

Original Research Article

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## Evaluation of Different Fungicides against *Alternaria macrospora* Zimm. inciting Leaf Blight in Cotton under *in vitro*

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### ABSTRACT

*Alternaria* leaf blight caused by *Alternaria macrospora* Zimm. is devastating foliar disease in cotton (*Gossypium* spp.) occur in all the major growing regions of cotton. The experiment of evaluation of fungicides was conducted against the *Alternaria macrospora* pathogen causal agent of leaf blight of cotton. The nine viz., azoxystrobin, chlorothalonil, difenoconazole, propiconazole, tebuconazole, trifloxystrobin, copper oxy chloride, mancozeb and propineb were screened under *in-vitro* and per cent inhibition of radial growth of mycelia was calculated. All the fungicides were found effective against *A. macrospora* and caused significant mycelial growth inhibition of test pathogen over untreated control. difenoconazole and propineb were effective fungicides in inhibition of 100% radial growth of pathogen among the systemic and contact fungicides respectively at lower fungicide concentration of 500 ppm. Five fungicides viz., azoxystrobin, propiconazole, trifloxystrobin, copper oxy chloride, mancozeb were recorded 100% inhibition at 750 ppm concentration. In 1000 ppm concentration all the fungicides were showed the 100% inhibition in all the fungicides except tebuconazole.

#### Keywords

*Alternaria macrospora*,  
Cotton, Fungicides,  
Inhibition

#### Article Info

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### Introduction

Cotton (*Gossypium* spp.) the “king of fibers” is a widely produced natural fiber in the world representing about 44 per cent of the global textile market (Cotton Australia, 2007). Cotton seed is a by-product of cotton fiber and can make up about 15 per cent of the total

financial returns to farmers. Seed is a valued raw material for oils for human consumption and high protein feed for livestock. Apart from the fiber production, it is also used for gossypol production due to its wide range of biological properties including anti-cancer, antimicrobial, anti-HIV, anti-oxidative and male contraceptive activities (Vander Jagt *et*

*al.*, 2000). It is being cultivated as a major crop in parts of the African Tropics, Australia, China, Egypt, India, Mexico, Pakistan, Soviet Union, the Sudan, United States and warmer regions of Central and South America. It is the major commercial crop among all cash crops in India and provides livelihood to more than 60 million people in cultivation, processing and textile industry.

India is the second largest producer of cotton in the world after China accounting for about 18 per cent of the world cotton production. It has the distinction of having the largest area under cotton cultivation in the world accounting 12.59 million hectares and constituting about 23 per cent of the world area under cotton cultivation with the production of 32.81 million bales and productivity of 443 kg lint per hectare during 2017-18 (Bodh *et al.*, 2019). Even though cotton ranks second in production, the productivity of is low due to many reasons. The productivity of cotton crop is affected by various biotic and abiotic stress. Among the various biotic stresses, diseases caused by, fungal pathogen inflicts major per cent loss in total yield annually (Ganesan *et al.*, 2009). The major diseases associated with the cotton are root rot, Fusarium wilt, Verticillium wilt, damping off, grey mildew, Alternaria blight, anthracnose, Cercospora leaf spot, bacterial blight and cotton leaf curl. Among all diseases, leaf blight caused by *Alternaria* spp. is most important and appear every year and has reduced the cotton production (Cui *et al.*, 2000). The Alternaria leaf blight was the most prevalent disease of cotton (Bellgard, 2001) a yield loss of 37 per cent was reported in India by Padaganur *et al.*, (1989). The infection by the Alternaria pathogen results in pre-mature defoliation in cotton (Rajasha *et al.*, 2012) and cause rapid and severe defoliation leading to yield losses (Zhao *et al.*, 2013). The prevailing weather condition during crop growth period leads to development of leaf

blight epidemics during short period of time. But, the sudden and curative effect of plant disease control almost exclusively achieved by application of chemical pesticides. Use of fungicides is the best method of controlling the diseases whenever there is outbreak of disease. Therefore, the different fungicides were evaluated under *in-vitro* to know the effective fungicides at lower concentration against the Alternaria leaf blight pathogen.

## **Materials and Methods**

### **Collection of sample and Isolation of pathogen**

The Alternaria infected diseased leaf samples were collected randomly from the cotton field and wrapped in aluminum foil. The samples were transported to the Plant Pathology Research Laboratory at Tamil Nadu Agricultural University (TNAU), Coimbatore within 12 hrs. of the collection of samples. The pathogen was isolated by tissue segment method (Rangaswami, 1958) on potato dextrose medium. Alternaria leaf blight infected leaves were collected from Department of Cotton, Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The diseased leaf portions were cut into small pieces of 0.5 to 1.0 cm, surface sterilized with 0.1 per cent mercuric chloride for one min. and washed in sterile distilled water thrice and blot dried with sterilized filter paper. Then the leaf bits were placed in Petri plate containing PDA medium. The hyphal tips of fungi growing from the pieces were transferred aseptically to PDA slants and the pathogen was identified based on true morphological characters. The pathogen was sub-cultured on PDA slants and allowed to grow for seven day at  $28 \pm 2$  °C. Such slants were preserved for further work in refrigerator at 4 °C and sub-cultured under aseptic condition periodically.

### **Establishment of Koch's postulates**

Pathogenicity of the isolated pathogen was carried out on cotton. Seeds of cotton variety MCU-5 were sown and 2-3 leaf stage old plants were used for the proving of Koch's postulates. Conidial suspension of the pathogens was prepared according to Sutton and Shane (1983). Fully grown culture plant of *Alternaria*, the conidia were harvested by flooding the plates with 20 ml of sterile distilled water. The surface of the culture was scrapped with a sterile scalpel. The dislodged spores were filtered through thin layers of cheese cloth to remove mycelial fragment. The inoculum load was adjusted to  $1 \times 10^7$  conidia per ml of sterile distilled water. One ml teepol AG (Alkaly benzene sodium salt) was added to 100 ml conidial suspension to increase the spreading quality of the suspension.

When the plants were in 2-3 leaf stage, injuries were made on the leaves and spore suspension of *Alternaria* pathogen was sprayed over the leaves at the rate of  $1 \times 10^7$  conidia per ml during cool evening hours and seedlings were maintained in glass house at  $28 \pm 2$  °C and at 80 per cent relative humidity (RH). The seedlings sprayed with sterile distilled water served as control. The plants were incubated for a period of two weeks and periodically observed for the expression of symptom and compared with the original symptoms. The pathogens were re-isolated from the infected leaves and the isolated cultures were compared with original cultures for identity and further used for evaluation of fungicides.

### **Evaluation of fungicides on fungal radial growth**

The efficacy of nine fungicides against the *Alternaria macrospora* pathogen was tested by poisoned food technique (Schmitz, 1930).

The detail of fungicides with different of mode of action evaluated against *A. macrospora* are given under Table 1. Potato Dextrose Agar (PDA) medium was amended with 1, 10, 25, 50, 100, 250, 500, 750 and 1,000 ppm of all the fungicides and poured separately into a sterile Petri plate and allowed for solidification. The twelve days old pathogen disc (9 mm dia.) was placed in the middle of the Petri plates and appropriate control was maintained without addition of fungicides. The treatments were replicated thrice and plate without fungicide considered as control. The plates were incubated at room temperature ( $28 \pm 2$  °C) and the diameter of colonies were recorded when the control plate reached full growth and per cent inhibition radial growth over control was calculated using the formula given by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = per cent inhibition.

C = growth of *A. macrospora* in non-poisoned food medium.

T = growth of *A. macrospora* in poisoned food medium.

### **Results and Discussion**

#### **Isolation and pathogenicity test of leaf blight pathogen**

The pathogen culture of *Alternaria macrospora* isolate CAM10 was isolated from the infected leaf sample and identified based on cultural and morphology of the pathogen. The pathogenicity test was conducted on cotton variety (MCU-5) at 2-3 leaf stage old seedlings maintained under glass house conditions. The results revealed that, after eight days of inoculation, symptom of *Alternaria* was developed on all inoculated

leaves whereas, uninoculated seedlings remained asymptomatic. In the present study, the symptoms produced by the pathogens on artificial inoculation on the leaves were similar to the symptoms observed under natural infection. In our study Koch postulate was proved (Fig. 1). Similar results were observed by Jadhav *et al.*, (2011), Verma *et al.*, (2007) and Dhiraj *et al.*, (2009). The development of symptoms showed that, *Alternaria macrospora* isolate CAM10 was pathogenic to cotton and same virulent isolate was used for the further studies of fungicide evaluation.

***In vitro* evaluation of fungicides against *A. macrospora* isolate CAM10**

*In vitro* evaluation forms a basis for dosage fixation in fungicide trials. Nine different fungicides viz., azoxystrobin, chlorothalonil,

difenoconazole, propiconazole, tebuconazole, trifloxystrobin, copper oxy chloride, mancozeb and propineb were screened for their fungitoxic action against *Alternaria macrospora* isolate CAM10 under *in vitro* at different concentrations such as 1, 10, 25, 50, 100, 250, 500, 750 and 1,000 ppm through poisoned food technique (Table 2; Fig. 2). The results revealed that increased per cent inhibition of radial growth of pathogen was observed in all the fungicides with increased concentrations. Hundred per cent inhibition of pathogen growth was recorded in the medium amended with difenoconazole and propineb at 500 ppm concentration, followed by azoxystrobin, propiconazole, trifloxystrobin, copper oxy chloride, mancozeb at 750 ppm concentration, whereas at 1000 ppm, the cent percent of inhibition was recorded in Chlorothalonil.

**Table.1** List of chemicals used for *in vitro* evaluation

S. No.	Chemical	Trade name	Formulation	Mode of action
1	Azoxystrobin	Amistar 25 SC	Liquid	Broad spectrum systemic
2	Difenoconazole	Score-25 SC	Liquid	Systemic
3	Propiconazole	Tilt-25 SC	Liquid	Systemic
4	Tebuconazole	Folicure 250 SC	Liquid	Systemic
5	Trifloxystrobin	Flint 50 WG	Granular	Systemic
6	Chlorothalonil	Bravo 75 WP	Wettable powder	Contact
7	Copper-oxy-chloride	Blitox50 WP	Powder	Contact
8	Mancozeb	Indofil M-45 75 WP	Powder	Contact
9	Propineb	Antracol 70 WP	Powder	Contact

**Table.2** Screening of fungicides against *A. macrospora* isolate CAM10 under *in-vitro*

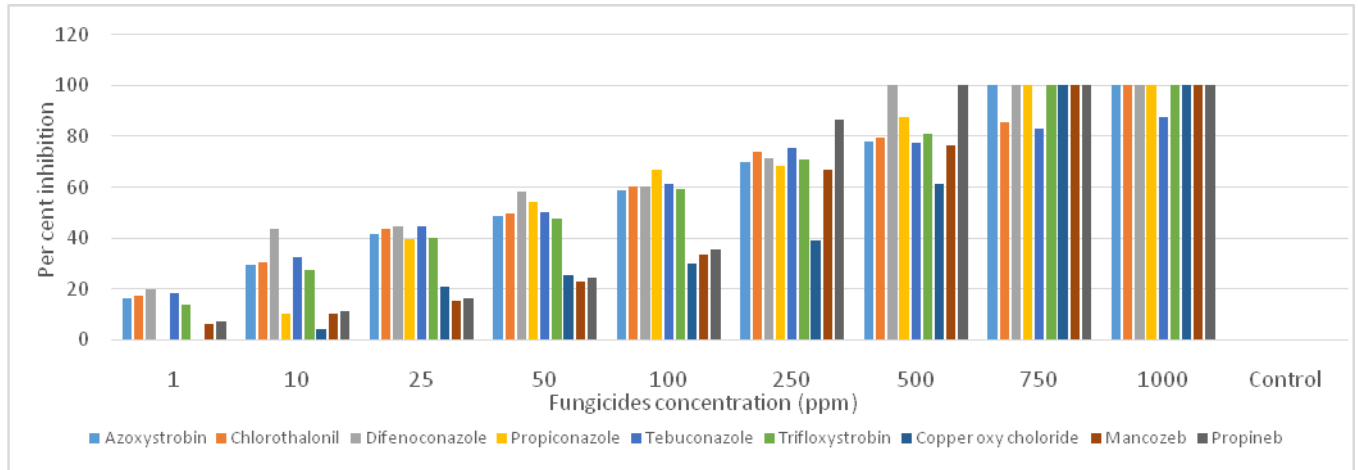
S. No.	Fungicide	Per cent inhibition of mycelial growth at different ppm of active ingredient*								
		1	10	25	50	100	250	500	750	1000
1	Azoxystrobin	16.30	29.26	41.48	48.52	58.52	70.00	78.15	100.00	100.00
2	Chlorothalonil	17.41	30.37	43.33	49.63	60.00	73.70	79.26	85.56	100.00
3	Difenoconazole	19.63	43.33	44.81	58.15	60.37	71.48	100.00	100.00	100.00
4	Propiconazole	0.00	10.00	39.63	54.07	66.67	68.15	87.78	100.00	100.00
5	Tebuconazole	18.15	32.59	44.44	50.37	61.48	75.19	77.41	82.96	87.78
6	Trifloxystrobin	13.70	27.41	40.00	47.78	59.26	71.11	81.11	100.00	100.00
7	Copper oxy chloride	0.00	4.07	20.74	25.19	29.63	38.89	61.11	100.00	100.00
8	Mancozeb	5.93	10.00	15.19	22.59	33.33	66.67	76.67	100.00	100.00
9	Propineb	7.04	11.11	16.30	24.07	35.19	86.67	100.00	100.00	100.00
10	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

\*Mean of three replications

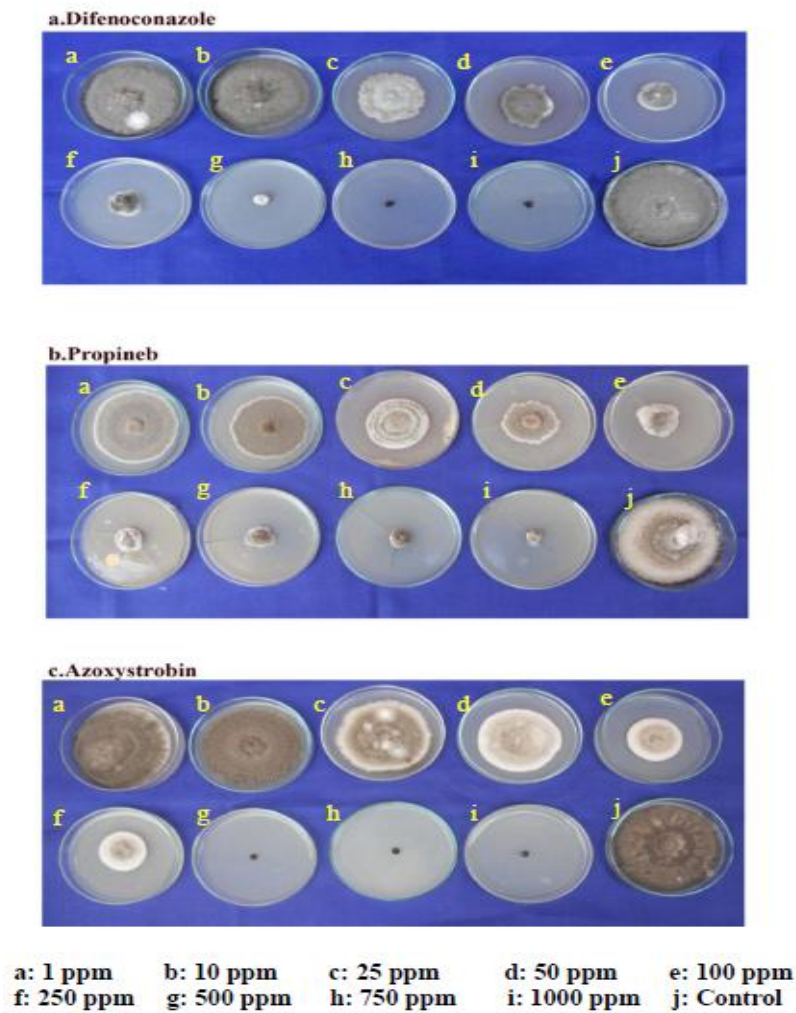
**Fig.1** Symptoms of leaf blight on cotton seedlings after challenge inoculation with *Alternaria*



**Fig.2** Screening of different fungicides at different concentration against the *A. macrospora*



**Fig.3** Screening of fungicides against the *Alternaria macrospora* isolate CAM10



The least per cent inhibition of 87.78 was recorded in the medium amended with tebuconazole at 1000 ppm concentration as compare to control. Among all the fungicides, difenoconazole and propineb were most effective at lowest concentration in complete inhibition of the radial growth of pathogen was observed from systemic and contact fungicides respectively (Fig. 3).

Results are in line with findings of Issiakhem and Bouznad (2010) who, reported that difenoconazole was effective than chlorothalonil in inhibiting the mycelial growth and conidial germination of *A. solani* and *A. alternata* in tomato. Further, Ramegowda *et al.*, (2007) reported that tridemorph and difenoconazole were effective in inhibiting the mycelial growth of *A. macrospora*. Difenoconazole, inhibits the C-14 demethylation of lanosterol or 24-methylenedihydrolanosterol, a biosynthesis step that occurs during the conversion of lanosterol to ergosterol, the final product of fungal sterol synthesis (Reuveni and Sheglov, 2002). Among five systemic and seven non-systemic fungicides were evaluated *in vitro* on *A. macrospora*, tridemorph and difenoconazole at 0.075, 0.05 and 0.025 per cent concentrations among systemic fungicides and iprodione and mancozeb among non-systemic fungicides at 0.15, 0.1 and 0.5 per cent concentrations were effective in inhibiting the mycelial growth (Ramegowda *et al.*, 2007). Hence, inhibition of demethylation by difenoconazole might be responsible for the inhibition of mycelia growth as well as sporulation of *A. macrospora*. Among the contact fungicides, Propineb was effectively inhibited the mycelial growth of *Alternaria*. It is preventive broad spectrum fungicide and interferes at different locations in the metabolism of the fungi. The combination of best systemic and contact fungicide should be preferred for the effective control of pathogen.

It is concluded that the study revealed that, difenoconazole and propineb fungicides were effective at very low concentration for management of leaf blight of cotton pathogen among the systemic and contact fungicides respectively.

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