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In Vitro Evaluation of Fungicides against *Alternaria* and *Corynespora* Leaf Spot Pathogens on Cotton

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ABSTRACT

An investigation to test *in vitro* efficacy of nine different fungicides against *Alternaria macrospora* and *Corynespora cassiicola* causing *Alternaria* and *Corynespora* leaf spots, respectively, in cotton was carried out during 2019-20. Among the different fungicides tested it was observed that propiconazole @ 0.1%, myclobutanil @ 0.1%, propineb @ 0.3%, zineb + hexaconazole @ 0.2% and fluxapyraxad + pyroxystrobin 0.06% completely inhibited the mycelial growth of *A. macrospora* whereas propiconazole @ 0.1%, myclobutanil @ 0.1%, metiram @ 0.2%, propineb @ 0.3%, zineb + hexaconazole @ 0.2%, trifloxystrobin + propineb @ 0.3%, metiram + pyraclostrobin @ 0.1% and fluxapyraxad + pyroxystrobin 0.06% (100%) completely inhibited the mycelial growth of *C. cassiicola* causing highest inhibition (100%) over control.

Keywords: *Alternaria leaf spot, Corynespora leaf spot, Cotton, fungicides and in vitro evaluation.*

Cotton was referred as "King of Fibres" or as "White Gold".It is one of the most important commercial crops grown in the state of Andhra Pradesh cultivated in an area of 5.24 lakh ha with a productivity of 584 kg lint/ha. In India, cotton occupies an area of 120.69 lakh ha with an annual production of 362.18 lakh bales of 170 kg and a productivity of 510 kg lint/ha. (ICAR-AICRP on Cotton, Annual Report, 2021-22). Fungal leaf spots caused by *Alternaria macrospora*, *Corynespora cassiicola*, *Myrothecium roridum*, *Helmintho - sporium gossypii* and *Cercospora gossypii* are of regular occurrence in cotton. Among these Alternaria and Corynespora leaf spots are major diseases in Andhra Pradesh. In India, Alternaria leaf spot was first reported by Rane and Patel in 1956 from Dharwad, Poona and Ahmednagar on Co-4-B-40 cotton variety. Later Srinivasan and Kannan (1974) reported from different parts of south India. Under congenial conditions Alternaria blight causes severe defoliation, cracking and breaking of stems and reduction in boll formation. The disease caused losses to the tune of 38.23% inLRA 5166 (Bhattiprolu and Prasada Rao, 2009) and 33.43% in Jayadhar varieties of cotton (Chattannavar *et al*, 2010). Yield losses up to 5-35% were reported due to Alternaria leaf spot (Zanjare *et al*., 2005, Chattannavar *et al*., 2006 and More *et al.,* 2010).Hagan and Sikora (2012) reported that Corynespora leaf spot caused loss of 100-200 lb/ac of lint in cotton.In India, Parakhia *et al*. (1989) first reported the occurrence of Corynespora leaf spot in Hybrid-4 and Hybrid-6 of cotton during 1984-85 in Junagadh district of Gujarat. Boll rot disease was observed on cotton *cv.* MCU 9 in Tamil Nadu, India in 1988, which was pathogenic to bolls and considered to be different from

the strain of *C. cassiicola* known elsewhere causing leaf spot of cotton (Lakshmanan *et al.,* 1990). Fulmer *et al.* (2012) reported that target spot damage on cotton varied upto 75%. The disease caused lint yield loss in susceptible cotton cultivars as high as 224 - 448 kg ha^{-1}equivalent to 5 to 40% (Conner *et al*., 2013; Hagan, 2014; Hagan *et al.,* 2015).Based on the importance of Alternaria and Corynespora leaf spots, *in vitro* experiment was conducted to find out the efficacy of fungicides against these pathogens.

Colony colour of *A. macrospora* on PDA was initially white that later turned to grey colour. The colony was woolly with a round to irregular margin (Plate 1A). Growth was moderately slow (8.0 cm in 15 days). The mycelium was dark brown and septate with muriform dark brown conidia and septate conidiophores. Colony growth of *C. cassiicola* on PDA was effuse, grey to light olive green with abundant aerial growth. On lower side, mycelium appeared greyish to black colour(Plate 1B). Mycelium was smooth, subhyaline, light brown in colour, thin-walled, branched and septate with a radial growth of about 9 cm in 8 days. Conidiophores were cylindrical, straight or curved and unbranched, 3-10 septate, smooth and pale brown. Conidia were variable obclavate to cylindrical, either singly or in chains, with truncate base and rounded apex with 5-14 pseudoseptate, subhyaline to pale, brown and smooth.

MATERIAL AND METHODS

The experiment was carried out in the Department of Plant Pathology, Agricultural College Bapatla. *Alternaria* and *Corynespora* infected cotton leaf samples were collected from Regional Agricultural Research Station, Lam during *kharif* 2019-20. The affected portion of the leaves were cut into small pieces and surface sterilized with 0.1 N sodium hypochlorite (NaOCl) solution for 30 seconds and then washed properly with sterile water for four times and transferred to the Petri plates (4 bits per Petri plate) containing Potato Dextrose Agar (PDA).

The efficacy of different fungicides against *A. macrospora* and *C. cassiicola* were evaluated *in vitro* by poisoned food technique (Nene and Thapliyal, 1993) using PDA as basal medium. Required quantity of test fungicides (Table 1) were added to the sterilized medium. Poisoned medium was equally distributed into three sterilized Petri plates under aseptic conditions in inoculation chamber. Each plate was inoculated in the centre with a 5 mm diameter of five days old fungal culture disc cut from the periphery of actively growing culture under aseptic conditions and incubated at 28±1°C in a BOD incubator. Three PDA plates with non poisoned medium inoculated with fungus served as control. Radial growth of the fungus was recorded daily in the control plate starting from the initiation of the fungal growth in correspondence to treatment plates till the fungal growth was full 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 the fungus in non-poisoned food medium and T = growth of the fungus in poisoned food medium.

RESULTS AND DISCUSSION

Efficacy of fungicides against Alternaria leaf spot

All the test fungicides significantly reduced radial growth of *A. macrospora as* compared to the control (7.87 cm) (Table 2). Propiconazole @ 0.1%, myclobutanil @ 0.1%, propineb @ 0.3%, zineb + hexaconazole @ 0.2% and fluxapyraxad + pyroxystrobin 0.06% completely inhibited the mycelial growth of the pathogen (100%) and were significantly superior to other treatments (Table 2).

The per cent of inhibition was ranged from 63.16% (metiram @ 0.2%) to 100% (propiconazole @ 0.1%, myclobutanil @ 0.1%, propineb @ 0.3%, zineb + hexaconazole @ 0.2% and fluxapyraxad + pyroxystrobin (0.06%) (Fig. 1). Metiram + pyraclostrobin @ 0.1%, trifloxystrobin + propineb @ 0.3% and pyraclostrobin @ 0.1% significantly reduced the radial growth and registered 86.40%, 81.32% and 78.02% reduction over control, respectively. Metiram @ 0.2% (63.16%) was found to be relatively less effective with radial growth of 2.90 cm.

These results are in accordance with Dighule *et al*. (2011), who reported propiconazole @ 0.1% was more effective against *A. macrospora in vitro*. Mancozeb, carbendazim, hexaconazole, propiconazole and carbendazim + mancozeb completely inhibited the growth of *A. macrospora* (Mohan Venkata Siva Prasad *et al*., 2018). Bodhke *et al.* (2019) found that propiconazole @ 0.1% and hexaconazole @ 0.1% resulted in 100 per cent inhibition against *A. macrospora*. 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* (Yamuna *et al*., 2020). Arun kumar (2008) reported that propiconazole and hexaconozole were highly effective in inhibition of mycelial growth against *A. alternata.* Mancozeb @ 0.3%, hexaconazole @ 0.2% and propiconazole @ 0.1% completely inhibited mycelial growth of *A. alternata* (Venkatesh *et al* 2015). Hexaconazole, propiconazole and zineb + hexaconazole recorded 100 per cent inhibition of *Alternaria* under *in vitro* conditions (Sangeetha *et al.,* 2018). Metiram 70% + Pyraclostrobin 20% WG (0.35 % concentration) was found most effective in inhibiting mycelial growth of *A. alternata in vitro* (Poonam Kumari *et al*., 2020).

Efficacy of fungicides against *Corynespora* leaf spot

All the test fungicides significantly reduced radial growth of *C. cassiicola* in comparison to the control (9.00 cm) (Table 2). Propiconazole @ 0.1%, myclobutanil @ 0.1%, metiram @ 0.2%, propineb @ 0.3%, zineb + hexaconazole @ 0.2%, trifloxystrobin + propineb @ 0.3%, metiram + pyraclostrobin @ 0.1% and fluxapyraxad + pyroxystrobin 0.06% (100%) completely inhibited the mycelial growth of the pathogen (100%) and were significantly superior to pyroxystrobin (Table 2). Pyraclostrobin @ 0.1% (38.11%) was found to be least effective with maximum radial growth of 5.57 cm.

The per cent of inhibition was ranged from 38.11% (pyraclostrobin @ 0.1%) to 100% (propiconazole @ 0.1%, myclobutanil @ 0.1%, metiram @ 0.2%, propineb @ 0.3%, zineb + hexaconazole @ 0.2%, trifloxystrobin + propineb @ 0.3 %, fluxapyraxad + pyroxystrobin (0.06%) and metiram + pyraclostrobin @ 0.1%) (Fig. 2).

Arvind *et al*. (2017) observed minimum inhibition of mycelial growth of *C. cassiicola* by mancozeb and pyraclostrobin in comparison to control at 50 ppm and 100 ppm concentrations. Carbendazim @ 0.1% completely inhibited the growth of the pathogen with maximum inhibition (100%) of mycelial growth (Yamuna *et al*., 2020). Ishwari *et al.* (2020) observed that propiconazole inhibited radial growth of *C. cassicola* with 94.44% inhibition against pathogen over control under *in vitro* conditions. Metiram + pyraclostrobin @ 0.2% completely inhibited the radial growth of mycelium against *C. cassicola* over control (Mushrif *et al.*, 2020).

Propiconazole inhibits the formation of critical fungal cell membrane ergosterols, primarily by blocking the action of 14-α-sterol demethylase, thus reduced

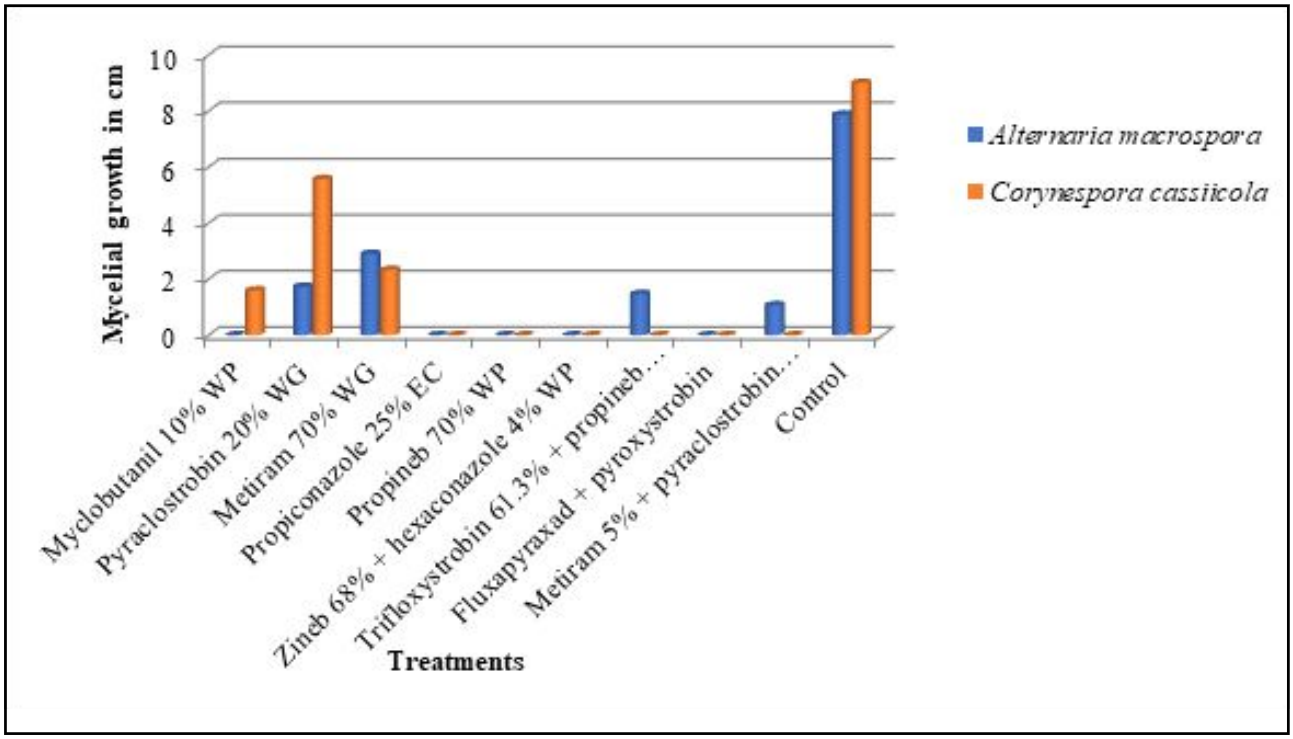


Fig 1. Effect of fungicides on mycelial growth of *Alternaria macrospora* and *Corynespora cassiicola*

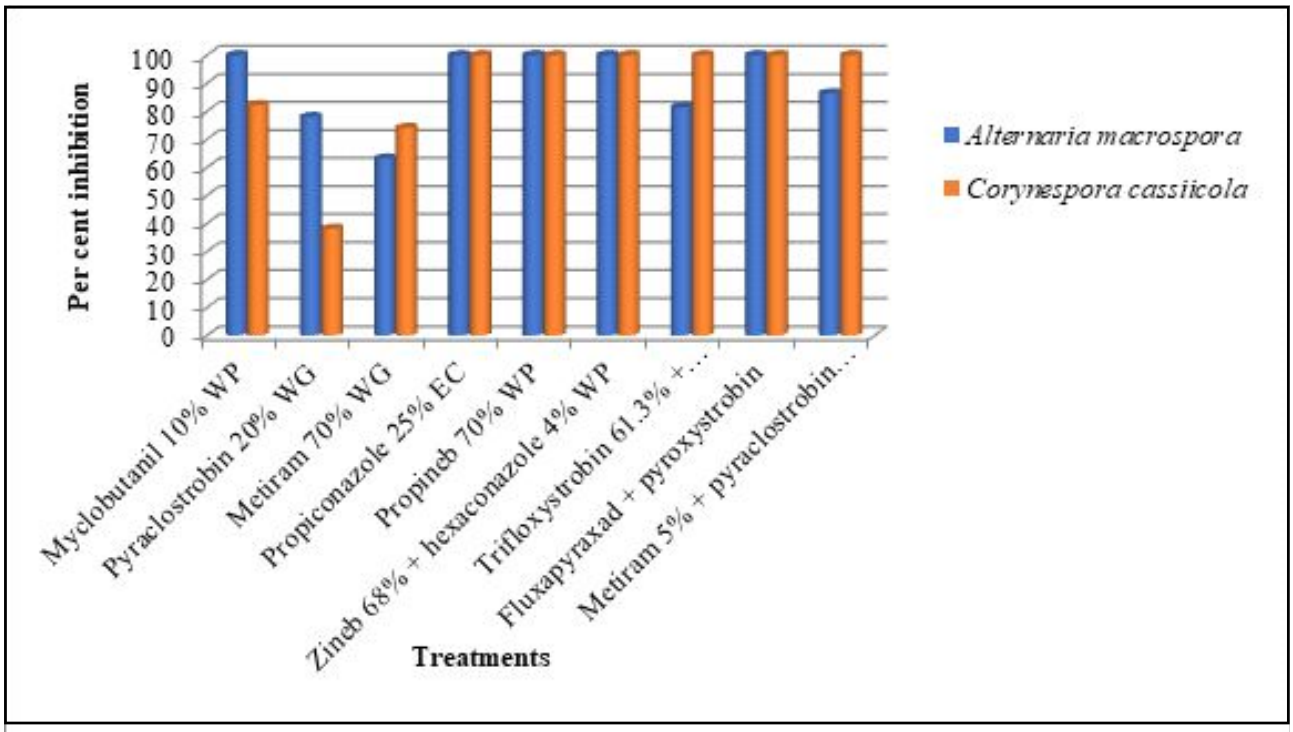
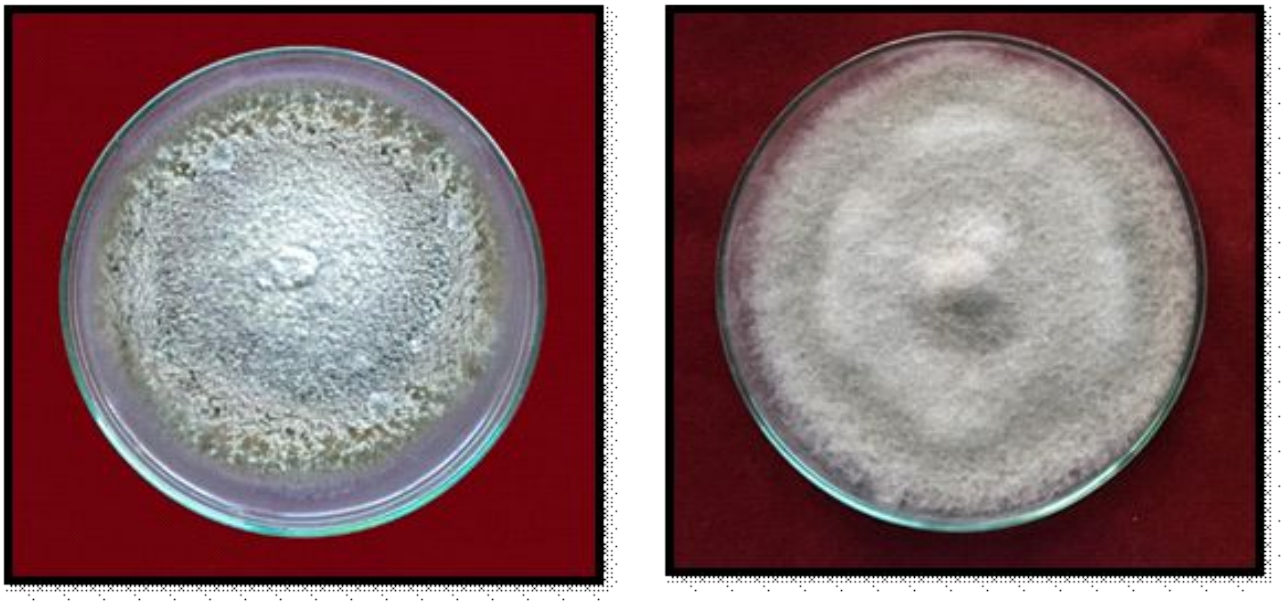
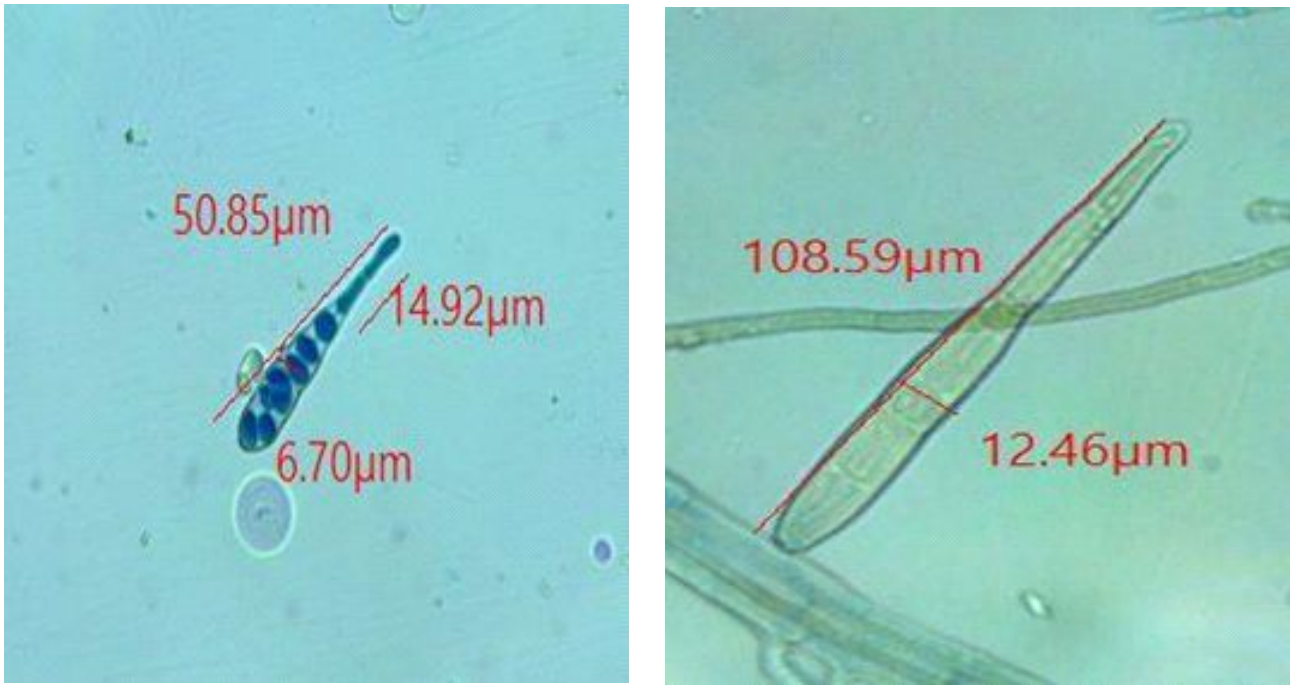


Fig 2. Effect of fungicides on per cent inhibition of *Alternaria macrospora* and *Corynespora cassiicola*



Alternaria macrospora *Corynespora cassiicola*

Plate 1. Pure cultures of fungal leaf spot pathogens of cotton



Alternaria macrospora *Corynespora cassiicola*

Plate 2. Morphological characters of fungal foliar pathogens of cotton

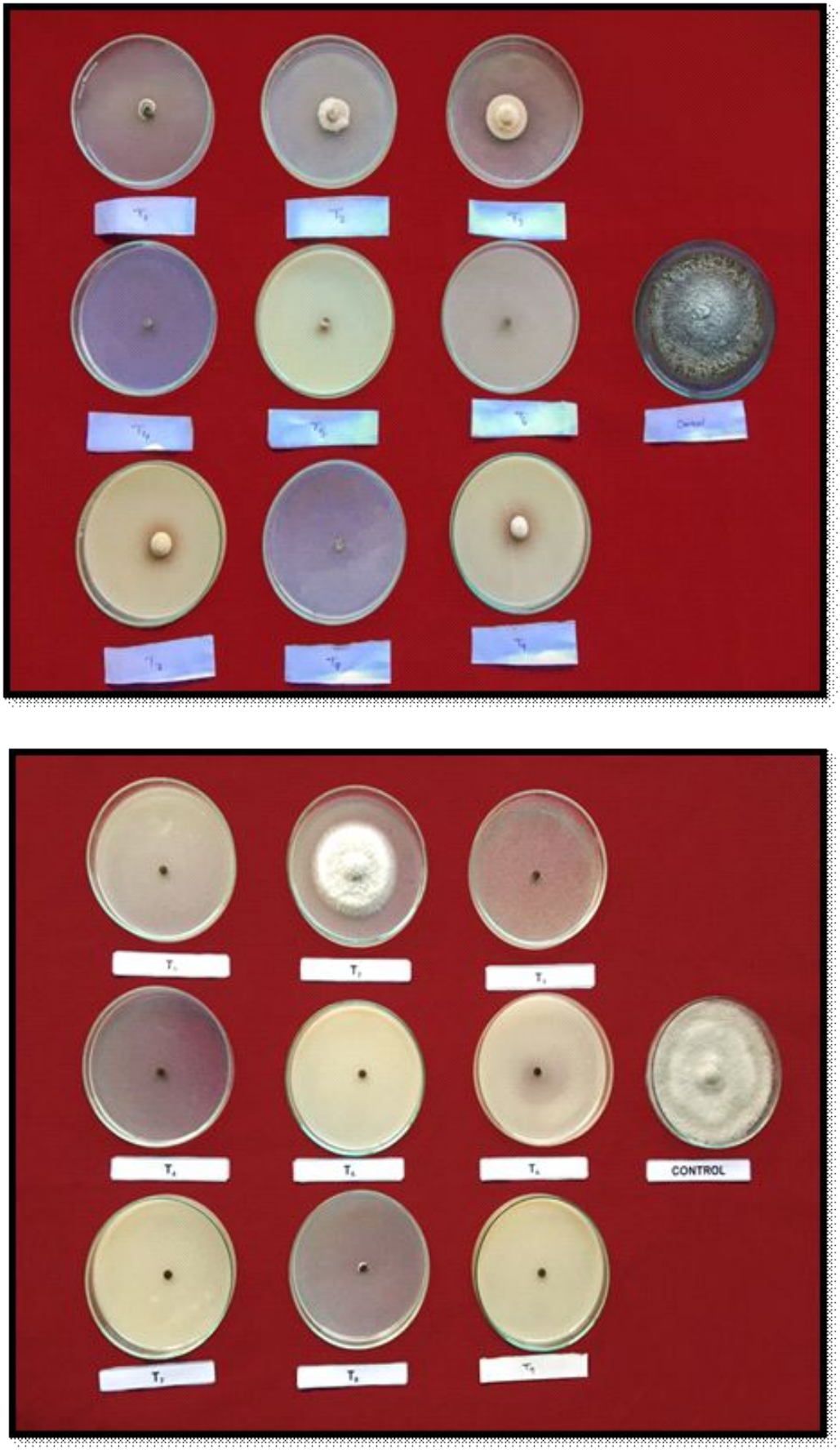


Plate 3. Efficacy of fungicides on mycelial growth of *Alternaria macrospora* and *Corynespora cassiicola*

Table 1. Efficacy of fungicides on mycelial growth of *Alternaria macrospora* and *Corynespora cassiicola*

Treatments	Common Name	Trade Name	Formulation	Conc. (%)	Mycelial growth (cm)*	
					Alternaria macrospora	*Corynespora cassiicola*
1	Myclobutanil	Index	10% WP	0.10%	0.00 (1.00)[a]	0.00 (1.00)[a]
2	Pyraclostrobin	Headline	20% WG	0.10%	1.73 (1.55)[b]	5.57 (2.36)[b]
3	Metiram	Polyram	70% WG	0.20%	2.90 (1.79)[b]	0.00 (1.00)[a]
4	Propiconazole	Tilt	25% EC	0.10%	0.00 (1.00)[a]	0.00 (1.00)[a]
5	Propineb	Antracol	70% WP	0.30%	0.00 (1.00)[a]	0.00 (1.00)[a]
6	Zineb + hexaconazole	Avatar	72% WP	0.20%	0.00 (1.00)[a]	0.00 (1.00)[a]
7	Trifloxystrobin + propineb	Flintpro	64.8% WG	0.30%	1.47 (1.50)[b]	0.00 (1.00)[a]
8	Fluxapyraxad + pyroxystrobin	Priaxor	50% SC	0.06%	0.00 (1.00)[a]	0.00 (1.00)[a]
9	Metiram + pyraclostobin	Carbrio Top	60% WG	0.30%	1.07 (1.39)[ab]	0.00 (1.00)[a]
10	Control	-	-	-	7.87 (2.96)[c]	9.00 (3.16)[c]

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

fungal growth. Pyraclostrobin blocks the mitochondrial electron transport and thus inhibits the fungus energy supply and results in the death of the target fungus.

CONCLUSION

In vitro evaluation of fungicides showed that propiconazole @ 0.1%, myclobutanil @ 0.1%, propineb @ 0.3%, zineb + hexaconazole @ 0.2% and fluxapyraxad + pyroxystrobin 0.06% completely inhibited the mycelial growth of *A. macrospora* and *C. cassiicola* with the highest inhibition (100%) over control.

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