

RESEARCH ARTICLE

Effect of growth and sporulation on different solid media and toxin production by *Alternaria* spp. causing leaf spot on cotton

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ARTICLE INFO

Received : 13.03.2013

Revised : 25.05.2013

Accepted : 29.05.2013

Key Words :

Sporulation, Media, Toxin, *Alternaria* spp., Leaf spot, Bt cotton, Non-Bt cotton

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ABSTRACT

In vitro physiological studies revealed that fungus grew well on PDA and malt extract media. Maximum mycelial growth was recorded on Potato dextrose agar medium at 25±2°C temperature and pH 7.0 for all the three *Alternaria* spp. The severe symptoms were produced by *Alternaria alternata* as compared to *Alternaria macrospora* and *Alternaria gossypina*. Thus, it was confirmed that the maximum toxin was produced by *Alternaria alternata* as compared to *Alternaria macrospora* and *Alternaria gossypina*. Non-Bt cotton plants were more susceptible as compared to Bt cotton plants.

How to view point the article : Meena, P.K. and Ratnoo, R.S. (2013). Effect of growth and sporulation on different solid media and toxin production by *Alternaria* spp. causing leaf spot on cotton. *Internat. J. Plant Protec.*, 6(2) : 293-295.

INTRODUCTION

Nutritional requirement of three *Alternaria* spp. viz., *A. alternata*, *A. macrospora* and *A. gossypina* were studied by different *in vitro* physiological tests. Out of seven solid media tested, maximum mycelial growth and sporulation were obtained on PDA followed by malt extract agar media and Richard's agar for growth and sporulation of *Alternaria alternata* and *Alternaria macrospora*. However, PDA was the best medium followed by Richard's agar media and Malt extract agar for growth and sporulation for *Alternaria gossypina*. Whereas, poor growth and sporulation were found on Brown's and Asthana and Hawker's media in all three *Alternaria* spp.

The toxin productions of three species of *Alternaria* were tested on Bt and non-Bt cotton plants in pots. After 10 day of inoculation the typical symptoms appeared on the leaves. The maximum symptoms were produce by *Alternaria alternata* as compared to *Alternaria macrospora* and *Alternaria gossypina*.

The Bt and non-Bt cotton tested were susceptible to the

pathogen and to its toxin. But the disease severity was low in Bt cotton compared to Non-Bt cotton because Bt cotton plants were well makeup with the gene capable of secreting protease enzymes and permitting the presence of fungi of Bt cotton plants.

MATERIAL AND METHODS

Growth and sporulation on different solid media :

Following different media were examined to assess their comparative suitability for supporting the growth and sporulation of three *Alternaria* spp. in laboratory.

- Potato dextrose agar (PDA) medium
- Richard's agar medium
- Czapek's dox agar medium
- Malt extract agar medium
- Sabouraud's medium
- Brown's medium
- Asthana and Hawker's medium

Different solid media were used and they were prepared with single distilled water. The medium in each Petri plate was

inoculated by 2 mm circular disc, cut from the fungal growth on 2% plain agar.

Sporulation under solid media was estimated by taking three 0.5 mm circular growth cylinders, from near the centre other two from middle and periphery of the colony. These were then put in 10 ml sterilized distilled water, crushed shaken vigorously and 0.1 ml of the suspension used for spore count.

Number of spores per microscopic field	Grading
Nil	Absent (-)
1-20	Poor (+)
21-40	Good (++)
41 and above	Abundant (+++)

For solid media studies, 25 ml of sterilized medium was poured in each sterilized Petri plate, inoculated and incubated at $25 \pm 2^\circ\text{C}$. After 7 days of inoculation, the growth was recorded by measuring colony diameter along with two diagonals axis passing through the centre of the colony. Sporulation was also recorded at the time of observations.

Toxin production (Liquid extracts of different cultures):

In order to find out the toxin production (liquid extracts of different cultures) of three isolates of *Alternaria* spp. were prepared on PDA liquid medium. In each conical flask (250 ml), 100 ml medium was poured in three flasks and sterilized in autoclave. Then the conical flasks were inoculated separately from one bit of 5 mm size culture of different species of *Alternaria*. Flasks were incubated at $25 \pm 2^\circ\text{C}$ in BOD incubator. Liquid cultures of different *Alternaria* spp. after 21 days of incubation were subjected to filtration through muslin cloth followed by filter paper and tested on Bt and non-Bt cotton plants in pots. Three pots were separately inoculated by different cultures of *Alternaria* spp. by spray inoculating technique.

RESULTS AND DISCUSSION

The experimental findings of the study have been presented in the following sub heads :

Effect of different solid media on growth and sporulation :

Fungi exhibit a great diversity in their nutritional requirements for their growth and sporulation which are greatly influenced by nature, concentration and total quality nutrients supplied (Cochrane, 1958 and Tandon, 1961). Semi-synthetic medium like PDA gave good growth and better sporulation than other, which could be attributed to the complex nutrients present in PDA.

In order to find out the most suitable medium for different *Alternaria* spp. seven different solid media were tested and mycelial growth and sporulation were recorded after 8 days of incubation (Table 1 and Fig. 1).

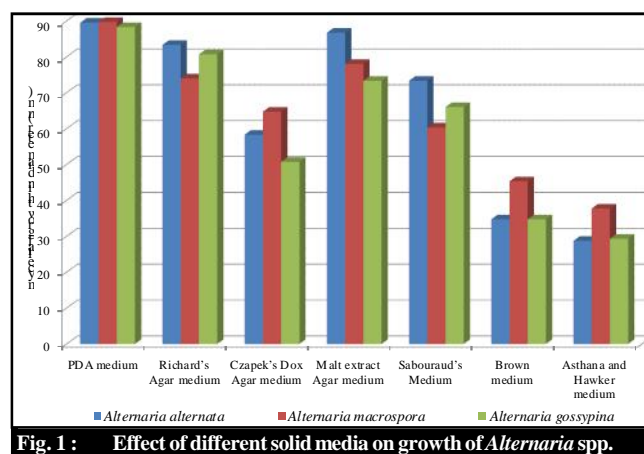


Fig. 1 : Effect of different solid media on growth of *Alternaria* spp.

The *Alternaria alternata* was capable to grow and sporulate on various media studied. Maximum mycelial growth (89.5 mm) and abundant sporulation was obtained on Potato

Table 1. Effect of different solid media on growth and sporulation of *Alternaria* spp.

Medium	<i>Alternaria alternata</i>		<i>Alternaria macrospora</i>		<i>Alternaria gossypina</i>	
	Mycelial growth in diameter (mm)	Sporulation	Mycelial growth in diameter (mm)	Sporulation	Mycelial growth in diameter (mm)	Sporulation
PDA medium	89.5	+++	89.7	+++	88.3	+++
Richard's Agar medium	83.3	+++	74.0	++	80.7	+++
Czapek's Dox Agar medium	58.3	++	64.7	++	50.7	++
Malt extract Agar medium	86.7	+++	78.0	+++	73.3	++
Sabouraud's medium	73.3	++	60.3	++	66.0	++
Brown's medium	34.7	+	45.3	+	34.7	+
Asthana and Hawker medium	28.7	+	37.7	+	29.3	+
SEM±	0.082		0.056		0.131	
CD 5%	0.248		0.171		0.397	
CD 1%	0.344		0.237		0.551	

*Average of three replications

Note: - +++ = Abundant, ++ = Good, + = Poor

dextrose agar followed by Malt extract agar medium (86.7 mm), Richard's agar medium (83.3 mm) and Sabouraud's medium (73.3 mm) and Czapek's dox medium (58.3 mm), sporulation of the pathogen was good whereas, poor growth and sporulation were seen on Brown medium and Asthana and Hawker's medium. The ability of the fungus to grow and sporulate on variety of culture media reveals the capability of the fungus to utilize various forms of nutrients for its growth and sporulation.

Alternaria macrospora was capable to grow and sporulate on various media studied. Maximum mycelial growth (89.7 mm) and abundant sporulation was obtained on potato dextrose agar followed by malt extract agar medium (78.0 mm) and Richard's agar medium (74.0 mm), Czapek's dox medium (64.7 mm) and Sabouraud's medium (60.3 mm). Sporulation of the pathogen was good whereas, poor growth and sporulation were seen on Brown medium and Asthana and Hawker's medium.

Alternaria gossypina was capable to grow and sporulate on various media studied. Maximum mycelial growth (88.3 mm) and abundant sporulation was obtained on potato dextrose agar followed by Richard's agar medium (80.7 mm) and malt extract agar medium (73.3 mm), Sabouraud's medium (60.0 mm) and Czapek's dox medium (50.7 mm). Sporulation of the pathogen was good whereas, poor growth and sporulation were seen on Brown's and Asthana and Hawker's medium. As already stated the ability of the fungus to grow and sporulate on variety of culture media reveals the capability of the fungus to utilize various forms of nutrients for its growth and sporulation. Many workers have found PDA as best and Brown's medium and Asthana and Hawker's media very poor for *Alternaria* spp. for growth and sporulation in laboratory studies. (Ionnidis and Main, 1973; Maheshwari *et al.*, 1999; Singh *et al.*, 2001; Pandey *et al.*, 2006; Waghunde and Patil, 2010).

Toxin production (liquid extracts of different cultures) :

The toxin production (liquid extracts of different cultures) of three species of *Alternaria* were prepared on PDA broth medium and tested on Bt and non-Bt cotton plants in pots. Three pots separately inoculated by different culture of *Alternaria* spp. by spray inoculation technique. After 10 day of inoculation, the typical symptoms appeared on the leaves. The severe symptoms were produced by *A. alternata* as compared to *A. macrospora* and *A. gossypina*. Therefore it was concluded that the maximum toxin was produced by *A. alternata* as compared to *A. macrospora* and *A. gossypina*.

A. macrospora produced toxin *in vitro* which induced

typical leaf spot symptoms in cotton. The Bt and non-Bt cotton tested were susceptible to the pathogen and to its toxin. The wild species of *Gossypium* tested differed in their reaction to the pathogen and the highly susceptible species were highly sensitive to the toxin. These results are in accordance with Krishnamohan and Vidhyasekaran (1989), Hiremath *et al.* (1991), Lee and Yu (1995), Vijayalakshmi (1996), Pangrikar *et al.* (2011).

REFERENCES

- Cocharne, V. W. (1958).** *Physiology of fungi*. John Wiley and Sons, New York.
- Hiremath, P.C., Savanur, R.D. and Reddy, B.M.R. (1991).** Extracellular enzyme production by *Alternaria macrospora* a causal agent of leaf and twig blight of cotton. *Curr. Res.-Univ. Agric. Sci. Bangalore, (KARNATAKA) INDIA.* **20(11)** : 245-246.
- Ionnidis, N. M. and Main, G. C. (1973).** Effect of culture medium on production and pathogenicity of *Alternaria alternata*. *Plant Dis. Repr.*, **57(1)** : 39-42.
- Krishnamohan, G. and Vidhyasekaran, P. (1989).** Possible involvement of toxin in *Alternaria* leaf spot development in cotton. *Indian Phytopath.*, **42(1)** : 99-102.
- Lee, H. and Yu, S. (1995).** Distribution of mycotoxin producing isolates in the genus *Alternaria*. *Korean J. Pl. Pathol.*, **11(2)**: 151-157.
- Maheshwari, S. K., Singh, D. V. and Sahu, A. K. (1999).** Effect of several nutrient media on the growth and sporulation of *Alternaria alternata*. *J. Mycopathol. Res.*, **37(1)** : 21-23.
- Pandey, B.N., Srivastava, S.P. and Srivastava, R.K. (2006).** Studies on effect of various culture media on growth, sporulation and morphological variations of *Alternaria alternata*(Fr.) Keissler. *Flora & Fauna (Jhansi)*, **12(2)**: 247-248.
- Pangrikar, P. P., Wadikar, M. S., Borde, V. U. and Chavan, A. M. (2011).** Production of Protease enzyme from rhizospheric fungi of Bt and non Bt cotton varieties. *J. Biosci. Discovery*, **2(2)**: 249-250.
- Singh, V., Kumar, P. and Sinha, A. P. (2001).** Influence of different media, pH and temperature on growth and sporulation of *Alternaria alternata* (Fr.) Keissler, causing *Alternaria* blight of chickpea. *Legume Res.*, **24(4)**: 238-242.
- Tandon, R. N. (1961).** Physiological studies on some pathogenic fungi. *Uttar Pradesh Science Research. Committee monographs* Allahabad, (U.P.) INDIA.
- Vijayalakshmi, M. (1996).** Toxigenic potential of *Alternaria macrospora* pathogenic to cotton. *Microbiol. Res.*, **151(3)**:239-241.
- Waghunde, R. R. and Patil, R. K. (2010).** Physiological studies of *Alternaria* fruit rot (*Alternaria alternata*) of aonla. *J. Pl. Disease Sci.*, **5(1)**: 73-75.