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Pathogenic Variability of *Alternaria* spp. Isolates Causing Leaf Blight of Cotton

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Cotton is one of the most ancient and important commercial crop in India. It is regarded as 'King of Fiber', back bone of our sprawling textile industry and fetching an export earning besides providing employment to farming community. *Alternaria,* a major foliar fungal pathogen showed wide variability with respect to morphological and cultural aspects were concerned. Conidial septation of ten isolates ranged from 1-2 vertical and 4-6 horizontal septa. Conidial size varied from 21.5 x 6.87µm (Haveri) to 49.38 x 12.82µm (Karlakatti). Out of ten isolates, two resembled *A. macrospora* and three resembled *A. alternata*. Maximum dry mycelial weight of *A. macrospora* was observed after sixteen days of incubation. These isolates were cultured on different solid media as part of variability study and the colony colour varied from grey to black, with white to black colony margin either irregular or smooth, raised to flat mycelial growth and sectoring was observed in few isolates.

Keywords: *Alternaria*, variability, isolates, Bt cotton.

Cotton is an important cash crop playing a significant role in the economy of the major developing countries including India. It is known as the 'King of fiber' and 'White Gold'. India has been recognized as the cradle of cotton industry and is the original home of domestication, diversification and development of particularly Asiatic cultivated cottons.. In India, leaf spot of cotton (*Alternaria macrospora* Zimm,) was reported for the first time by Uppal *et al.* (1935) which is a major contributing factor from the recent past for low productivity of cotton in Karnataka. The fungus derives food and energy from the substrate upon which they grow in nature. In order to culture the fungus in the laboratory, there is no universal substrate or artificial medium upon which all the fungi can grow and reproduce. Hence the present study was carried out to identify surface medium for the growth and sporulation by using different solid media.

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MATERIALS AND METHODS

Leaves were collected from infected fields and used for isolation of the fungus *in vitro*. The isolation of the fungus was made by following standard tissue isolation technique. Identification of the fungus was carried out based on the morphological characters of the isolated fungus. Selection of basal medium for growth and sporulation of the fungus was done by using potato dextrose agar and studied morphological characters like length and width of conidia, number of horizontal and vertical septa and beak length were measured under 40x using Differential Image Contrast microscope. Later the measurements were compared with the standard descriptions given by Ellis (1971) regarding *Alternaria macrospora* and *Alternaria alternata* for identification of

Table 1(a). Morphological variability of isolates of *Alternaria* spp

Name of the isolate	Number of horizontal septa	Number of vertical septa	Size of conidia (Length x Breadth) (µm)	Beak length (µm)	Overall length of conidia (µm)	Descriptions of Ellis M.B. regarding *Alternaria macrospora* Zimm. Number of horizontal septa	Number of vertical septa	Beak length	Overall length (µm)	Re *A* *m*
Karlakatti	5	1	49.38 x 12.82	29.15	78.53					No F
Yamkanmardi	6	1	32.8 x 17.17	32.84	65.64					No F
Marewada	4	1	47.95 x 15.26	33.52	81.47					No F
Unkal	5	2	28 x 12.31	63.02	91.02	4-9	1-5	Equal or twice the length of conidia	90-180	C Re
Chandanamatti	6	1	48.24 x 15.79	27.47	75.71					No F
Saundatti	4	2	21.97 x 13.02	74.03	96					C Re
Jagalur	6	1	26.99 x 7.61	30.98	57.97					No F

Table 1(b). Morphological variability of isolates of *Alternaria* spp

S. No.	Name of the isolate	Number of horizontal septa	Number of vertical septa	Size of conidia (Length x Breadth) (µm)	Beak length (µm)	Overall length of conidia (µm)	Descriptions of Ellis M.B. regarding *Alternaria alternata* (Fr.) Keissler				Resemblance towards *Alternaria alternata* (Fr.) Keissler
							Number of horizontal septa	Number of vertical septa	Beak length	Overall length (µm)	
A_2	Amminbhavi	6	1	33.29 x 12.54	26.37	56.37					Complete Resemblance
A_7	Haveri	5	2	21.5 x 6.87	25.32	46.82	1-8	0-4	Short or more than one third the length of conidia	20-63	Complete Resemblance
A_9	Gadag	5	2	30.22 x 7.4	24.04	56.26					Complete Resemblance

Table 2. Effect of incubation period on dry mycelial weight of *Alternaria macrospora*

Incubation period (days)	Mycelial dry weight (mg)
2	36.53
4	77.56
6	93.70
8	169.88
10	192.42
12	229.17
14	272.55
16	287.31
18	275.90
20	267.53
22	262.30
24	258.20
26	234.15
28	207.65
30	192.74
S. Em.±	0.57
CD at 1%	2.20

Alternaria spp. Later cultural study was carried out by inoculating the pathogen on Potato Dextrose Broth and dry mycelial weight was recorded at regular intervals inorder to know the number of days required for maximum growth of the fungus. The isolates were grown on 8 different solid media to select best media for growth *viz.*, Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), Czapek's Dox Agar (CDA), Host Extract Agar (HEA), Oat Meal Agar (OMA), Corn Meal Agar (CMA), Sabouraud's -Dextrose Agar (SDA) and V8 Juice Agar (V8JA)and to find the difference in colony characters such as radial growth, type of colony margin, colour of margin, mycelial growth, sectoring and sporulation. The best media was found and used as a basal media for further studies. Radial growth (mm) = (length + breadth of grown mycelium) / 2.

RESULTS AND DISCUSSION

Isolates collected during survey *viz.*, Karlakatti (A_1), Amminbhavi (A_2), Yamkanmardi (A_3), Marewada (A_4), Unkal (A_5), Chandanamatti (A_6), Haveri (A_7), Saundatti (A_8), Gadag (A_9) and Jagalur (A_{10}) were isolated and pure culture was maintained and were stored in the refrigerator at 5°C for further studies. The isolates subjected to various morphological variability tests showed that, conidia were septated by 1-2 vertical and 4-6 horizontal septa (Figure 1). The isolates, A_3, A_6 and A_{10} showed maximum horizontal septa of 6 followed by 5 horizontal septa in isolates, A_1 and A_5. Whereas minimum horizontal septa (4) was observed in the isolates, A_4 and A_8. The isolates, A_5 and A_8 showed maximum of 2 vertical septa and isolates, A_1, A_3, A_4, A_6 and A_{10} showed minimum of 1 vertical septa. The isolates, A_1, A_6 and A_4 showed maximum size of 49.38 x 12.82 mm, 48.24 x 15.79 mm and 47.95 x 15.26 mm, respectively. The least size of the conidia (21.97x 13.02 mm) was observed in isolate, A_8 (Table 1a). By comparing with *Alternaria macrospora* structural figure described by Ellis M.B. revealed that out of 7 isolates, only two isolates *viz.*, A_5 and A_8 showed complete resemblance with *Alternaria macrospora* and other isolates *viz.*, A_1, A_3, A_4, A_6 and A_{10} showed no resemblance with *Alternaria macrospora* morphologically.

Results revealed that isolate A_2 showed 6 horizontal and 1 vertical septa, whereas isolates, A_7 and A_9 showed 5 horizontal and 2 vertical septa (Table 1b). Maximum conidial size (33.29 x 12.54 mm) was observed in isolate, A_2, whereas isolate, A_7 showed minimum conidial size of 21.5 x 6.87 mm. Isolate, A_9 showed conidial size of 30.22 x 7.4 mm. When the isolates were compared with *Alternaria alternata* structural figure described by Ellis M.B., all the three isolates *viz.*, A_2, A_7 and A_9 showed complete resemblance morphologically.

Conidiophores of *Alternaria macrospora* arise singly or in groups, straight or flexuous, tapering towards the apex and septate. They are pale brown in colour, 4-9 µm thick and upto 180 µm in length. Conidia are solitary or in chains of two, straight or curved with the body of the conidium ellipsoidal tapering to a narrow beak and equal in length or upto twice as long as body. They are reddish brown in colour with four to nine transverse septa and several longitudinal septa (Ellis, 1971). Several attempts are made to classify *Alternaria* genera, several re-descriptions and revised criteria of these genera (Joly, 1964) resulted in a growing number of new species.

There was significant difference among the incubation periods. The dry mycelial weight of *Alternaria macrospora* gradually increased (36.53

Table 3. Cultural variability of growth and sporulation of ten isolates of *Alternaria* spp. on different solid media

S. No.	Isolates	Radial growth (mm)								Mean
		PDA	PCA	CDA	HEA	OMA	CMA	SDA	V8JA	
A_1	Karlakatti	77.50 +++	63.77 ++	71.23 ++	90.00 ++++	82.50 +++	43.60 ++	82.50 ++	42.50 +++	69.20
A_2	Amminbhavi	60.33 +++	82.50 ++++	65.00 +++	90.00 ++++	90.00 +++	25.00 +++	45.47 ++	37.50 +++	61.98
A_3	Yamkanmardi	75.00 +++	61.00 +++	73.37 +++	90.00 +++	90.00 +++	34.27 +++	86.00 +++	35.00 ++	68.08
A_4	Marewada	90.00 ++++	90.00 ++++	90.00 ++++	90.00 +++	90.00 +++	31.34 ++	90.00 ++++	56.27 ++	78.45
A_5	Unkal	77.17 +++	73.33 +++	83.33 +++	90.00 ++++	90.00 +++	36.17 ++	71.27 ++	34.60 +++	69.48
A_6	Chandanamatti	76.77 +++	84.83 +++	77.83 ++	90.00 +++	90.00 +++	65.83 +++	85.00 ++++	82.30 +++	81.57
A_7	Haveri	81.27 +++	90.00 ++++	75.43 +++	90.00 ++++	90.00 ++++	83.83 +++	82.43 +++	39.92 +++	79.11
A_8	Saundatti	73.33 +++	69.83 +++	75.33 +++	90.00 +++	90.00 ++++	44.33 +++	81.67 +++	40.17 +++	70.58
A_9	Gadag	90.00 ++++	90.00 ++++	71.42 +++	90.00 +++	90.00 ++++	90.00 ++++	90.00 ++++	36.08 ++++	80.94
A_{10}	Jagalur	73.50 +++	86.38 +++	56.46 ++	90.00 +++	90.00 +++	67.67 +++	90.00 ++++	33.77 +++	73.47
	Mean	77.49	79.16	73.94	90.00	89.25	52.20	80.43	43.81	73.29
		Isolates (I)	Media (M)	I x M						
	S. Em.±	0.27	0.24	0.78						
	CD at 1%	1.09	0.97	3.09						

++: Moderate sporulation +++: Good sporulation ++++: Excellent sporulation PDA - Potato dextrose agar
PCA – Potato carrot agar CDA – Czapeck's dox agar HEA – Host extract agar OMA – Oat meal agar CMA – Corn meal agar
SDA – Sabouraud's dextrose agar V8JA – V-8 Juice agar

Table 4. Cultural diversity of ten isolates of *Alternaria* spp

S. No.	Isolates Media	Colony charcters – colour, margin, type of margin, mycelia growth							
		PDA	PCA	CDA	HEA	OMA	CMA	SDA	V8JA
A_1	Karlakatti	Dark grey, irregular margin, raised mycelium	Whitish grey, irregular margin. raised mycelium	Ashy white, irregular margin, raised mycelium	Grayish black, smooth margin, raised mycelium	Grey white, irregular margin, raised mycelium	Black, irregular margin, distrorted mycelium	Dark grey, irregular margin, flat mycelium	Black, irregular margin, raised mycelium
A_2	Amminbhavi	Grey, irregular margin, raised mycelium	Grey, irregular margin. Flat mycelium	Grey, smooth margin, raised mycelium	Grayish black, smooth margin, raised mycelium	Grayish black, irregular margin, raised mycelium	Black, irregular margin, distrorted mycelium	Grey, irregular margin, raised mycelium	Grey, smooth margin, flat mycelium
A_3	Yamkanmardi	Grey white, irregular margin, raised mycelium	Grey, irregular margin. raised mycelium	Grey, irregular margin, raised mycelium	Black, irregular margin, raised mycelium	Grey, irregular margin, raised mycelium	Black, irregular margin, distorted mycelium	Grayish black, irregular margin, raised mycelium	Black, irregular margin, flat mycelium
A_4	Marewada	Grayish black, smooth margin, flat mycelium	Grey, smooth margin. Flat mycelium	Grayish black, irregular margin, raised mycelium	Black, smooth margin, raised mycelium	Black, smooth margin, raised mycelium	Black, irregular margin, distorted mycelium	Black, irregular margin, raised mycelium	Black grey, irregular margin, raised mycelium
A_5	Unkal	Black white, irregular margin, raised mycelium	Grey, smooth margin. raised mycelium	Grey, smooth margin, raised mycelium	Grey, irregular margin, raised mycelium	Grey, irregular margin, raised mycelium	Grey, distorted margin, raised mycelium	Grayish black, irregular margin, raised mycelium	Black, irregular margin, raised mycelium
A_6	Chandanamatti	Black grey, smooth margin. Flat mycelium	Whitish grey, irregular margin. raised mycelium	Grey, irregular margin. raised mycelium	White, smooth margin. flat mycelium	Black, irregular margin. raised mycelium	Grey, irregular margin. distorted mycelium	White grey, irregular margin. raised mycelium	Grey black, irregular margin. raised mycelium
A_7	Haveri	Black, irregular margin. raised mycelium	Grey, irregular margin. Flat mycelium	Grayish black, irregular margin. raised mycelium	Grey, smooth margin. Raised mycelium	Grey, irregular margin. raised mycelium	Grey, irregular margin. Distorted mycelium	Black, irregular margin. raised mycelium	Grey black, irregular margin. raised mycelium
A_8	Saundatti	Grey, irregular margin. raised mycelium	Grey, irregular margin. raised mycelium	Ashy grey, irregular margin. raised mycelium	Grayish black, smoothmargin. raised mycelium	Grey white, irregular margin. raised mycelium	Black, irregular margin. raised mycelium	Black grey, irregular margin. Flat mycelium	Black, irregular margin. raised mycelium
A_9	Gadag	White grey, smooth margin. Flat mycelium	Grey, smooth margin. Flat mycelium	Grey, smooth margin. Flat mycelium	White, smooth margin. Flat mycelium	Grey black, smooth margin. Flat mycelium	Grey, smooth margin. distorted mycelium	White grey, smooth margin. Flat mycelium	White grey, irregular margin. Flat mycelium
A_{10}	Jagalur	Grayish black, irregular margin. Raised mycelium	Grayish black, irregular margin. Flat mycelium	Grey black, smooth margin, raised mycelium	Black, smooth margin. Raised mycelium	Grey, smooth margin. Flat mycelium	Black, irregular margin, distorted mycelium	Black, smooth margin. Flat mycelium	Grey, smooth margin. Flat mycelium

PDA - Potato dextrose agar, PCA – Potato carrot agar, CDA – Czapeck's dox agar, HEA – Host extract agar, OMA – Oat meal agar, CMA – Corn meal agar, SDA – Sabouraud's dextrose agar, V8JA – V-8 Juice agar

mg) from third day of inoculation and reached maximum (287.31 mg) on sixteenth day. The data showed a declining trend from eighteenth day (275.90 mg) to thirtieth day (192.74 mg) (Table 2). The isolates exhibited variability in cultural characters when grown on 8 different solid media (Figure 2). Among ten isolates, A_4 and A_9 showed maximum radial growth (90 mm) on many of the media *viz.,* PDA, PCA, HEA, OMA and SDA tested. Whereas, Isolate, A_2 showed least mean radial growth (61.98 mm).

Among the eight solid media, HEA (90 mm) and OMA (89.25 mm) showed maximum radial growth in all isolates. Majority of the isolates showed moderate to excellent sporulation (Table 3). SDA (80.43 mm) and PCA (79.17 mm) were on par with each other. Whereas, PDA recorded mean radial growth of 77.49 mm and V8JA (43.81 mm) showed least radial growth.

The isolates grown on different media showed varied colony characters (Table 4). A_1, A_2, A_5, A_7 and A_9 isolates showed grey colony on most of the media, whereas isolates, A_3, A_6, A_8 and A_{10} showed grey and black colour colonies. The colony margin varied from grey to black in all isolates. Irregular margin was seen predominantly in the isolates *viz.,* A_1, A_3, A_5, A_6, A_7 and A_8, whereas isolates, A_9 and A_{10} showed smooth margin among the media tested. Several workers observed diversity in cultural characteristics such as growth rate, type of growth, colony colour and sporulation among different isolates of *Alternaria* spp. infecting sesame, sunflower and cotton (Ramegowda**,** 2007).

CONCLUSIONS

Morphological variability study of ten isolates revealed that, two resembled with *Alternaria macrospora,* five showed no resemblance with *Alternaria macrospora* and three isolates resembled with *Alternaria alternata* morphologically. Pathogen required sixteen days to attain maximum dry mycelial weight. Among the ten isolates, A_6 recorded maximum mean radial growth (90 mm) on the media. Among the eight media tested, HEA and OMA showed maximum radial growth in all the ten isolates. Majority of the isolates showed moderate to excellent sporulation with flat to raised, irregular to smooth grey colonies with presence or absence of sectoring. Though several cotton varieties and hybrids are being released from time to time, none of them has shown complete resistance to the disease. This indicates the existence of variability among the pathogens which may be attributed to weather conditions of particular location, varieties and hybrids and ability of pathogen to adopt themselves to various situation. Once genus is narrowed by morphology, symptomatology and host-specificity, then it can be used to differentiate species (Chakrabarty *et al.,* 2007). Therefore, study of variability among the isolates will be helpful for designing Integrated Disease Management strategies.

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