

Original Research Article

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Cultural Variability of Different Isolates of Cotton *Alternaria* spp. on Different Media

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ABSTRACT

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Cotton (*Gossypium hirsutum* L.) is the one of the most important commercial crops of the world, which belongs to the family Malvaceae. India is the largest cotton growing country in the world with an area of around 12.35 M ha accompanied by United States and China with production of 36.1 M bales and productivity of 524 kg lint/ha. Cotton crop is known to suffer from number of fungal, bacterial and viral diseases. In India foliar diseases have been estimated to cause yield losses up to 38 per cent. Cotton is under persistent threat of foliar diseases like grey mildew, *Alternaria* leaf spot, *Myrothecium* leaf spot, bacterial leaf blight, rust *etc.* Cultural variability among the twelve isolates were studied by growing isolates on different media *i.e.*, Cotton leaf extract agar, Potato dextrose agar, Oat meal agar. Among the media tested, cotton leaf extract agar supported good growth of the fungus with excellent radial growth and high sporulation followed by potato dextrose agar and while least sporulation was observed on Oat meal agar.

Introduction

Cotton is the most essential natural fiber crop in the world for textile produce, accounting for about 50% of all fibers used in the textile industry. It is grown all over the world in about 80 countries. Cotton is unique among agricultural crops, because it is the main natural fiber crop, and also provides edible oil. It is one of the agro-industrial crops which are produced in both developing and developed countries (Bedane and Arkebe, 2019).

India is the largest cotton growing country in the world with an area of around 12.35 M ha followed by United States and China with production of 36.1 M bales and productivity of 524 kg lint/ha (Cotton Association of India, 2018-2019). India's share in global cotton exports is around 25 percent In India, Maharastra (26.63%), Gujarat (17.96%), Andhra Pradesh (13.75%) and also Madhya Pradesh are the leading cotton producing states. Cotton in India provides direct livelihood to 6 million farmers and about 40 - 50 million people are employed in cotton trade and its processing (Chitte *et al.*, 2019).

The word “cotton” is derived from Arabic word (*qutnor qutun*). This was the usual word for cotton in medieval Arabic. Cotton (*Gossypium* spp.) belongs to the botanical family Malvaceae. Cotton is referred to as “King of Fibres” and also known as “White Gold” (Prasad *et al.*, 2018).

There are four cultivated species of cotton viz., *Gossypium arboreum*, *G. herbaceum*, *G. hirsutum* and *G. barbadense*. The first two species are diploid ($2n=26$) and are native to old world. The last two species are tetraploid ($2n=52$). *G. hirsutum* is the predominant species which alone contributes about 90 per cent to the global production. Perhaps, India is the only country in the world where all the four cultivated species are grown on commercial scale (Chitte *et al.*, 2019).

The various species of cotton grown as agricultural crops are native to subtropical parts of the world. Cotton can be found as perennial tree like plants in tropical climate but is normally cultivated as a shrubby annual in temperate climates.

Cotton is a heat loving crop. During germination it requires 32-34 °C and 25-27 °C during the vegetative stage. Average temperature of 21-22 °C is required for the crop (Sangameshwari *et al.*, 2019). It is grown between latitudes 30° N and 30° S.

Materials and Methods

Cotton leaf extract agar (MA)

Agaragar	:	20 g
Dextrose	:	20 g
Maizeleaves	:	200 g
Distilled water:		1000 ml

Two hundred grams of cotton leaves were boiled in 500 ml of distilled water in a 1000 ml beaker for 30 min. The extract was filtered

through a double layered muslin cloth. To another 500 ml of distilled water in another 1000 ml beaker, 20 g of agar agar was added and melted till it gets dissolved. Both the solutions were mixed in another 1000 ml beaker into which 20 g of dextrose was added. The final volume of the medium was made up to 1000 ml by addition of sterile distilled water. The pH of the media was adjusted 6.8 by 1 N NaOH or 1 N HCl. Two hundred and fifty ml of the media was then transferred to 500 ml conical flask and were sterilized in an autoclave at 121°C and 15 *Psi* for 20 minutes. The sterilized media was stored in a refrigerator for further use.

Potato dextrose agar (PDA) medium

PDA medium was prepared using the following components for culturing the fungi in the laboratory.

Potato (peeled, washed and sliced):	200 g
Dextrose	: 20 g
Agaragar	: 20 g
Distilledwater	: 1000 ml

Two hundred gram of potatoes were taken, peeled and cut into small pieces and boiled for 20 to 30 min. After boiling extract was filtered through muslin cloth. Dextrose and agar were added in equal amount and boiled until properly dissolved. Sterilization was done 121 °C for 15 min. Twenty ml of media (PDA) was poured into sterilized Petriplates and kept for solidification.

Oat Meal Agar (OMA)

Oatflakes	:	30 g
Agar-agar	:	20 g
Distilled water:		1000 ml (volume to make up)

Oat flakes were boiled in 500 ml distilled water for 30 min and filtered through muslin cloth. Agar agar was melted in 500 ml

distilled water separately. Both the solutions were mixed thoroughly and the volume was made up to 1000 ml and was sterilized was done 121 °C and 15 psi for 15 min.

Twenty ml of media (PDA) was poured into sterilized petriplates and kept for solidification. After solidification, each treatment was replicated thrice. 5 mm discs of the *Alternaria* spp. were cut using a cork borer and a single disc was placed on the slat. Each set of the experiment was replicated twice and the plates were incubated at 27 ± 1 °C for 12 days. After 15 days, the observation of diameter of radial growth, type of colony margin, colour of margin, mycelial growth, sectoring and sporulation were recorded. Sporulation was graded as per (Sangeetha K. D., 2014)

Results and Discussion

Cultural characteristics of different isolates of *Alternaria* spp. on Cotton leaf extract agar

The data pertaining to cultural characteristics of different isolates *Alternaria* spp. was recorded twelve days after incubation on medium at 25 ± 1 °C.

Colony colour

The colony colour of fungus was recorded based on dominant spectral colour from Munsell's soil colour chart (1951), twelve days after incubation on medium. The colony colour varied from grey to black colour.

Based on the colony colour, all the twelve isolates were grouped into four categories *i.e.*, white, black, ashy and greyish. Isolate N1A, A2A and isolate A4A showed white. Isolates N2A, N3A, N4A and M3A showed black while isolates, A3A, M1A and M4A showed greyish.

Isolates M2A and A1A showed ashy colour.

Colony margin colour

The margin colour of culture medium of all the twelve isolates were grouped into three categories *i.e.*, white, black, greyish colour.

There was significant difference among the isolates with regard to colony margin in the culture medium.

The isolates N1A, A2A and M3A showed white margin. Isolates N2A, A3A, N3A, N4A and M4A showed black margin while Isolates M1A, A1A, A4A and M2A exhibited greyish margin.

Type of margin

All twelve isolates showed varied type of margin from raised to flat. Isolates M1A, M3A and M4A had flat type of margin whereas, isolates like N1A, N4A, A3A, N3A, N2A, A2A, M2A, A1A and A4A had raised type of margin.

Radial growth

All the isolates showed full plate growth 90.00 mm except one isolate A2A with least radial growth of 85.33 mm.

Sporulation

All the twelve isolates of *Alternaria* spp. were classified into three groups based on the sporulation. Excellent sporulation was observed in isolate N2A. The isolates, N1A, A1A, N4A, A4A and M2A recorded very good sporulation. Good sporulation was observed in isolate N3A while, poor sporulation was seen in isolates M1A, M3A, M4A and A2A.

Table.1 The sporulation grade for *Alternaria* spp

Sl. No.	Score	Grade	Description No. of spores/microscopic field (10×)
1	++++	Excellent sporulation	> 30 spores/microscopic field
2	+++	Good sporulation	21-30 spores/microscopic field
3	++	Moderate sporulation	11-20 spores/microscopic field
4	+	Poor sporulation	1-10 spores/microscopic field
5	-	No sporulation	< 1 spores/microscopic field

Table.2 Cultural characteristics of different isolates of *Alternaria* spp. on cotton leaf extract agar

S.NO	Isolate	Colony color	Colony colourmargin	Type of margin	Radial growth	Sporulation
1.	N1A	White	White	Raised	90.00	+++
2.	N2A	Black	Black	Raised	90.00	++++
3.	N3A	Black	Black	Raised	90.00	++
4.	N4A	Black	Black	Raised	90.00	+++
5.	M1A	Greyish	Greyish	Flat	90.00	+++
6.	M2A	Ashy	Greyish	Raised	90.00	+++
7.	M3A	Black	White	Flat	90.00	+
8.	M4A	Greyish	Black	Flat	90.00	+
9.	A1A	Ashy	Greyish	Raised	90.00	+++
10.	A2A	White	White	Raised	85.33	+
11.	A3A	Greyish	Black	Raised	90.00	+++
12.	A4A	White	Greyish	Raised	90.00	+++
				C.D.	0.989	
				SE(m)±	0.337	
				C.V.	0.651	

Table.3 Cultural characteristics of different isolates of *Alternaria* spp. on potato dextrose agar

S.NO	Isolate	Colony colour	Colony colour margin	Type of margin	Radial growth	Sporulation
1.	N1A	White	White	Flat	90.00	+++
2.	N2A	Black	Black	Flat	90.00	++++
3.	N3A	Black	Black	Flat	82.00	+
4.	N4A	Black	Black	Raised	90.00	+++
5.	M1A	Greyish	Greyish	Raised	90.00	++++
6.	M2A	Black	Greyish	Raised	78.33	++
7.	M3A	Ashy	Greyish	Flat	90.00	++
8.	M4A	Greyish	Black	Flat	90.00	+++
9.	A1A	White	Greyish	Flat	90.00	+++
10.	A2A	Black	Black	Raised	90.00	+
11.	A3A	Ashy	White	Flat	90.00	++++
12.	A4A	White	White	Raised	42.00	++
				C.D.	1.787	

Table.4 Cultural characteristics of different isolates of *Alternaria* spp. on oat meal agar

S.NO	Isolate	Colony colour	Colony colour margin	Type of margin	Radial growth	Sporulation
1.	N1A	Ashy	White	Raised	28.33	++++
2.	N2A	Black	Black	Raised	90.00	+++
3.	N3A	Greyish	White	Flat	90.00	+
4.	N4A	Black	Black	Flat	90.00	++
5.	M1A	Ashy	Greyish	Raised	50.33	++++
6.	M2A	Black	Black	Raised	27.33	+++
7.	M3A	White	Greyish	Flat	90.00	++
8.	M4A	Ashy	White	Flat	90.00	++
9.	A1A	Ashy	Greyish	Raised	90.00	+++
10.	A2A	Black	Black	Flat	28.00	+
11.	A3A	Black	Black	Raised	56.00	++++
12.	A4A	White	White	Raised	34.33	+++
				C.D.	1.719	
				SE(m)±	0.585	
				C.V.	1.592	

Cultural characteristics of different isolates of *Alternaria* spp. on Potato dextrose agar

Colony colour

Based on the colony colour, all the twelve isolates were grouped into four categories (as described earlier in 4.2.1.1). The isolates N1A, A1A and A4A showed white. The isolates N4A, N3A, N2A and M2A showed black colour. Whereas, the isolate M1A and M4A has showed greyish black colour, and isolate A2A, M3A and A3A exhibited ash colour.

Colony margin colour

The margin colour of culture medium of all the twelve isolates were grouped into three categories *i.e.*, white, black and greyish colour.

Isolate N1A, A3A, A2A and A4A showed white margin. Isolates like M1A, M2A, M3A, A1A showed greyish colour margin, whereas, isolates like N4A, N2A, M4A and N3A exhibited black margin.

Type of margin

All twelve isolates showed varied type of margin from raised to flat. Isolates N4A, M1A, A2A, M2A and A4A had raised type of margin. Whereas isolates like N1A, A3A, N3A, N2A, M4A, A1A and M3A showed flat type of margin.

Radial growth

The highest radial growth was observed in N1A, N2A, N4A, M1A, M3A, M4A, A1A, A2A and A3A isolates with 90.00 mm followed by N3A isolate (82.00 mm) and M2A (78.33 mm), least radial growth was observed in A4A isolate (42.00 mm).

Sporulation

Excellent sporulation was observed in isolate N2A and isolate A3A. While the isolates, N1A, N4A, A1A and M1A, M4A recorded very good sporulation. Good sporulation was observed in isolate A4A and isolate M3A while, poor sporulation was seen in isolates N3A, A2A, and M2A.

Cultural characteristics of different isolates of *Alternaria* spp. on oat meal- agar

Colony colour

Based on the colony colour, all the twelve isolates were grouped into four categories as described earlier in 4.2.1.1. The isolates A1A, A4A and M3A showed white colour. Isolates like N4A, A3A, N2A, A2A and M2A showed black whereas isolates like N1A and M4A showed ashy. Isolates like M1A and N3A showed greyish.

Colony margin colour

Isolates N1A, M4A and A4A showed white margin. Isolates M1A, A1A and M3A showed greyish colour margin whereas isolates like N4A, A3A, N3A, N2A, A2A, M2A exhibited black margin.

Type of margin

All twelve isolates showed varied type of margin from raised to flat. Isolates N1A, A3A, M1A, M2A, N2A, A4A and A1A had raised type of margin. Whereas isolates like N4A, N3A, M4A, A2A and M3A showed flat type of margin.

Radial growth

The highest radial growth was observed in isolates N2A, N3A, N4A, M3A, M4A, A1A (90.00 mm) followed by A3A (56.00 mm), M1A (50.33 mm), A4A (34.33 mm), N1A (28.33 mm). Lowest radial growth was observed in isolate M2A (27.33 mm).

Sporulation

Excellent sporulation was observed in isolate M1A, A3A and isolate N4A. While the isolates, N1A, N2A, A4A and M4A recorded very good sporulation. Good sporulation was observed in isolate A1A, M2A and isolate M3A while, poor sporulation was seen in isolates N3A, A2A.

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