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Survey, surveillance and cultural characteristics of Alternaria leaf blight of cotton

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ABSTRACT : The roving survey was undertaken in 4 districts *viz.*, Parbhani, Nanded, Osmanabad, and Beed of Marathwada region revealed that the average Alternaria blight was ranged from 14.60 to 30.03 per cent. *Bt* cotton grown in Nanded was found to be more affected with *Alternaria* blight (overall incidence 30.03%), followed by Parbhani (25.20%), Beed (21.06%) and Osmanabad (14.60%). In Nanded, maximum disease incidence (32.60%) was recorded in Degloor thesil and this was followed by thesil of Naigaon (30.00%) and Nanded (27.50%). Results revealed that all the 11 culture media tested encouraged better growth of *Alternaria macrospora*. However, potato dextrose agar gave significantly highest radial mycelial growth of 88.18 mm. The second and third best culture media found were Czapek's dox agar (81.28 mm) and Richards agar (79.75 mm). Ashby's manitol agar was found least suitable for the growth of test pathogen (39.92 mm).

Key words: Alternaria leaf blight, cotton, cultural character, survey

Cotton is one of the most important commercial crop, playing a key role in economical, social and political status of the world. India occupies first place in area and second place in cotton production after USA in the world. Among the several factors responsible for reduction in yield and quality deterioration of cotton, diseases are the major one and of these Alternaria blight is one of the most important and destructive disease of cotton, inflicting yield losses to the tune of 20-30 per cent (Mayee and Mukewar, 2007).

Keeping in view the economic importance of cotton and losses incurred by Alternaria blight (*Alternaria macrospora*) disease, present investigations were carried out on the aspects *viz.,* survey, isolation of the pathogen, pathogenicity and identification of the pathogen.

MATERIALS AND METHODS

Survey and surveillance: An roving survey was undertaken during August-September, 2010 in 4 districts *viz.*, Nanded, Parbhani, Osmanabad, and Beed of Marathwada region to record the incidence of Alternaria blight on cotton .For recording the blight incidence, farmer's cotton field were randomly selected. Incidence of bacterial blight disease was examined as severe, moderate, traces and free on the basis of percentage of severity of disease. Similarly, the per cent disease incidence of bacterial blight was calculated by using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of plant infected}}{\text{Total number of plant examined}} \times 100$$

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of all disease rating}}{\text{Number of ratings x Maximum disease grade}} \times 100$$

Isolation and purification : Alternaria blight typical symptoms were collected from farmers' field and brought to the laboratory. All samples collected from different locations were subjected to isolation on potato dextrose agar medium (PDA). The plates were labeled and incubated in an inverted position at 26+2^{0}C and examined daily. After 7-8 days of incubation the well isolated colonies of fungus showing light brown colour were transferred to another petriplate. Purification of fungal culture was carried out on PDA medium in petriplates under

aseptic conditions and well isolated true to type colonies from these plates were transferred to PDA slants.

Pathogenicity: In order to confirm pathogenic nature of isolated fungal culture, seedling of cotton variety NHH 44 were raised in pots in glass house/screen house. These seeds were sown in sterilized soil: compost: sand mixture (2:1:1) at the rate of 5 seeds/pot (30 cm dia) and on germination 2 seedlings / pot were maintained. Before inoculation, leaves were injured by rubbing carborandum powder to have small injuries for development of symptoms.These seedlings were inoculated at the stage of 4-6 true leaves by sparyers spore suspension ($2x10^6$ spores/ml) with an automizer. For this purified culture was multiplied in conical flask (250ml containing sterilized PDA broth,100 ml/flask). These flasks were kept on mechanical shaker for 72 h at slow speed. This growth of fungus was then used for inoculation. Immediately pots were watered and covered with polyethene bags for 48 h to maintain humidity. Intermittently pot were watered and the polythene bags were also taken out for few min to avoid in rise in temperature. Observations were recorded daily and sufficient number of untreated control was maintained.

Cultural studies: Growth characters of the isolated pathogen was studied by growing it on different culture media namely Conn's Agar, Yeast Extract, Yeast manitol Agar, Oat Meal Agar, V-8 Juice Agar, Ashby's Mannitol Agar, Richards Synthetic Agar, Czapak dox Agar, Beijerinckia Medium, Jensens Medium and Potato dextrose agar medium. These agar media were prepared by following standard laboratory procedure, sterilized by autoclaving, poured into the sterile petriplates, (10 plates of each medium) and allowed to cool down and solidify. Then, the plates were inoculated by fungus. Each set of experiment was replicated thrice with completely randomized design and the plates were incubated at 27+ 1^0C for 7 days. The colony dia in the culture plates and cultural character such as colony dia, colour, type of margin, growth were recorded.

RESULTS AND DISCUSSION

Survey and surveillance : Results (Table 1) indicated that the disease (*A. macrospora*) was found to occur and distributed widely in the four districts of Marathwada region. *Bt* cotton crop grow in the district of Nanded was found to be affected more with Alternaria blight (overall incidence 30.03%), followed by the districts of Parbhani (25.20%), Beed (21.06%) and Osmanabad (14.60%). In Nanded district, maximum disease incidence (32.60%) was recorded in Degloor tahsil and followed by Naigaon (30.00%) and Nanded tahsils (27.50%). In the Parbhani district, overall average incidence of blight disease was 25.20 per cent; however it was maximum in Gangakhed thasil (28.16%), followed by Pathri (26.16 %) and Parbhani (21.28%).

In Beed district, overall average incidence of disease was 21.06 per cent; however it was maximum in Wadvani thasil (23.85 %), followed by Majalgaon thasil (20.33%) and Beed tahsil (19.00%) However, the blight incidence was found to be comparatively minimum with overall incidence of 14.60 per cent in the district of Osmanabad.

Thus, of the 4 districts of Marathwada region surveyed for recording disease incidence; maximum incidence was found in the district of Nanded, followed by Parbhani, Beed and Osmandbad. More *et al.,* (2010) reported that the cotton *Alteraria* blight disease appeared regularly in Marathwada region, causing losses in yield.

Isolation: The test pathogen (*A. macrospora*) was isolated successfully on the basal culture medium Potato dextrose agar, from the foliage showing typical symptoms of Alternaria blight. The pathogen produced circular, white gray flat colonies with raised centre and concentric rings.

Pathogenicity: Healthy growing, one

Table 1. Incidence of Alternaria blight of cotton

Districts	Thasils	Locations	Average incidence (%)	**Mean incidence (%)**
Parbhani	Parbhani	7	21.28	**25.20**
	Pathri	6	26.16	
	Gangakhed	6	28.16	
Nanded	Nanded	4	27.50	**30.03**
	Naigaon (B)	3	30.00	
	Degloor	5	32.60	
Osmanabad	Osmanabad	4	13.50	**14.60**
	Washi	5	14.60	
Beed	Beed	5	19.00	**21.06**
	Majalgaon	6	20.33	
	Wadvani	7	23.85	

month old seedlings of cotton cv. NHH 44 were inoculated. After two weeks of incubation, typical symptoms of disease on foliage of cotton seedling were observed. The test pathogen was reisolated from the artificially diseased cotton seedlings on the PDA medium and its morphological, cultural and microscopic observations were made which were found similar to that of the test pathogen isolated from naturally *Alternaria* infected cotton plants. Thus, pathogenicity of test pathogen was proved and the pathogen was identified and confirmed as *A.macrospora* .

Identification of culture: The pathogen produced circular, white gray flat colonies with raised centre and concentric rings. Based on typical symptoms of Alternaria blight produced on the foliage of naturally and artificially diseased cotton plants, cultural characteristics, the spores and mycelium and pathogenicity test; the pathogen under investigation was identified and confirmed as *A. macrospora* Zimm. The pathogenic culture isolated from the leaf spot of cotton was identified based on colony characteristics and spore characters with the help of relevant monograph Illustrated book (Dematiaceous Hyphomycets M.B. ellis) and CMI description. Isolation, pathogenicity and identification of *A.macrospora* causing leaf spot in cotton were successfully attempted and reported earlier by several workers (Ramegowda and Naik, 2008).

Cultural studies: Cultural characteristics *viz.*, mycelial growth, colour of the colony and concentric rings produced by *A. macrospora* were studied *in vitro* using ten synthetic and one non synthetic culture media. All the media tested encouraged better growth of the test pathogen.

The result (Table 2), revealed that of the 11 cultures tested, Potato dextrose agar was found most suitable and encouraged maximum

Table 2. Effect of different culture media on growth and cultural characteristics of *Alternaria macrospora* Zimm.

Culture media	Colony dia. (mm)*	Colony characteristics
Potato dextrose agar	88.18	Circular white grey colony, flat throughout but with raised circle at midway and periphery, centre raised with concentric rings.
Richard's agar	79.75	Irregularly circular and pinkish white colony, forming 3 concentric rings, with raised centre
Czapek's dox agar	81.28	Nearly circular and pinkish white colony, cushion like flat with concentric rings
Oat meal agar	70.79	Circular white colony with cushion like topography with concentric rings.
Conn's agar	49.60	Circular white colony with little raised rings at periphery
Yeast manitol agar	42.10	Greyish circular colony, slightly raised at the centre
Beijerinkia media	40.22	Dark brown, irregular colony
V8 Juice agar	43.76	Loose to dense mycelia mat, whitish red, black centre
Yeast extract agar	44.14	Circular light brown colony
Jensens medium	43.91	Irregular light brown colony
Ashby's manitol agar	39.92	Greyish circular colony, slightly raised at the centre
SE +	0.58	
CD (p=0.05)	1.72	

*Average of three replications

radial mycelial growth (88.18 mm) of the test pathogen. The second best medium was Czapek's dox agar (81.28 mm). This was followed by Richards agar (79.75 mm), Oat meal agar (70.79 mm), Conn's agar (49.60 mm), Yeast extract agar (44.14 mm), Jensens medium (43.91 mm), V8 juice agar (43.76 mm), Yeast manitol agar (42.10 mm), Beijerinckia medium (40.22mm). Ashby's manitol agar was found least suitable for the growth of test pathogen(39.92 mm).

All the culture media tested exhibited a wide range of colony morphology, colony colour and number of concentric rings. The mycelial growth produced on all the culture media tested was mostly abundant and dense (Table 2).

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