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Management of anthracnose disease of cotton (*Gossypium* spp.) incited by *Colletotrichum gossypii* using fungicides

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ABSTRACT

Anthracnose, caused by *Colletotrichum gossypii*, poses a significant increasing hazard to cotton (*Gossypium* spp.) crops. The present study was carried out during rainy (*Kharif*) seasons of 2020 and 2021 at Cotton Research Station, Sirsa, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana aimed to assess the efficiency of novel fungicides over *in vitro* as well as *in vivo* condition against *C. gossypii*, the causative agent of anthracnose disease in cotton. This experiment was carried on *Bt* (RCH 773) and non *Bt* (H1098i) cotton susceptible cultivars in randomized block design (RBD) with four replications for each treatment.. The highest growth inhibition was observed when using the fungicide Azoxystrobin 18.2% + Difenoconazole 11.4% SC (90.48%), followed by Metiram 55% + Pyraclostrobin 5% WG (84.57%). Minimum disease intensity was observed with the fungicide Azoxystrobin 18.2% + Difenoconazole 11.4% SC in cotton cultivars i.e. *Bt* (7.01%) and non *Bt* (8.20%) during the years of evaluation with the maximum seed cotton yield of 2590 kg/ha as well as 2105 kg/ha, respectively. The results of this experiment reported that all the fungicides tested have great potential in managing anthracnose disease of cotton and recorded fruitful results as compared to untreated check.

Keywords: Anthracnose, *Colletotrichum gossypii*, Cotton, Fungicide, Seed cotton yield

Cotton (*Gossypium* spp.) is one of the most ancient and essential fibre crop after food grains, serving as a globally significant industrial commodity and extensively cultivated cash crop in tropical and subtropical regions worldwide. Cotton is globally recognised as 'White Gold' and 'King of Fibres,' holding significant importance as a fundamental raw material for textile industries. It is a member of the Malvaceae family, classified within the genus *Gossypium*. The major cotton growing states of India are Gujrat, Maharashtra, Telangana, Andhra Pradesh, Madhya Pradesh, Karnataka, Haryana, Rajasthan, Punjab and Odisha (Yadav *et al.* 2025). The overall area dedicated to cotton cultivation in India being 130.61 lakh hectares, yielding a total yield of 343.47 lakh bale. In Haryana, cotton is grown over an area of about 6.83 lakh hectares with total production of 14.50 lakh bale (Anonymous 2024). Regardless of the promising yield potential of cotton, various abiotic and biotic factors adversely affect the lint quality and seed cotton yield. In India, several fungal, bacterial, viral and nematode diseases were reported to affect the cotton crop from germination to maturity stage (Tanweer 2013). In India, various foliar diseases are responsible for causing 20–30% yield losses (Mayee and Mukewar 2007). The fungus *Colletotrichum* is cosmopolitan in nature which includes over 900 species, and it infects various crop plants. It was also proved that anthracnose of cotton was caused by *Glomerella gossypii* (Barre 1909, Edgerton 1912, Cauquil 1960). The anthracnose infection of cotton, induced by *Colletotrichum gossypii*, is highly detrimental, wide spread disease throughout the country and gaining importance from economic point of view. All the plant parts including stems, leaves and fruits are affected and grayish brown spots with acervuli in concentric rings can be observed frequently on infected tissues (Saxena *et al.* 2016). Kiran *et al.* (2020) also observed that dark and sunken necrotic spots are symptoms of anthracnose disease. Anthracnose disease is of immense importance as it causes both, direct and indirect losses to quantity and quality of final produce. The *Colletotrichum gossypii* fungus in association with other pathogens i.e. *Colletotrichum indicum*, *Diplodia gossypina* and *Phytophthora* spp. are responsible for causing boll rot which leads up to 21% bolls destruction (Lagiere 1970).

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With the passage of time, it becomes serious obstruction for cotton production and yield losses up to 48.4% were also reported (Yadav *et al.* 2024). This disease is also responsible for deteriorating the lint quality and seed germination. The present study was conducted to assess the effectiveness of several fungicides under both *in vitro* and *in vivo* settings for the management of anthracnose disease in cotton.

MATERIALS AND METHODS

Source of pathogen and isolation: The present study was carried out during rainy (*Kharif*) seasons of 2020 and 2021 at Cotton Research Station, Sirsa, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. The isolation of *Colletotrichum gossypii* and pure culture was obtained through hyphal tip method. The fungus had been recognised as *C. gossypii* based on its cultural and physical traits, and molecular identification was done by using ITS and TUB region primers as well as species specific SNP primer pair for further confirmation. The pure culture was preserved on PDA slants at 5 ± 1°C in a refrigerator for subsequent experiments.

In vitro *assessment of fungicides*: The efficacy of seven fungicides (Kresoxim methyl 44.3% SC, Pyraclostrobin 20 WG, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Metiram 55% + Pyraclostrobin 5% WG, Copper oxychloride 50 WP, Propiconazole 25% EC, and Carbendazim 50% WP) at concentrations of 100, 500, and 1000 ppm was assessed against *C. gossypii* utilising the poisoned food technique. Sterilised PDA, modified with fungicide at the specified concentration, was dispensed into sterilised Petri plates in aseptic conditions and permitted to harden, with three replications of each treatment alongside an untreated control. Each Petri dish was injected with a 5 mm diameter fungal mycelial disc from an actively growing pure culture of *C. gossypii.* Mycelial growth was observed at 90 mm in control plates maintained at 25 ± 2°C, and the percentage of growth inhibition was determined using the formula provided by Vincent (1927):

$$\text{Growth inhibition (\%)} = \frac{\text{Fungal growth in control} - \text{Fungal growth in treatment}}{\text{Fungal growth in control}} \times 100$$

Evaluation of fungicides against anthracnose disease of cotton under field conditions: This experiment was carried on *Bt* (RCH 773) and non *Bt* (H1098i) cotton susceptible cultivars in randomized block design (RBD) with four replications for each treatment. The foliar sprays of four fungicides (Kresoxim methyl 44.3% SC @0.1%, Azoxystrobin 18.2% + Difenoconazole11.4% SC @0.1%, Copper oxychloride 50 WP @0.25% and Carbendazim 50% WP @0.1% concentration) twice, first at 75 days after sowing (DAS) and 90 DAS were done. These selected four fungicides are recommended in cotton crop by Central Insecticides Board and Registration Committee (CIB & RC). Disease reactions were recorded by using 0–9 scale (Table 1) and the per cent disease incidence and per cent disease intensity were calculated by formulae given by Mayee and Datar (1986):

$$\text{Per cent Disease Incidence} = \frac{\text{No. of infected leaves}}{\text{Total no. of leaves observed}} \times 100$$

$$\text{Per cent Disease Intensity} = \frac{\text{Sum of all disease rating}}{\text{No. of leaves observed} \times \text{maximum disease rating}} \times 100$$

Statistical analysis: Statistical analysis of the data was carried out using OPSTAT software of CCSHAU, Hisar, Haryana. The critical difference (CD) was calculated at 5% level of significance (p=0.05) for comparison of difference between the means of treatment (Anonymous 2022).

$$\text{CD} = \sqrt{\frac{2\,\text{Error Variance}}{n}} \times t \text{ at 5\% probability level}$$

Where, CD, Critical difference; n, The quantity of observations for which the coefficient of determination (CD) is to be computed; $t_{0.05\%}$, The percentage point value of the 't' distribution for the degrees of freedom at a 5% level of significance.

RESULTS AND DISCUSSION

In vitro *evaluation of fungicides*: An examination of the data (Table 2) indicated that all evaluated fungicides at doses of 100, 500, and 1000 ppm considerably suppressed the radial development of *C. gossypii* in comparison to the untreated control. The synergistic formulation of Azoxystrobin 18.2% and Difenoconazole 11.4% SC shown superior fungicidal efficacy at concentrations of 100, 500, and 1000 ppm, achieving complete inhibition of radial growth of *C. gossypii* at 1000 ppm, with an average growth inhibition of 90.48%. The combined product of Metiram

Table 1 Disease severity rating scale

Rating scale	Disease reaction	Disease symptoms
0	Immune	No observable symptoms on the plant.
1	Resistant	Minor lesions on foliage, constituting less than 1 percent of the leaf area affected
3	Moderately Resistant	Medium-sized lesions on leaves encompassing 1-10 percent of the affected area
5	Moderately Susceptible	Large spots; merging to cover 11-25 percent of leaf area
7	Susceptible	Large spots coalescing, encompassing 26-50 percent of the leaf area.
9	Highly Susceptible	Spots on leaves encompassing over 51 percent of the leaf area

Table 2 Growth inhibition of *C. gossypii* by fungicides under *in vitro* condition

Treatments	Per cent fungal growth inhibition*			
	Concentrations (ppm)			
	100	500	1000	Mean
T_1, Kresoxim methyl 44.3% SC	60.93 (51.29)	77.04 (61.35)	88.15 (69.86)	75.37 (60.84)
T_2, Pyraclostrobin 20 WP	47.20 (43.39)	74.81 (59.86)	84.44 (66.74)	68.83 (56.67)
T_3, Azoxystrobin 18.2% + Difenoconazole11.4% SC	78.70 (62.49)	92.74 (74.36)	100.00 (89.39)	90.48 (75.42)
T_4, Metiram 55% + Pyraclostrobin 5% WG	71.85 (57.94)	87.41 (69.21)	94.44 (76.35)	84.57 (67.83)
T_5, Copper oxychloride 50 WP	11.11 (19.43)	55.19 (47.96)	70.37 (56.99)	45.56 (41.46)
T_6, Propiconazole 25% EC	67.41 (55.17)	79.07 (62.76)	94.07 (75.92)	80.18 (64.61)
T_7, Carbendazim 50% WP	32.96 (35.02)	71.85 (57.94)	78.33 (62.23)	61.05 (51.73)
T_8, Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	46.27 (40.59)	67.26 (54.18)	76.25 (62.19)	
	Treatment	Concentration	Treatment × Concentration	
CD (p=0.05)	(0.78)	(0.48)	(1.35)	
SEM ±	0.35	0.21	0.60	

*Average of three replications. Values in brackets are transformed using the Arcsine function.

55% + Pyraclostrobin 5% WG inhibited 94.44% radial growth of *C. gossypii* followed by Propiconazole 25% EC (94.07 %) and Kresoxim methyl 44.3% SC (88.15 %) at 1000 ppm concentration with 84.57, 80.18 and 75.37% mean growth inhibition, respectively.

Copper oxychloride 50 WP exhibited the least efficacy in inhibiting mycelial growth, achieving just 11.11% inhibition at a concentration of 100 ppm, resulting in a mean growth inhibition of 45.56%, the lowest above all treatments.

An increase in the concentration of every single fungicide significantly enhances mycelial growth inhibition. The present results corroborate the results of Rony *et al.* (2021), who documented the inhibitory properties of Difenoconazole, Carbendazim, and Copper oxychloride at doses of 100, 200, 300, 400, and 500 ppm. Difenoconazole completely suppressed mycelial growth at a dosage of 500 ppm. The results were in conformity with various researchers (Datar 1990, Mistry *et al.* 2008, Gawade and Suryawanshi 2009, Jagtap *et al.* 2012, Ali *et al.* 2021) who studied the effects of fungicides including Kresoxim methyl, Azoxystrobin, Metiram and Pyraclostrobin against various anthracnose pathogens.

Assessment of fungicides for the management of anthracnose disease in cotton in field conditions: The assessment of fungicides towards anthracnose disease in Bt and non-*Bt* cotton cultivars during the *kharif* seasons of 2020 and 2021 showed that all fungicides effectively mitigated the illness and enhanced cotton output in both years (Table 3 and 4). Among the four treatments, the combined use of Azoxystrobin 18.2% and Difenoconazole 11.4% SC at a concentration of 0.1% had the highest efficacy, resulting in a disease intensity of 7.01% in *Bt* cotton and 8.20% in non-*Bt* cotton, with mean disease control rates of 78.21% and 75.80%, respectively. The application of Kresoxim methyl 44.3% SC at a concentration of 0.1% achieved disease control rates of 56.51% in *Bt* cotton and 53.41% in non-*Bt* cotton. The application of Copper oxychloride 50 WP at a concentration of 0.25% resulted in the highest disease intensity of 24.41% and 25.28%, along with the lowest disease control rates of 24.41% and 26.74% in *Bt* and non-*Bt* cotton cultivars, respectively. The maximum seed cotton yield, specifically 2590 kg/ha for *Bt* cotton and 2105 kg/ha for non-*Bt* cotton cultivars, was seen with the treatment combination consisting of Azoxystrobin 18.2% + Difenoconazole 11.4% SC. The treatment with Kresoxim methyl 44.3% SC recorded 2317 kg/ha and Carbendazim 50% WP recorded 2218 kg/ha seed cotton yield in *Bt* cotton both years, which exhibited 21.84 and 16.68% increase in yield over untreated check. Conversely, the application of Kresoxim methyl 44.3% SC yielded 2077 kg/ha, while Carbendazim 50% WP yielded 1858 kg/ha of seed cotton in non-*Bt* cotton over both years, resulting in yield increases of 24.01% and 13.34% over the control, respectively. The application of copper oxychloride 50 WP at 0.25% in the *Bt* cotton cultivar resulted in the lowest disease control (24.12%) and minimum yield (2100 kg/ha), whereas the non-*Bt* cotton cultivar exhibited the least disease control (21.09%) and minimum yield (1693.02 kg/ha) among all treatments, although it outperformed the untreated control. The results of this study also conform to the findings of Kumar *et al.* (2015) who reported that Difenoconazole and Keroxim methyl reduced the disease intensity by 72.33 and 71.00%, respectively. Campo-Arana *et al.* (2019) shown that foliar treatments of Azoxystrobin alternating in Mancozeb were the most effective in disease management, with a 44.97% reduction compared to the untreated control. The results obtained by Ali *et al.* (2021) were also in conformity with our experiment which revealed that Pyraclostrobin 5% + Metiram 55% WDG controlled the anthracnose disease by 74.57% under field conditions. Recently, Sandipan *et al.* (2022) concluded that foliar sprays with Azoxystrobin 18.2% + Difenoconazole 11.4% SC recorded 41.90% disease

Table 3 Effect of fungicides upon anthracnose disease along with seed cotton production in *Bt* cotton

Treatments	Disease incidence* (%)			Disease intensity* (%) (PDI)			Disease control (%)	Cotton yield (kg/ha)			Increase in seed cotton yield (%)
	2020	2021	Pooled mean	2020	2021	Pooled mean		2020	2021	Pooled mean	
T_1, Kresoxim methyl 44.3% SC @0.1%	45.25 (42.24)	43.73 (41.37)	44.49 (41.81)	14.80 (22.67)	13.18 (21.27)	13.99 (21.97)	56.51	2211	2423	2317	21.84
T_2, Azoxystrobin 18.2% + Difenoconazole1 1.4% SC @0.1%	30.70 (33.56)	28.77 (32.35)	29.74 (32.96)	7.23 (15.56)	6.78 (15.06)	7.01 (15.31)	78.21	2569	2611	2590	36.21
T_3, Copper oxychloride 50 WP @0.25%	60.53 (51.07)	58.87 (50.09)	59.70 (50.58)	25.45 (30.28)	23.37 (28.90)	24.41 (29.59)	24.12	2058	2143	2100	10.45
T_4, Carbendazim 50% WP @0.1%	52.15 (46.21)	50.71 (45.38)	51.43 (45.80)	18.65 (25.56)	16.79 (24.17)	17.72 (24.87)	44.92	2163	2274	2218	16.68
T_5, Control	72.03 (58.08)	70.76 (57.29)	71.40 (57.69)	33.78 (35.52)	30.56 (33.54)	32.17 (34.53)		1817	1987	1902	
CD (p=0.05)	(4.18)	(4.08)		(2.59)	(2.01)			295.99	296.09		
SEM ±	2.23	2.13		1.09	0.79			95.01	95.04		
CV	8.54	8.43		10.94	8.73			8.78	8.31		

Values in brackets are transformed using the Arcsine function.

Table 4 Effect of fungicides on anthracnose disease and seed cotton yield in non *Bt* cotton

Treatments	Disease incidence* (%)			Disease intensity* (%) (PDI)			Disease control (%)	Cotton yield (kg/ha)			Increase in seed cotton yield (%)
	2020	2021	Pooled mean	2020	2021	Pooled mean		2020	2021	Pooled mean	
T_1, Kresoxim methyl 44.3% SC @0.1%	52.55 (46.45)	50.25 (45.12)	51.40 (45.79)	16.56 (23.99)	15.02 (22.79)	15.79 (23.39)	53.41	1822	2077	1949	24.01
T_2, Azoxystrobin 18.2% + Difenoconazole11.4% SC @0.1%	40.75 (39.63)	38.37 (38.25)	39.56 (38.94)	8.53 (16.97)	7.87 (16.28)	8.20 (16.63)	75.80	2009	2201	2105	33.93
T_3, Copper oxychloride 50 WP @0.25 %	67.10 (54.99)	63.98 (52.51)	65.54 (53.75)	28.20 (32.06)	25.28 (30.17)	26.74 (31.12)	21.09	1646	1740	1693	7.70
T_4, Carbendazim 50% WP @0.1 %	60.15 (50.86)	57.77 (49.46)	58.96 (50.16)	20.50 (26.91)	16.38 (23.86)	18.44 (25.39)	45.59	1705	1858	1782	13.34
T_5, Control	76.10 (60.75)	74.05 (59.38)	75.08 (60.07)	36.68 (37.25)	31.10 (33.87)	33.89 (35.56)		1514	1630	1572	
CD (p=0.05)	(4.87)	(4.31)		(2.81)	(2.21)			191.47	227.67		
SEM ±	2.61	2.33		1.23	0.87			61.46	76.71		
CV	8.78	8.23		11.16	9.13			7.67	8.07		

Values in brackets are transformed using the Arcsine function.

control and the highest seed cotton yield of 2308.67 kg/ha as compared to water sprayed untreated control.

The application of chemical fungicides is one of the most promising, widely adopted and effective methods to manage plant disease. In the present investigation, all the seven fungicides evaluated were found effective in inhibiting mycelial growth of *C. gossypii* at all the three concentrations and the foliar applications of four fungicides on cotton cultivars (*Bt* and non *Bt*) concluded that, all the fungicides significantly controlled the anthracnose disease and increased the cotton yield in both the years. An integrated disease management module is also needed to be developed with combination of identified potent fungicides along with other management practices.

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