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CULTURAL, MORPHOLOGICAL AND PATHOGENIC VARIABILITY IN CAUSING ALTERNARIA LEAF SPOT (*ALTERNARIA BRASSICICOLA*) OF CABBAGE

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ABSTRACT

The investigation was undertaken to study the cultural, morphological and pathogenic variability in four isolates of causal agent of Alternaria leaf spot (*Alternaria brassicicola*). The isolates were procured from four major cabbage growing districts, Udaipur, Jaipur, Bundi and Ajmerof Rajasthan. These exhibited considerable variations in cultural and morphological characteristics. Pathogenic variability with inoculation on pot-grown plants of cabbage cultivars, Golden acre, Gayatri, Supper Gayatri, Kank and vilina resulted in 68.0 to 88.05, 64.02 to 84.08, 48.0 to 52, 47.0 to 64.02 and 7.98 to 31.99 PDI, respectively. The isolates also exhibited significant variations in symptoms and latent period. Based on the disease severity, the four isolates were distinguished into pathogenic groups, where isolate from Udaipur district was found most virulent and predominant by causing the typical leaf spot symptoms with shortest latent period and highest disease severity. All the isolates caused susceptible (S) reaction on two cultivar (Supper Gayatri and Kank), moderately susceptible reaction (MS) and moderately resistant reaction (R) on one cultivar (Vilina).

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INTRODUCTION

Cabbage (*Brassica oleracea*var *capitata* L.), as belongs to the family Cruciferae and is an important ancient vegetable crop. It covers 4% of total area under vegetable. The disease responsible for significant reduction in quantity and quality in yield of cabbage and seeds. The initial symptoms of the disease are small, circular, dark spots on older leaf surfaces the tan-coloured centers of lesions may eventually fall out, producing a hole, or under wet conditions, may become covered with masses of black spores. In storage, spots enlarge and soft-rot bacteria may enter lesions. The disease is transmitted through the seeds. The pathogen can over-season on crop debris. Weeds from the family Cruciferae may also harbour the fungus. Spores of *Alternaria* can be spread by wind and water. The disease is most damaging under wet, warm (20-30.5°C) conditions (Verma and Saharan, 1994). Studied severity of leaf spot of cabbage, caused by *A. brassicicola*. Severity of *Alternaria* leaf spot of cabbage differs among growing regions as also between individual crops within a region (Singh *et al.*, 1992). This may be due to existence of variability among isolates of spp. Many reports on the existence of morphological variability within the isolates of other spp have been reported by earlier (Verma*et al.,* 2006). Studies of liquid media revealed that *Alternaria solani* growth was best on Potato Dextrose Broth (34.1 mg) followed by Czapeck's medium (58 mm) and sporulation was maximum on Potato Dextrose Agar (13.2×106 spores/mL). The pathogen also sporulated maximum at temperature 25ºC and RH 100% (Somappa *et al.*, 2013). A range of maximum temperature 23.83 to 29.63ºC was found to be most appropriate for *Alternaria* leaf blight disease caused by *Alternaria brassicae* development. While, it was found to be decreased with the higher range of minimum temperature. Maximum relative humidity (80.33 to 90.55 %) and minimum relative humidity (52 to 58 %) were the most suitable ranges for lesion development (Biswas, 2013). The disease is considered to be a major constraint in sustainable cabbage production and various control strategies include use of fungicides, biological agents, botanicals and their combinations. However, several factors including pathogenic variability influence the efficacy of these management practices. Variability in spp. infecting various crops have been reported but detailed holistic study on cultural, morphological and pathogenic variability in prevalent in different cabbage growing districts of Rajasthan is not available. In view of this variability in causing *Alternaria* leaf spot of cabbage was studied and the results reported in this paper.

MATERIALS AND METHODS

Identification of the pathogen

Cultural characters of all isolates were studied by growing them on PDA at 28 ± 1º C. The colonies frequently showed sectoring. Each sector was carefully separated by transferring on to fresh PDA plates by hyphal tip culture technique and by single spore method using a dummy objective and maintained on PDA slants for further studies and designated as Udaipur (UDR), Jaipur (JPR), Bundi (BND) and Ajmer (AJR) isolate. The sporulating cultures of *A. brassicicola* were identified on the basis

of morphological characters of somatic and reproductive structures with the help of standard literature (Rao, 1964).

Pathogenicity tests

Pathogenicity of the 4 isolates of *Alternaria* isolated from cabbage was tested by spray inoculation technique on pot grown plants of cabbage. The plants were raised in autoclaved sand: soil: FYM (3:1:1) mixture and surface sterilized with (0.1% $HgCl_2$ for two minutes) seeding were sown @ 1-3 per pot. For preparation of the inoculum the pure culture of different isolates was grown on PDA for 10 days on 28 ± 1ºC in Petri plates so as to allow profuse sporulation. The spores were harvested by flooding the plate with sterile distilled water and gently scrapping the colony with the help of a sterilized plastic loop and the conidial suspension was strained through muslin cloth. Final concentration of the spores was maintained 1x 10^4 conidia ml^{-1}. Sixty days-old-plants were spray inoculated with the suspension in a hand held atomizer. The inoculated plants were kept in humid chamber for 24 hours and then transferred to cage house and high humidity was maintained through out the disease development period by frequent irrigations.

As the Infection started, disease symptoms first appeared as small dark-brown turned black spots on the leaves. Later on spots enlarged, a definite zonation or concentric rings became evident after 48-89 hours of inoculation and the centre of affected parts become darker. As the disease progress, the spots became numerous and the spotted leaves turned yellow and die prematurely. These typical leaf spot symptoms appeared 7 days after inoculation. Re-isolation was done from infected plant parts collected 10 days after inoculation. The resulting cultures were compared with the original ones to confirm the pathogenicity (Rao, 1964) and all the isolates were identified as *A. brassicicola*.

Variability among four isolates of *A. brassicicola*

Four isolates of *A. brassicicola* represent from different districts in Rajasthan (UDR, JPR, BND and AJR) were studied for their morphological and cultural variability like: growth rate, type of growth, pigmentation and rate of sporulation, size of conidia.

The four isolates of *A. brassicicola* were grown on potato dextrose agar (PDA) medium. The autoclaved medium was dispensed in Petri plate and allowed to solidify. Five mm disc of the individual isolate of *A. brassicicola* removed from the periphery of seven day old culture was aseptically placed in the centre of the PDA plate, *i.e.* one disc per plate, keeping three plates as three replications for each isolates. The plates were incubated at 28 ± 1ºC under 12 hrs light and 12 hrs darkness. After seven days of incubation, variations in growth pattern and colony growth (diameter) of fungi in all isolates were recorded. Spore production by each isolate was determined by removing agar-plugs (5 mm diameter) from three linear spots across the centre of the colony, which were suspended in 10 ml sterile water in glass test tube and agitated twice for about 10 seconds each time on a vortex shaker to dislodge conidia. The number of conidia in the resultant was determined using a heamocytometer, and expressed as number of conidia per mm^2 of medium. For spore size (length and width) mounts were prepared in aniline-blue lacto-phenol and measurements were taken by measuring spores of each isolate using stage and ocular micrometer. For liquid media studies, 50 ml of the medium was poured in each/50 ml conical flask replicated thrice, autoclaved, inoculated and incubated at 28 ± 2ºC.

Comparative pathogenic potential in the isolates of *A. brassicicola*

The pathogenic potential of four isolates of *A. brassicicola* was tested on pot grown plants of a set of cabbage cultivar (varieties/ land races *viz.*, Golden acre, Supper gayatri, Gayatri, Kanak, Vilina). The seeds were procured from local market. The surface sterilized seeds were sown in Sand: Soil: FYM (3:1:1) mixture keeping five replications for each four isolates in completely randomized design (CRD) having 1-3 plants in each pot. Five pots were separately inoculated by the individual culture of *A. brassicicola* (UDR, JPR, BND and AJR) by spray inoculating technique and suitable uninoculated control pots were also maintained for each land race of cabbage. Observations for latent period were started 24 hrs after inoculation and detailed symptoms produced by each isolate were recorded. The disease severity was recorded on standard 1 to 5 disease rating scale as givenbelow:

Per cent of above ground parts infected	**score**
Free from disease	0
1 to10% area of leaf	1
11 to 20% area of leaf	2
21 to 35% area of leaf,	3
36 to 60% area of leaf, stem	4
More than 60% area of leaf, stem	5

Fifteen plants for each isolate were screened and PDI*A. brassicicola* isolates were calculated. The per cent disease incidence up to 1 to 20% was considered as resistant reaction (R), up to 21 to 35% as moderately resistant (MR), 36 to 60% as moderately susceptible (MS) and more than 60% as susceptible.

RESULTS AND DISCUSSION

Cultural and morphological character of four isolates of the *A. brassicicola*

The four isolates of *A. brassicicola* collected from different areas showed variation in colony diameter, size and colour of the colony and rate of sporulation on PDA at 28 ± ºC after 7 days of inoculation. Among *A. brassicicola* isolates, maximum colony diameter (88 mm) was of UDR isolate, followed by 77.2 mm in BND, 76.8 mm in JPR, and minimum 75 mm was observed in AJR isolate. The maximum number of conidia 12.6 × 10^4 conidia / mm^2 were produced in isolate UDR, followed by 11.6 × 10^4 conidia / mm^2 in BND, 10.2 × 10^4 conidia / mm^2 in JPR, while the minimum sporulation 8.8 × 10^4 conidia / mm^2 was recorded in the isolate AJR (Table- 1).

The colony character/colony colour/pigmentation of the culture of individual isolates are given below:

UDR

Cottony, dark brown with dirty white margin aerial at centre, submerged, regular growth and pigmentation brownish. Pale olivaceous brown. Conidia in long narrow chains, beaks were not visible, mostly 2-6 transverse septa, one longitudinal

Table 1: Mycelial growth, dry weight and sporulation of *Alternaria brassicicola* isolates on solid and liquid media

Isolates	Agar media Average mycelial growth in diameter (mm*)	Average dry mycelial weight (g*)	Rate of sporulation** ($x10^4/mm^2$ medium)
Udaipur isolate (UDR)	88.0	0.8	12.6
Jaipur isolate (JAR)	76.8	0.7	10.2
Bundi isolate (BND)	77.2	0.7	11.6
Ajmer isolate (AJM)	75.0	0.6	8.8
SEm±	0.51	0.06	0.57
CD at 5%	1.67	0.21	1.72
CD at 1%	2.43	0.30	2.83
CV%	1.96	28.29	1.80

*Average of three replications. **Mean of 15 replications, three from each of the plate

Table 2: Variation in conidial morphology of four isolates of *A. brassicicola*

S.No.	Isolate	Conidial morphology (μm) Length Mean	Range	Width Mean	Range
1.	UDR	34.60±1.58	31.5-38.8	9.5±0.43	8.6-10.6
2.	JPR	36.70±1.75	33.8-47	10±0.47	9.2-11.4
3.	BND	37.25±3.36	26-41	12±1.15	8-12.6
4.	AJR	26.50±1.30	24-30	6.7±0.32	6-7.5

*Mean no. of 50 conidia and ± S.D. of mean value

Table 3: Latent period (hours) of four isolates of *Alternaria brassicicola* on five cabbage cultivars in pot condition

S. No.	Isolates Cabbage Cultivars	Latent period in hours* Golden acre	Gayatri	Supper Gayatri	Kank	Vilina	Mean
1.	UDR	48	52	68	56	80	60.8
2.	JAR	70	76	77	78	82	76.6
3.	BND	65	68	72	74	86	73.0
4.	AJM	78	86	88	79	89	84.0
	Mean	65.2	70.5	76.2	71.7	84.2	
		SEm±	CD (5%)	CD (1%)			
	Isolate	0.87	2.50	3.34			
	Cultivar	0.97	2.79	3.73	CV % 4.84		
	Isolate × cultivar	1.95	5.59	7.47			

*Mean of three replications

septum in mid cell, measuring 31.5-38.8x 8.6-10.6 μm.

JPR

Irregular, black to dark brown smooth growth and pigmentation dull blackish brown. Conidia obclavate, long septation, smooth walled, olivaceous brown, with darker septation, 3-7 transverse septa, one longitudinal septum, measuring 33.8-47x 9.2-11.4 μm.

BND

White aerial at center, dull black and brown colour, regular smooth growth and pigmentation light brown. Conidia with darker septation, 3-6 transverse septa, 2 longitudinal septa, more rounded at tips, and dark brown and measuring 26-41x 8-12.6 μm.

AJR

Light brown, thin flat and smooth regular growth and pigmentation light brownish. Conidia obclavate, thick walled, small in size, light olivacious brown, 2-5 transverse septa, measuring 24-30x 6-7.5 μm

Pathogenic variability

The four isolates of *A. brassicicola* were evaluated for their pathogenic variability on five cabbage cultivars employing Inoculation spraying technique (In pots).

Variability in pathogenic virulence of isolates of *A. brassicicola* was studied on different cabbage cultivars *viz.,* Golden acre, Gayatri, Supper Gaytri, Kank and Vilina. The observations for latent period in hrs for development of first chlorotic or necrotic lesion were started 2nd day after inoculation. Observations on disease severity were recorded on 0-5 disease rating scale after 14 days of inoculation as described in Materials and Methods. The longest latent period (89hrs) was by isolate AJM on variety Vilina Hrs and shortest latent period (48hrs) was of isolate Udaipur on variety Golden acre. Among the cultivars, Golden acre also showed shortest mean latent period (65.2 hrs) across the four isolates, followed by Gayatri and Kank (70.5 and 71.7 hrs, respectively) and longest mean latent period (84.2 hrs) by Vilina, followed by Supper gayatri (76.2 hrs) . The shortest mean latent period across the five cultivars was 60.8 hrs by isolate UDR, followed by 73.0 hrs by BND. The mean latent period for JPR was 76.6 hrs. The longest mean latent period 84.0 hrs was in case of isolate AJR.Isolate UDR of *A. brassicicola* showed shortest latent period (48 hrs) in Golden acre, followed by in Gayatri (52 hrs) and longest latent period 80 hrs in Vilina. The latent periods with UDR isolate was 56 and 68 hrs in Kank and Supper Gayatri,

Table 4: Disease severity (PDI) and virulence index of four isolates of *Alternaria brassicicola* on five cabbage cultivars in pot condition

S. No.	Isolates	Cabbage cultivars					
		Golden Acre PDI	Gayatri PDI	Supper Gayatri PDI	Kank PDI	Valina PDI	Mean PDI
1.	UDR	88.05 (S)(69.7)	84.08(S) (66.08)	48.00 (MS)(43.85)	64.02(S)(53.14)	31.99(MR)(34.44)	72.41(58.3)
2.	JAR	80.03 (S)(63.4)	72.03(S)(58.07)	48.00(MS)(28.3)	52.00(MS)(46.15)	11.95(R)(20.33)	63.68(52.9)
3.	BND	75.00 (S)(60.00)	76.04(S)(60.69)	52.00(MS)(46.15)	47.00(MS)(43.28)	11.95(R)(20.33)	62.99(52.5)
4.	AJM	68.00 (S)(55.55)	64.02(S)(53.14)	48.00(MS)(43.85)	52.00(MS)(46.15)	7.98(R)(16.41)	58.12(49.7)
	Mean	78.24 (S)(62.2)	74.39(S)(59.60)	49.00(MS)(44.43)	53.80(MS)(47.18)	15.05(R)(22.83)	
		SEm±	CD at 5%	CD at 1%			
Isolate		0.52	1.53	2.08			
Cultivar		0.45	1.32	1.80	CV %	0.21	
Isolate × cultivar		0.16	3.42	4.65			

*Mean of three replications; Figures in parentheses are √arcine percent angular transformed values

respectively. The Jaipur isolate JPR showed shortest latent period (70 hrs) in Golden acre, followed by 76, 77 and 78 hrs in Gayatri, Supper Gayatri and Kank, respectively. It showed longest latent period (82 hrs) in Vilina. BND showed shortest latent period (65 hrs) in Golden acre followed by 68 in Gayatri, 72 hrs in Supper Gayatri and 74 hrs in Kank. It showed longest latent period (86 hrs) in Vilina. The shortest latent period of isolate from Ajmer (AJM) was 78 hrs in Golden acre and longest in Vilina (89 hrs) while it latent period was 79 hrs in Kank, 88 hrs in Supper Gayatri and 86 hrs in Gayatri (Table-3). All the isolates caused susceptible (S) reaction on two cultivar (Supper Gayatri and Kank), moderately susceptible reaction (MS) and moderately resistant reaction (R) on one cultivar (Vilina). However, the disease severity due to these varied with the isolate x cultivar interaction. Golden acre Udaipur isolate caused 88.05 PDI, Gayatri (PDI 84.08) and Kank (PDI 64). This isolate exhibited moderate susceptible (MS) reaction to Supper Gayatri with PDI 48.0, and moderately resistance (MR) reaction with PDI 31.9 in Vilina. JAR isolate caused susceptible (S) reaction with PDI 80.03 in Golden acre, PDI 72.03 in Gayatri and PDI 52.0 in Kank, moderately susceptible (MS) reaction with PDI 48.0 on Supper Gayatri and it gave resistance (R) reaction with PDI 11.95 in Vilina. Isolate BND exhibited susceptible (S) reaction with PDI 76.04, 75.0 in Gayatri and Golden acre. This isolate exhibited moderate susceptible (MS) reaction to Supper Gayatri with PDI 48.0 and it gave resistance (R) reaction with PDI 11.95 in Vilina. . Isolate AJR exhibited susceptible (S) reaction with PDI 68.0, 64.02 in Golden acre and GayatriKank. This isolate caused moderate susceptible (MS) reaction with PDI 52.0 and 48.0 in kank and Supper Gayatri. It caused resistance (R) reaction with PDI 7.98 in Vilina.

The isolates of *A. brassicicola* showed considerable variation in colony characteristics, rate of sporulation and also in conidial morphology. The maximum colony diameter and rate of sporulation after 7 days was 88 mm and 12.6×10^4 conidia / mm in Udaipur isolate, while it was least (75 mm and 8.8×10^4 conidia / mm) in Ajmer isolate(AJR). Among the four isolates, size of conidia ranged from 26.50 (24-30) to 36.60 (33.5-47) μm in length and 6.7 (6-7.5) to 12 (8-12.6) μm in width. The maximum mean length 37.25μm and width12 (8-12.6) μm was of Bundi isolate and the length of conidia 36.70 (33.8-47μm) maximum under isolates of Jaipur. The micrometrical data of present study confirm the similar line of work as published literature by (Rao, 1964). Kumar *et al.* (2003) reported variability in *A. brassica*ecause leaf spots disease in Cole crops. Variability has also been reported by (Verma*et al.* 2007) and (Kumar *et al.*, 2008).

The pot culture studies were carried out to screen cabbage cultivars for disease resistance against the isolates of *A. brassicicola*. The isolates exhibited variation in latent period (time in hrs after inoculation to development of spot). The shortest latent period (48hrs) observed in cultivar Golden acre for Udaipur isolate, while longest latent period (89hrs) was observed in Villina cultivar against Ajmer isolate. Across the five cultivars, mean latent period was 60.8 hrs of Udaipur isolate followed by 73 hrs by Bundi isolate. Longest mean latent period (84.0 hrs) was recorded in Ajmer isolate. Of the four isolatesof*A. brassicicola*, Udaipur isolate was the most virulent, as it caused susceptible (S) reaction (PDI 88.05, 84.08 and 64.02 on Golden acre, Gayatri, Kank cultivars, respectively) and moderate susceptible (MS) reaction with PDI 48.0 in Supper Gyatri and in Vilina (PDI 31.9). Ajmer isolate was less virulent and showed susceptible (S) reaction with PDI 68.0, 64.02 in Golden acre, Gayatri cultivars, respectively and moderate susceptible (MS) reaction 52.0, 48.0 in Kank, Supper Gayatri cultivars, respectively. Cultivar Vilina showed resistance (R) reaction with PDI 7.98 against Ajmer isolate. The data and studies on varietal screening for disease resistance against leaf spot of cabbage is not available so far in current literature. However, Varalakshmi*et al.* 2009 reported IIHR 73-3-20, IIHR 250-4-4-16-27, IIHR 264-3 and IIHR 392 as resistant cauliflower germplasms against Alternaria leaf spot. These observations also suggest pathogenic variability in population of Alternaria blight pathogen.However, several factors including pathogenic variability influence the efficacy of these management practices.

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