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In vitro evaluation of fungicides against fungal foliar pathogens of cotton

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Abstract
The fungicides, individually and combination products *viz*., hexaconazole, carbendazim, propiconazole, azoxystrobin, hexaconazole+captan, trifloxystrobin+tebuconazole, kresoxim methyl and mancozeb were evaluated *In vitro* against fungal foliar pathogens of cotton including *Alternaria*, *Corynespora*, *Helminthosporium* and *Myrothecium* by employing poisoned food technique. Hexaconazole @ 0.2%, propiconazole @ 0.1%, hexaconazole+captan @ 0.1% and mancozeb @ 0.3% showed 100 per cent inhibition against *Alternaria* and *Helminthosporium*. Trifloxystrobin+tebuconazole also caused 100 per cent inhibition of *Helminthosporium* but 78.86 per cent inhibition in case of *Alternaria*. Carbendazim @ 0.1% was most effective against *Corynespora* with 100 per cent inhibition of mycelial growth followed by hexaconazole+captan (82.83%), hexaconazole (79.03%) and trifloxystrobin+tebuconazole (75.74%), mancozeb (75.60%) and propiconazole (75.25%). Hexaconazole and propiconazole showed 100 per cent inhibition against *Myrothecium* followed by mancozeb (86.82%), hexaconazole+captan (76.48%) and trifloxystrobin+tebuconazole (76.20%), respectively. Fungicides with broad spectrum activity against leaf spot fungi indicate their usefulness in integrated disease management of cotton.

Keywords: Cotton, hexaconazole, leaf spot fungi, mancozeb, propiconazole

Introduction
Cotton (*Gossypium spp*.), referred as 'King of Fibre' and 'White Gold', is the most extensively cultivated commercial crop which plays a key role in economic development. In India, cotton was cultivated in an area of 122.38 lakh ha with an annual production of 361 lakh bales of 170 kg and a productivity of 501 kg lint/ha during 2018-19. Andhra Pradesh stood 4th in area (6.66 lakh ha) but 7th in production (20.0 lakh bales) and 5th in productivity (617 kg/ha) (ICAR-AICRP on Cotton, 2019) [1]. Cotton crop is affected by number of foliar, wilt and rot pathogens, in which foliar diseases account for 20 to 30% yield losses (Mayee and Mukewar, 2007) [2]. Losses due to leaf spots such as Alternaria leaf spot (Chattannavar *et al*., 2006 [3], Myrothecium leaf spot (Taneja *et al*., 1989) [4] and Helminthosporium leaf spot (Bhattiprolu, 2010) [5] were up to 26%, 15%, 32%, respectively. Hagan and Sikora, 2012 [6] recorded 100-200 lb/acre losses due to Corynespora leaf spot. Sandipan *et al.* (2017) [7] estimated losses due to Alternaria leaf spot (26.6%) and Myrothecium leaf spot (29.1%). In view of the economic importance of these fungal foliar diseases in cotton, certain new fungicides and combination fungicides were evaluated *In vitro* against these pathogens of cotton.

Materials and Methods
Eight fungicides, as detailed below were evaluated at recommended doses *In vitro* against foliar fungal pathogens (*Helminthosporium*, *Corynespora*, *Alternaria* and *Myrothecium*) by poisoned food technique (Nene and Thapliyal, 1993) [8].

Treatment details

Treatments	Common Name	Trade Name	Formulation	Concentration (%)
T_1	Hexaconazole	Contaf	5% EC	0.2
T_2	Carbendazim	Bavistin	50% WP	0.1
T_3	Propiconazole	Tilt	25% EC	0.1
T_4	Azoxystrobin	Amistar	23% SC	0.05
T_5	Hexaconazole+captan	Taqat	75% WP	0.1
T_6	Kresoxim methyl	Ergon	44.3% SC	0.1
T_7	Trifloxystrobin+tebuconazole	Nativo	75% WG	0.05
T_8	Mancozeb	Dithane M-45	75% WP	0.3
T_9	Control (unsprayed)	-	-	-

Stock solution of 100000ppm concentration was prepared using 10 ml of sterilized distilled water. Desired concentration of fungicide was obtained by diluting stock solution using the following formula.

$C_1V_1 = C_2V_2$ 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) and V_2 = volume of PDA in which fungicide is to be amended (ml).

Poisoned medium (20 ml) was poured in to sterilized Petri plate under aseptic conditions in inoculation chamber and allowed to solidify. Each plate was inoculated aseptically in the centre with a 5 mm diameter of five day old fungal culture disc cut from the periphery of actively growing culture and incubated at 28 ± 1^0C.

PDA plates with non poisoned medium inoculated with fungus served as control. Radial growth of the fungus was recorded daily in control plates starting from the initiation of the fungal growth in correspondence to treatment plates till the full growth of fungus was observed in control. Per cent inhibition of growth over control was calculated using the formula given by Vincent (1927) [9].

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

I = per cent inhibition, C = growth of thfungus in non poisoned food medium and T = growth of the fungus in poisoned food medium.

Results and Discussion

Efficacy of fungicides on radial growth of *Alternaria macrospora*

All the test fungicides significantly reduced radial growth of *A. macrospora* compared to control (8.80 cm) (Table 1). Hexaconazole @ 0.2%, propiconazole @ 0.1%, hexaconazole+captan @ 0.1% and mancozeb @ 0.3% caused 100 per cent inhibition of mycelial growth of *A. macrospora* and found significantly superior to other treatments. Trifloxystrobin+tebuconazole @ 0.05% also significantly reduced the radial growth and registered 78.86% reduction over control (Plate 1, Fig 1)

Kresoxim methyl 0.1%, carbendazim 0.1% and 0.05% azoxystrobin were found to be less effective fungicides with radial growth of 5.60, 6.13 and 7.46 cm respectively. Mycelial inhibition ranged from 15.22% (azoxystrobin @ 0.05%) to 100% (hexaconazole @ 0.2%, propiconazole @ 0.1%, hexaconazole+captan @ 0.1% and mancozeb @ 0.3%) (Table 1, Fig 1).

Fungicides *viz*., carbendazim, hexaconazole+ captan, hexaconazole (Prasad *et al*., 2018) [10], mancozeb (Pareek *et al*., 2012; Bhat *et al*., 2017; Vijayalakshmi *et al*., 2018) [11-13], mancozeb+carbendazim (Pareek *et al*., 2012; Prasad *et al*., 2018) [11, 10], propiconazole (Prasad *et al*., 2018 [10]; Vijayalakshmi *et al*., 2018) [13] and trifloxystrobin+tebuconazole @ 0.05% (Singh *et al*., 2018) [14] were found most effective in inhibiting *Alternaria* infecting different crops. In cotton azoxystrobin (Prasad *et al*., 2018) [10] was significantly least effective against *Alternaria In vitro*, whereas mancozeb completely inhibited the growth of *Alternaria* even at a lower concentration of 500 ppm (Vihol *et al*., 2009) [15]. Sahani and Mahapatra (2014) [16] tested the efficacy of different fungicides against *A. Sesami* under *In vitro* conditions and reported that propiconazole (0.1%) showed 100 per cent inhibition followed by difenoconazole (87.41%).

Efficacy of fungicides on radial growth of *Corynespora cassiicola*

All the test fungicides significantly reduced radial growth of *C. cassiicola* compared to control (8.73 cm) (Table 1). Carbendazim @ 0.1% completely inhibited the mycelial growth of the pathogen (100%) and found significantly superior to other treatments. Hexaconazole @ 0.2%, hexaconazole+captan @ 0.1% and trifloxystrobin+tebuconazole @ 0.05% also significantly reduced the radial growth and registered 79.03%, 82.81% and 76.74% reduction over control, respectively (Plate 2 and Fig 1).

Mancozeb @ 0.3% significantly reduced the radial growth and registered 75.60% reduction over control which was statistically on a par with 0.1% propiconazole (75.25%) and 0.05% trifloxystrobin+tebuconazole. Kresoxim methyl @ 0.1% and azoxystrobin @ 0.05% were found to be less effective with radial growth of 4.46 and 3.50 cm, respectively. Mycelial inhibition ranged from 48.91% (kresoxim methyl @ 0.1%) to 100% (carbendazim @ 0.1%) (Fig 1).

Table 1: Efficacy of fungicides on mycelial growth of fungal foliar pathogens of cotton

Treatments		Conc. (%)	Mycelial growth (cm)*			
			Alternaria macrospora	*Corynespora cassiicola*	*Helminthosporium gossypii*	*Myrothecium roridum*
T_1	Hexaconazole 5% EC	0.20	0.00 (1.00)[a]	1.83 (1.68)[c]	0.00 (1.00)[a]	0.00 (1.00)[a]
T_2	Carbendazim 50% WP	0.10	6.13 (2.67)[d]	0.00 (1.00)[a]	5.03 (2.45)[b]	5.06 (2.46)[f]
T_3	Propiconazole 25% EC	0.10	0.00 (1.00)[a]	2.16 (1.77)[d]	0.00 (1.00)[a]	0.00 (1.00)[a]
T_4	Azoxystrobin 23% SC	0.05	7.46 (2.90)[e]	3.50 (2.12)[e]	7.00 (2.82)[d]	2.50 (1.87)[d]
T_5	Hexaconazole+Captan 75% WP	0.10	0.00 (1.00)[a]	1.50 (1.58)[b]	0.00 (1.00)[a]	1.66 (1.63)[c]
T_6	Kresoxim methyl 44.3% SC	0.10	5.60 (2.56)[c]	4.46 (2.33)[f]	5.63 (2.57)[c]	4.80 (2.40)[e]
T_7	Trifloxystrobin+Tebuconazole 75% WG	0.05	1.86 (1.69)[b]	2.03 (1.74)[cd]	0.00 (1.00)[a]	1.70 (1.64)[c]
T_8	Mancozeb 75% WP	0.30	0.00 (1.00)[a]	2.13 (1.76)[d]	0.00 (1.00)[a]	0.93 (1.39)[b]
T_9	Control (unsprayed)	-	8.80 (3.13)[f]	8.73 (3.11)[g]	9.00 (3.16)[e]	7.06 (2.84)[g]
	SEm (±)		0.01	0.02	0.01	0.02
	CD ($p \leq 0.05$)		0.03	0.07	0.03	0.05
	CV (%)		1.03	2.26	0.91	1.6

*Mean of the three replications Treatment means with same alphabet do not differ significantly
Figures in the parenthesis are square root transformed values

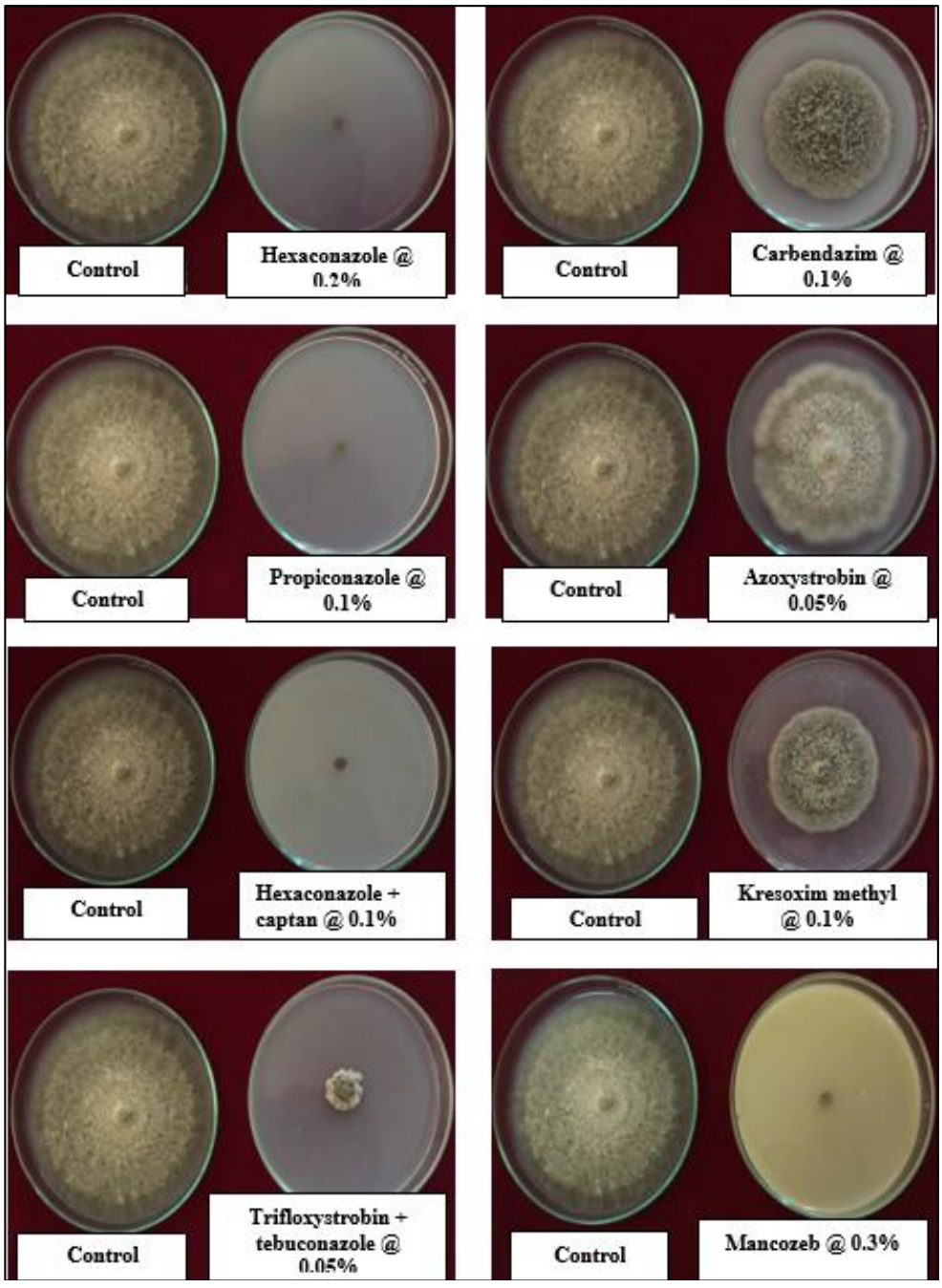

Plate 1: Efficacy of fungicides on radial growth of *Alternaria macrospora In vitro*

Efficacy of fungicides on radial growth of *Helminthosporium gossypii*

All the fungicides significantly reduced radial growth of *H. gossypii* compared to control (9.00 cm) (Table 1). Hexaconazole @ 0.2%, propiconazole @ 0.1%, hexaconazole+captan @ 0.1%, trifloxystrobin+tebuconazole @ 0.05% and mancozeb @ 0.3% completely inhibited the mycelial growth of the pathogen (100%) and found significantly superior to other treatments (Fig 1, Plate 3). Carbendazim @ 0.1% and kresoxim methyl @ 0.1% significantly reduced the radial growth and registered 44.11% and 37.44% reduction over control respectively. Azoxystrobin @ 0.05% was found to be the least effective fungicide with radial growth of 7.00 cm. Mycelial inhibition ranged from 22.22% (azoxystrobin @ 0.05%) to 100% (hexaconazole @ 0.2%, propiconazole @ 0.1%, hexaconazole+captan @ 0.1%, trifloxystrobin+tebuconazole @ 0.05% and mancozeb @ 0.3%) (Fig 1). The present result was in accordance with Dighule *et al*. (2011) [17], who reported that propiconazole @ 0.1% and mancozeb @ 0.3% were effective against *Helminthosporium gossypii In vitro.*

Efficacy of fungicides on radial growth of *Myrothecium roridum*

All the test fungicides significantly reduced radial growth of *M. roridum* compared to control (7.06 cm) (Table 1). Hexaconazole @ 0.2% and propiconazole @ 0.1% completely inhibited the growth of the pathogen and found significantly superior to other treatments (Fig 1, Plate 4). Mancozeb @ 0.3% also significantly reduced the radial growth and registered 86.82% reduction over control. Hexaconazole+captan @ 0.1% showed 76.48% reduction over control and found statistically on a par with trifloxystrobin+tebuconazole @ 0.05% (76.20%). Carbendazim @ 0.1% and azoxystrobin @ 0.05% were found to be less effective fungicides with radial growth of 5.06 and 2.50 cm respectively. Mycelial inhibition ranged from 28.32% (carbendazim @ 0.1%) to 100% (hexaconazole @ 0.2%, propiconazole @ 0.1%) (Fig 2).

Plate 2: Efficacy of fungicides on radial growth of *Corynespora cassiicola In vitro*

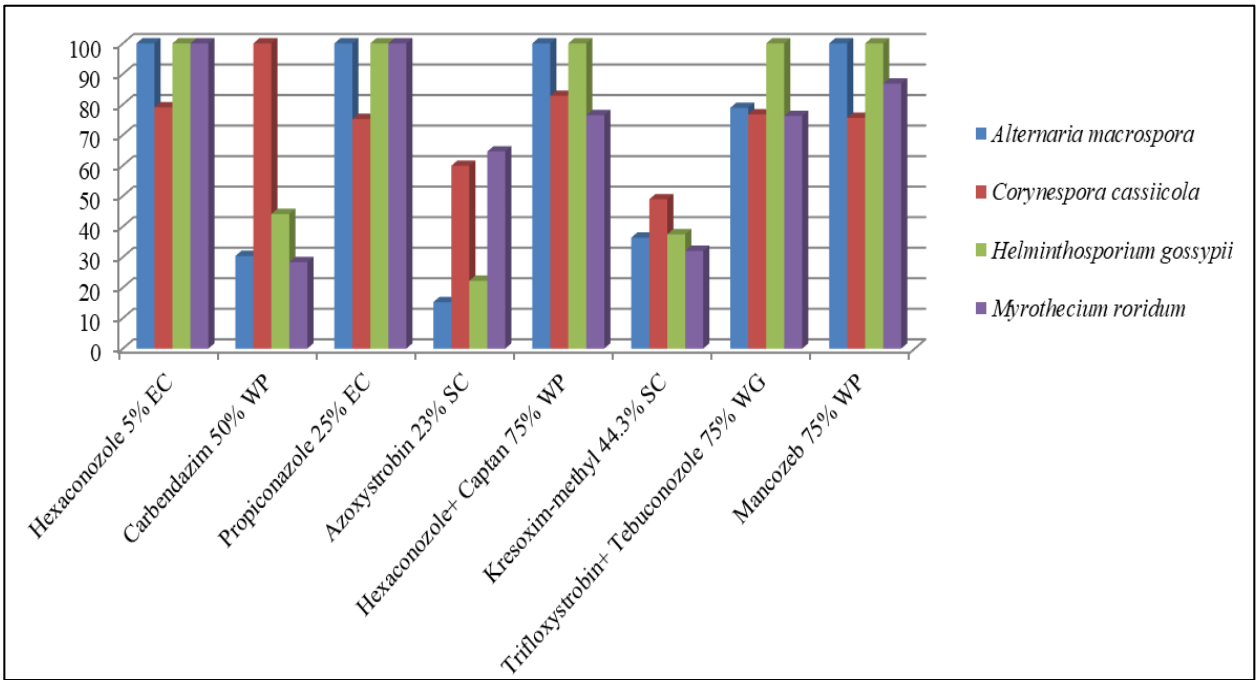

Fig 1: Effect of fungicides on per cent inhibition of fungal foliar pathogens

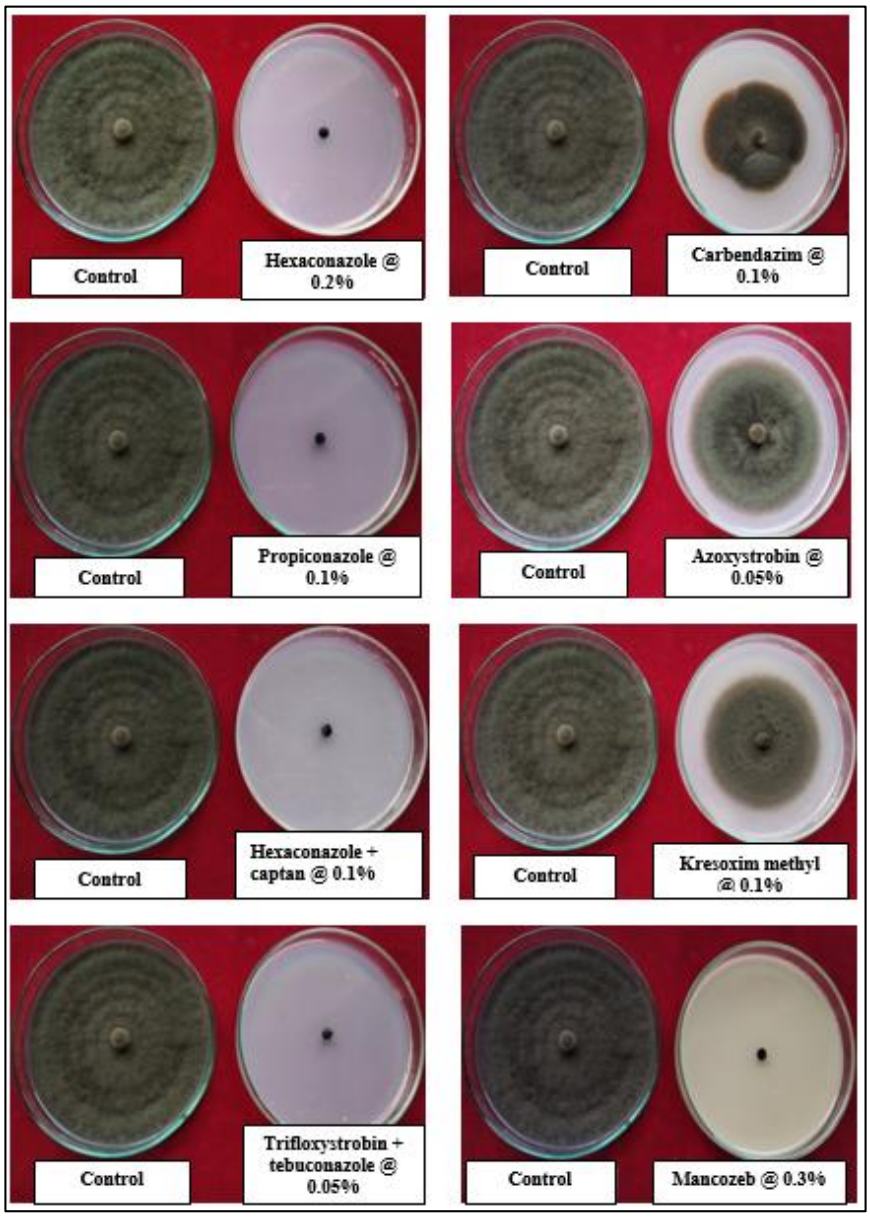

Plate 3: Efficacy of fungicides on radial growth of *Helminthosporium gossypii In vitro*

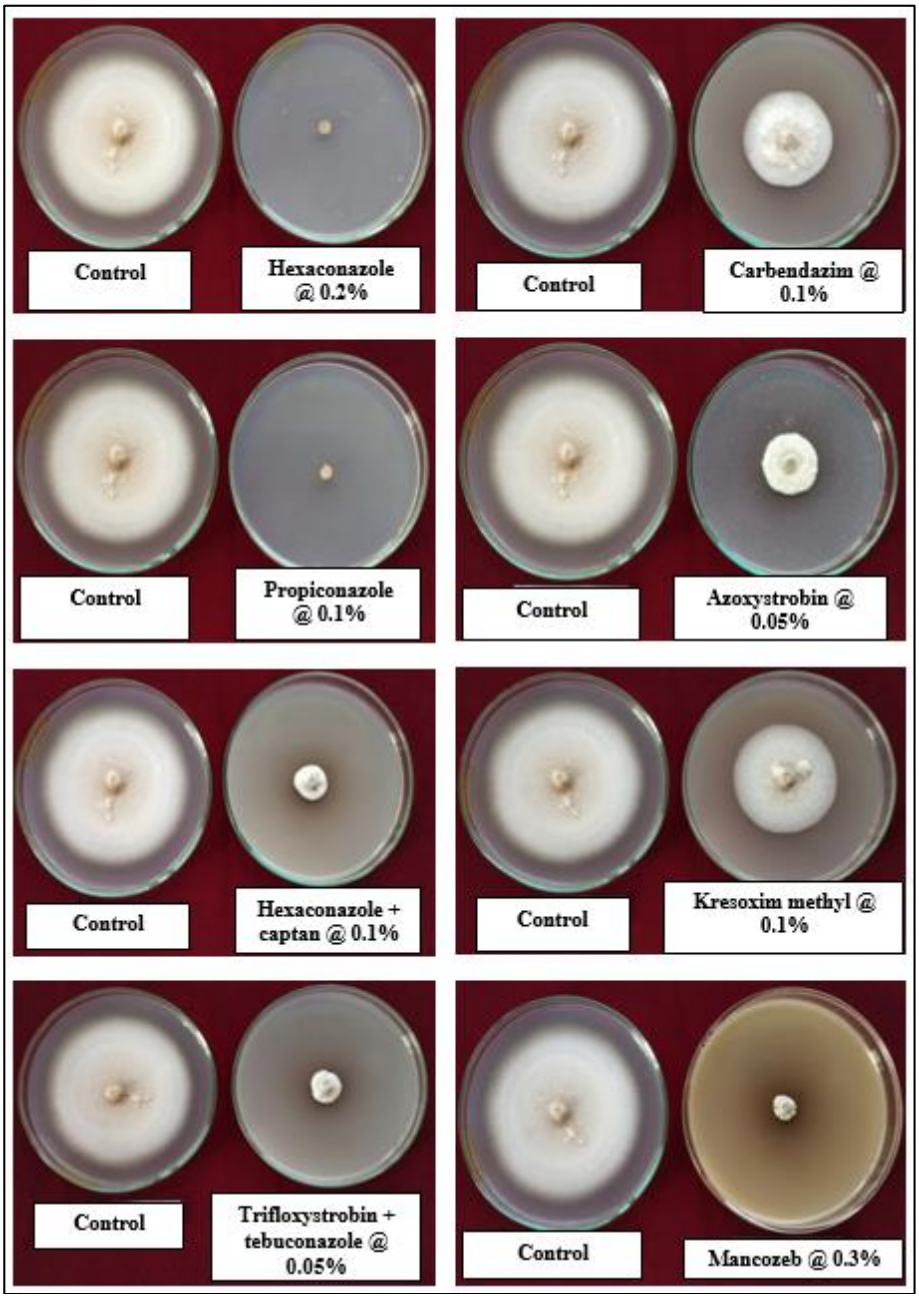

Plate 4: Efficacy of fungicides on radial growth of *Myrothecium roridum In vitro*

Mourya *et al.* (2009) [18] observed minimum radial growth with thiophanate methyl and propiconazole against *M. roridum in vitro* while Tomar and Shashtri (2006) [19] found propiconazole @ 0.1% as the most effective fungicide with 100% inhibition over control. Dighule *et al.* (2011) [17] observed that propiconazole @ 0.1% and copper oxychloride @ 0.25% were effective against *M. roridum*. Tebuconazole @ 0.05% was effective in controlling *M. roridum* followed by propiconazole @ 0.05% *In vitro* (Divyasikamani *et al.* 2013) [20].

Conclusion

The fungicides and/or combination products *viz*., hexaconazole @ 0.2%, propiconazole @ 0.1%, hexaconazole+captan @ 0.1% and mancozeb @ 0.3% showed broad spectrum activity against fungal foliar pathogens of cotton, *Alternaria*, *Corynespora*, *Helminthosporium* and *Myrothecium*, which were statistically on a par with carbendazim @ 0.1% and trifloxystrobin+tebuconazole @ 0.05%. Hence these fungicides are useful in the integrated disease management in cotton.

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