	118.22	148.20	136.00	109.34	118.00	163.86	89.99
Gpr – C - A	165.00	173.00	167.00	140.00	160.00	182.99	135.00
Gha – C - A	130.33	149.00	109.33	111.76	103.99	163.00	99.34
Jau – C - A	113.67	148.00	140.00	120.00	116.33	151.33	116.34
Kha – C - A	160.40	172.67	152.00	132.00	153.00	180.00	130.50
Kus – C - A	110.33	160.00	118.33	122.67	138.17	150.50	148.67
Luk – C - A	125.00	173.00	141.67	114.33	139.00	177.68	105.00
Mau – C - A	148.33	160.55	141.67	131.67	142.00	177.00	122.00
Mir – C - A	114.00	160.67	124.00	140.00	142.00	170.00	130.33
Mah – C - A	166.33	174.17	173.68	146.50	162.00	192.00	148.67
Rai – C - A	118.84	148.00	136.00	109.00	140.00	150.00	118.00
Son – C - A	102.93	136.00	117.00	130.00	120.00	125.00	103.00
Sid – C - A	156.67	160.67	149.00	131.83	149.00	178.33	129.00
Sul – C - A	141.00	110.55	130.00	131.67	119.80	138.00	119.00
Vns – C - A	105.00	110.00	124.00	121.67	109.80	118.00	109.80
CD at 5%	4.11	6.13	6.13	5.20	3.67	4.17	3.13
SE (d)	2.02	3.02	3.02	2.59	1.81	2.05	1.54
SE (m)	1.43	2.13	2.13	1.83	1.28	1.45	1.09
CV	1.88	2.43	2.43	2.58	1.67	1.55	1.66

DAI=Days afterInoculation

been reported earlier by Meena *et al,* (2005, 2012) and Singh *et al,* (2007). They reported that different isolates of A. brassicae and different species of Alterania manifested variability in radial growth at different temperatures. The present study stated that the temperature range of 25^{0}C - 30^{0}C, was optimum and 25^{0}C was most favorable for mycelial growth of *A. brassicae.*

Effect of different nutrient media on dry weight and sporulation of Alternaria brassicae isolates

Twenty isolates recorded maximum mycelial dry weight on CLA ranging from 118.0 mg – 192.0 mg followed by two isolates on Czapek dox agar (CZPA) medium i.e. 136 mg by Son-C-A and 160.00 mg by Kus-C-A. Only one isolate, Sul-C - A recorded its maximum dry weight i.e. 141.0 mg on PDA (Table 5). The maximum dry weight was recorded in case of Azm-C-A and Mah-C-A, both registering192.0 mg in CLA while the minimum dry weight of 90.33 mg was recorded by Bl-C-A on GPA medium. Most of the isolates exhibited minimum dry weight on GPA medium. As dry weight is greatly influenced by the composition of the nutrient media, it was a crucial yard-stick to assess the variability of different isolates of *A. brassicae.* Although the dry weight variations were not very high, but the indications supported the findings of Meena *et al,* (2012) who reported that maximum dry mycelial biomass of *A. brassicae* was recorded in Czapek's medium followed by Elloot's medium while least dry mycelial biomass was observed in Brown's medium. Out of twenty three isolates, twenty recorded maximum conidial sporulation on CLEA

Table 6: Spore Density of *Alternaria brassicae* isolates on different nutrient media.

Isolates	Average number of conidia per ml x 10^3 at 7 DAI						
	PDA	CZPA	WFPA	YEA	RA	CLEA	GI
Azm – C - A	4.50	1.44	2.33	4.23	7.50	8.24	3.
Bl – C - A	2.82	3.20	1.37	4.25	5.50	8.00	3.
Brh – C - A	6.43	1.58	1.34	4.00	8.06	8.35	3.
Bar – C - A	4.20	1.58	2.00	4.30	6.80	7.80	3.
Bas – C - A	7.43	3.84	3.69	4.83	8.11	9.35	3.
Bh – C - A	5.30	0.98	1.92	4.20	7.06	7.58	3.
Deo – C - A	4.00	0.91	2.95	3.98	6.50	6.30	3.
Fai – C - A	4.03	1.52	2.20	4.05	4.50	5.19	3.
Gon – C - A	3.40	3.00	3.39	3.20	4.19	5.37	3.
Gpr – C - A	8.40	4.76	4.81	6.00	9.43	10.24	4.
Gha – C - A	2.82	1.88	1.92	2.01	4.96	6.47	1.
Jau – C - A	2.10	2.14	2.98	1.87	4.96	5.69	2.
Kha – C - A	8.30	4.44	4.50	5.88	9.10	10.20	4.
Kus – C - A	1.77	2.42	1.09	4.40	7.49	6.19	5.
Luk – C - A	1.82	2.84	2.50	4.44	8.04	8.39	1.
Mau – C - A	7.66	3.93	3.82	4.88	8.62	9.68	4.
Mir – C - A	2.87	3.81	2.42	4.55	4.23	3.76	3.
Mah – C - A	8.40	5.00	4.98	6.23	9.50	10.50	5.
Rai – C - A	2.33	2.00	2.32	4.20	5.00	4.62	2.
Son – C - A	1.86	2.81	1.40	2.40	3.12	2.63	2.
Sid – C - A	8.20	4.00	4.12	5.30	8.33	9.80	4.
Sul – C - A	1.67	2.63	1.51	1.19	2.26	8.20	3.
Vns – C - A	5.40	1.47	1.98	3.83	7.45	7.95	3.
CD at 5%		0.33	1.04	0.65	0.80	0.80	0.
SE (d)		0.16	0.51	0.32	0.39	0.39	0.
SE (m)		0.11	0.36	0.22	0.28	0.28	0.
CV		8.68	25.5	10.15	7.71	7.71	9.

DAI=Days afterInoculation

(Cauliflower Leaf Extract Agar) ranging from 5.19x10^3 / ml to 10.5 x10^3 / ml followed by three isolates on Richard's Agar (RA) medium (Table 6). The Mah – C – A, isolate showed maximum sporulation on CLEA, RA, and PDA medium. On CZPA, WFPA, YEA and GPA medium, most of the isolates were low sporulating and the lowest sporulation was recorded by isolate Deo-C -A on CZPA medium (0.91x10^3/ml). In fact, majority of the isolates recorded lowest sporulation on GPA medium. Sharma *et al,* (2013) reported that all the isolates did not grow and sporulate abundantly on the same nutrient medium but Potato Dextrose Agar, Cauliflower (Host) Agar medium and Carrot Potato Agar were good for all the cultures. Hence, our findings that the medium based on the host i.e. cauliflower as well as PDA did support the growth and sporulation of all the *Alternaria* isolates are further validated.

All the twenty three isolates of *Alternaria brassicae* though different at cultural and morphological level were found to be pathogenic in nature. The variability of isolates highlighted in the present study in the light of their growth at different pH, temperature, dry mycelial weight and sporulation, urged the use of molecular markers to assess the genetic relationship among the isolates. Saha *et al,* (2014) used sixteen random primers in RAPD-PCR technique for a better understanding of the genetic diversity of A. brassicae isolates. However, to understand the details of the gene functionality in

pathogen under such conditions, in-depth study like characterization of internal transcriber tracer regions, toxins etc. needs to be studied. More number of isolates, may be included in the later investigation to have further clarity regarding the diversity and variability among the isolates of the pathogen.

REFERENCES

Castro, M. E. A., Zambolim, L., Chavos, G. M., Cruz, C. D. and Matsuoka, K. 2000. Pathogenic variability of *Alternaria solani,* the causal agent of tomato early blight. *Summa Phytopathologica.* **26:** 24- 28.

Goyal, P., M. Chahar, A.P. Mathur, A. Kumar and C. Chattopadhyay, 2011. Morphological and cultural variation in different oilseed *Brassica* isolates of *Alternaria brassicae* from different geographical regions of India. *Indian J. Agric. Sci.*, **81:** 1053-1058.

Lapis, D.B. and E.T. Ricaforte. 1974. *Alternaria* leaf spot of crucifers in the Philippines. *Philipp. Agric.* **58:** 121-137

Meena, P.D., A. Rani, R. Meena, P. Sharma, R. Gupta and P. Chowdappa, 2012. Aggressiveness, diversity and distribution of *Alternaria brassicae* isolates infecting oilseed *Brassica* in India. *Afr. J. Microbiol. Res.,* **6:** 5249-5258.

Meena, P.D., C. Chattopadhyay, V.R. Kumar, R.L. Meena and U.S. Rana, 2005. Spore behaviour in atmosphere and trends in variability of *Alternaria brassicae* population in India. *J. Mycol. Plant Pathol.,* **35:** 511-511.

Pandey, K.K, Pandey P.K. and Satpathy, S. 2002. Integrated management of disease and insects of tomato, chilli and cole crops. *IIVR Technical bulletin,* **9** : p 7

Pandey, K.K., Pandey, P.K., Kalloo, G., Banerjee, M.K. 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. *J Gen Plant Pathol.* **69**:364–371

Saha, Sujoy, Ruchi, Garg,Venkataravanappa, V., Mishra, P. K. and Rai, A. B. 2014. *Molecular and Cultural Characterization of Alternaria brassicae Infecting Cauliflower in Uttar Pradesh, India Proceedings of the National Academy of Sciences, India* Section B: Biological Sciences DOI 10.1007/s40011-014-0472

Sharma, M. Swati, Deep, Bhati D. S., Chowdappa,P., Selvamani R. and P. 2013. Morphological, cultural, pathogenic and molecular studies of *Alternaria brassicae* infecting cauliflower and mustard in India. *African Journal of Microbiology Research.* **7** : 3351 - 3363

Simmons, E.G. 1967. Typification of *Alternaria*, *Stemphylium* and *Ulocladium. Mycologia,* **59**: 67- 92.

Singh, D., R. Singh, H. Singh, R.C. Yadav and N. Yadav 2007. *Cultural and morphological variability* in *Alternaria brassicae isolates of Indian mustard (Brassica juncea L. czern and coss.).* Proceeding of the 12th International Rapeseed Congress, 158-160.