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Evaluation of fungicides and bio-agents against *Alternaria macrospora* incitant of Alternaria blight of cotton

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Abstract
Alternaria blight of cotton is an important disease of cotton inflicting heavy losses in now days. The present investigation was carried out to test the efficacy of fungicides and bio-agents *in vitro*. Among the fungicides tested, Mancozeb, carbendazim + mancozeb, tebuconazole, propiconazole, hexaconazole and tebuconazole + trifloxystrobin showed 100 percent inhibition while pyraclosterbin and trifloxystrobin restricted the growth to the extent of 50.95 and 75.11% respectively. Bio-efficacy of bio-agent tested by dual culture technique and results revealed that maximum mycelial growth inhibition of was recorded in *Trichoderma harzianum*, *Bacillus* subtilis, *Pseudomonas fluorescens* recorded 71.83, 71.06, and 64.66 per cent inhibition against *Alternaria macrospora* respectively. Least inhibition was noticed in *Mythelobacterium i.e* 42.76%.

Keywords: Cotton, fungicides, bio-agent, alternaria**,** *Trichoderma* spp

Introduction
Cotton is one of the most important fiber and cash crop of India. India is the largest cotton growing country in the world with in area around country 10.5 M ha and shares in global cotton exports around 25%. During the year 2017-18, Gujarat, Maharashtra and Telangana were the major cotton growing states covering around 71% (86.4 lakh hectare) in area under cotton cultivation and 65% (246 lakh bales) of cotton production in India (AICCIP, 2017). There has also been a manifold improvement in production, productivity and quality with virtual increase in area.

Cotton is one of the important fiber crop grown in India. The crop suffers severely due to the attack of Alternaria leaf spot. The losses by leaf spot are always considered to be limiting factor for yield and quality of cotton. Recently, the Alternaria leaf spot caused by *Alternaria macrospora* was found to be in severe form in many cotton growing countries. The present investigation was taken under parameter to find out the effective chemical for the management of Alternaria leaf spot in *in vitro* condition and evaluation of antagonistic activity of four bio-agents.

Materials and Methods
Isolation and maintenance of pure cultures
The sample of Alternaria leaf spots were collected from Cotton Research Unit, Akola. These leaf spot specimen of cotton were first cleaned with tap water to remove the adhering soil particle etc. The affected portion was cut into small bits with healthy tissue. The small bits were surfaced sterilized with 0.1% mercuric chloride solution for 1 minute and then thoroughly washed with three change of distilled sterilized water in order to remove the trace of disinfectant. They were blot dried and placed on sterilized filter paper to absorb the excess water. Three-four bits were then aseptically transferred to each sterilized Petri plates containing solidified PDA medium. All the above operations were carried out in sterilized condition (under laminar air flow unit). Then plates were incubated at 27±2 °C for 10 days. The fungal growth, which developed around each bit, was then transferred to PDA medium slant for sub culturing. The isolated fungus was purified by single spore isolation method. The isolate obtained were maintained on PDA slant by sub culturing. The culture was stored in refrigerator at 10 °C and sub cultured at subsequent interval as and when required.

***In vitro* evaluation of fungicides**

Poisoned food technique was used to evaluate the efficiency of fungicides against pathogens. Potato dextrose agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flask, autoclaved 1.05kg/cm^2 for 15 min. Then before solidification of media different fungicides with desired concentration were incorporated aseptically in different flasks. These flasks shaken thoroughly and poured in Petri plate's 20 ml/plate likewise three plates for each treatment were poured. One set of three plates was poured without any fungicides to serve as a control.

Table 1: List of fungicides against fungal pathogen.

SN	Common Name	Formulation	Concentration used	Trade Name
11	Mancozeb	75 WP	0.25%	Indofil M- 45
T2	Carbendazim12%+ Mancozeb 63%	75 WP	0.2%	Saaf
T3	Propiconazole	25 EC	0.1%	Tilt
T4	Tebuconazole	250 EC	0.1%	Folicur
T5	Pyraclosterbin	20 WG	0.1%	Headline
T6	Hexaconazole	5 SC	0.1%	Contaf
T7	Tebuconazole 50%+ Trifloxystrobin 25%	75 WG	0.1%	Nativo
88	Trifloxystrobin	50 WG	0.1%	Flint

After solidification of medium, the plates inoculated with eight days old pathogens separately. Six mm diameter mycelial disc selected from peripheral growth of the plate by sterilized cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelia growth touch the surface of medium. The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogens on medium was recorded and percent inhibition in each treatment was calculated by using following formula. (Vincent, 1927).

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

Where,

I = Per cent inhibition of mycelial growth

C = Growth of mycelium in control (mm)

T = Growth of mycelium in treatment (mm)

Efficacy of bio-agents against fungal pathogen

The lawn culture of test fungi and bio-agents *i.e. Trichoderma harzianum, Pseudomonas fluorescence*, *Bacillus subtilis* and Mythelobacterium were prepared. Autoclaved medium was poured in the sterilized Petri plates and allowed to solidify for obtaining leveled surface. The plates were inoculated with the culture of test fungi and bio-agents after solidification of media and then plates were incubated at room temperature for ten days.

Bacterial bio-agent *Pseudomonas fluorescens* was prepared by inoculating a loopful culture in sterilized conical flask containing hundred ml of nutrient broth. Broth culture was incubated at room temperature for three days. Five mm disc of one week old test fungus and bio-agent lawn culture was cut with the help of cork borer lifted and transferred in Petri plates, containing autoclaved solidified medium. In each Petri plates, four discs of bio-agents were inoculated at four peripheral points of the plates and the test fungi was placed in centre of Petri plates. In case of *Pseudomonas fluorescens*, a three days old culture was streaked around the disc of test fungus. The test fungi grown in same condition on medium without bio-agents served as control. The treatments were replicated thrice. All these plates were incubated at room temperature for four, five and ten days. After an expiry of ten days incubation period the radial mycelial growth of test fungi was measured in treated and controlled plates on 4th, 5th and 10th days and inhibition per cent was calculated.

Results and Discussion

***In vitro* evaluation of fungicides against Alternaria leaf spot pathogen**

Fungi toxic activities of different fungicides was assayed against *Alternaria macrospora* and observed on 4, 7 and 10 days after incubation and the data are presented in Table 2. On fourth day mancozeb @ 0.25%, carbendazim 12% +mancozeb 63% @ 0.20%, tebuconazole @ 0.10%, propiconazole @ 0.10%, hexaconazole @ 0.10% and tebuconazole 5% + trifloxystrobin 25% @ 0.10% exhibited antifungal properties as cent per cent inhibition was achieved while on 7th day also similar trend was noted except pyraclosterbin @ 0.10% and trifloxystrobin @ 0.10% showed 25.45 mm, 12.65 mm radial mycelial growth respectively. The observation on 10th day was subjected to statistical analysis and revealed significant differences among the fungicides in their fungistatic abilities. Mancozeb, carbendazim + mancozeb, tebuconazole, propiconazole, hexaconazole and tebuconazole + trifloxystrobin showed cent percent inhibition while pyraclosterbin and trifloxystrobin restricted the growth to the extent of 50.95 and 75.11% respectively. Pyraclosterbin was ineffective as 29.43 mm growth and only 50.95% inhibition was recorded after 10th day of incubation.

Table 2: Efficacy of fungicides against *Alternaria macrospora* by poisoned food technique

Treatments No.	Fungicide	Concentrations (%)	Mycelial growth (mm)			Growth inhibition (%)
			4th	7th	10th	
T1	Mancozeb	0.25	00.00	00.00	00.00	100.00
T2	Carbendazim 12% +Mancozeb 63%	0.20	00.00	00.00	00.00	100.00
T3	Propiconazole	0.10	00.00	00.00	00.00	100.00
T4	Tebuconazole	0.10	00.00	00.00	00.00	100.00
T5	Pyraclosterbin	0.10	17.67	25.45	29.43	50.95
T6	Hexaconazole	0.10	00.00	00.00	00.00	100.00

T7	Tebuconazole 50%+Trifloxystrobin 25%	0.10	00.00	00.00	00.00	100.00
T8	Trifloxystrobin	0.10	7.80	12.65	14.93	75.11
T9	Control	-	38.25	56.58	60.25	00.00
	'F` test	-	-	-	Sig.	-
	SE(m)±	-	-	-	1.20	-
	CD (P=0.01)	-	-	-	4.64	-

*****Mean of four replications

The present findings are in agreement with the report of Bhaskar and Sharma (1973) who reported that mancozeb and Zineb were better than other fungicide tested against *Alternaria macrospora*.

Unser *in vitro* studies, 100 % inhibition recorded in mancozeb, carbendazim + mancozeb, propiconazole, tebuconazole and hexaconazole. This finding are almost similar to Meena and Ratoo (2014) who observed maximum (>60 %) per cent inhibition of mycelial growth of *Alternaria macrospora* in a treatment of mancozeb, hexaconazole, copper oxychloride, propiconazole, captafol, tebuconazole and carbendazim + mancozeb. No related information could be traced during perusal of literature in respect to combi product of tebuconazole 50% + trifloxystrobin 25% efficacy against *A. macrospora*. Thus it provides additional information as effective fungicides for management of *A. macrospora*.

***In vitro* evaluation of bio-agents against Alternaria leaf spot pathogen**

Antagonistic abilities of different bio-agents was assayed against *Alternaria macrospora* and observed at 10 DAI (Table 3). The results revealed that *Trichoderma harzianum, Bacillus* subtilis, *Pseudomonas fluorescens* recorded 71.83, 71.06, and 64.66 per cent inhibition against *Alternaria macrospora* respectively. Least inhibition was noticed in *Mythelobacterium i.e* 42.76%.

Table 3: Efficacy of bio-agents against *Alternaria macrospora* by dual culture technique (DAI)

Treatments	Bio-agents	Mycelial growth (mm)			Growth inhibition (%)
		4th	7th	10th	
T1	*Trichoderma harzianum*	16.25	18.75	22.00	71.83
T2	*Bacillus subtilis*	17.00	19.25	22.60	71.06
T3	*Pseudomonas fluorescens*	20.95	24.80	27.60	64.66
T4	*Mythelobacterium*	34.65	39.40	44.70	42.76
T5	Control	48.90	63.60	78.10	00.00
	' F, test			Sig	
	SE(M)±			0.31	
	CD(p=0.01)			1.39	

*Mean of four replications

Tested bio-agents showed significant effect compared to control. The present results are coincides with the results of Dalpati *et al*. (2010) who reported that *T. harzianum* was found superior as compared to *Pseudomonas fluorescence*, *Bacillus subtilis*, and *Trichoderma viride* as inhibited the growth by 76.66% followed by *Bacillus subtilis* (73.65%). These results are also in accords with Mandhare *et al.,* (2002) and Chidambaram *et al.,* (2002) who reported effectiveness and antagonistic properties of *Trichoderma species* against *Alternaria alternata* and *Alternaria macrospora*.

Conclusions

The salient features of the present investigation are as under

1. Maximum inhibition of *Alternaria macrospora* was recorded in Mancozeb, carbendazim + mancozeb, tebuconazole, propiconazole, hexaconazole and tebuconazole + trifloxystrobin.
2. Bio-agents *Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas fluorescens* were found most effective against *Alternaria macrospora.*

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