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STUDIES ON VARIABILITY IN *Alternaria alternata* (KESSLER) CAUSING LEAF BLIGHT OF ISABGOL (*Plantago ovata)*

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ABSTRACT

All the five isolates of *Alternaria alternata* isolated from different agro climate zone of Rajasthan were tested for their variability in terms of cultural, conidial, pathogenic characteristics and toxin production. All the five isolates differed in cultural characters i.e. dark black colored and very fast mycelial growth with smooth margins (90.00 mm), light black with white at centre and fast growing (80.00 mm), dark brown and medium mycelium growth with smooth margins (75.00 mm), black colored, medium flat mycelial growth with smooth margins (68.00 mm) and white with slightly black in colour with slow mycelial growth (65.00 mm) were observed in Aa-1, Aa-2, Aa-3, Aa-4 and Aa-5 respectively. The variability in conidial morphology of five different isolates was simple, septate, pale to dark brown in colour, often geniculate with one conidial scar. In respect of pathogenic variability, showed significant variations in terms of disease intensity and incubation periods. The isolates Aa-1 was highly pathogenic on Isabgol cv. RI-89 under artificial inoculation conditions showing 52.12% disease intensity followed by Aa-3 ,Aa-2, Aa-4 and Aa-5 isolates. The variability in toxin production was reflected in terms of time taken in inducing wilting symptoms of Isabgol cuttings. Isolate Aa-1 was highly toxic followed by isolates Aa-2, Aa-3, Aa-4 and Aa-5.

Key words: *Alternaria alternata*, Cultural, Spore Morphology, pathogenic, Toxin variability and *Plantago ovata*

INTRODUCTION

Isabgol, *Plantago ovata*, belongs to a large genus of herbs distributed mostly in the temperate regions and a few in the tropics. It comprises about 800 species, of

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which 10-14 are natives of India. In India, it is mainly cultivated in Mehsana and Banaskantha districts of Gujarat and adjoining districts of Rajasthan and to a limited extent in Haryana. Presently, Rajasthan is a dominating state of Isabgol production. Isabgol is cultivated in about 2,27,705 hectares of land in Rajasthan with the production of 1,39,998 tons (Anon., 2009-10). Isabgol growing districts of Rajasthan are Jalore, Barmer, Jodhpur, Bikaner, Pali, Sirohi, Chittorgarh and Udaipur. Its seeds and husk are used in the indigenous medicine for many centuries. Seed is the important plant part which has medicinal values. The husk has the property of absorbing and retaining water and therefore it works as an anti-diarrhoea drug.

Mandal (2010) reported that a number of plant diseases like wilt (*Fusarium oxysporum* Schlechtemend snyd and Hnns.), damping off (*Pythium ultimum* Trow.), leaf blight (*Alternaria alternata (*Fr.) Keissler), downy mildews (*Peronospora plantaginis* Underwood, *P eronospora alta* Fuckel and *Pseudopernospora plantaginis*) and powdery mildew (*Erysiphe cichoracearum* D.C.) blight (*Alternaria*) attack Isabgol. In recent years, the diseases become serious problem of the medicinal plant. It has been found that downy mildew infected the plants are more prone to be attacked by *Alternaria alternata*. It causes considerable damage every year and sometimes become very severe which results in total yield. The present investigations were conducted to find variability in *Alternaria alternata* (Kessler) causing leaf blight of Isabgol.

MATERIAL AND METHODS

Isolation, Purification and Identification of *A. alternata*

Five isolates of *A. alternata* were collected from five different Agro climate zones of Rajasthan i.e., R.C.A. farm (Udaipur), Kapasan (Chittorgarh), Mandore (Jodhpur), Sumerpur (Pali) and Keshwana (Jalore). On the basis of morphological, cultural and pathogenic characteristics, the isolates were identified as *Alteraria alternata* (Fr.) Keissler. Pathogenicity test was performed according Koch's postulates for all the five isolates. The identity of R.C.A. farm (Udaipur) isolate was confirmed by Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi-110012 (The ITCC Code no.6317, 2008).

Cultural variability

For cultural variability, five isolates of the pathogen were grown on potato dextrose agar (PDA) medium to record their growth pattern. The plates were inoculated with 5 mm discs cut from PDA culture of the isolates. The discs were placed at the centre of the Petri plates containing PDA. All inoculated plates were incubated at 25 ± 1ºC temperature in BOD incubator. Each isolate was replicated thrice (Petri dishes). The growth rate was measured and colony characters, pigmentation, growth habit and sporulation were recorded after 24 hrs of incubation till the growth of the pathogen in Petri plate completes.

Variability in spore morphology

Purified culture of each isolate was prepared following single spore method. For this purpose, a conidial suspension was prepared in sterilized distilled water from 10 days old culture on PDA and flooded on 2% plain agar in Petri plates. The excess suspension was drained off and the Petri plates were incubated in inverted position at 25 $\pm 1^0$C. After eight hours of incubation, a single germinating spore was marked with the help of dummy objective and then transferred individually with a piece of plane agar medium to PDA slants by inoculating needle under aseptic conditions. These monoconidial isolates were maintained on PDA slants and used to study spore morphology. Observations on variation in conidial dimension of five isolates of *A. alternata* were recorded with the help of Ocular and Stage Micrometer.

Pathogenic variability

Pathogenic variability is the genetic characters of fungi which may vary amongst isolates. Healthy seeds of Isabgol variety RI-89 were surface sterilized with 0.1% $HgCl_2$ and were sown in pot containing sterilized soil @ 10 seeds per pot and replicated thrice (three pots). Leaves, stems and branches of six weeks old Isabgol plants were randomly selected, and these were injured gently by delicate brush and ten days old culture suspensions of individual isolates were sprayed with an automizer in early morning, when dew deposition was observed on the leaves of the plants. Simultaneously, un-inoculated check was maintained by spraying sterilized distilled water on the plants. The inoculated plants were observed daily to record the incubation period for disease development. The disease intensity was calculated with the help of disease rating scale (1-5) where,

1=1-20% infection or 1-20% leaves of the plant are infected, 2= 21-40% infection or 1-40% leaves of the plant are infected, 3= 41-60% infection or 41-60% leaves of the plant, 4= 61-80% infection or 61-80% leaves of the plant are infected and 5= 81-100% infection or 81-100% leaves of the plant are infected.

Per cent disease index (PDI) was calculated data following standard formula (Wheeler (1969), as given:

$$PDI = \frac{\Sigma\, 1x\ n + 2 \times n + 3x\ n + 4x\ n + 5 \times n}{N} X \frac{100}{\text{Maximmum score (5)}}$$

Where, n = Number of plants in each score

N= Total number of plants checked

Toxin variability

For determining toxin variability, 25 ml Richards' medium having pH 6.5 was poured in 100 ml sterilized flasks were inoculated with 5 mm diameter fungal discs of 10 days old culture of different isolates of *A. alternata* grown on PDA and incubated at 25 ± 1ºC for 15 days. The culture filtrate was obtained by filtration through Whatman No.42 filter paper. The culture filtrates obtained from 15 days old

cultures of *A. alternata* were centrifuged at 600 rpm for 20 min. The clear supernatant was collected in clean sterilized conical flasks and pellet sedimented at the bottom of the centrifuge tube was discarded. The clear supernatant solutions served as samples of crude toxin produced by different isolates were used to study toxin variability using detached (Salvik,1974).Observations were recorded regarding toxicity symptom expressions like necrosis, leaf drooping, wrinkling and drying of leaves at regular intervals of6, 12, 18, 20 and 24 hrs.

RESULTS AND DISCUSSION

Cultural variability

Considerable variability in all five in terms of colony characters like dark black colourd and very fast mycelial growth with smooth margin, light black with white at centre and fast growing, dark brown and mycelial growth with smooth margin, black colored, flat mycelial growth with smooth margin, and white with slightly black in colored with slow mycelial growth were observed in Aa-1, Aa-2, Aa-3, Aa-4 and Aa-5 respectively. The average radial growth of isolate Aa-1 was highest i.e. 90.00 mm while, in isolate Aa-2, Aa-3, Aa-4 and Aa-5, it was comparatively less i.e. 80.00 mm, 75.00 mm, 68.00 mm and 65.00 mm respectively on 7^{th} day of incubation under uniform environments and medium. Sporulation was recorded in all five isolates but very good sporulation was observed in Aa-1. In view of the results obtained for cultural variation, it is clear that all the five isolates differed with respect to mycelial growth of *A. alternata* attained after 7^{th} day for sporulation and colony characters. (Table 1) The results are also in similarity with the results obtained by Verma et al. (2007), Raja and Reddy (2007) and Tetarwal et al. (2008).

Variability in spore morphology

Clear variations among the isolates of *A. alternate* was observed (Table 2) The spore characteristics of individual isolates are described below:

Aa-1: Conidia were simple, obclavate, pale to dark brown formed in chains. Conidia have both transverse and vertical septa measuring 23-31 x 14-19 µm (with beak) and 6-18 x 6-11 µm (without beak).

Aa-2: Conidia were light brown to dark brown, obclavate, measuring 22-28 x 13-16 µm (with beak) and 9-11 x 7-10 µm (without beak).

Aa-3: Conidia dark brown colored, long beak and both transverse and vertical septa were present. The size of conidia measuring 29-36 x 14-23 µm (with beak) and 7-21 x 8-11 µm (without beak).

Aa-4: Conidia obclavate, shorten beak and light brown to dark brown in colour measuring 20-24 x 14-17 µm (with beak) and 8-12 x 6-9 µm (without beak).

Aa-5: Conidia were light brown and measuring 18-31 x 12-22 µm (with beak) and 10-15 x 7-9 µm (without beak).

The findings of the present investigation supported with the findings of Raja and Reddy (2007) collected the samples of leaf spot and fruit rot caused by *Alternaria alternata* from brinjal growing areas and it was found that The size of conidia varied from 35.2 -43.5 µm and 12.4-13.9 µm wide, with average beak length of 9.6 -12.4 µm. Horizontal and vertical septations of conidia varied form 1.8 and 0.3, respectively and conidia were produced in chain.

Pathogenic variability

The isolates of the pathogen collected from different geographical areas may show difference in virulence. The isolates Aa-1 was found to be highly pathogenic on Isabgol cv. RI-89 under artificial inoculation conditions, which showing 52.12% disease intensity followed by Aa-3 (47.56%), Aa-2 (41.40%), Aa-4 (38.20%) and Aa-5 (35.48%). However, data were recorded 4-5 days of incubation in Aa-1 followed by Aa-3 (4-6), Aa-2 (4-6), Aa-4 (5-7) and Aa-5 (5-7). The seedlings grown applying sterilized distilled water without inoculation did not produce any blighted symptoms and grew healthy (Table 3). The pathogenic variability have also been carried out by Verma et al. (2007) and Kumar et al. (2008) on *A. solani.* They recorded pathogenic variability among different isolates of *A. solani*. Tetarwal et al. (2008) observed variability among six isolates of *A. alternata* infecting Senna (*Cassia angustifolia*)

Toxin variability

The details of the experimental results are presented in Table 4. The culture filtrate is assumed as 100 per cent toxin concentration. This was simply an indicator test for toxin production. The symptoms like drooping of leaves, blackening of leaves was initiated at 6 hours and continued up to twenty-four hours, finally leading to wilting and necrosis, thus revealing the existence of variation among the isolates in producing toxic metabolites in the culture medium, which was reflected in terms of inducing wilting of Isabgol cuttings. The results indicated that Aa-1 isolate showed very severe toxic effect where initial toxicity symptom expression was within six hours, leading to complete and severe necrosis of leaves with distinct black colourations. Similarly, severely toxic , moderately toxic, slight toxic and least toxic effect were observed in cultural filtrate toxin of Aa-2, Aa-3, Aa-4 and Aa-5 isolates, respectively. This suggests that the toxin has active role in causing disease as well as mortality. Such phytotoxic effects produced by culture filtrate were also reported by Reddy and Chaudhary (1990) where, they observed that, when pigeon pea seeds were soaked in culture filtrate of six *Fusarium udum* isolates for 6, 12, 24 h, no germination occurred after 24 h and radial length was also decreased with increasing in soaking time. Maiero et al. (1991) stated that *A. solani* produced phytotoxic metabolites, and tomato seedlings exposed to culture filtrates for 20 h exhibited marginal and inter veinal leaf necrosis and subsequently wilting.

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Table 1: Cultural variability among five isolates of *Alternaria alternata* on PDA

Sl. No.	Isolates	Location of collection of isolates	Radial mycelial growth in (mm)	Sporulation	Colony characters
1.	Aa-1	Udaipur	90.00	+ + + +	Dark black colourd, very fast mycelial growth with smooth margin.
2.	Aa-2	Chittorgargh	80.00	+ + +	Light black with white at centre and fast growing.
3.	Aa-3	Jodhpur	75.00	+ + +	Dark brown and medium mycelial growth with smooth margin.
4.	Aa-4	Pali	68.00	+ +	Black colourd, flat mycelial growth with smooth margin.
5.	Aa-5	Jalore	65.00	+ +	White with slightly black in colour and slow mycelial growth.
	SEm±		0.865		
	CD at 5%		2.821		
	CV%		2.46		

*Average of three replications

Note: ++ = Fair, +++ = Good, ++++ = very good.

Table 2: Variation in conidial morphology of five isolates of *Alternaria alternata*

S. No.	Isolates	Conidial morphology with beak (µm)				Conidial morphology without beak (µm)			
		Length		Width		Length		Width	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.	Aa-1	28.05± 1.68	23-31	17.18± 1.00	14-19	12.93± 1.76	6-18	8.73±0.69	6-11
2.	Aa-2	24.84± 1.26	22-28	15.23± 0.76	13-16	10.22± 0.51	9-11	8.47±0.42	7-10
3.`	Aa-3	32.19± 1.69	29-36	20.08± 1.48	14-23	16.67± 4.02	7-21	9.55±0.46	8-11
4.	Aa-4	22.22± 0.99	20-24	15.23±0.76	14-17	9.30± 0.72	8-12	7.88±0.44	6-9
5.	Aa-5	28.01± 2.90	18-31	18.95±1.91	12-22	12.07± 1.00	10-15	8.43±0.44	7-9
	SEm±	0.26		0.19		0.34		0.05	
	CD at 5%	0.74		0.55		0.97		0.16	
	CV%	9.80		11.35		8.40		6.84	

* Mean no. of 25 conidia and ± S.D. of mean value

Table 3: Pathogenic variability of five isolates *Alternaria alternata* under artificial inoculation conditions.

S.No.	Isolates	Disease Intensity (%)*	Incubation periods (days)*
1.	Aa-1	52.12 (52.12)	4-5 days
2.	Aa-2	41.40 (41.39)	4-6 days
3.`	Aa-3	47.56 (47.56)	4-6 days
4.	Aa-4	38.20 (38.19)	5-7 days
5.	Aa-5	35.48 (35.48)	5-7 days
6.	Spray with water (Control)	0.00 (0.00)	-
	SEm±	0.569	
	CD at 5%	1.792	
	CV%	2.41	

*Average of three replications

Figures in parentheses are angular transformed values

Table 4: Toxin variability among five isolates based on their culture filtrates (crude toxin) toxicity symptoms on Isabgol leaves.

S.No.	Isolates	Toxicity symptoms observed	Grade
1.	Un-inoculated broth	Did not show any toxicity.	Non toxic
2.	Aa-1	Initial toxicity symptom expression in six hours, leading to complete and severe necrosis of leaves with distinct black colourations.	Very Severely Toxic
3.	Aa-2	Initial toxicity symptom expression in twelve hours, leading to complete leaf drooping, wrinkling, drying and brittling of leaves.	Severely Toxic
4.	Aa-3	Initial toxicity symptoms expression in eighteen hours, leading to complete wrinkling and necrosis.	Moderately Toxic
5.	Aa-4	Initial toxicity symptoms expression in twenty hours, leading to slight necrosis.	Slight Toxic
6.	Aa-5	Initial toxicity symptoms expression after twenty-four hours, leading to least toxic.	Least toxic