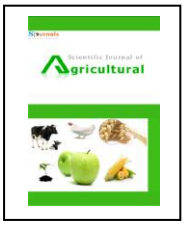
Sjournals
Scientific Journals
Scientific Journal of Agricultural (2013) 2(1) 29-35
ISSN 2322-2425

Contents lists available at Sjournals
Scientific Journal of Agricultural
Journal homepage: www.Sjournals.com



Original article

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

G.P. Jagtap, S.M. Jogdand, D. Utpal*

Department of Plant Pathology, College of Agriculture, Marathwada Krishi Vidyapeeth, Parbhani – 431 402 (MS)

*Corresponding author; Department of Plant Pathology, College of Agriculture, Marathwada Krishi Vidyapeeth, Parbhani – 431 402 (MS)

ARTICLE INFO

Article history:
Received 03 December 2012
Accepted 28 December 2012
Available online 28 January 2013

Keywords:
Survey
Alternaria leaf blight
Cotton

ABSTRACT

The roving survey undertaken in four districts viz., Parbhani, Nanded, Osmanabad, and Beed of Marathwada region revealed that average *Alternaria* blight incidence was ranged from 14.60 to 30.03 per cent. 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 district 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 this was followed by Naigaon tahsil (30.00%) and Nanded tahsil (27.50%). Results revealed that all the 11 culture media tested encouraged better growth of *A. 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).

1. Introduction

Cotton is one of the most important commercial crops, 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. In India, area, production and productivity of cotton were about 103.10 lakh ha., 295 lakh bales and 486 kg lint/ha;

whereas in Maharashtra were 35.03 lakh bales and 306 kg lint/ha production and productivity, respectively (Anonymous, 2010).

Among the several factors responsible for reduction in yield and quality deterioration of the cotton, diseases are the major one and of these diseases *Alternaria* blight is one of the most important and destructive disease of cotton, inflicting yield losses to the tune of 20-30 per cent (Srinivasan, 1994; Chauhan *et al*., 1997 and Mayee and Mukewar, 2007).

Keeping in view economic importance of cotton and losses incurred by *Alternaria* blight (*Alternaria macrospora*) disease, present investigations on the aspects *viz.,* survey, isolation of the pathogen, pathogenicity and identification of the pathogen and effect of different culture media were undertaken at the Department of Plant Pathology, College of Agriculture, M.K.V. Parbhani, during the year 2010-11.

2. Materials and methods

2.1. Survey and surveillance

An roving survey was undertaken during Aug-Sept, 2010 in four districts *viz*., Nanded, Parbhani, Osmanabad, and Beed of Marathwada region to record the incidence of *Alternaria* blight on cotton .For recording the blight incidence,farmers cotton field were randomly selected.

Incidence of bacterial blight disease was exmined as severe, moderate, trace and free on the basis of percentage of severity of disease. Similarly, the per cent disease incidence of bacterial blight was calculated by using formula.

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

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

2.2. Isolation

Cotton plants naturally infected and *Alternaria* blight with 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) in the laboratory. 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 plate. Purification of fungal culture was carried out on PDA medium in Petri plates under aseptic conditions and well isolated true to type colonies from these plates were transferred to PDA slants.

2.3. Pathogenicity

In order to confirm pathogenic nature of isolated fungal culture, seedling of cotton variety NHH- 44 was raised in pots in glass house/screen house. These seeds were sown in sterilized soil: compost: sand mixture (2:1:1) at the rate 5 seeds/pot (30 cm diameter) on germination 2 seedlings per pot were maintained. These seedlings were inoculated at the stage of 4-6 true leaves with spore suspension ($2x10^{6}$ spores/ml.). Inoculation was carried out by spraying the suspension 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 hrs at slow speed. This growth of fungus was then used for inoculation. Before inoculation, leaves were injured by rubbing carborandum powder to have small injuries for development of symptoms. Immediately pots were watered and entire seedlings with pots were covered with polyethene bags for 48 hrs to maintain humidity. Intermittently pot were watered and the polythene bags were also taken out for a few minutes to avoid rise in temperature. Observations were recorded by observing the plants daily and sufficient number of untreated control was maintained for comparison.

2.4. Cultural studies

A growth character of the isolated Alternaria macrospora was studied by growing it on different agar culture media. The media used were 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 Petri plates, (ten 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$\pm$ 1^0C for seven days. The colony diameter in the culture plates and cultural character such as colony diameter, colour, type of margin, growth were recorded.

3. Results and discussion

3.1. Survey and surveillance

Results (Table 1 and Fig.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 district 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 this was followed by Naigaon tahsil (30.00%) and Nanded tahsil (27.50%). In the Parbhani district, overall average incidence of blight disease was 25.20 per cent; however it was maximum in Gangakhed tahsil (28.16%), followed by Pathri tahsil (26.16 %) and Parbhani tahsil (21.28%).

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

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

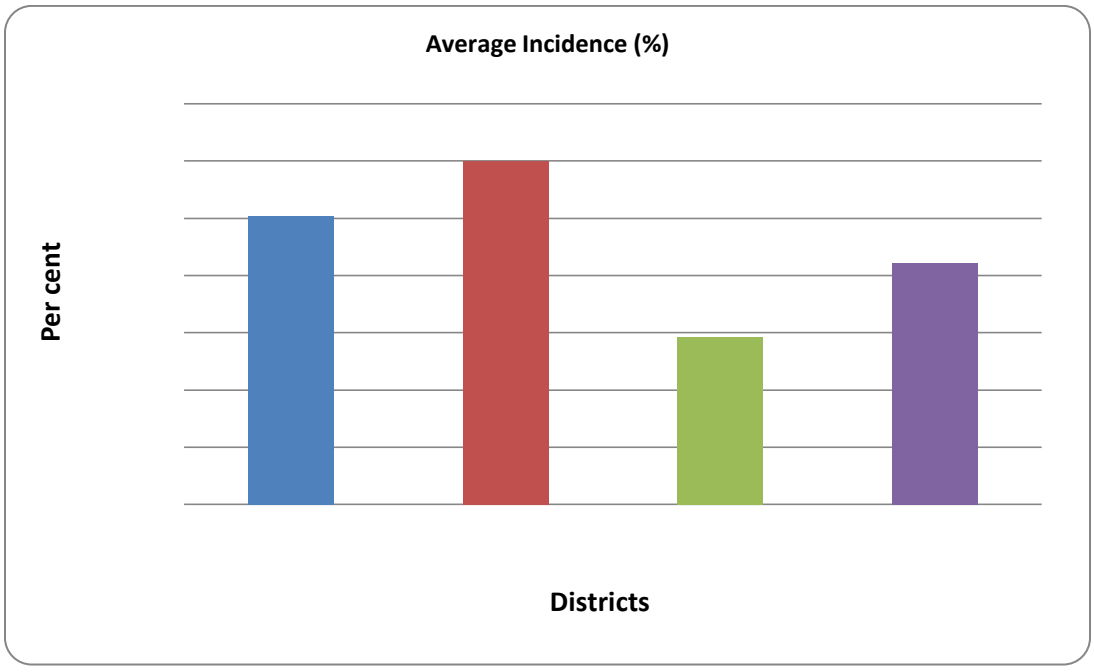


Fig. 1. Districtwise Incidence of Alternaria blight disease of cotton

Table 1
Incidence of *Alternaria* blight (*A. macrospora* Zimm) of cotton in four major districts of Marathwada region.

Districts	Tahsil	No. of locations	Average incidence (%)	Overall Av. 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	

3.2. Pathogenicity

Healthy growing, one month old seedlings of cotton cv. NHH- 44 was spray inoculated with the spore suspension (2 $X10^6$ spores/ml) of the test pathogen and incubated in the screen house, where high relative humidity (80%) and optimum temperature (28 ± 2^0C) were maintained for further development of *Alternaria* blight symptoms. After two weeks of incubation, typical symptoms of *Alternaria* blight on foliage of artificially inoculated 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 (PLATE III) which was found similar to that of the test pathogen isolated from naturally *Alternaria* infected cotton plants. Thus, pathogenicity of test pathogen was proved (PLATE II) and the pathogen was identified and confirmed as *Alternaria macrospora*.

3.3. Isolation and identification of culture

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 (PLATE- I). 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 *Alternaria 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 (Zimmermann, 1904; Ling and Yang, 1941; Mukewar, 1993; and Ramegowda and Naik, 2008).

3.4. 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 (PLATE IV).

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

except on the media, Ashby's manitol agar, Beijerinckia medium and Yeast manitol agar. Colours of the colonies produced were white grey, pinkish white, white, dark brown, whitish red, light brown, and grey. The most prominent concentric rings were produced on culture media *viz*., Potato dextrose agar, Richard's agar, Czapek's dox agar and Oat meal agar whereas unclear and sparse concentric rings were produced on the culture media *viz*., Conn's agar, Yeast manitol agar, Beijerinckia medium, V8 juice agar, Yeast extract agar, Jensens medium and Ashby's manitol agar (PLATE IV). The result of the present study obtained in respect of the effect of various culture media on cultural characteristics of *Alternaria macrospora,* are in consonance with those reported earlier by several worker (Deshpande, 1973; Fencelli and Kimati, 1990; Mazzonetto *et al.,*1996; Pria *et al.,* 1997; Naik *et al.;* 2008).

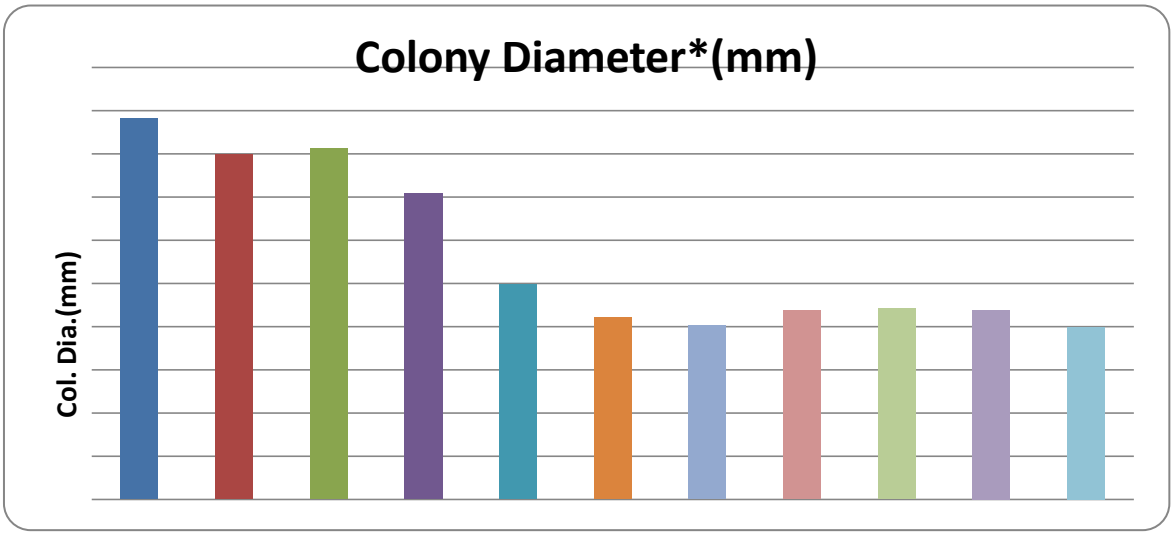


Fig. 2. Effect of different culture media on radial growth of *A. macrospora*

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

Treatments	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 three 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 dike 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 $\pm$	0.58	
CD	1.72	

*Average of three replications

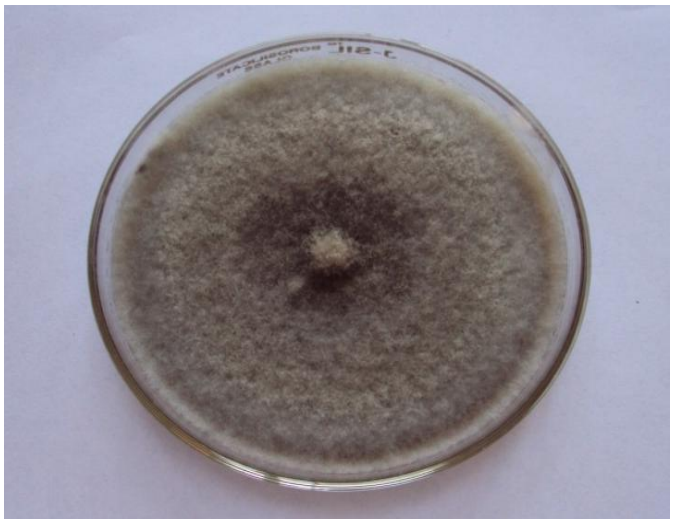


Plate 1. Pure cultue of *A. macrospora*.

Plate 2. Pathogenicity of *A. macrospora*.

Plate 3. Microphotograph of the mycelium and spores of *A. macrospora*.

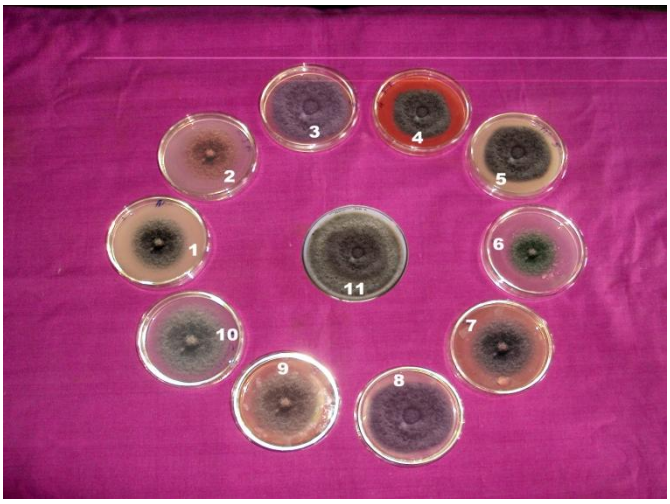


Plate 4. Effect of different culture media on mycelial growth of *A. macrospora*.

1: Ashby's manitol agar 2: Jensens medium
3: Richard's Agar 4: Yeast manitol Agar
5: Oat meal Agar 6: Yeast extract agar
7: V8 Juice Agar 8: Czapek's dox Agar
9: Beijerinkia media 10: Conn's Agar
11: Potato Dextrose Agar

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