Morphological and cultural variability in isolates of Alternaria spp. causing leaf blight of cotton

K. D. SANGEETHA AND S. A. ASHTAPUTRE

Department of Plant Pathology, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India E-mail: sudheendra67@gmail.com

(Recevied: July, 2014 ; Accepted: April, 2015)

Abstract: Cotton is the most important cash crop, back bone of sprawling textile industry and fetching an export earning besides providing employment to Indian population. *Alternaria*, a major foliar fungal pathogen showed wide range of variability in morphology as well as in culture. Septation of conidia of ten isolates ranged from 1-2 vertical and 4-6 horizontal. Size of the conidia varied from 21.5 x 6.87 (Haveri) to 49.38 x 12.82 µm (Karlakatti). Measurements of all isolates were compared with standard measurements of *Alternaria macrospora* Zimm. and *Alternaria alternata* (Fr.) Keissler. 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 for variability study. The colony colour varied from grey to black, with white to black colony margin *viz.*; irregular or smooth, raised to flat mycelial growth and sectoring was observed in few isolates.

Key words: Alternaria macrospora, Cotton, Isolates, Variability

Introduction

Cotton is one of the most ancient and important fibre crops next only to food grains and is the principal raw material for a flourishing textile industry. Cotton known as the 'King of fibre' and called as 'White Gold' and is the most vital crop of commerce to many countries including India having largest cotton area of 11.61 million hectares with a production of 33.4 million bales and productivity of 489 kg/ha. Karnataka state has an area of 5.16 lakh hectares and a production of 12 lakh bales with productivity of 572 kg/ha (Anon., 2012). The low productivity of cotton in Karnataka is attributed to many factors, one of which is the losses due to diseases. Among all the foliar diseases, Alternaria leaf spot disease has the prime importance. Conidiophores of Alternaria macrospora Zimm. arise singly or in groups. They are erect, simple straight or flexuous, cylindrical or 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, obclavate or 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 resulted in a growing number of new species. Results of a lifetime study on Alternaria taxonomy based upon morphological characteristics were summarised by Simmons (2007), in which 275 Alternaria species were recognised. Though, several cotton varieties and hybrids are being released from time to time, none of them has shown absolute resistance to this disease. This indicates the existence of variability among the isolates of this pathogen. Therefore, the present study was undertaken to understand the different aspects of Alternaria spp. with respect to its morphological and cultural variability. But not much work has been done on these aspects in the past. In addition, it helps in comprehensive understanding of the causal organism.

Material and methods

Morphological characters such as length and width of conidia, number of horizontal and vertical septa and length of beak were measured under 40 X using DIC microscope and the pathogen was cultured on Potato Dextrose Agar. All the above mentioned measurements were compared with the descriptions given by Ellis (1971) regarding *A. macrospora* and *Alternaria alternata* (Fr.) Keissler for identification of *Alternaria* spp.

To study the effect of incubation period on dry mycelial weight of A. macrospora, 30 ml of potato dextrose broth (PDB) was poured into each of 150 ml conical flasks and then sterilized. The growth of the fungus was studied at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 days of inoculation. The flasks filled with medium were then inoculated with 5 mm disc of Alternaria spp. from actively growing culture and incubated at $25\pm2^{\circ}$ C. Each treatment was replicated three times. Three flasks were harvested separately at a time, starting from the third day onwards upto 30th day by leaving gap of 48 hours between the two successive harvests. The cultures were filtered through previously weighed Whatman No. 42 filter paper, which were dried to a constant temperature at 60°C in hot air oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to get rid of the salts likely to be associated with the mycelial mass.

The filter paper along with the mycelial mat was dried to a constant temperature at 60°C for 8 hr, cooled and weighed immediately on an analytical balance. The difference between final and initial weight of filter discs were taken as the weight of the mycelia. The data were analysed statistically.

The isolates collected were grown on various solid media to find the difference in colony characters among them. The different solid media used for the study of growth of *Alternaria* spp. included Potato Dextrose Agar, Potato Carrot Agar, Czapek's Dox Agar, Host Extract Agar, Oat Meal Agar, Corn Meal Agar, S-Dextrose Agar and V8 Juice Agar. The composition and preparation of these synthetic and non-synthetic media were obtained from Ainsworth and Bisby's "Dictionary of the fungi" (Hawksworth *et al.*, 1983).

The potato dextrose agar (PDA) was prepared with 200 g of peeled potatoes which were cut into small bits and boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose 20 g and agar 20 g each were dissolved in the potato extract and the final volume was made upto 1000 ml with distilled water and sterilized as described earlier and preserved for further use.

The potato carrot agar (PCA) preparation was done with grated vegetables which were boiled for one hour in distilled water. The mixture was strained through fine sieve to which agar-agar was added. It was boiled over water-bath till agar dissolved. It was then sterilized at 121°C for 15 minutes. The Czapek's dox agar (CDA) medium was prepared by all the ingredients except agar-agar were mixed in 500 ml water. Agar-agar was melted in 500 ml distilled water. The two solutions were mixed thoroughly and the volume was made up to 1000 ml and was sterilized. Similarly, host extract agar (HEA) medium was prepared by cotton plant leaves which were boiled in 500 ml water, both the solutions were mixed and the volume was made up to 1000 ml water, both the solutions were mixed and the volume was made up to 1000 ml and was sterilized.

The oat meal mgar (OMA) medium was prepared by boiling oat flakes in 500 ml distilled water for 30 minutes 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. The corn meal agar (CMA) was prepared with 60 g of dehydrated corn flakes which were boiled for 15 minutes in 500 ml of distilled water and filtered. Twenty gram of agar-agar was melted separately and both the solutions were mixed. The volume was made up to 1000 ml and sterilized at 121°C for 15 minutes. Similarly, to prepare Sabouraud's dextrose agar (SDA), all the ingredients except agar-agar were dissolved one by one in 400 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made up to 1000 ml before sterilization. The V-8

Table 1. Morphological variability of isolates of Alternaria spp

juice agar (V8JA) was prepared by dissolving all the ingredients of V8JA medium except agar-agar one by one in 400 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made up to 1000 ml before sterilization.

Twenty ml of media (PDA) was poured into sterilized petriplates and kept for solidification. After solidification, 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 $27\pm1^{\circ}$ C for 12 days. After 15 days, the observation on diameter of radial growth, type of colony margin, colour of margin, mycelial growth, sectoring and sporulation were recorded. The sporulation was graded as follows.

Score	Grade	No. of spores/
		microscopic field (10 X)
++++	Excellent sporulation	> 30
+++	Good sporulation	21-30
++	Moderate sporulation	11-20
+	Poor sporulation	1-10
_	No sporulation	< 1

Results and discussion

The isolates collected during survey from the northern Karnataka were grown on PDA and were subjected to various morphological variability tests and the results are presented hereunder. Table 1 showed that, conidia of different isolates were septated by 2 vertical and 4-6 horizontal septa. The isolates, A₃, A₆ and A₁₀ showed maximum horizontal septa of 6 followed by 5 horizontal septa in isolates, A₁ and A₅; Whereas, minimum horizontal septa (4) was observed in the isolates, A₄ and A₈ The isolates, A₅ and A₈ 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, 48.24 x 15.79 and 47.95 x 15.26 µm, respectively. The least size of the conidia (21.97 x 13.02 μ m) was observed in isolate, A₈. By comparing with A. macrospora as per structural figure described by Ellis (1971) revealed that out of 7 isolates, only two isolates viz., A_5 and A_8 showed

Sl.	Name of	Number of	Number	Size of	Beak	Overall		Descriptions of			Resemblance
No.	the isolate	horizontal	of vertical	conidia	length	length		Alternari	ia macrospora		towards
		septa	septa	(Length x	(µm)	of conidia	Number of	Number	Beak length	Overall	Alternaria
				Breadth)		(µm)	horizontal	of vertical		length	macrospora
				(µm)			septa	septa		(µm)	
$\overline{A_1}$	Karlakatti	5	1	49.38 x 12.82	29.15	78.53					No Resemblance
A_3	Yamkanmard	li 6	1	32.80 x 17.17	32.84	65.64					No Resemblance
A_4	Marewada	4	1	47.95 x 15.26	33.52	81.47					No Resemblance
A ₅	Unkal	5	2	28.00 x 12.31	63.02	91.02	4-9	1-5	Equal or twice the length	90-180	Complete Resemblance
									of conidia		
A_6	Chandanama	atti 6	1	48.24 x 15.79	27.47	75.71					No Resemblance
A ₈	Saundatti	4	2	21.97 x 13.02	74.03	96					Complete Resemblance
A_{10}	Jagalur	6	1	26.99 x 7.61	30.98	57.97					No Resemblance

Morphological and cultural variability.....

complete resemblance with *A. macrospore* and other isolates *viz.*, A_{1} , A_{3} , A_{4} , A_{6} and A_{10} showed no resemblance with *A. macrospora*, morphologically.

Results in Table 2 reveals 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. Maximum conidial size (33.29 x 12.54 µm) was observed in isolate, A_2 , whereas, isolate, A_7 showed minimum size of 21.5 x 6.87 µm. Isolate, A_9 showed conidial size of $30.22 \times 7.4 \mu$ m. When the isolates were compared with *A. alternata*; as per structural figure described by Ellis (1971), all the three isolates *viz.*, A_2 , A_7 and A_9 showed complete resemblance, morphologically, which is in accordance with the findings of Anil (2013) who reported that out of their 12 isolates, only four showed complete resemblance with *A. macrospora*, morphologically.

There was significant difference among the incubation periods. The dry mycelial weight of *A. macrospora* gradually increased (36.53 mg) from third day of inoculation and reached maximum (287.31 mg) on 16th day (Table 3). The data showed a declining trend from 18th day (275.90 mg) to 13th day (192.74 mg). The decline in dry mycelial weight may be due to increase in cell death than the new cells formed. This result is in agreement with the findings of Padmanabhan and Narayanaswamy (1977)

Table 2. Morphological variability of isolates of Alternaria spp.

who reported that the maximum growth of *A. macrospora* was attained 14 days after incubation in Czapeck's dox medium.

Fungi secure food and energy from the substrate upon which they live in nature. In order to culture fungus in the laboratory, it is necessary to furnish essential elements and compounds in the medium for their growth and other life processes. All media are neither equally good for all fungi, nor there can be a universal substrate or artificial medium upon which all fungi can grow. So, different media were tried for A. macrospora and A. alternata in the present investigation. The isolates exhibited variability in cultural characters such as radial growth, type of colony margin, colour of margin, mycelial growth, sectoring and sporulation in different solid media tested viz., PDA, PCA, CDA, HEA, OMA, CMA, SDA and V8JA. The present study revealed that the isolates, A₄ and A₉ showed maximum radial growth (90 mm) on many of the media viz., PDA, PCA, HEA, OMA and SDA. Among the eight media tested, HEA and OMA showed maximum radial growth in all test isolates. Majority of the isolates showed moderate to excellent sporulation (Table 4).

The results revealed that there was significant difference between the isolates, medias and their interaction effect. Isolate, A_c has recorded highest mean radial growth (81.57 mm), which

	1	0	2		1	1						
Sl.	Name of	Number of	Number	Size of	Beak	Overall		Descriptions of				
No.	the isolate	horizontal	of	conidia	length	length		Alternaria alternata				
		septa	vertical	(Length x	(µm)	of	Number of	Number o	f Beak	Overall	Alternaria	
			septa	Breadth)		conidia	horizontal	vertical	length	length	alternata	
				(µm)		(µm)		septa	septa	(µm)		
$\overline{A_2}$	Amminbhav	/i 6	1	33.29 x 12.54	26.37	56.37					Complete	
-									Short or more		Resemblance	
A_7	Haveri	5	2	21.50 x 6.87	25.32	46.82	1-8	0-4	than one third the	20-63	Complete	
,									length of conidia		Resemblance	
A _o	Gadag	5	2	30.22 x 7.40	24.04	56.26					Complete	
											Resemblance	

Table 3. Effect of incubation period on dry mycelial weight of Alternaria macropora

пистини пистозроги	
Incubation period	Mycelial dry
(days)	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
C.D. at 1%	2.20

was at par with A_9 (80.94 mm), A_7 (79.11 mm) and A_4 (78.45 mm) isolates and were significantly superior over other isolates. Isolates, A_{10} (73.47 mm), A_8 (70.58 mm), A_5 (69.48 mm), A_1 (69.2 mm) and A_3 (68.08 mm) were next in order and were at par with each other. A_2 isolate showed least mean radial growth (61.98 mm). With respect to the different media concerned, HEA medium expressed significantly highest radial growth (90 mm) in all the isolates and was superior over other media followed by OMA (89.25 mm). SDA (80.43 mm) and PCA (79.17 mm) were at par with each other. PDA recorded mean radial growth of 77.49 mm, whereas, least radial growth was observed in V8JA (43.81 mm) followed by CMA (52.20 mm). With respect to interaction effect, isolates A_4 and A_9 recorded maximum radial growth (90.00 mm) on both, HEA and OMA.

The isolates grown on different media showed varied colony characters (Table 5). The results revealed that isolates A_{1} , A_{2} , A_{5} , A_{7} and A_{9} showed grey colony colour on most of the media, whereas isolates A_{3} , A_{6} , A_{8} and A_{10} showed both grey and black colour colonies. Only isolate A_{3} produced black colour colony. The colony margin colour of the isolates varied from white to

Karnataka J. Agric. Sci., 28(2): 2015

Table 4. Cultural variability of growth and sporulation of ten isolates of Alternaria spp. on different solid media

Sl.	Isolates				Radial gr	rowth (mm)	Radial growth (mm)							
No.	•	PDA	PCA	CDA	HEA	OMA	СМА	SDA	V8JA					
A_1	Karlakatti	i 77.50	63.77	71.23	90.00	82.50	43.60	82.50	42.50	69.20				
		+++	++	++	++++	+++	++	++	+++					
A_2	Amminbh	avi 60.33	82.50	65.00	90.00	90.00	25.00	45.47	37.50	61.98				
		+++	++++	+++	++++	+++	+++	++	+++					
A_3	Yamkanm	ardi 75.00	61.00	73.37	90.00	90.00	34.27	86.00	35.00	68.08				
		+++	+++	+++	+++	+++	+++	+++	++					
A_4	Marewad	a 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				
5		+++	+++	+++	++++	+++	++	++	+++					
A.	Chandana	matti 76.77	84.83	77.83	90.00	90.00	65.83	85.00	82.30	81.57				
6		+++	+++	++	+++	+++	+++	++++	+++					
A	Haveri	81.27	90.00	75.43	90.00	90.00	83.83	82.43	39.92	79.11				
7	114,011	+++	++++	+++	++++	++++	+++	+++	+++	///11				
Δ	Saundatti	73 33	69.83	75 33	90.00	90.00	44 33	81.67	40.17	70 58				
18	Saundatti	15.55		15.55	J0.00	J0.00	++.55	01.07 +++	+0.17	70.50				
٨	Cadag	00.00	00.00	71.42	00.00	00.00	00.00	00.00	26.09	80.04				
\mathbf{A}_{9}	Gadag	90.00	90.00	/1.42	90.00	90.00	90.00	90.00	30.08	60.94				
•	T	++++	++++	+++	+++	++++	++++	++++	++++	72 47				
A_{10}	Jagalur	/3.50	86.38	56.46	90.00	90.00	67.67	90.00	33.77	/3.4/				
		+++	+++	++	+++	+++	+++	++++	+++					
	Mean	77.49	79.16	73.94	90.00	89.25	52.20	80.43	43.81	73.29				
			Isolates (I))		Medias (M)			I x M					
	S.Em.±		0.27			0.24			0.78					
	C.D. at 19	%	1.09			0.97			3.09					
++	: Moderate spo	orulation -	+++ : Good sp	orulation	-	++++ : Exceller	nt sporulation							
PD.	A - Potato dext	rose agar PC	A – Potato car	rot agar CDA	-Czapeck's	dox agar	HEA – H	Host extract	agar					
OM	IA – Oat meal a	agar CM	IA – Corn mea	lagar SDA	- Sabouraud'	s dextrose agar	V8JA-V	V-8 Juice ag	ar					
		0		0		U		L.	, ,					
Tab	le 5. Cultural d	iversity of ten iso	plates of Altern	<i>aria</i> spp.										
Sl.	Media	Characters	PDA	PCA	CDA	HEA	OMA	CMA	SDA	V8JA				
No.	Isolates													
A_1	Karlakatti	Colony colour	Dark grey	Ashy	Ashy	Grayish black	Grey	Black	Dark grey	Black				
		Colony margin	Grey	Grey	White	Black	White	Black	Grey	Black				
		colour Type of	Irregular	Irregular	Irregular	Smooth	Irregular	Irregular	Irregular	Irregular				
		margin	Medium	Medium	Medium	Medium	Medium	Distorted	Flat	Raised				
		Mycelial growth	h raised	raised	raised	raised	raised							
		Sectoring	Absent	Absent	Present	Absent	Present	Present	Absent	Absent				
A_2	Amminbhavi	Colony colour	Grey	Whitish grey	Grey	Grayish black	Grayish black	Black	Grey	Grey				
		Colony margin	Grey	Grey	Grey	Black	Grey	Black	Grey	Black				
		colour Type of	Irregular	Smooth	Smooth	Smooth	Irregular	Irregular	Irregular	Smooth				
		margin	Raised	Medium	Medium	Medium	Raised	Distorted	Raised	Flat				
		Mycelial growth	h	raised	raised	raised								
		Sectoring	Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent				
Α,	Yamkanmardi	Colony colour	Grey	Ashy	Grey	Black	Grey	Black	Greyish blac	k Black				
5		Colony margin	White	Grey	Grey	Black	Black	Black	White	Black				
		colour	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular				
		Type of margin	Raised	Medium	Raised	Raised	Raised	Distorted	Raised	Flat				
		Mycelial growth	h Absent	raised	Absent	Absent	Absent	Present	Absent	Absent				
		Sectoring		Absent										
Α.	Marewada	Colony colour	Grevish black	Black	Gravish black	Black	Black	Black	Black	Black				
4		Colony margin	Black	Grev	Grev	Black	Grev	Grev	Black	Grev				
		colour	Smooth	Smooth	Irregular	Smooth	Smooth	Irregular	Irregular	Irregular				
		Type of margin	Flat	Flat	Medium	Raised	Medium	Distorted	Raised	Raised				
		Mycelial growth	h Absent	Present	raised	Present	raised	Absent	Absent	Absent				
		Sectoring	1100011	1 resent	Absent	11000111	Absent	1 105011	100000	1 105011				
Δ	Unkal	Colony colour	Black	Grevish white	Grev	Grev	Grev	Grev	Gravish blac	k Black				
· 1 5	Unixai	Colony margin	White	White	Grev	Rlack	Grey	Rlack	Grey	Rlack				
		colour	Irragular	Irragular	Smooth	Irragular	Irragular	Irragular	Irragular	Irromler				
		Type of marcin	Madium	Daisad	Roised	Poioed	Flot	Distortad	Doised	Madium				
		Mycoliol mouth	h roised	Dracant	Abcont	Abcont	Aboot	Aboont	Abcont	roiced				
		Sectoria -	A here t	Fiesent	Ausein	Ausent	Ausent	Absent	Absent	Dressed				
		Sectoring	Absent							Present				

Morphological and	l cultural variabi	lity
-------------------	--------------------	------

Sl.	Media	Characters	PDA	PCA	CDA	HEA	OMA	CMA	SDA	V8JA
No.	Isolates									
A ₆	Chandanam	attiColony colour	Black	Whitish grey	Grey	White	Black	Grey	White	Grey
0		Colony margin	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Black
		colour	Smooth	Irregular	Irregular	Smooth	Irregular	Irregular	Irregular	Irregular
		Type of margin	Flat	Medium	Medium		Medium			
		Mycelial growth		raised	raised	Flat	raised	Distorted	Raised	Raised
		Sectoring	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
A_7	Haveri	Colony colour	Black	Grey	Grayish black	Grey	Grey	Grey	Black	Grey
,		Colony margin	Grey	Grey	Grey	Grey	Grey	Black	Black	Black
		colour	Irregular	Irregular	Irregular	Smooth	Irregular	Irregular	Irregular	Irregular
		Type of margin	Raised	Flat	Raised	Raised	Raised	Distorted	Raised	Raised
		Mycelial growth Sectoring	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
A ₈	Saundatti	Colony colour	Grey	Grey	Ashy	Grayish black	Grey	Black	Black	Black
		Colony margin	Grey	Grey	Grey	Black	White	Black	Grey	Black
		colour	Irregular	Irregular	Irregular	Smooth	Irregular	Irregular	Irregular	Irregular
		Type of margin	Medium	Medium	Medium	Medium	Medium	Distorted	Flat	Raised
		Mycelial growth	raised	raised	raised	raised	raised			
		Sectoring	Absent	Present	Present	Absent	Present	Present	Absent	Absent
A ₉	Gadag	Colony colour	White	Grey	Grey	White	Grey	Grey	White	White
		Colony margin	Grey	Grey	Grey	Grey	Grayish black	Grey	Grey	Grey
		colour	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Irregular
		Type of margin	Flat	Flat	Flat	Flat	Flat	Distorted	Flat	Flat
		Mycelial growth Sectoring	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
A ₁₀	Jagalur	Colony colour	Grayish	Grayish	Grey	Black	Grey	Black	Black	Grey
10	-	Colony margin	black	black	Black	Black	Grey	Black	Black	Grey
		colour	Grey	Grey	Smooth	Smooth	Smooth	Irregular	Smooth	Smooth
		Type of margin	Irregular	Irregular	Medium	Medium				
		Mycelial growth	Medium	Flat	raised	raised	Flat	Distorted	Flat	Flat
		Sectoring	raised Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent
PD	A - Potato dez	xtrose agar	PCA – Potat	o carrot agar	CDA	-Czapeck's doz	x agar	HEA	– Host extra	act agar
OMA – Oat meal agar		l agar	CMA - Corn	meal agar	SDA	– Sabouraud's d	V8JA – V-8 Juice agar			

black. Isolates A_4 , A_6 , A_7 and A_9 showed grey colony margin on most of the media; whereas, isolates A_2 , A_8 and A_{10} shared colony margin colour of both, grey and black. Isolates A_1 , A_3 and A_5 showed white colony margin along with grey and black. The type of margin of the isolates varied from irregular to smooth. 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. The isolates A_2 and A_4 shared characters of both types of margin.

Different isolates showed varied mycelial growth characters from flat to raised. The isolates, A_3 and A_7 showed raised mycelial growth; whereas, medium-raised mycelial growth was observed in isolates, A_1 and A_8 . The flat mycelial growth was observed in isolate A_9 . The isolates, A_2 and A_5 showed both raised and medium-raised mycelial growth; whereas, isolate A_{10} showed both medium-raised and flat mycelial growth. All the three characters *viz.*, raised, medium-raised and flat mycelial growth was observed in isolates A_4 and A_6 . With respect to media

References

Anil, G. H., 2013, Studies on leaf blight of Bt cotton caused by *Alternaria* spp. M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad, Karnataka (India). concerned, CMA medium showed distorted growth in all isolates. Sectoring was absent in isolates A_2 , A_3 , A_6 , A_7 and A_9 ; whereas, isolates A_1 , A_4 , A_5 , A_8 and A_{10} showed characters of both, presence and absence of sectoring among the media tested. Several workers observed the 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 (Savitha, 2004; Mesta, 2006 and Ramegowda, 2007).

The diversity in morphological and cultural characters observed in the present investigation emphasizes the variability in *Alternaria* spp. isolates. Variability among the isolates may be attributed to weather conditions of a particular location, varieties and hybrids and ability of pathogen to adopt themselves to various situations. Therefore, study of variability among the isolates will be helpful in identifying the resistant donors against the disease as well as for developing disease management strategies.

- Anonymous, 2012, Agricultural Statistics at a Glance, Ministry of Agriculture, Government of India.
- Ellis, M. B., 1971, *Dematiaceous Hypomycetes*, Commonwealth Mycological Institute, Kew, Surrey, England, pp. 495-496.

Karnataka J. Agric. Sci., 28(2): 2015

- Hawksworth, D. L., Suttan, B. C. and Ainsworth, G. C., 1983, Ainsworth and Bisbys Dictionary of the fungi. VII Ed. Commonwealth Mycological Institute, Kew, Surrey, England, p. 445.
- Mesta, R. K., 2006, Epidemiology and management of *Alternaria* blight of sunflower caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara. *Ph. D. Thesis*, Univ. Agric. Sci., Dharwad, Karnataka (India).
- Padmanabhan, P. and Narayanaswamy, P., 1977, Growth studies of *Alternaria macrospora* Zimm. an incitant of leaf spot disease of cotton. *Madras Agric. J.*, 64: 258-261.
- Ramegowda, G., 2007, Disease scenario in *Bt* cotton with special reference to Alternaria leaf spot. *M. Sc. (Agri.) Thesis,* Univ. Agric. Sci., Dharwad, Karnataka (India).
- Savitha, A. S., 2004, Variability and toxin studies of *Alternaria* spp., the incitant of blight of sesame caused by *Alternaria sesami*. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad, Karnataka (India).
- Simmons, E. G., 2007, *Alternaria- An identification Manual.* CBS Biodiversity Series 6, CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.