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In Vitro Efficacy of Fungicides against Helminthosporium and Myrothecium Leaf Spot Pathogens on Cotton

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ABSTRACT

An investigation to test *in vitro* efficacy of nine different fungicides against *Helminthosporium gossypii* and *Myrothecium roridum* causing Helminthosporium and Myrothecium leaf spots, respectively, in Cotton was carried out at Regional Agricultural Research Station, Lam, during 2019-20. Among the different fungicides tested it was observed that propiconazole @ 0.1%, propineb @ 0.3%, zineb + hexaconazole @ 0.2% trifloxystrobin + propineb @ 0.3% and metiram+ pyraclostrobin @ 0.1% completely inhibited mycelial growth of *H. gossypii* and *M. roridum* and resulted in the highest inhibition (100%) over control.

Keywords: *Cotton, Fungicides, Helminthosporium leaf spot, In vitro evaluation and Myrothecium leaf spot.*

Cotton is referred as "King of Fibres" or as "White Gold". It is a member of Malvaceae family and most important fibre crop enjoying pre-eminent status among the cash crops in the country. In India, it occupies an area of 125.84 lakh ha with an annual production of 360 lakh bales of 170 kg and a productivity of 486 kg lint/ha. Andhra Pradesh stood 7th in area (5.86 lakh ha) but 6th in production (20.0 lakh bales) and 4th in productivity (584 kg/ha). (ICAR-AICRP (Cotton) Annual Report 2019-20). Fungal pathogens are of considerable importance, causing major diseases. Leaf blight (*Alternaria macrospora*) caused losses to the tune of 38.23% in cotton variety LRA 5166 (Bhattiprolu and Prasada Rao, 2009) and 33.43% in variety Jayadhar (Chattannavar *et al.*, 2010). Target spot (*Corynespora cassicola*) caused loss of 448kg lint/ha during 2013 (Hagan *et al.* 2015). Helminthosporium leaf spot (*Helminthosporium gossypii*) recorded 33.76% yield losses (Bhattiprolu, 2010) whereas 15-20% losses in seed cotton were reported due to Myrothecium leaf spot (*Myrothecium roridum*) (Umamaheswari *et al*., 2018). Grey mildew (*Ramularia areola*) and rust (*Phakopsora gossypii*) caused 38.38% and 34.05% losses in Bt cotton hybrids, respectively (Bhattiprolu, 2012, 2015).

Sporadic occurrence of Helminthosporium leaf spot in Dharwad district of Mysore state was reported by Rane and Patel (1956) and leaf spots appeared with purple margins. The culture of *H. gossypii* was initially light grey that turned to dark black, with velvety surface. The mycelium was fluffy, thick, dark brown, profusely branched and septate (Plate 1A). The conidia were curved, fusoid, obclavate, dark coloured, with 3-4 dark pseudosepta and were clearly bent producing germ tubes at polar ends measuring on an average about 55.76×13.0 µm borne on light brown to medium brown conidiophores that were often branched (Plate 2A).

Myrothecium leaf spot was first reported from Delhi (Munjal, 1960). Circular brown spots with sporodochia in spherical rings developed in Myrothecium leaf spot. The fungus in culture was white and fluffy with hyaline, septate mycelium showing typical zonations (Plate 1B). The conidia were cylindrical, one-celled with rounded ends, biguttulate, initially hyaline later turned to pale green, each oil globule being restricted at the tips. They were light to olive green in colour with deep coloured wall and measured 6.62 ìm length and the 2.91 ìm width (Plate 2B).

MATERIAL AND METHODS

The experiment was carried out in the laboratory, Department of Plant Pathology, Agricultural College Bapatla. Myrothecium and Helminthosporium 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 using Potato Dextrose Agar (PDA) for *H. gossypii* and Potato Sucrose Agar (PSA) for *M. roridum*.

The poisoned medium was equally distributed into three Petri plates, which were treated as three replications. Each plate was inoculated in the centre with a 5 mm discs from the periphery of actively growing colony (seven days old culture of *H. gossypii* and 16 days old culture of *M. roridum* with sterilized cork borer transferred to the centre of each plate containing poisoned medium. Control was maintained by placing fungal discs in plates containing untreated (not poisoned) medium. All the inoculated Petri plates were incubated at 28±2ºC in BOD incubator. The observation was recorded after fifteen days of inoculation. Per cent inhibition in the growth of the organism in different chemical treatments over the control was calculated. The percentage inhibition of radial growth 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

Poisoned food technique was employed for the evaluation of nine fungicides under *in vitro* conditions against *H. gossypii* and *M. roridum*. The results in Table 2 and 3 revealed that all the fungicides were capable of inhibiting the mycelial growth of test fungus at recommended dosage in comparison to control.

Efficacy of fungicides against Helminthosporium leaf spot

Per cent growth inhibition of *H. gossypii* by various fungicides tested ranged from 57.93 to 100% (Fig 2), after 7 DAI. Propiconazole @ 0.1%, myclobutanil @ 0.1%, propineb @ 0.3%, zineb + hexaconazole @ 0.2%, trifloxystrobin + propineb @ 0.3% and metiram + pyraclostrobin@ 0.3% completely inhibited the mycelial growth of *H. gossypii* (100%) and were significantly superior to other treatments (Table 2 and Fig 1). Metiram @ 0.2% and pyraclostrobin @ 0.1% were found to be less effective fungicides with radial growth of 3.83 (57.93%) and 3.07 cm (65.93%) respectively (Plate 3).

The present results are in accordance with Dighule *et.al*. (2011), who reported that

Table 1. List of fungicides used in Poisoned food technique *in vitro*

Treatments	Common Name	Trade Name	Formulation	Conc. (%)
1	Myclobutanil	Index	10% WP	0.10%
2	Pyraclostrobin	Headline	20% WG	0.10%
3	Metiram	Polyram	70% WG	0.20%
4	Propiconazole	Tilt	25% EC	0.10%
5	Propineb	Antracol	70% WP	0.30%
6	Zineb + hexaconazole	Avatar	72% WP	0.20%
7	Trifloxystrobin + propineb	Flintpro	64.8% WG	0.30%
8	Pyroxystrobin + fluxapyraxad	Priaxor	50% SC	0.06%
9	Metiram + pyraclostobin	Carbrio Top	60% WG	0.30%
10	Control	-	-	-

Table 2. Efficacy of fungicides on mycelial growth of *Helminthosporium gossypii*

Treatments	Common name	Conc. (%)	Mycelial growth (cm)*	Inhibition Percentage (%)*
1	Myclobutanil 10% WP	0.10%	0.00 (1.00)[a]	100.00 (89.96)
2	Pyraclostrobin 20% WG	0.10%	3.07 (1.90)[c]	65.93 (56.05)
3	Metiram 70% WG	0.20%	3.83 (2.01)[b]	57.93 (47.49)
4	Propiconazole 25% EC	0.10%	0.00 (1.00)[a]	100.00 (89.96)
5	Propineb 70% WP	0.30%	0.00 (1.00)[a]	100.00 (89.96)
6	Zineb 68% + hexaconazole 4% WP	0.20%	0.00 (1.00)[a]	100.00 (89.96)
7	Trifloxystrobin 61.3% + propineb 3.5% WG	0.30%	0.00 (1.00)[a]	100.00 (89.96)
8	Pyroxystrobin 25% + fluxapyraxad 25% SC	0.06%	0.60 (1.26)[b]	93.33 (76.06)
9	Metiram 5% + pyraclostrobin 55% WG	0.30%	0.00 (1.00)[a]	100.00 (89.96)
10	Control	-	9.00 (3.16)[d]	-
	SEm (±)		0.15	-
	CD (P≤ 0.05)		0.43	
	CV (%)		1.02	

*Mean of the three replications; Treatment means with same alphabet do not differ significantly

Figures in the parenthesis in mycelial growth and inhibition percentage are square root transformed values and arcsin transformed values, respectively

Table 2. Efficacy of fungicides on mycelial growth of *Myrothecium roridum*

Treatments	Common Name	Conc. (%)	Mycelial growth (cm)*	Inhibition Percentage (%)*
1	Myclobutanil 10% WP	0.10%	2.13 (1.63)[b]	73.33 (63.55)
2	Pyraclostrobin 20% WG	0.10%	0.00 (1.00)[a]	100.00 (89.96)
3	Metiram 70% WG	0.20%	1.67 (1.56)[b]	79.17 (65.32)
4	Propiconazole 25% EC	0.10%	0.00 (1.00)[a]	100.00 (89.96)
5	Propineb 70% WP	0.30%	0.00 (1.00)[a]	100.00 (89.96)
6	Zineb 68% + hexaconazole 4% WP	0.20%	0.00 (1.00)[a]	100.00 (89.96)
7	Trifloxystrobin 61.3% + propineb 3.5% WG	0.30%	0.00 (1.00)[a]	100.00 (89.96)
8	Pyroxystrobin 25% + fluxapyraxad 25% SC	0.06%	0.00 (1.00)[a]	100.00 (89.96)
9	Metiram 5% + pyraclostrobin 55% WG	0.30%	0.00 (1.00)[a]	100.00 (89.96)
10	Control	-	8.00 (2.85)[c]	-
	SEm (±)		0.16	-
	CD (P≤ 0.05)		0.48	-
	CV (%)		1.26	-

*Mean of the three replications; Treatment means with same alphabet do not differ significantly
Figures in the parenthesis in mycelial growth and inhibition percentage are square root transformed values and arcsin transformed values, respectively

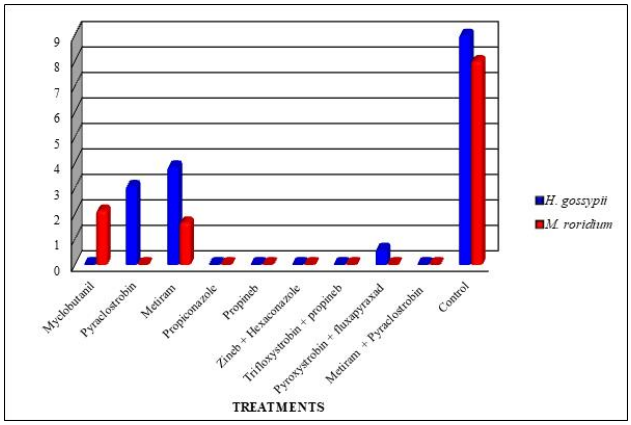

Fig 1. Effect of fungicides on mycelia growth of *Helminthosporium gossypii* and *Myrothecium roridum*

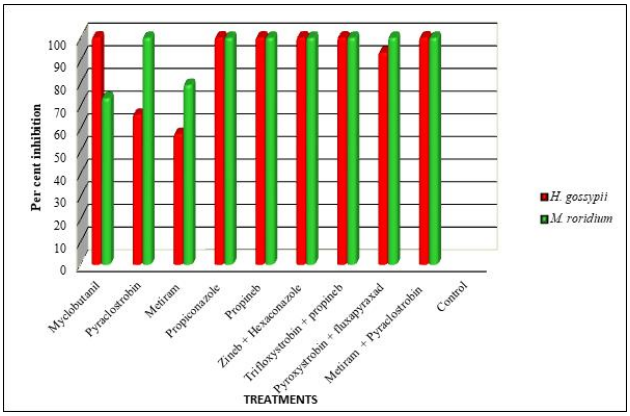

Fig 2. Effect of fungicides on per cent inhibition of *Helminthosporium gossypii* and

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

A= *Helminthosporium gossypii* **B=*Myrothecium roridum***

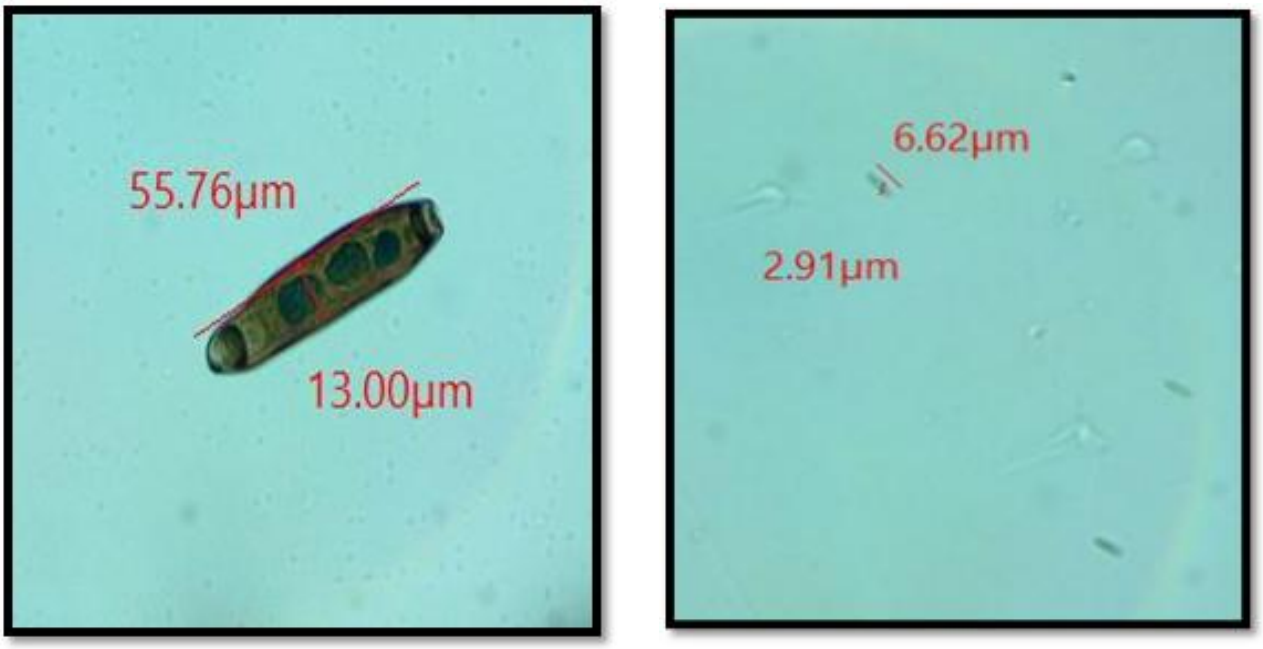

Plate 2. Morphological characters of isolated fungal foliar pathogens of cotton

A= *Helminthosporium gossypii* **B=*Myrothecium roridum***

Plate 3. Efficacy of fungicides on mycelial growth of *Helminthosporium gossypii*

Plate 4. Efficacy of fungicides on mycelial growth of *Myrothecium roridum*

propiconazole @ 0.1% and mancozeb @ 0.3% to be effective against *H. gossypii in vitro*.

Efficacy of fungicides against Myrothecium leaf spot

Per cent growth inhibition of *M. roridum* by various fungicides tested ranged from 73.33 to 100% (Fig 2), after 15 DAI. Propiconazole @ 0.1%, pyraclostrobin @ 0.1%, propineb @ 0.3%, zineb + hexaconazole @ 0.2%, trifloxystrobin + propineb @ 0.3%, pyroxystrobin + fluxapyraxad 0.06% (100%) and metiram + pyraclostrobin @ 0.1% completely inhibited the growth of *M. roridum* (100%) and were significantly superior to other treatments (Plate 4). Myclobutanil @ 0.1% and metiram @ 0.2% significantly reduced the radial growth with 73.33% (2.13 cm) and 79.17% (1.67 cm) reduction over control, respectively (Table 3 and Fig 1).

Similar results were obtained by Tomar and Shastry (2006) who reported propiconazole @ 0.1% as the most effective fungicide with 100% inhibition over control against *M. roridum*. Minimum radial growth, minimum disease incidence and maximum seed cotton yield were recorded with thiophanate methyl and propiconazole treatments against Myrothecium leaf blight both *in vitro* and *in vivo* (Mourya *et al.*, 2009). Complete inhibition of mycelial growth was recorded in hexaconazole 4% + zineb 68% WP by 100% (Umamaheswari *et al.*, 2018).

CONCLUSION

The present study on evaluation of fungicides against *H. gossypii* and *M. roridum* under *in vitro* conditions showed that propiconazole @ 0.1%, propineb @ 0.3%, zineb + hexaconazole @ 0.2%, trifloxystrobin + propineb @ 0.3% and metiram+ pyraclostrobin @ 0.1% completely inhibited mycelial growth of *H. gossypii* and *M. roridum* with the highest inhibition (100%) over control.

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