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In Vitro Efficacy of Fungicides against Alternaria and Corynespora Leaf Spot Pathogens Infecting Cotton

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ABSTRACT

An experiment was conducted to test *in vitro* efficacy of eleven different fungicides against *Alternaria alternata* and *Corynespora cassiicola* causing Alternaria and Corynespora leaf spots, respectively, in Cotton, at Department of Plant Pathology, Agricultural College, Bapatla, during 2020-21. Among the eleven fungicides tested *in vitro*, it was observed that hexaconazole @ 0.2% showed complete inhibition of mycelial growth of *A. alternata* and *C. cassiicola* followed by propiconazole @ 0.1% (100 and 99.75%, respectively), metiram + pyraclostrobin @ 0.3% (98.67 and 98.61%, respectively), pyroxystrobin + fluxapyraxad @ 0.06% (98.67 and 97.50%, respectively).

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

Cotton (*Gossypium* spp.), is one of the most important fibre and cash crop in our country and worldwide. It belongs to the family Malvaceae, and generally referred as "White Gold" or as "King of Fibres". Cotton production is one of the key factor in the economy of many developing countries in Asia, Africa and America. In addition to the textile industry, cotton is also used in manufacturing of fishing nets, paper industry and production of mattresses, automobile cushions, furniture, pharmaceuticals, ropes, cords etc. In India, it occupies premier position with 70% share in the textile industry. In India, cotton occupies an area of 129.57 lak ha with an annual production of 371 lakh bales of 170 kg and a productivity of 487 kg lint/ha. In Andhra Pradesh, it occupies an area of 5.24 lakh ha with an annual production of 18 lakh bales productivity of 584 kg lint ha^{-1} (ICAR-AICRP on Cotton, Annual Report, 2020-21). Among the foliar diseases, *Alternaria* blight (*Alternaria macrospora*) was reported to cause yield loss of 26% (Sandipan *et al.,* 2017). C*orynespora cassicola* was reported to cause leaf spot on cotton from different parts of the world (Jones, 1961; Fulmer *et al.,* 2012 and Price *et al.,* 2015). In India, it was first reported from the Junagadh district of Gujarat in cotton Hybrid-4 and Hybrid-6 during 1984-1985 (Parakhia *et al.,* 1989) and yield loss to the time of 100 to 200 lb per acre of lint was reported Hagan and Sikora, 2012. Pratyusha *et al.* (2021) found thatAlternaria was being reported as internally as well as externally seed borne. Seed treatment with fungicides may protect the seeds from seed borne infections and improve seed germination. Up to 2015-2016, Alternaria was major disease but due to favourable conditions for *Corynespora* damage has surpassed *Alternaria* damage. Both the leaf spot diseases are causing maximum losses in cotton. Under favourable conditions, losses caused by Alternaria leaf spot range from 26.59% to 38.23% (Monga *et al*., 2013; Bhattiprolu and Prasadrao, 2009). The fungal

colony was initially white in colour on PDA that later turned to grey colour. The fungal colony was woolly with a round to irregular margin with moderately slow growth. The mycelium was dark brown and septate with muriform dark brown conidia and septate conidiophores. The conidia, on an average, measured 31.78×13.12 µm with 4-5 transverse septa and 1-2 longitudinal septa with short beak.

MATERIALS AND METHODS

The experiment was carried out in the laboratory, Department of Plant Pathology, Agricultural College Bapatla. Alternaria and Corynespora infected cotton leaf samples were collected from Regional Agricultural Research Station, Lam during *kharif* 2020-21.The affected portion of the leaves were cut into small pieces and surface sterilized with 0.1N sodium hypochlorite (NaOCl) solution for 30 seconds and then washed properly with sterile water for four times and transferred to the Petri plates (4bits per Petri plate) containing Potato Dextrose Agar (PDA).

Eleven fungicides, as detailed in Table 1, were evaluated at recommended doses *in vitro* against foliar fungal pathogens (*A. alternata* and *C. cassiicola*) using poisoned food technique (Nene and Thapliyal, 1993). 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 disc cut from the periphery of actively growing colony (seven days old culture of *A. alternata* and *C. cassiicola* with sterilized cork) 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. Radial growth of the fungus was recorded daily in the control plate starting from 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).

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

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

The results revealed that all the fungicides were capable of inhibiting the mycelia growth of test fungus at recommended dosage in comparison to control (Table 2).

Efficacy of Fungicides on Radial Growth of *Alternaria alternata*:

All the test fungicides significantly reduced the radial growth of *A. alternata* compared to the control (7.50 cm) (Table 2 and Fig. 1). Per cent inhibition varied from 35.47% to 100% in different treatments. Hexaconazole @ 0.2% and propiconazole @ 0.1% completely inhibited the mycelial growth of the pathogen and were significantly superior to other treatments.Fluxapyroxad+ pyraclostrobin @ 0.06% and metiram + pyraclostrobin @ 0.3% were significantly on par (0.1cm) with 98.67% inhibition (Table 2; Fig. 2; Plate 1)

Myclobutanil 10% WP registered 94.40% inhibition followed by captan + hexaconazole @ 0.1% and hexaconazole + zineb @ 0.2% with 93.33% inhibition each. Among the fungicides tested, chlorothalonil @ 0.2%, mancozeb @ 0.3% and propineb @ 0.3% were found to show inhibition that ranged below 50%.

The present results were in accordance with various research works across the world while working with different fungicides. Yamuna *et al.*

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

Treatments	Common Name	Trade Name	Formulation	Conc. (%)
1	Hexaconazole	Contaf	5% SC	0.20%
2	Myclobutanil	Index	10% WP	0.10%
3	Propiconazole	Tilt	25% EC	0.10%
4	Propineb	Antracol	70% WP	0.30%
5	Captan + Hexaconazole	Taqat	75% WP	0.10%
6	Hexaconazole + Zineb	Avatar	72% WP	0.20%
7	Fluxapyroxad + Pyraclostrobin	Priaxor	50% SC	0.06%
8	Mancozeb	Indofil M-45	75% WP	0.30%
9	Metiram + Pyraclostrobin	Carbrio Top	60% WG	0.30%
10	Chlorothalonil	Kavach	75% WP	0.20%
11	Copper Oxy Chloride	Blitox 50 W	50% WP	0.30%
12	Control	-	-	-

(2020) observed hexaconazole @ 0.2% and propiconazole @ 0.1% showed 100 per cent inhibition against *A. macrospora*. Bodhke *et al.* (2019) stated that propiconazole @ 0.1% and hexaconazole @ 0.1% resulted in 100 per cent inhibition against *A. macrospora*. Poonam Kumari *et al.* (2020) reported metiram + pyraclostrobin @ 0.3% was significantly superior in inhibiting the growth of *A. alternata* over the individual chemicals and control under *in vitro* condition. Copper oxy chloride @ 2000 ppm was recorded with 6.46 mm radial growth and 92.8 per cent inhibition under *in vitro* trails (Pranaya *et al.*, 2020).

Efficacy of Fungicides on Radial Growth of *Corynespora cassicola*: At 12 DAI, all the test fungicides (Table 1) significantly reduced radial growth of *C. cassiicola* in comparison to the control (7.20 cm) (Table 2 and Fig. 1). Hexaconazole @ 0.2% completely inhibited the mycelia growth of the pathogen with maximum inhibition (100%) followed by propiconazole @ 0.1% (99.72%) and metiram + pyraclostrobin@ 0.3% (98.61%), fluxapyroxad + pyraclostrobin @ 0.06%(97.50%) (Plate 2).

Chlorothalonil @ 0.2% was found to be least effective fungicide with maximum radial growth of 6.12 cm and 15.00% inhibition. Copper oxy chloride @ 0.3% (0.48cm) and myclobutanil @ 0.1% (0.54cm) were on par with 93.33% and 92.50% inhibition, respectively. Captan +hexaconazole @ 0.1% (1.14cm) registered 84.17% inhibition whereas hexaconazole + zineb @ 0.2% (3.78cm), mancozeb 75% WP (4.52cm) and propineb @ 0.3% (5.34cm) recorded 47.50, 37.22 and25.83% inhibition, respectively (Table 2 and Fig. 2).

Arvind *et al*. (2017) reported that hexaconazole had completely inhibited the growth of the *C. cassiicola* compared to control at both 50 and 100 ppm concentrations. Yamuna *et al.* (2020) noticed mancozeb @ 0.3% significantly reduce radial growth of *C. cassiicola* mycelium and reported 75.60% reduction over control. Ishwari *et al.* (2020) observed that propiconazole inhibited radial growth of *C. cassiicola* with 94.44% inhibition against pathogen over control under *in vitro* conditions. Similar results were obtained by Mushrif *et al.* (2020) where metiram + pyraclostrobin @ 0.2% completely inhibited the radial growth of mycelium against *C. cassiicola* over control.

Table 2. *In vitro* efficacy of fungicides on mycelia growth of *Alternaria* and *Corynespora* leaf spot pathogens in cotton (12 DAI)

T.No	TREATMENTS	CONC (%)	Mycelial Growth		Inhibition over control	
			A. alternata	*C. cassiicola*	*A. alternata*	*C. cassiicola*
1	Hexaconazole 5% EC	0.20%	0.00 (1.00)^{a}	0.00 (1.00)^{a}	100	100
2	Myclobutanil 10% WP	0.10%	0.42 (1.19)^{e}	0.54 (1.24)^{ef}	94.4	92.5
3	Propiconazole 25% EC	0.10%	0.00 (1.00)^{a}	0.02 (1.01)^{ab}	100	99.72
4	Propineb70% WP	0.30%	4.84 (2.42)^{jk}	5.34 (2.52)^{j}	35.47	25.83
5	Captan 70% + Hexaconazole 5% WP	0.10%	0.50 (1.22)^{ef}	1.14 (1.46)^{g}	93.33	84.17
6	Hexaconazole 4% + Zineb 68% WP	0.20%	0.50 (1.22)^{efg}	3.78 (2.19)^{h}	93.33	47.5
7	Fluxapyroxad 167 g/l + Pyraclostrobin 333 g/l SC	0.06%	0.10 (1.04)^{abc}	0.18 (1.08)^{cd}	98.67	97.5
8	Mancozeb 75% WP	0.30%	4.82 (2.41)^{j}	4.52 (2.35)^{i}	35.73	37.22
9	Metiram 55% + Pyraclostrobin 5% WG	0.30%	0.10 (1.04)^{abcd}	0.10 (1.04)^{abc}	98.67	98.61
10	Chlorothalonil 75% WP	0.20%	3.92 (2.22)^{i}	6.12 (2.67)^{k}	47.73	15
11	Copper oxy chloride 50 % WP	0.30%	0.80 (1.34)^{h}	0.48 (1.22)^{e}	89.33	93.33
12	Control		7.50 (2.92)^{l}	7.20 (2.86)^{l}	0	0
	SEm (±)		0.02	0.02		
	CD (P≤ 0.05)		0.06	0.06		
	CV (%)		2.78	2.89		

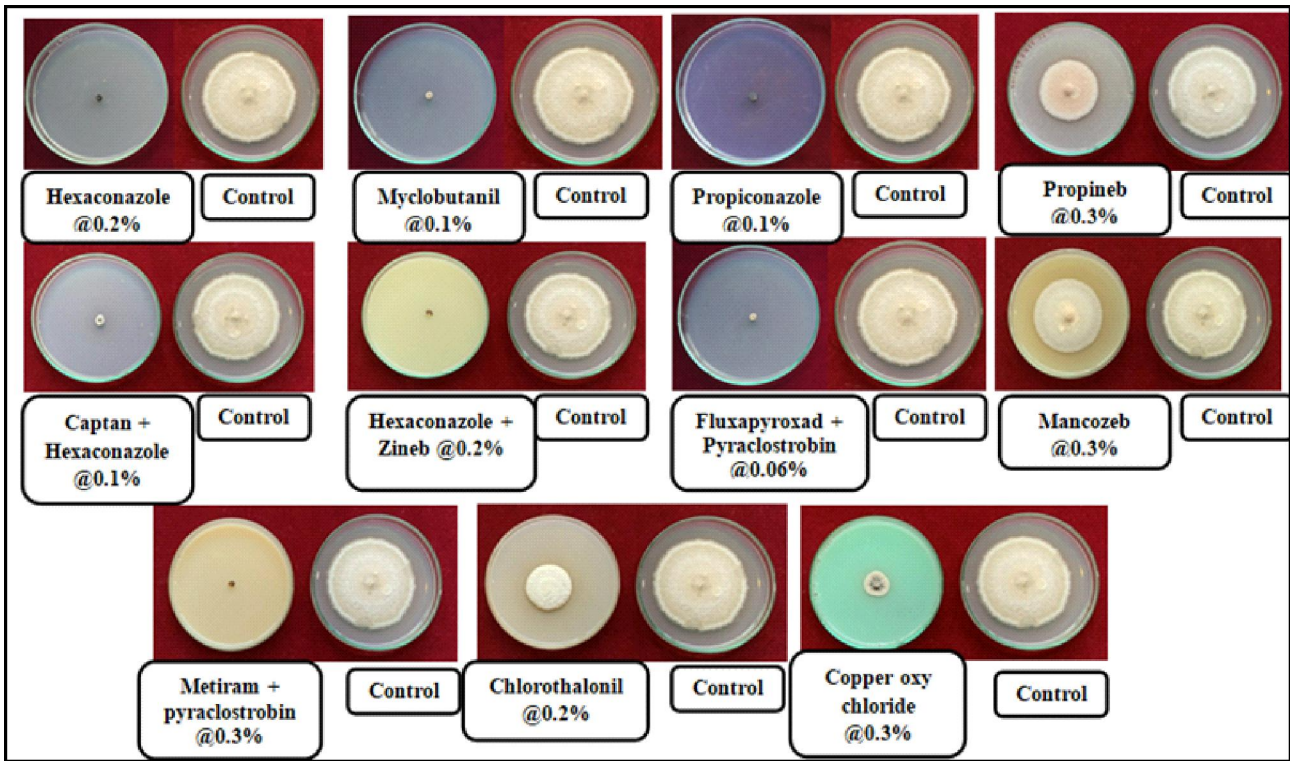


Plate 1. Efficacy of fungicides on mycelial growth of *Alternaria alternata*

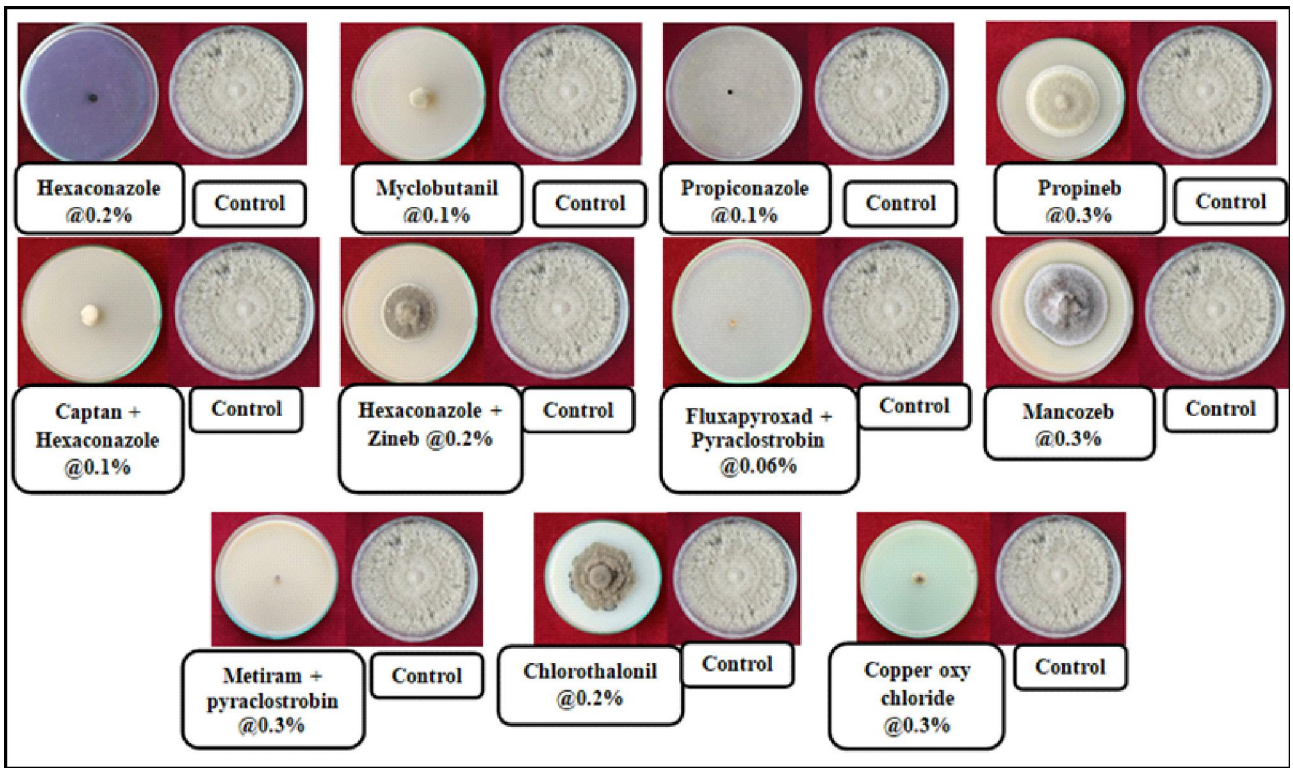


Plate 2. Efficacy of fungicides on mycelial growth of *Corynespora cassiicola* at 12 DAI

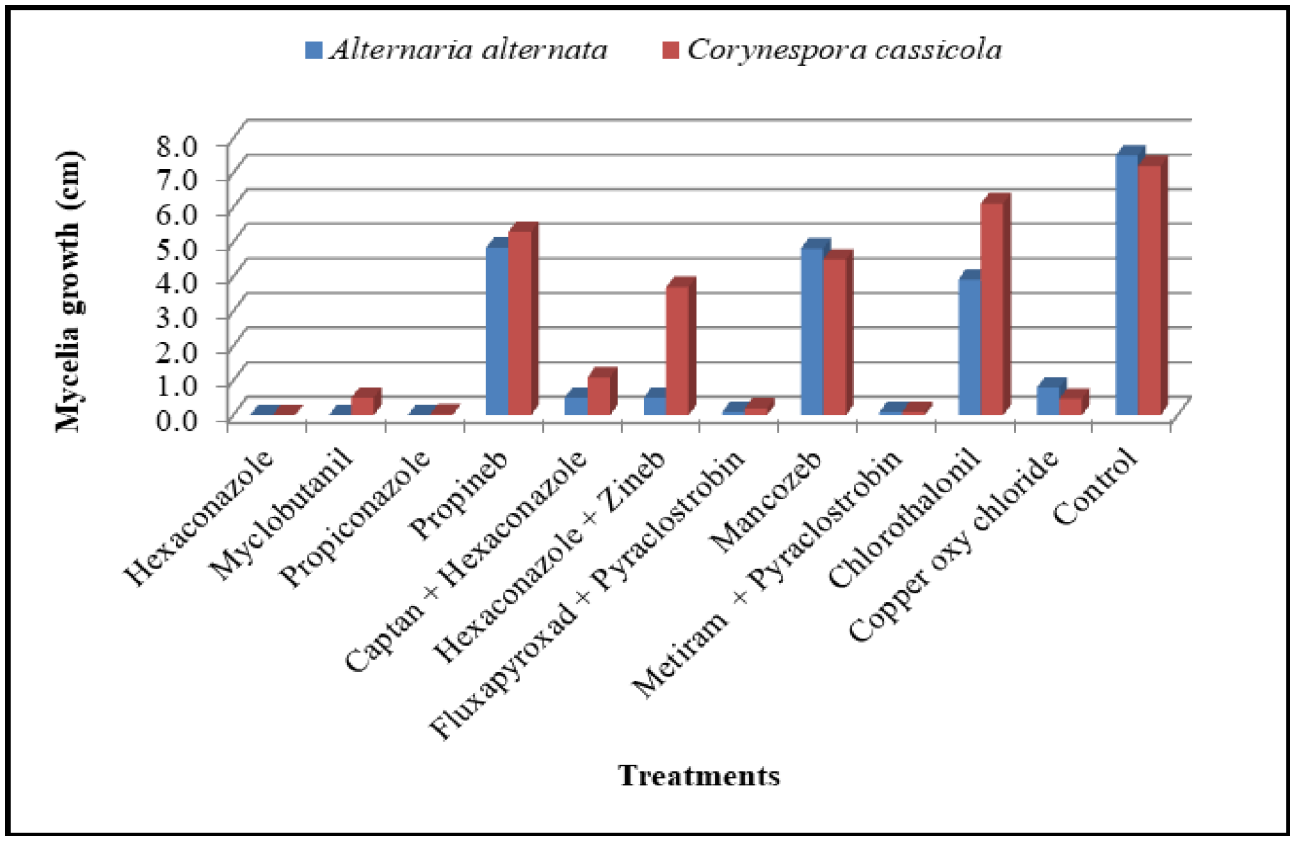


Fig. 1. Efficacy of fungicides on mycelial growth of Alternaria and Corynespora leaf spot pathogens in Cotton

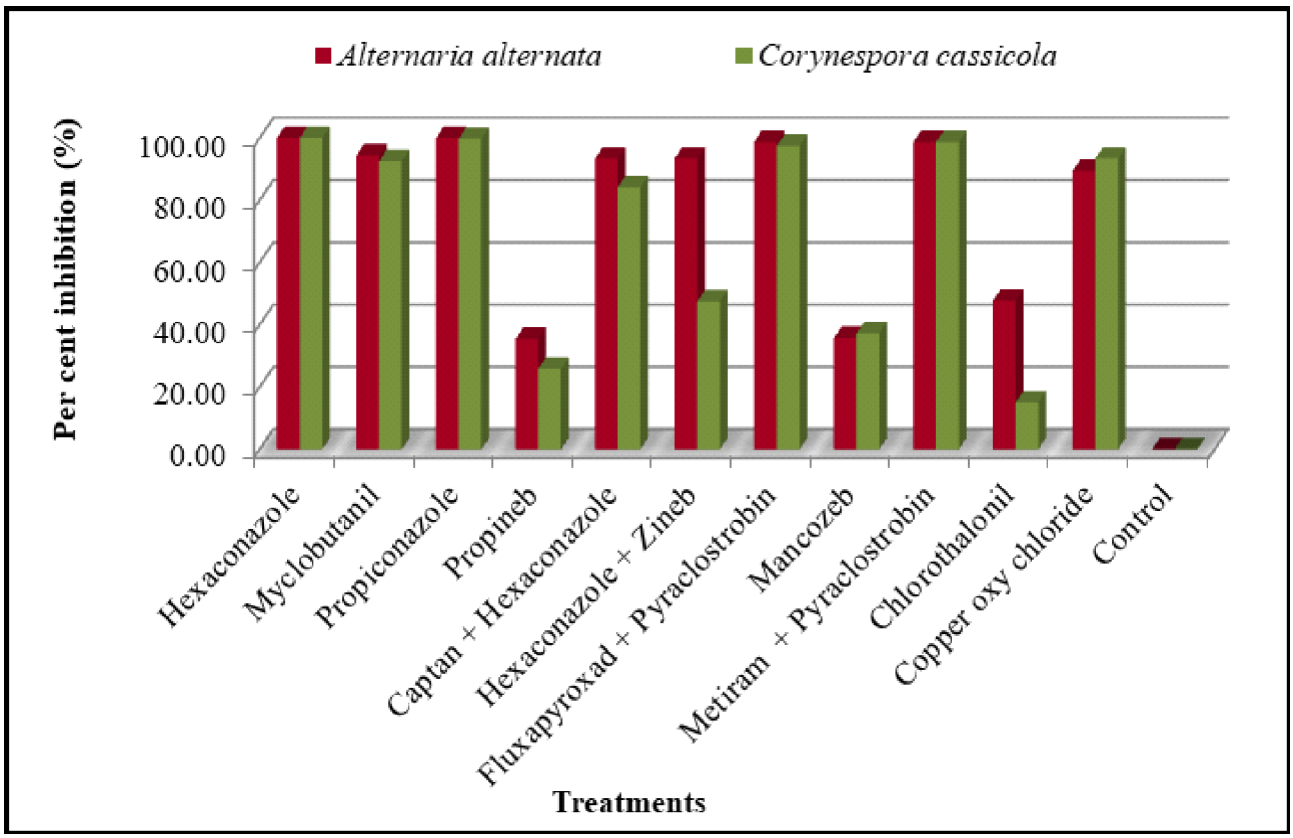
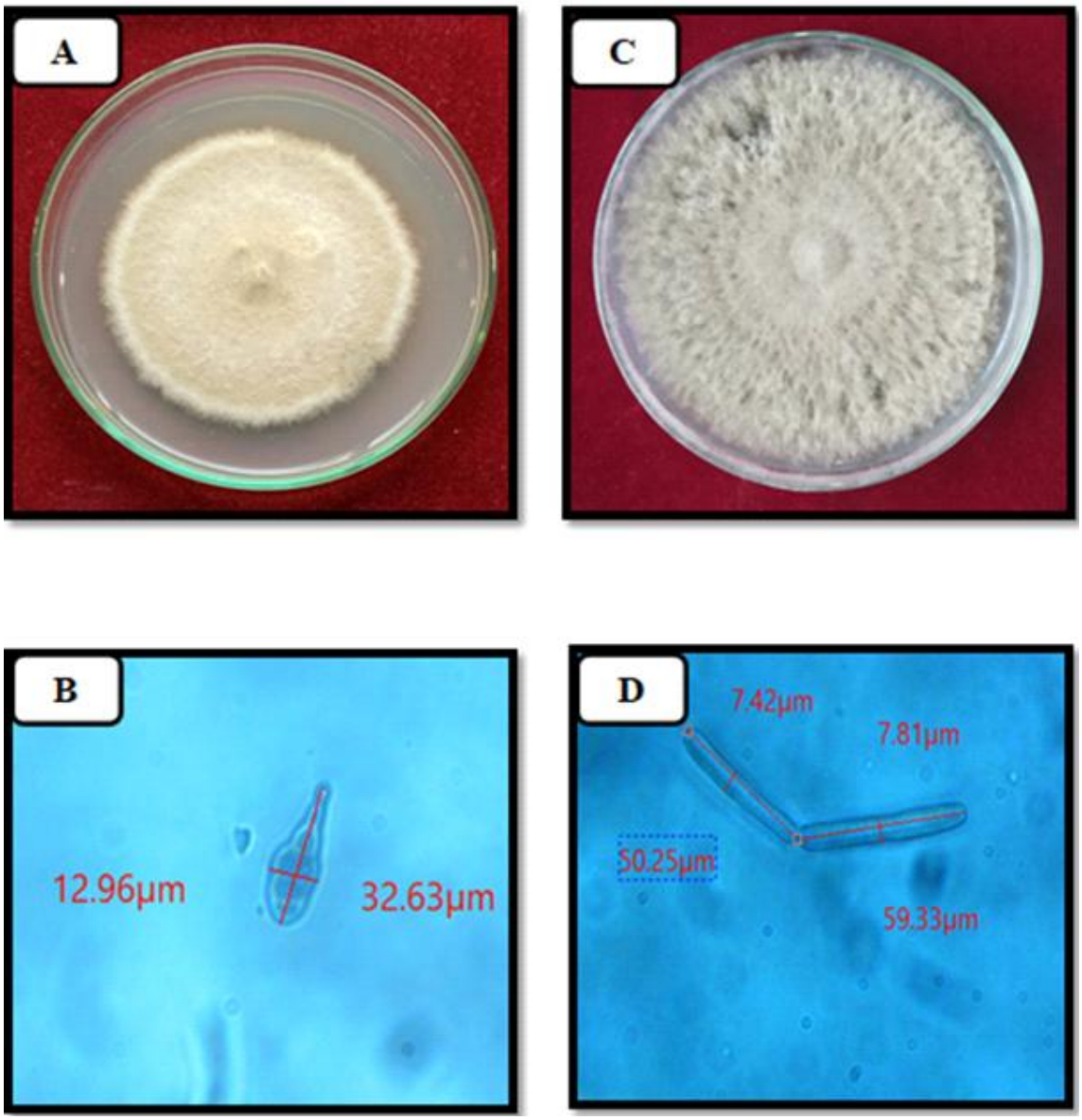


Fig. 2. *In vitro* efficacy of fungicides against Alternaria and Corynespora pathogens of cotton



A = *Alternaria alternata* **B = *Alternaria alternata***

C = *Corynespora cassiicola* **D = *Corynespora cassiicola***

Plate 3. A- Pure culture of *Alternaria alternata*;
B- Conidial characters of *Alternaria alternata*
C- Pure culture of *Corynespora cassiicola* (400X);
D- Conidial characters of *Corynespora cassiicola* (400 X)

CONCLUSION

In vitro evaluation of fungicides revealed that hexaconazole @ 0.2% (100%) completely inhibited the mycelial growth of both the pathogens, *A. alternata* and *C. cassiicola* followed by propiconazole @ 0.1% (100 and 99.75%, respectively), metiram + pyraclostrobin @ 0.3% (98.67 and 98.61%, respectively), fluxapyroxad + pyraclostrobin @ 0.06% (98.67 and 97.50%, respectively).

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