INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 10 | ISSUE 2 | OCTOBER, 2017 | 281-290

● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/10.2/281-290

Studies on morphological and cultural variability of *Alternaria* spp. causing leaf blight in cotton

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ARITCLE INFO

Received : 14.06.2017
Revised : 11.08.2017
Accepted : 23.08.2017

KEY WORDS :
Alternaria, Cotton, Culture, Morphology, Variability

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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 variability in morphology as well as in culture. Septation of twelve isolates conidia ranged from 1-7 vertical and 3-9 horizontal. Raladoddi isolate showed maximum horizontal septa (9) and Kanakapura showed maximum vertical septa. Size of the condia varied from 132.24 x 9.10 to 14.98 x 2.56, maximum size was measured in Raladoddi isolate. Measurements of all isolates were compared with standard measurements of *Alternaria macrospora* given by Ellis (1971), out of twelve isolates eight resembled *A. macrosora*. These isolates cultured on potato dextrose agar (PDA) for variability, the colony margin varied from irregular to soft, with a colour of brown, light gray and light pink. The maximum (1.99 µm) width of mycelia was found in Tagalladoddi isolate.

How to view point the article : Anil, G.H., Ashtaputre, S.A. and Rao, M.S. L. (2017). Studies on morphological and cultural variability of *Alternaria* spp. causing leaf blight in cotton. *Internat. J. Plant Protec.*, **10**(2) : 281-290, **DOI : 10.15740/HAS/IJPP/10.2/281-290**.

INTRODUCTION

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There are hundreds of textile fibre crops but the most important have been cotton (*Gossypium* spp.), flax, Ramie (*Boehmeria nivea*), jute (*Cochorus* spp.) and sisal (*Agave* spp.). Cotton, although under pressure from synthetic fibres, has made resurgence worldwide and remains the most improved crop species producing lint plus oil and meal from seed (Nosberger *et al*., 2001).

Cotton is the most important cash crop of India. It is the backbone of our sprawling textile industry contributing 7.00 per cent to our GDP, fetching an export earning besides providing employment in the production, promotion, processing and trade of cotton. Cotton

accounts for 45 per cent of the world fibre and supplies 10 per cent world edible oil (Rathore, 2005).

Four species of *Gossypium* have been cultivated for fibre. Of these species, the tetraploid *Gossypium hirsutum* and *Gossypium barbadense* currently dominate commercial cotton production, with *Gossypium hirsutum* accounting for over 90 per cent of the production. *Gossypium hirsutum* and *Gossypium barbadense* differ significantly in agronomic and fibre attributes and their commercial end uses. The area under cotton during 2011-12 in India was 110.00 lakh ha with production and productivity of 325 lakh bales and 503 kg lint per ha, respectively. In the year 2011, in Karnataka, cotton was grown over an area of 4.66 lakh ha with 10.15 lakh bales production and 370 kg productivity (Anonymous, 2012).

The low productivity of cotton is attributed to many factors, one of which is the losses due to diseases although insect pests continue to be a major constraint. A large number of fungal, bacterial, viral and nematode diseases have been reported on cotton crop right from early stage to maturity. There is a need to understand different aspects of the major fungal foliar pathogen *Alternaria* spp. with respect to its cultural, pathogenic and genetic variability since 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

Collection and isolation of *Alternaria* spp. :

During survey, leaves of cotton plant showing the symptoms of *Alternaria* blight were collected from different cotton growing areas and *Alternaria* spp. were isolated from these infected leaves by standard tissue isolation technique in the laboratory.

Single spore isolation :

Ten ml of clear, filtered two per cent water agar was poured into sterile Petri plates and allowed to solidify. Dilute spore suspension was prepared in sterilized distilled water from 15 days old culture. One ml of such suspension was spread uniformly on agar plate. These plates were incubated at 27 ± 1°C for 12 hrs. Then such plates were examined under microscope to locate single isolated and germinated conidium and marked with ink on the surface of the plates. The growing hyphal tip portion was transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at 27 ± 1°C. Such culture tubes were used for further studies.

Pathogenicity test :

The leaves of cotton were taken and washed in $NaOCl_2$ (0.2%) washed in sterile changes of water. In a moist chamber, the leaves were inoculated with spore and mycelial suspension (1x 10^6 spores/ ml) of the fungus by using atomizer. After inoculation, the moisture in Petri plates was maintained. After 48 hr of inoculation, the observations were made for symptom development. Suitable control was maintained by spraying with distilled water. The culture thus, obtained was compared with the original culture to confirm the identity.

Morphological and cultural variability of *Alternaria* spp.:

The study has been carried out to know the variation in the pathogen of different areas causing leaf blight in cotton. Both morphological and genomic variations were assessed to know the variation among different isolates collected.

Morphological variability of *Alternaria* spp. :

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 measurements given by Ellis (1971) regarding *Alternaria macrospora* for identification of *Alternaria* species.

The composition and preparation of the PDA media was obtained from Ainsworth and Bisby's "Dictionary of the Fungi" by Hawksworth *et al.* (1983). The composition of media is given below.

Potato dextrose agar (PDA) :

Peeled potato	200 g
Dextrose	20 g
Agar-agar	20 g
Distilled water	1000 ml (volume to make up).

Two hundred grams of peeled potatoes were cut into small pieces and boiled in distilled water and the

Table A: The sporulation was graded as follows

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

extract was cooled by filtering through muslin cloth. Dextrose 20.0g and agar 20.0g of each were dissolved in potato extract and the final volume was made upto 1000 ml with distilled water and sterilized as described earlier and preserved for further use.

Cultural diversity among different isolates

The twelve isolates collected were grown on PDA to find the difference in colony characters among them. Single spore was placed in center of media to get pure colony. 15 days after sub culturing the observation of mycelia growth, type of colony margin, colour of margin, sporulation and mycelial width (µm) were recorded.

Twenty ml of media (PDA) was poured into sterilized Petri plates and kept for solidification. After solidification, 5 mm discs of the *Alternaria* sp. were cut using a cork borer and a single disc is placed on slat. Each set of experiment replicated thrice and the plates were incubated at 27 ± 1° C for 12 days. The sporulation was graded as follows.

Statistical analysis :

Statistical analysis was carried out as per the procedures given by Panse and Sukhatme (1985). Actual data in percentage were converted to angular values, before analysis according to the table given by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Morphological variability of *Alternaria* spp.:

The different isolates collected during survey from the northern Karnataka were subjected to morphological variability tests and the results are presented in the Table 1. The study showed that, conidia of different isolates were septated by 1-7 vertical and 3-9 horizontal septa. The isolates A_3, A_5, A_7, A_8 and A_{12} showed maximum horizontal septa upto 7-9. Whereas minimum horizontal septa (1-6) was observed in most of the remaining isolates (Plate 1). The vertical septa ranged from 1-7. The isolates A_5, A_7 and A_{10} showed maximum of 6-7 vertical septa and isolate A_1, A_2 A_3 and A_8 showed minimum of 1-3 vertical septa. The isolates A_{11} and A_7 showed maximum size of 34.60 x 7.40 µm and 33.55 x 9.10 µm, respectively. The least size of the conidia of 11.29 x 2.56 µm and 14.45 x 3.62 µm was observed in isolates of A_1 and A_2, respectively. By comparing with *Alternaria macrospora* structural figure described by Ellis (1971). revealed that out of 12 isolates collected, four isolates showed complete resemblance with *Alternaria macrospora viz.*, A_4, A_7, A_{10} and A_{11} and other four isolates showed resemblance with *Alternaria macrospora viz.*, A_5, A_6, A_9 and A_{12} and remaining four isolates were found to be different from *Alternaria macrospora* morphologically.

The location and identity of *Alternaria* isolates collected from different cotton growing area of northern Karnataka has been represented in Table 1. The isolates of Kadagamannadoddi, Kanakpura, Tagalladoddi, Raladoddi, Navalli, Chandrabanda, Shaktinagar and Murkumbi showed the existence of *Alternaria macrospora*.

Cultural characters of different isolates of *Alternaria* spp. :

Twelve isolates were grown on PDA to know the diversity among them. The disc of *Alternaria* culture was placed on the media and incubated at 25ºC for 15 days. The colony characters and measurements were recorded after 15 days of incubation. The results are presented in Table 3. Results showed that the mycelial width was larger in A_6 (1.99 µm) followed by A_9 (1.85 µm), A_5 (1.68 µm) and A_{10}. The least mycelial width was found in A_4 (0.92 µm) followed by A_3 (1.10 µm) and A_1 (1.15 µm). The

Table 1: Comparison between isolates of *Alternaria* spp. collected from various parts of northern Karnataka

Sr. 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 macrospora* Zimm.				Resemblance towards *Alternaria macrospora* Zimm.
							Number of horizontal septa	Number of vertical septa	Beak length	Overall length (µm)	
A_1	Dharwad	4	3	11.29 x 2.56	3.11	14.98	4-9	Several septa	Equal or twice the length of conidia	90-180	No resemblance
A_2	Agasikoppa	3	1	14.45 x 3.62	6.22	20.67	4-9	Several septa	Equal or twice the length of conidia	90-180	No resemblance
A_3	Chicknasabi	7	3	24.65 x 4.82	7.27	31.92	4-9	Several septa	Equal or twice the length of conidia	90-180	No resemblance
A_4	Kadagammanadoddi	6	4	27.87 x 5.33	77.79	105.66	4-9	Several septa	Equal or twice the length of conidia	90-180	Complete resemblance
A_5	Kanakapura	8	7	24.32 x 7.55	52. 55	76.87	4-9	Several septa	Equal or twice the length of conidia	90-180	Resemblance
A_6	Tagalladoddi	6	5	26.22 x 7.65	55.63	81.85	4-9	Several septa	Equal or twice the length of conidia	90-180	Resemblance
A_7	Raladoddi	9	6	33.55 x 9.10	98.69	132.24	4-9	Several septa	Equal or twice the length of conidia	90-180	Complete resemblance
A_8	Annigeri	7	2	15.66 x 3.31	4.18	19.66	4-9	Several septa	Equal or twice the length of conidia	90-180	No resemblance
A_9	Navali	6	5	24.44 x 6.28	46.65	51.09	4-9	Several septa	Equal or twice the length of conidia	90-180	Resemblance
A_{10}	Chandrabanda	6	6	29.85 x 8.95	83.21	113.06	4-9	Several septa	Equal or twice the length of conidia	90-180	Complete resemblance
A_{11}	Shaktinagar	6	4	34.60 x 7.40	83.97	118.57	4-9	Several septa	Equal or twice the length of conidia	90-180	Complete resemblance
A_{12}	Murkumbi	8	5	31.42 x 7.40	45.17	86.59	4-9	Several septa	Equal or twice the length of conidia	90-180	Resemblance

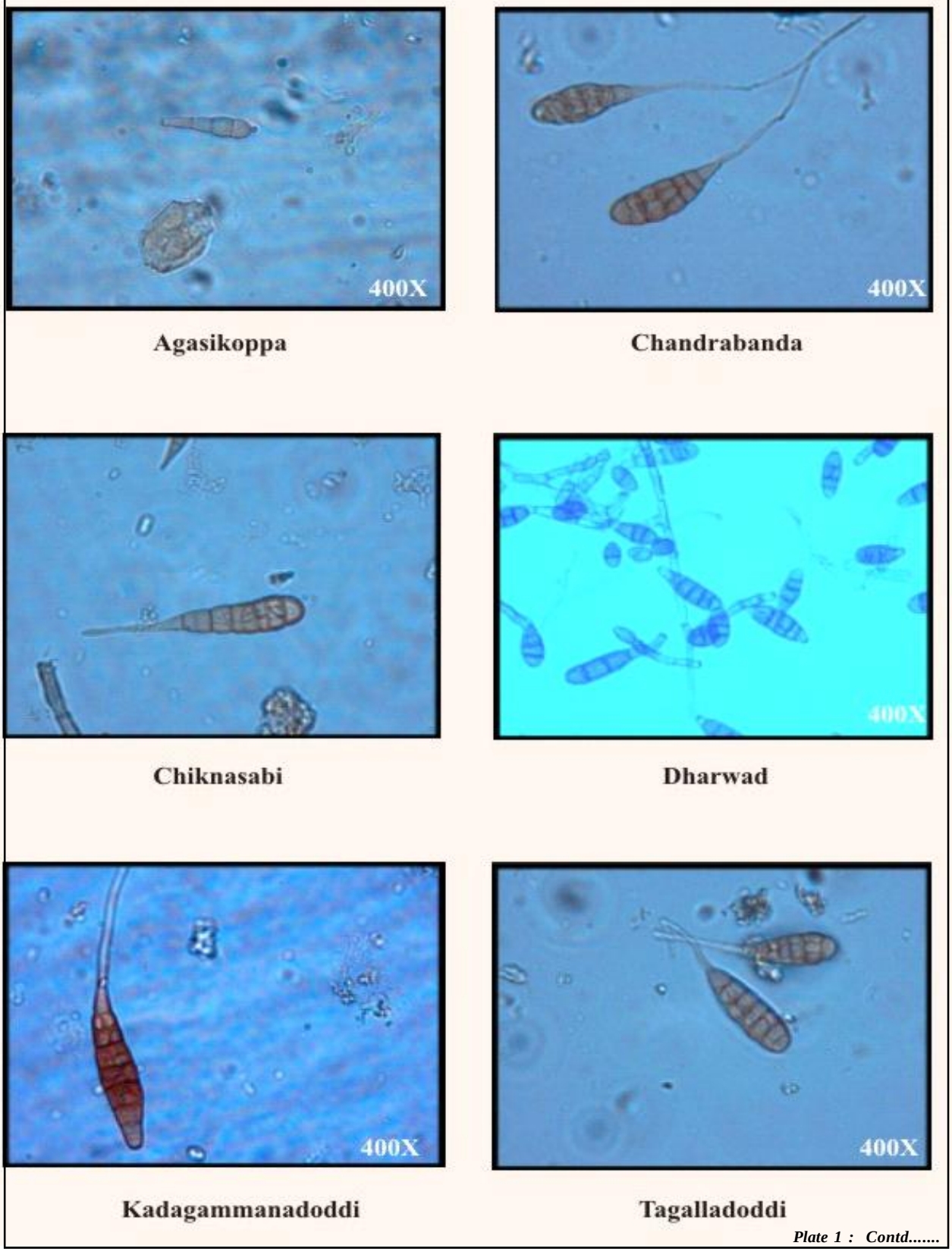

Plate 1 : Contd.....

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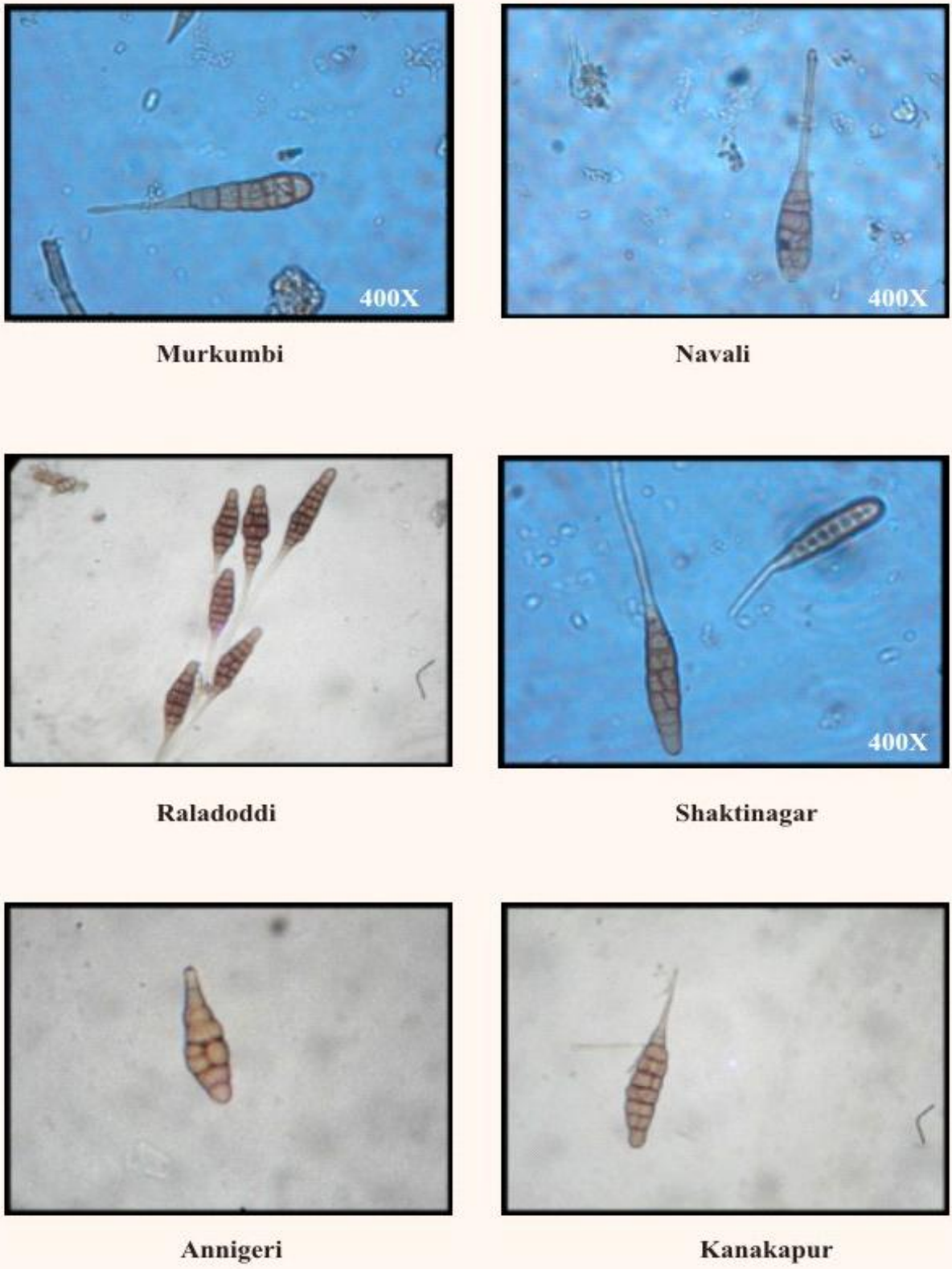

Plate 1 : Morphological variability of twelve isolates of *Alternaria* spp. collected causing leaf blight on cotton

Table 2: Source of different *Alternaria* sp. isolates collected and their identity

Isolates	Location	District	Identity
A_1	ARS Dharwad	Dharwad	*Alternaria* sp.
A_2	Agsikoppa	Bagalkot	*Alternaria* sp.
A_3	Chicknasbi	Bagalkot	*Alternaria* sp.
A_4	Kadagammanadoddi	Raichur	*Alternaria macrospora*
A_5	Kanakpura	Koppal	*Alternaria macrospora*
A_6	Tagalladoddi	Raichur	*Alternaria macrospora*
A_7	Raladoddi	Raichur	*Alternaria macrospora*
A_8	Annigeri	Gadag	*Alternaria* sp.
A_9	Navalli	Koppal	*Alternaria macrospora*
A_{10}	Chandrabanda	Raichur	*Alternaria macrospora*
A_{11}	Shaktinagar	Raichur	*Alternaria macrospora*
A_{12}	Murkumbi	Belgaum	*Alternaria macrospora*

Table 3: Cultural characters of different isolates of *Alternaria* spp.

Sr. No.	Isolate	Mycelial growth	Colour of margin	Type of margin	Sporulation	Mycelial width (µm)
A_1	Dharwad	Flat	Light grey	Irregular	+++	1.152
A_2	Agasikoppa	Raised	Light grey	Irregular	++	1.65
A_3	Chicknasabi	Raised	Whitish pink	Irregular	++++	1.10
A_4	Kadagammanadoddi	Flat	Light brown	Smooth	++++	0.92
A_5	Kanakapura	Raised	Light brown	Smooth	+++	1.68
A_6	Tagalladoddi	Flat	Light grey	Smooth	++++	1.99
A_7	Raladoddi	Raised	Light brown	Smooth	+++	1.12
A_8	Annigeri	Raised	Light grey	Irregular	+++	1.32
A_9	Navali	Flat	Light brown	Smooth	+++	1.85
A_{10}	Chandrabanda	Raised	Whitish pink	Smooth	+++	1.62
A_{11}	Shaktinagar	Flat	Light brown	Irregular	++	1.17
A_{12}	Murkumbi	Flat	Whitish pink	Smooth	+++	1.55

+ + + +: Excellent sporulation [> 30 spores/microscopic field (10 X)]
+ + +: Good sporulation [21-30 spores/microscopic field (10 X)]
+ +: Moderate sporulation [11-20 spores/microscopic field (10 X)]

margin of colony was light grey in A_1, A_2, A_6 and A_8 isolates, whitish pink in A_3, A_{10} and A_{12} isolates and light brown in rest of the isolates (Table 2).

The isolates A_1, A_2, A_3, A_8 and A_{11} showed irregular margin whereas isolates A_4, A_5, A_6, A_7, A_9, A_{10} and A_{12} showed smooth colony margin. Different isolates showed varied mycelia growth characters. Isolates A_2, A_3, A_5, A_7, A_8, A_{10} and A_{12} showed raised growth, whereas, the isolates A_1, A_4, A_6, A_9 and A_{11} flat growth on PDA (Plate 2). Different isolates had poor to excellent sporulation behaviour. The isolates A_3, A_4 and A_6 showed excellent sporulation, A_1, A_5, A_7, A_8, A_9, A_{10} and A_{12} showed good sporulation whereas A_2 and A_{10} showed moderate sporulation, poor sporulation or no sporulation was not observed in any of the isolates. Similar results were also

Table 4: Inoculation studies of *Alternaria* spp. on cotton plants

Isolate	Cotton
Dharwad	+
Agsikoppa	+
Chicknasabi	+
Kadagammanadoddi	+
Kanakapura	+
Tagalladoddi	+
Raladoddi	+
Annigeri	+
Navali	+
Chandrabanda	+
Shaktinagar	+
Murkumbi	+

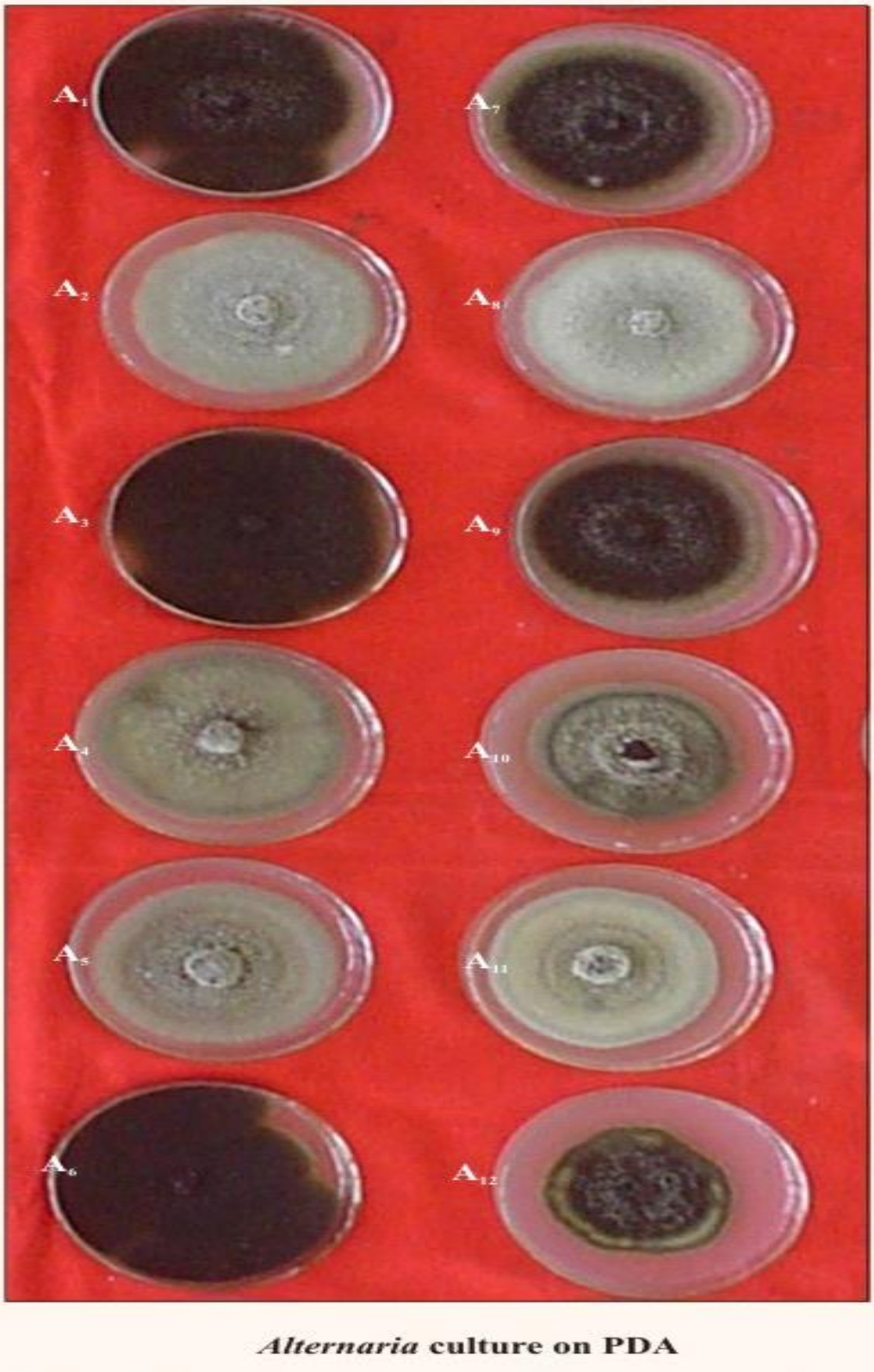

Plate 2 : Cultural growth of different isolates of *Alternaria* spp.

obtained by Jadhav *et al.* (2011) and Ramegowda (2007).

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