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In Vitro Evaluation of Fungicides against *Alternaria macrospora* Causing Leaf Spot in Cotton

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A B S T R A C T

Eleven fungicides *viz*., thiram, captan, copper oxychloride, mancozeb, chlorothalonil, carbendazim, hexaconazole, propiconazole, combination products of carbendazim + mancozeb, captan + hexaconazole and azoxystrobin (each at recommended dose, 500 ppm below their recommended dose and 500 ppm above their recommended dose) were evaluated *in vitro* against *Alternaria macrospora* causing leaf spot of cotton. All the treatments significantly inhibited mycelial growth of *Alternaria macrospora* over untreated control. Among different fungicides, mancozeb, carbendazim, hexaconazole, propiconazole and carbendazim + mancozeb at all the test doses completely inhibited (100%) the growth of *A. macrospora* followed by captan + hexaconazole (89.74%), thiram and captan (78.89%) and azoxystrobin showed least inhibition (56.81%) compared to untreated control.

Introduction

Cotton (*Gossypium* spp.) is the most important commercial crops of the world, which belongs to the botanical family Malvaceae. Cotton is referred to as "King of Fibres" and also known as "White Gold". India is the largest cotton growing country in the world with an area around 10.5 M ha followed by United States and China. The cotton production of India is 35.1 million bales and productivity is 568 kg ha^{-1}. India's share in global cotton exports is around 25%. In India, Andhra Pradesh stands seventh in area with 4.49 lakh ha, eighth in production with 13.10 lakh bales and second in productivity with 719 kg ha^{-1} during 2016-17 (AICCIP, 2017).

Cotton crop is affected by fungal, bacterial and viral diseases. Among fungal diseases, leaf spot/blight caused by *Alternaria macrospora* Zimm., is the most commonly occurring disease in Andhra Pradesh.

Under congenial conditions the disease causes severe defoliation, cracking and breaking of stems and reduction in boll formation. The disease caused losses to the tune of 38.23% in

LRA 5166 (Bhattiprolu and Prasada Rao, 2009) and 33.43% in Jayadhar (Chattannavar *et al.,* 2010). Based on the importance of Alternaria and its regular occurrence in Andhra Pradesh, the present investigation was carried out with a view to find out the efficacy of fungicides against *A. macrospora.*

Materials and Methods

Effect of Fungicides on fungal growth

The details of fungicides evaluated against *A. macrospora* are given under Table 1. All the fungicides were tested at three concentrations (with recommended dose, 500 ppm below their recommended dose and 500 ppm above their recommended dose) on active ingredient basis. Thus the fungicides @ 0.3% were tested at 2500, 3000 and 3500 ppm and those @ 0.2% tested at 1500, 2000 and 2500 ppm and the fungicides @ 0.1% tested at 500, 1000 and 1500 ppm.

Selected fungicides were evaluated *in vitro* against *A. macrospora* by employing poisoned food technique (Nene and Thapliyal, 1993). Ten ml stock solution of 1,00,000 ppm concentration was prepared in sterilized distilled water. The desired concentration of fungicide in the medium was obtained by using the formula:

$C_1V_I = C_2V_2$

Where,

C_1 = concentration of the stock solution (ppm)

V_1 = volume of the stock solution to be added (ml)

C_2 = desired concentration (ppm)

V_2 = volume of PDA in which fungicide is to be amended (ml)

Twenty ml of the poisoned medium was poured in to sterilized Petri plate (9.0 cm diameter) under aseptic conditions in Laminar air flow inoculation chamber and allowed to solidify. Each plate was inoculated in the centre with five mm diameter disc cut from the periphery of actively growing seven days old *A. macrospora* culture under aseptic conditions and incubated at 28 ± 1^0C in a BOD incubator. Three replications were maintained for each treatment. PDA plates containing non poisoned medium inoculated with *A. macrospora* served as control. Radial growth of *A. macrospora* was recorded daily in control plate starting from the initiation of the fungal growth in correspondence to treatment plates until full growth of fungus was obtained in control. Per cent inhibition of 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

All the fungicides at three concentrations tested significantly reduced radial growth of *A. macrospora* compared to check (9.00 cm) (Table 2). Mancozeb, carbendazim, hexaconazole, propiconazole and carbendazim + mancozeb completely inhibited the growth of the pathogen at three test concentrations. Decrease in radial growth with increased concentration was observed among the other

fungicides *viz*., captan + hexaconazole, thiram and captan with a mean radial growth of 0.92 cm, 1.9 cm and 1.9 cm, being significantly superior followed by copper oxychloride (COC) and chlorothalonil with 2.21 cm and 2.48 cm mean radial growth. The significantly least effective fungicide was azoxystrobin with a mean radial growth of 3.89 cm (Table 2 and Plate 1).

Significant decrease with increase in concentration was recorded with all fungicides with the least radial growth at > 500 ppm of recommended dose (1.02 cm) and the highest at < 500 ppm of recommended dose (1.41 cm).

The mean radial growth for each concentration was significantly lesser than that of the next higher concentration (Fig. 1).

Fig.1 Effect of fungicides on growth of *Alternaria macrospora in vitro*

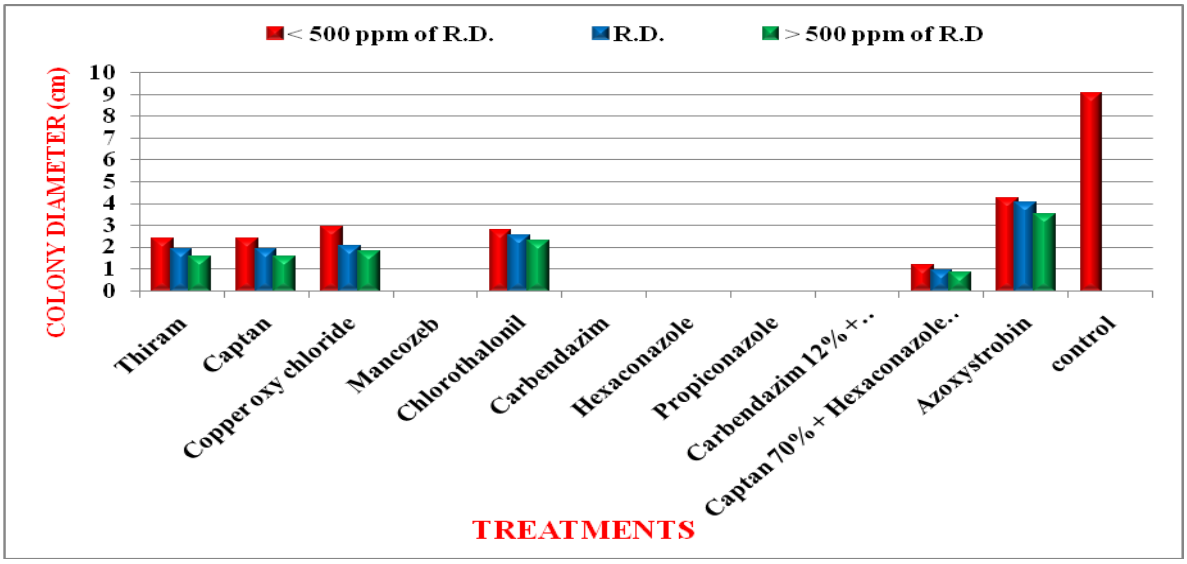

Fig.2 Effect of fungicides on per cent growth inhibition of *Alternaria macrospora in vitro*

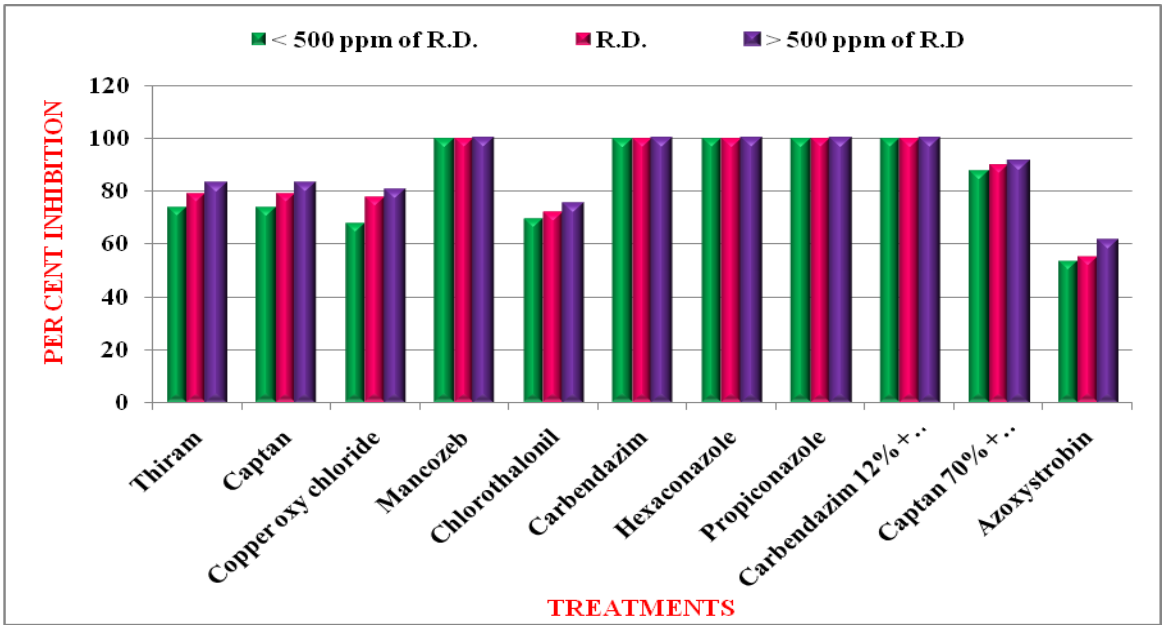

Plate.1 Effect of fungicides on radial growth of *Alternaria macrospora*

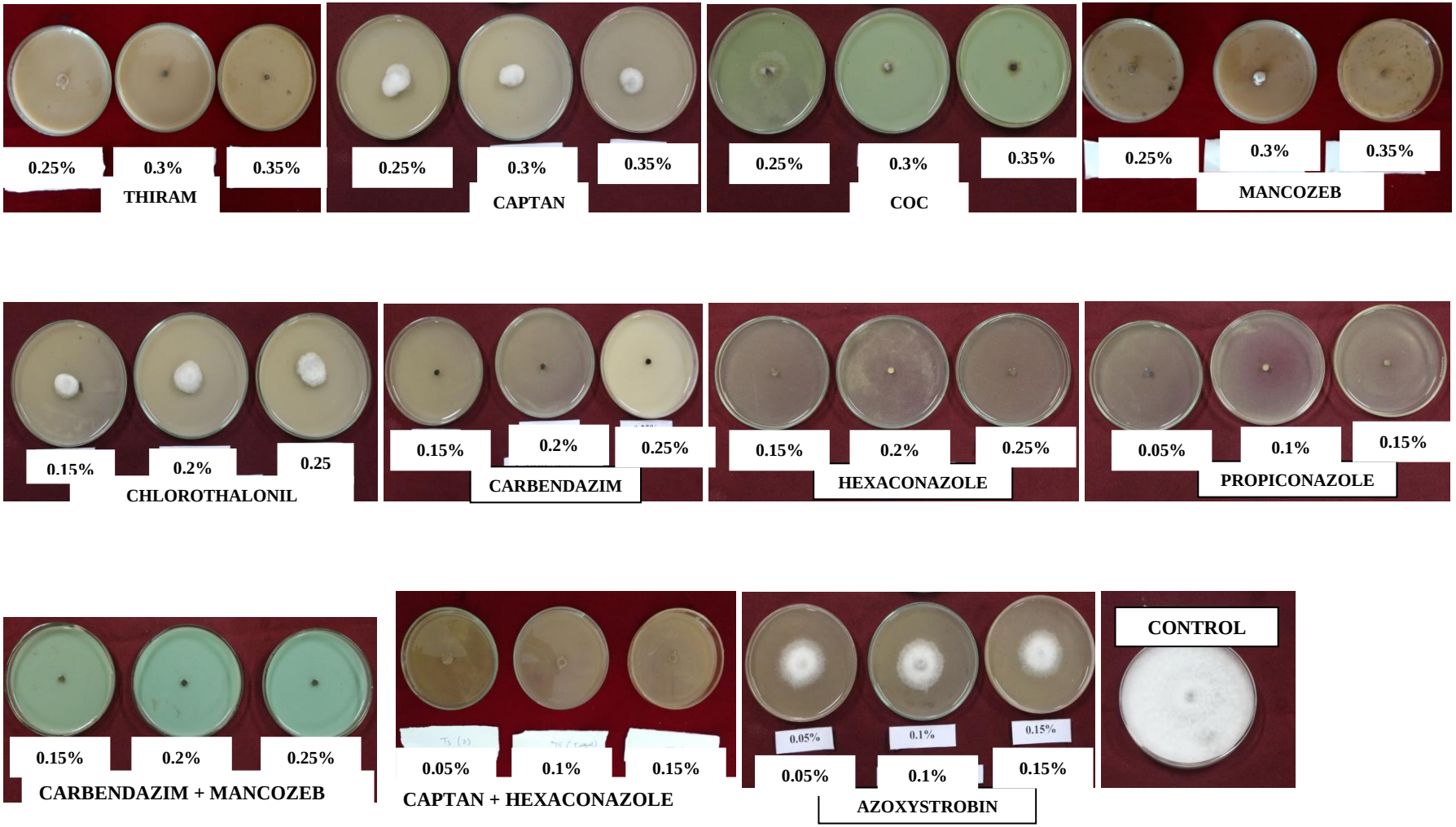

Table.2 *In vitro* evaluation of fungicides at different concentrations against *Alternaria macrospora*

S. No.	Fungicides / Concentrations	R.D.	Colony diameter (cm)*				Per cent inhibition			
			< 500 ppm of R.D.	R.D.	> 500 ppm of R.D	mean	< 500 ppm of R.D.	R.D.	> 500 ppm of R.D	Mean
1	Thiram	0.30%	2.33 (1.53)	1.87 (1.36)	1.50 (1.22)	1.9 (1.37)	74.11	79.22	83.33	78.89
2	Captan	0.30%	2.33 (1.53)	1.87 (1.36)	1.50 (1.22)	1.9 (1.37)	74.11	79.22	83.33	78.89
3	Copper oxy chloride	0.30%	2.89 (1.70)	2.00 (1.41)	1.74 (1.32)	2.21 (1.48)	67.88	77.77	80.66	75.44
4	Mancozeb	0.30%	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	100.00	100.00	100.00	100.00
5	Chlorothalonil	0.20%	2.73 (1.65)	2.50 (1.58)	2.22 (1.49)	2.48 (1.57)	69.66	72.22	75.33	72.40
6	Carbendazim	0.20%	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	100.00	100.00	100.00	100.00
7	Hexaconazole	0.20%	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	100.00	100.00	100.00	100.00
8	Propiconazole	0.10%	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	100.00	100.00	100.00	100.00
9	Carbendazim 12% + Mancozeb 63%	0.20%	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	100.00	100.00	100.00	100.00
10	Captan 70% + Hexaconazole 5%	0.10%	1.10 (1.05)	0.90 (0.95)	0.77 (0.88)	0.92 (0.96)	87.77	90.00	91.44	89.74
11	Azoxystrobin	0.10%	4.18 (2.04)	4.00 (2.00)	3.48 (1.86)	3.89 (1.97)	53.55	55.55	61.33	56.81
	Mean		1.41 (1.19)	1.19 (1.11)	1.02 (1.05)		84.28	86.73	88.67	
	Check		9.00 (3.0)							
		Fungicide		**Concentration**	**F X C**	**Check vs Others**				
	SEm ±	0.01		0.01	0.02	0.02				
	CD (P ≤ 0.05)	0.03		0.02	0.06	0.06				
	CV (%)	3.09								

*Figures in parentheses are square root transformed values R.D. – Recommended dose

Table.1 Fungicides evaluated against *Alternaria macrospora in vitro*

S. No.	Name of the fungicide	Recommended dose	Trade name	Active ingredient
1	Thiram	0.3%	Thiram (Bayer)	42-S% WP
2	Captan	0.3%	Captaf (TATA)	50% WP
3	Copper oxychloride	0.3%	Blitox (TATA)	50% WP
4	Mancozeb	0.3%	Dithane M- 45 (Indofil)	75% WP
5	Chlorothalonil	0.2%	Kavach (Syngenta)	75% WP
6	Carbendazim	0.2%	Zoom (UPL)	50% WP
7	Hexaconazole	0.2%	Contaf (TATA)	5% EC
8	Propiconazole	0.1%	Tilt (Syngenta)	25% EC
9	Carbendazim 12% + Mancozeb 63%	0.2%	Saaf (UPL)	75%
10	Captan 70% + Hexaconazole 5%	0.1%	Taaqat (TATA)	80% WP
11	Azoxystrobin	0.1%	Amistar (Syngenta)	25% SC

Interaction between fungicides and concentrations revealed that inhibition of growth at recommended dose of chemicals ranged between 55.55% in azoxystrobin and 100% in mancozeb, carbendazim, hexaconazole, propiconazole and carbendazim + mancozeb with inhibition by the next superior chemical captan + hexaconazole being 90.0%. A similar trend was observed for radial growth of *A. macrospora* at > 500 ppm and < 500 ppm of recommended dose concentrations with obvious lesser growth (Fig. 2).

Among the different fungicides tested, thiram was found most effective against *A. macrospora*, with maximum growth inhibition (Gholve *et al.*, 2014). Mancozeb and hexaconazole were the best fungicides for control of all the three species of *A. alternata*, *A. macrospora* and *A. gossypina* (Meena and Ratnoo, 2014). Ghazanfar *et al.*, (2016) reported that mancozeb significantly reduced the growth of *Alternaria solani* compared to other treatments. Sanjeev *et al.*, (2017) reported that among different fungicides tebuconazole showed significant reduction in mycelia growth of *A. alternata in vitro.* It was concluded that among the fungicides evaluated *in vitro*, mancozeb, carbendazim, hexaconazole, propiconazole and combination product of carbendazim 12% + mancozeb 63% at their recommended doses as well as below and above their recommended doses showed complete inhibition of mycelial growth of *A. macrospora*.

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