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Integrated Management of *Corynespora cassiicola* (Berk. and Curt.) Wei. Causal Agent of Target Spot Disease in Cotton

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ABSTRACT

Laboratory experiments were conducted during during *kharif* (July 2019–February 2020 and July 2020–February 2021) in the Department of Plant Pathology, Agricultural College, Bapatla and field experiments with selected treatments were carried out at Regional Agricultural Research Station (RARS), Lam, Guntur, Andhra Pradesh to develop an integrated management strategy against Corynespora leaf spot of cotton. Evaluation of fungicides, biocontrol agents, salicylic acid, potash and phosphate salts revealed that carbendazim, carbendazim+mancozeb and propiconazole at 250, 500, 750 and 1000 ppm and salicylic acid at 5.0 and 6.5mM completely inhibited the mycelial growth of cotton isolate of *Corynespora cassiicola*. Both phosphorous acid (79.98%) and ammonium dihydrogen phosphate (48.72%) showed reduction of mycelial radial growth in poisoned food technique. *Trichoderma* isolate T 18001 grew fast and exhibited deformation, demelanization, protoplast aggregation and finally lysis of mycelia in dual cultures. *Pseudomonas fluorescens* (PF1), *Bacillus subtilis* BS2, Actinomycetes (ACT2) were found to be effective with 39.23, 40.26 and 41.03% inhibition, respectively, as against control (3.90 cm) and induced shrinkage of *Corynespora* mycelia, reduction of interseptal length, demelanization in mycelia, excessive production of chlamydospores and finally, hyphal lysis at the zone of interaction. Propiconazole at 0.1% resulted in the lowest per cent disease index (12.4), highest yield (2931.7 kg ha^{-1}) and BC ratio of 1.46 followed by carbendazim+mancozeb at 0.1% (13.9 PDI, 2851.1 kg ha^{-1} and 1.42) and were significantly superior to other treatments as against control (27.3 PDI, 2265.6 kg ha^{-1} and 1.13), respectively.

KEYWORDS: Biocontrol agents, *Corynespora cassiicola*, cotton, fungicides

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1. INTRODUCTION

Cotton (*Gossypium* spp.) is the most important commercial crops of the world, which belongs to the botanical family "*Malvaceae*". Cotton is referred to as "King of Fibres" and also known as "White Gold". As the largest source of natural fibre, cotton is used as raw material in textile, pulp and paper industries and oil extracted from cotton seed is used in food, chemicals, cosmetics, pharmaceuticals etc. Cotton seed cake is used as cattle feed (Proto et al., 2000). The cotton production in India during 2024–25 was estimated to be 299.26 lakh bales of 170 kg from 113.6 l ha with a productivity of 448 kg lint ha^{-1} by the Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, New Delhi. The area under cotton during the current year in the country decreased by 10.46% and cotton production decreased by 7.98% compared to last year. Andhra Pradesh stood 8th both in cultivated area (4.53 l ha) and production (9.41 l bales) and 9th in productivity with 353 kg lint ha^{-1} in India (Anonymous, 2025). In India, cotton is cultivated under rainfed conditions and the cultivation is highly fluctuating due to biotic as well as abiotic stresses and competition from other crops in recent years. Biotic stress on cotton caused by pests and diseases contribute to 10–30% of annual yield loss worldwide (Hagan et al., 2015; Bowen et al., 2018). In India, foliar diseases (fungal, bacterial and viral) have been estimated to cause yield losses up to 20 to 30% (Mayee and Mukewar, 2007). Among all fungal foliar diseases, Corynespora leaf spot caused by *Corynespora cassiicola* has been increasing its prevalence and severity in cotton growing areas of South-Central United States, Central India and South India (Butler et al., 2016; Salunkhe et al., 2019 and Siva Prasad et al., 2022). Corynespora leaf spot has been observed in Andhra Pradesh since 2017 which attained major status. On infected cotton leaves initially, minute pinhead size light orange to brick red minute spots appeared that gradually enlarged and became circular to oval or irregular concentric spots with tan to light brown centre with yellow halo around the margin. These spots enlarged and concentric zonations were formed resulting in target board symptom. The disease resulted in premature defoliation during boll formation and boll maturation stage (Bowen et al., 2018). Corynespora target spot caused 224–448 kg ha^{-1} equivalent to 5% to 40% loss of lint in susceptible cotton cultivars (Conner et al., 2013; Hagan et al., 2015). Fungal foliar diseases including Corynespora leaf spot could cause losses to the tune of 16.14% in Jaadoo BG II, 20.34% in RCH 2 BG II and 26.28% in L 1060 (Roshan et al., 2022a). Carbendazim at 0.1%, hexaconazole at 0.2%, fluxapyroxad+pyraclostrobin at 0.06% and metiram+pyraclostrobin at 0.3% induced 97.50 to 100% inhibition of *C. cassiicola* (Mahesh et al., 2021; Mounika et al., 2024). Roshan Baba et al. (2022c) observed that propiconazole at 0.1% and zineb+hexaconazole at 0.2% caused 85.90% and 85.57% reduction in Per cent disease index (PDI) of Corynespora leaf spot followed by propineb at 0.3% (80.41%). Efficacy of potassium, sodium and phosphate salts was observed against soil borne or foliar pathogens (Kashyap and Dhiman, 2009, Kaiser et al., 2011). Efficacy of *Trichoderma*, *Pseudomonas* and *Bacillus* against foliar pathogens of cotton and other crops was reported earlier (Bhattiprolu, 2010, Bhattiprolu and Prasada Rao, 2013, Bhattiprolu et al., 2016, Chang et al., 2023, Sharma et al., 2024). In view of its importance of the disease the present investigation was conducted to develop an integrated management strategy against Corynespora leaf spot.

2. MATERIALS AND METHODS

Laboratory experiments were conducted during (June to September) 2019 in the Department of Plant Pathology, Agricultural College, Bapatla and field experiments with selected treatments were carried out at Regional Agricultural Research Station (RARS), Lam, Guntur, Andhra Pradesh (latitude of 16.3437555 and longitude of 80.4409937) during *kharif* seasons (July 2019–February 2020 and July 2020–February 2021) to test the efficacy of fungicides, salicylic acid, potash and phosphate salts and biocontrol agents.

2.1. *Effect of fungicides on the growth of Corynespora cassiicola in vitro*

Six fungicides viz., Carbendazim, Mancozeb, Carbendazim 12%+Mancozeb 63%, Propiconazole, Pyraclostrobin and Azoxystrobin were tested at 250, 500, 750 and 1000 ppm by employing poisoned food technique (Nene and Thapliyal, 1993). Briefly poisoned medium (20 ml) was poured in to sterilized Petri plates under aseptic conditions in inoculation chamber and were allowed to solidify. Each plate was inoculated in the centre with five-day old fungal culture disc of 5 mm diameter cut from the periphery of actively growing culture under aseptic conditions and incubated at 28±1 °C in a BOD incubator. Potato Dextrose Agar (PDA) plates containing 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.

2.2. Effect of salicylic acid, potash and phosphate salts on the growth of Corynespora cassiicola in vitro

Fungicidal activity of salicylic acid, potash and phosphate salts was evaluated against *C. cassiicola*. Salicylic acid at different concentrations viz., 0.5 mM, 2.0 Mm, 3.5 mM, 5 Mm and 6.5 Mm whereas potash and phosphate salts at 0.1%, 0.2%, 0.4%, 0.8%, 1.0% and 2.0% were tested using poisoned food technique (Nene and Thapliyal, 1993). All the potash and phosphate salts viz., phosphorous acid (H_3PO_4), potassium chloride (KCl), potassium sulphate (K_2SO_4), monopotassium phosphate, dipotassium phosphate, potassium nitrate and ammonium dihydrogen phosphate were also tested at 2% concentration by employing detached leaf technique.

2.3. Isolation of biocontrol agents

Selective media were used for isolation of *Pseudomonas* (Pseudomonas Agar (For Fluorescein), *Bacillus* (Bacillus Differentiation Agar), Actinomycetes (Actinomycete Isolation Agar) and Methylotrophic bacteria (Ammonium Mineral Salt Agar Medium, Whittenbury et al., 1970) by adopting leaf imprinting technique (Aneja, 2003). Phyllospheric bacteria were isolated from fresh healthy cotton leaves by serial dilution method. Pure cultures were maintained on nutrient agar slants for further evaluation against *C. cassiicola*.

2.4. Evaluation of Antagonistic Potential of phyllospheric bacterial isolates against C. cassiicola

Nine isolates of bacteria (PF1, PF2, PF3, BS1, BS2, MB1, MB2, ACT1 and ACT2) were evaluated for their antagonistic potential by dual culture technique against *C. cassiicola* (Whittenbury et al., 1970). One day old bacterial cultures and five-day old *C. cassiicola* were used for the dual culture. A loopful of each bacterial isolate was streaked at two ends of the Petri plate leaving 1 cm from the periphery and a mycelial disc of five mm diameter were inoculated on to PDA plate at the centre. The plates were incubated at 25 °C. The experiment was laid in three replications and 10 treatments including pathogen control. Observations on radial growth were recorded for every 24 h until zone of inhibition was clearly seen. The per cent growth inhibition of the test pathogen over control was calculated according to the formula,

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

2.5. Evaluation of Antagonistic potential of trichoderma isolates against C. cassiicola

Five *Trichoderma* isolates collected from Plant Pathology Lab, Agricultural College, Bapatla (T 19012- *Trichoderma virens* strain 1, T-19001- *T. harzianum* strain 1, T 19023-*T. asperellum* strain 1, T 19020- *T. viride* strain 1 and T 19007- *T. asperellum* strain 2) and two native isolates of RARS, Lam (T 18001- *T. harzianum* strain 2 and T 18002- *T. asperellum* strain 3) were evaluated for their antagonistic potential against *C. cassiicola* using dual culture technique (Dhingra and Sinclair, 1985). Culture discs (0.5 cm) each of the fungal antagonist and the pathogen were taken from the margin of the actively growing cultures and transferred to PDA medium contained in 9 cm Petri plates on opposite sides approximately at 1 cm from the wall of the plate. A control having the test pathogen alone was kept for comparison. The Petri plates were incubated at 25±1 °C till the control plate was completely covered by *C. cassiicola*. Each treatment was replicated thrice. Colony diameter of the test fungus as well as each antagonist up to the zone of inhibition was recorded and the per cent growth inhibition of the test pathogen over control was calculated according to the formula given by Vincent (1927).

2.6. Field evaluation against corynespora leaf spot

Field trial was conducted at RARS, Lam, Guntur, Andhra Pradesh during *kharif*, 2019–2020 and 2020–2021. Susceptible hybrid Jaadoo BG II was sown at spacing of 105×60 cm^2. The experiment was laid out in a split-plot design with three main plots (ST with fungicide combination of carboxin+thiram (F) 2 g kg^{-1}; ST with biocontrol agent *Trichoderma* sp. (BCA) at 10^8 CFU ml^{-1} and ST with compatible F+BCA) and eight subplots viz. S_1- Propiconazole at 0.1%, S_2- Carbendazim 12%+mancozeb 63% at 0.1%, S_3- *Trichoderma* sp. at 10^8 CFU ml^{-1} (T 18001), S_4- *Pseudomonas* at 10^8 cfu ml^{-1} (PF1), S_5- *Bacillus* at 10^8 cfu ml^{-1} (BS2), S_6- Actinomycetes at 10^8 cfu ml^{-1} (ACT2), S_7- Salicylic acid at 5mM and S_8- Control and replicated thrice. Three sprays were given at 15 days interval with the first spray initiated as soon as the disease appeared for the first time. The biocontrol agents were added with 0.1% Tween-20 to ensure uniform spread of inoculum (Dhingra and Sinclair, 1985). Seven days after last spraying the disease in different treatments was recorded using 0–4 scale (Sheo, 1988) and PDI was calculated by using the formula given by Wheeler (1969). Treatment wise yield data was recorded and benefit cost ratio was calculated by dividing gross returns with gross expenditure for each treatment in comparison to untreated control.

3. RESULTS AND DISCUSSION

3.1. Efficacy of fungicides on the radial growth of C. cassiicola

All the six fungicides significantly reduced the radial growth of *C. cassiicola* at four test concentrations (250 ppm, 500 ppm, 750 ppm and 1000 ppm) compared to control (8.97 cm) (Table 1). Carbendazim, carbendazim+mancozeb and propiconazole completely inhibited the growth of the pathogen at all concentrations tested. However, decrease in radial growth with increased concentrations was observed with respect to other fungicides. Mancozeb with a mean radial growth of 1.96 cm was found significantly superior over other two fungicides pyraclostrobin (2.90 cm) and azoxystrobin (3.86 cm) (Table 1 and Plate 1). Significant reduction in mean radial growth with increase in concentration was recorded with the least radial growth at 1000 ppm concentration (1.23 cm) and the highest with 250 ppm concentration (1.71 cm). Significant lower radial growth was observed with that of next higher concentration. Interaction between fungicide and concentrations revealed that inhibition of radial growth at 250 ppm concentration ranged between 50.61% in azoxystrobin and 100% inhibition in carbendazim, carbendazim+mancozeb and propiconazole followed by next superior chemical, mancozeb (72.13%). A similar trend was observed for radial growth inhibition at 500, 750 and 1000 ppm.

Table 1: Effect of fungicides on radial growth of *C. cassiicola in vitro*

Fungicides/ Concentrations (ppm)	Colony diameter (cm)					Inhibition (%)				
	250	500	750	1000	Mean	250	500	750	1000	Mean
Carbendazim	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	100.00	100.00	100.00	100.00	100.00
Mancozeb	2.50(1.87)	2.50(1.87)	1.48(1.58)	1.37(1.54)	1.96(1.72)	72.13	72.19	83.50	84.73	78.14
Carbendazim+ Mancozeb	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	100.00	100.00	100.00	100.00	100.00
Propiconazole	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	100.00	100.00	100.00	100.00	100.00
Pyraclostrobin	3.33(2.08)	2.90(1.97)	2.80(1.95)	2.57(1.89)	2.90(1.97)	62.88	67.77	68.78	71.35	67.70
Azoxystrobin	4.43(2.33)	3.87(3.14)	3.67(2.16)	3.47(2.11)	3.86(2.44)	50.61	56.86	59.10	61.31	56.97
Mean	1.71(1.55)	1.54(1.51)	1.32(1.45)	1.23(1.42)		80.94	82.80	85.23	86.23	
Control			8.97 (3.16)							
	Fungicide	Concentration		F × C	Check vs Others					
SEm±	0.003	0.003		0.006	0.006					
CD (p=0.05)	0.01	0.01		0.02	0.02					
CV (%)			0.88							

Figures in parentheses are square root transformed values

Jones and Jones (1985) reported mancozeb and chlorothalonil to be the most effective fungicides against *C. cassiicola* isolate of tomato under *in vitro* conditions. Fluxapyroxad and prothioconazole provided the greatest mycelial growth inhibition of *C. cassiicola* from soybean (Teramoto et al., 2017). The fungicides, carbendazim, carbendazim+mancozeb, hexaconazole and mancozeb showed the complete inhibition of mycelium growth at 250 ppm and 500 ppm against the *C. cassiicola* from rubber (Manju et al., 2019). Propiconazole at 0.1%, myclobutanil at 0.1%, metiram at 0.2%, propineb at 0.3%, zineb+hexaconazole at 0.2%, trifloxystrobin+propineb at 0.3%, metiram+pyraclostrobin at 0.1% and fluxapyraxad+pyroxystrobin at 0.06% completely inhibited the mycelial growth of cotton isolate of *C. cassiicola* (Roshan Baba et al., 2022b).

3.2. *Efficacy of salicylic acid on radial growth of C. cassiicola*

Significant reduction in pathogen growth was observed (0.0 to 5.43 cm) with salicylic acid at five different concentrations as against control (5.75 cm) and inhibition ranged from 5.57 to 100%. Salicylic acid at 5.0 mM and 6.5 mM concentrations completely inhibited the radial

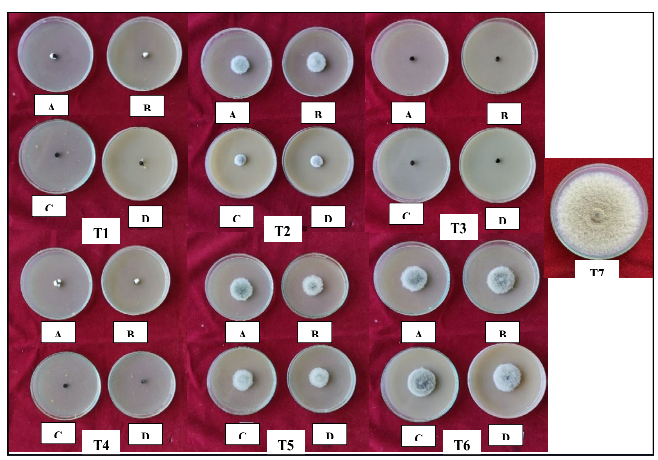

Plate 1: Effect of fungicides on radial growth of *Corynespora cassiicola in vitro;* T_1: Carbendazim, T_2: Mancozeb. T_3: Carbendazim+Mancozeb. T_4: Propiconazole, T_5: Pyraclostrobin, T_6: Azoxystrobin, T_7: Control, A-250 ppm, B-500 ppm, C-750 ppm and D-1000 ppm

growth of *C. cassiicola* followed by 3.5 mM and 2.0 mM concentrations with 32.17 and 21.22% radial growth inhibition, respectively. The least radial growth was observed at 0.5 mM concentration with 5.57% inhibition compared to control. No sporulation was observed at 5.0 mM and 6.5 mM while decreased concentration had enhanced sporulation (Table 2 and Plate 2).

Amborabe et al. (2002) reported that 2.0 mM concentration of salicylic acid reduced the mycelial growth of Eutypalata causing die back in grapevine. Bhattiprolu et al. (2014) observed that salicylic acid application reduced Alternaria leaf spot severity to the tune of 45.47%. Rakesh et al. (2017) reported inhibition of radial growth (41.2%) of *Alternaria* mycelium under *in vitro* condition using salicylic acid.

Table 2: Effect of salicylic acid on the radial growth of *Corynespora cassiicola in vitro*

Sl. No.	Salicylic acid	Colony diameter (cm)	Per cent inhibition	Sporulation at 6 DAI ($n\times10^4$ spores mm^{-2})
1.	0.5 mM	5.43 (2.53)^d	5.57	0.17±0.03
2.	2.0 mM	4.53 (2.35)^c	21.22	0.16±0.06
3.	3.5 mM	3.9 (2.21)^b	32.17	0.13±0.01
4.	5.0 mM	0.00 (1.00)^a	100.00	NIL
5.	6.5 mM	0.00 (1.00)^a	100.00	NIL
6.	Check	5.75 (2.60)^e		0.24±0.04
	SEm±	0.01		
	CV (%)	1.12		
	CD (p=0.05)	0.03		

DAI: Days after inoculation; Figures in parentheses are arc sine transformed values; Figures indicated with the same alphabets are statistically not significant

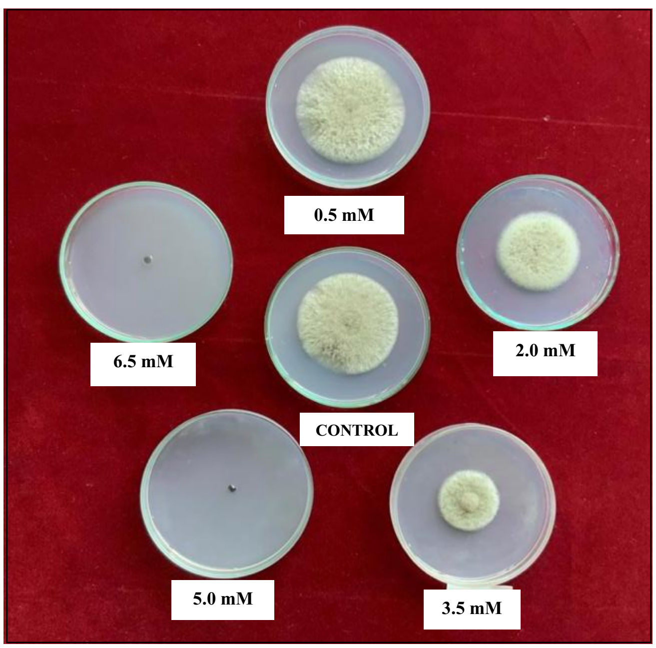

Plate 2: Effect of fungicides on radial growth of *Corynespora cassiicola in vitro*

3.3. *Effect of potassium and phosphate salts on radial growth of C. cassiicola*

Significant variation among the treatments and also with respect to different concentrations was observed. Among the salts, H_3PO_3 showed complete reduction of radial growth at 0.2, 0.4, 0.8, 1.0 and 2.0% over control, however, at lower concentration (0.1%) radial growth of *C. cassiicola* (7.17 cm) was high compared to control (5.97 cm) (Table 3). Radial growth of *C. cassiicola* increased with increase in the concentration of KCl, K_2SO_4 and KH_2PO_4; KCl recorded 7.09 cm mean radial growth followed by KH_2PO_4 (6.87 cm) as against control (5.97 cm). K_2HPO_4 showed reduction of radial growth at all concentrations in comparison to control. K_2HPO_4 resulted in mean radial growth of 5.02 cm compared to control (5.97 cm) with 15.86% mean per cent radial growth inhibition. KNO_3 salts initially increased the radial growth with increase in concentration upto 0.8% and then decreased with increase in concentration but the mean radial growth was higher (6.98 cm) when compared to control (5.97 cm). $NH_4H_2PO_4$ salt showed reduction in radial growth with increase in concentrations viz., 3.05 cm with mean radial growth of test pathogen as against control (5.97 cm) with 48.72% mean percent radial growth inhibition (Plate 3).

These results were in accordance with Kashyap and Dhiman (2009) where potash and phosphate salts, KH_2PO_4 and H_3PO_3 significantly reduced Alternaria leaf blight in cauliflower.

Abdel-Kader et al. (2012) obtained similar results for the antifungal activity of K_2HPO_4. Arslan (2015) reported the antifungal activity of K_2HPO_4 and KH_2PO_4 against soil borne pathogens and showed significant reduction of radial

Table 3: Effect of potash and phosphate salts on radial growth of *Corynespora cassiicola in vitro*

Treatments/ Concentrations	Colony growth (cm)						
	0.1%	0.2%	0.4%	0.8%	1%	2%	Mean
H_3PO_3	7.17(2.86)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	1.2(1.31)
KCl	6.40(2.72)	6.57(2.75)	6.67(2.77)	7.47(2.91)	7.70(2.95)	7.73(2.96)	7.09(2.84)
K_2SO_4	4.67(2.38)	6.47(2.73)	6.60(2.76)	7.13(2.85)	7.40(2.90)	7.57(2.93)	6.64(2.76)
KH_2PO_4	6.10(2.66)	6.63(2.76)	6.93(2.82)	7.10(2.85)	7.13(2.85)	7.33(2.89)	6.87(2.81)
K_2HPO_4	6.10(2.66)	5.40(2.53)	4.97(2.44)	4.67(2.38)	4.53(2.35)	4.47(2.34)	5.02(2.45)
KNO_3	6.77(2.79)	6.97(2.82)	7.30(2.88)	7.47(2.91)	6.73(2.78)	6.63(2.76)	6.98(2.82)
$NH_4H_2PO_4$	2.63(1.91)	2.60(1.90)	2.60(1.90)	2.57(1.89)	3.47(2.11)	4.50(2.35	3.05(2.01)
Mean	5.69(2.57)	4.93(2.35)	5.01(2.37)	5.20(2.40)	5.28(2.42)	5.46(2.46)	
Check							

	Treatment	Concentrations	T × C	Check vs Others
SEm±	0.006	0.006	0.01	0.01
CD (p=0.05)	0.02	0.02	0.04	0.03
CV (%)		1.05		

Treatments/Concentrations	Inhibition (%)						
	0.1%	0.2%	0.4%	0.8%	1%	2%	Mean
H_3PO_3	-20.1	100	100	100	100	100	79.98
KCl	-7.20	-10.05	-11.73	-25.13	-28.98	-29.48	-18.76
K_2SO_4	21.78	-8.38	-10.55	-19.43	-23.95	-26.80	-11.22
KH_2PO_4	-2.18	-11.06	-16.08	-18.93	-19.43	-22.78	-15.08
K_2HPO_4	-2.18	9.55	16.75	21.78	24.12	25.12	15.86
KNO_3	-13.40	-16.75	-22.28	-25.13	-12.73	-11.10	-16.90
$NH_4H_2PO_4$	55.95	56.45	56.45	56.95	41.87	24.62	48.72
Mean	4.67	17.11	16.08	12.87	11.56	8.51	
Check							

Figures in parentheses are square root transformed values; ' - ' sign indicates growth excess than check, i.e., growth is promoted

Plate 3: Efficacy of potassium and phosphate salts against radial growth of *Corynespora cassiicola*

growth at 3% concentrations. Khiareddine et al. (2016) recorded the effect of potash salts against soil borne and foliar plant pathogens *in vitro*.

Complete inhibition of the fungal linear growth was achieved at the highest concentration (2%) of the potassium bicarbonate and dipotassium phosphate (Abd-El-Kareem et al., 2020).

In case of detached leaf technique H_3PO_3 salt and $NH_4H_2PO_4$ showed phytotoxicity effect (Table 4). Discolouration of leaf from green to light greenish brown colour was observed immediately after application of H_3PO_3 whereas $NH_4H_2PO_4$ showed discolouration 24 h after application. Remaining salts viz., K_2SO_4, KCl, KH_2PO_4, K_2HPO_4 and KNO_3 showed high per cent disease index (30.00 to 41.33%) when compared to control (24.33%). Based on these results, potash and phosphate salts were not

Table 4: Effect of potassium and phosphate salts against *Corynespora cassiicola* using detached leaf technique

Sl. No.	Treatments	Phytotoxicity	Lesion Number	PDI	Colour of the leaf
1.	H_3PO_3	+	0.0[e]	0.00 (0.00)[e]	Greenish brown
2.	KCl	-	4.8±0.45[c]	33.44 (35.32)[c]	Light green
3.	K_2SO_4	-	5.8±0.44[b]	30.00 (33.15)[c]	Light green
4.	KH_2PO_4	-	5.6±0.55[b]	33.67 (35.45)[c]	Light green
5.	K_2HPO_4	-	7.2±0.84[a]	50.00 (44.98)[a]	Light green
6.	KNO_3	-	7.0±0.71[a]	41.33 (39.99)[b]	Light green
7.	$NH_4H_2PO_4$	+	0.0[e]	0.00 (0.00)[e]	Greenish brown
8.	Control	-	4±0.71[d]	24.33 (29.54)[d]	Green
	SEm±		0.24	0..91	
	CV (%)		12.74	5.74	
	CD (p=0.05)		0.71	2.71	

Figures in parentheses are arc sine transformed values; Figures indicated with the same alphabets are statistically not significant

utilized for further field assessment studies.

3.4. Effect of Trichoderma isolates on the growth of C. cassiicola

The data revealed that all the *Trichoderma* spp. reduced the growth of *C. cassiicola* compared to control. Among the different *Trichoderma* isolates, T 19007 significantly reduced the growth of test pathogen (3 DAI and 4 DAI). At 3 DAI, T 19007 isolate was observed with 1.10 cm radial growth with 26.67% inhibition while T 18001, T 19001, T 19020, T 19023, T 19012 and T 18002 recorded 1.23 cm, 1.27 cm, 1.27 cm, 1.27 cm, 1.40 cm and 1.40 cm radial growth with 18.00%, 15.33%, 15.33%, 15.33%, 6.67% and 6.67% inhibition, respectively. T19001, T 19023 and T 19020 were statistically on par with T 19012 and T 18002 isolates (Table 5).

At 4 DAI, T 19007 isolate recorded 1.10 cm radial growth with 47.62% inhibition of *C. cassiicola*; next best isolate, T 18001 showed 1.23 cm radial growth with 41.23% inhibition which was on a par with T 19001 and T 19020 (1.27 cm and 1.30 cm) with 39.52; 38.09% inhibition, respectively, followed by T 19012 isolate with 1.40 cm radial growth and 33.33% inhibition. T 18002 isolate showed lesser radial growth of 1.53 cm with 27.14% inhibition. Evaluation of efficacy of *Trichoderma* isolates (Table 6) revealed that isolate T 18001 was the best, as it grew fast on test pathogen with good sporulation.

Table 5: Effect of Trichoderma isolates on the radial growth of *Corynespora cassiicola* in dual culture

Sl. No.	*Corynespora* dual culture with	Radial growth of *C. cassiicola* (cm)		Per cent inhibition		Zone of inhibition	Lysis of *C. cassiicola*	Over-growth of *Trichoderma* on *C. cassiicola*	*Trichoderma* sporulation upon *C. cassiicola*	*Trichoderma* sporulation at ZI	Pigmentation in *C. cassiicola*
		3 DAI	4 DAI	3 DAI	4 DAI						
1.	T 19012	1.4(1.18)[a]	1.40(1.18)[c]	6.67	33.33	-	+	+	++	-	+
2.	T 19001	1.27(1.12)[b]	1.27(1.12)[d]	15.33	39.52	-	+	+	+++	-	+
3.	T 19023	1.27(1.13)[b]	1.40(1.18)[c]	15.33	33.33	-	+	+	-	+	+
4.	T 19020	1.27(1.13)[b]	1.30(1.14)[d]	15.33	38.09	-	+	+	-	-	+
5.	T 19007	1.10(1.05)[c]	1.10(1.05)[e]	26.67	47.62	-	+	+	-	-	+
6.	T 18001	1.23(1.11)[b]	1.23(1.11)[d]	18.00	41.23	-	+	+	+	-	+
7.	T 18002	1.40(1.18)[a]	1.53(1.24)[b]	6.67	27.14	-	+	+	-	-	+
8.	Monoculture (*C. cassiicola*)	1.50(1.22)[a]	2.10(1.45)[a]								
	SEm±	0.01	0.01								
	CV (%)	2.09	1.83								
	CD (p=0.05)	0.04	0.04								

DAI: Days after inoculation; Figures in parentheses are arc sine transformed values; Figures indicated with the same alphabets are statistically not significant; + sign indicates the presence of character and – sign indicates the absence of character

Table 6: Radial growth of *Trichoderma* isolates dual cultured with *Corynespora cassiicola in vitro*

Sl. No.	Treatment	Radial growth (cm)			Per cent inhibition	
		2 DAI	3 DAI	4 DAI	3 DAI	4 DAI
1.	T 19012-D	3.13	5.00	5.10	9.09(17.54)[e]	32.00 (34.4)[a]
	T 19012-C	3.10	5.50	7.50		
2.	T 19001-D	3.00	4.90	5.20	10.91(19.30)[d]	26.76 (31.14)[e]
	T 19001-C	3.00	5.50	7.10		
3.	T 19023-D	3.20	5.00	5.30	12.28(20.50)[c]	29.33 (32.78)[c]
	T 19023-C	3.20	5.70	7.50		
4.	T 19020-D	3.20	4.83	5.07	10.31(18.71)[d]	32.4 (34.52)[a]
	T 19020-C	3.20	5.40	7.50		
5.	T 19007-D	4.20	5.20	5.40	27.28(31.47)[b]	28.00 (31.94)[d]
	T 19007-C	4.20	7.20	7.50		
6.	T 18001-D	4.30	5.17	5.30	30.93(33.78)[a]	29.33 (32.78)[c]
	T 18001-C	4.30	7.50	7.50		
7.	T 18002-D	2.77	4.80	5.00	4.00(11.53)[f]	31.50 (34.13)[b]
	T 18002-C	2.80	5.00	7.30		
	SEm±				0.20	0.03
	CV (%)				1.58	0.16
	CD (*p*=0.05)				0.61	0.09

D: Dual culture; C: Check; DAI: Days after inoculation; Figures in parentheses are arc sine transformed values; Figures indicated with the same alphabets are statistically not significant

3.5. Interaction of Trichoderma isolates with C. cassiicola in dual culture

All the seven isolates of *Trichoderma* spp. inhibited the growth of test pathogen *C. cassiicola* (Plate 4). Primarily *Trichoderma* hyphae coiled around the test pathogen resulting in wrinkling of the mycelia and reduction in inter septal length. Finally, protoplast aggregation was observed (Plate 5). Coiling of *Trichoderma* mycelia on conidia, conidial aggregation and penetration of conidia as well as the mycelia of *C. cassiicola* was noticed. Khiareddine et al. (2016) isolates showed lysis of mycelia and pigmentation on mycelia besides overgrowth of *Trichoderma* on mycelia. Abnormalities were observed in the mycelia of *C. cassiicola* incubated with cell-free culture filtrate of *T. spirale* T76-1 (Burhanah et al. 2019a).

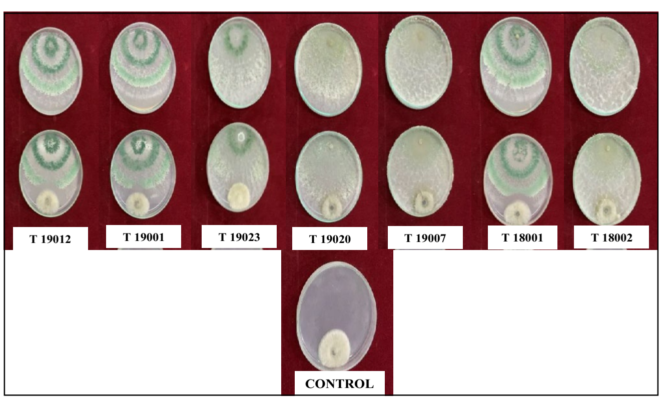

Plate 4: Antagonistic efficacy of *Trichoderma* isolates on radial growth of *Corynespora cassiicola*

Sandamali et al. (2017) observed the antagonistic activity of native *Trichoderma* isolates against the *Corynespora* pathogen with 51.3% inhibition. Burhanah et al. (2019a) reported that *T. spirale* T76-1 inhibited the mycelial growth of *C. cassiicola*

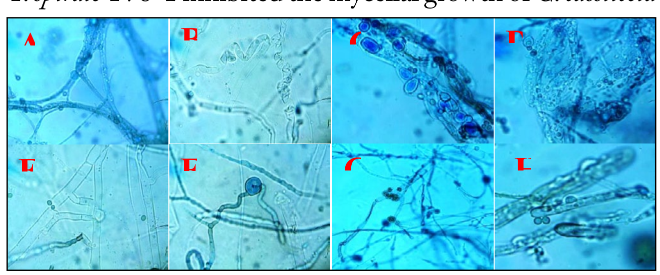

Plate 5: Interaction of *Trichoderma* isolates with *Corynespora cassiicola* in dual culture (400x); A: Abnormalities in *Corynespora* mycelium; B: Wrinkling of *Corynespora* mycelium; C, D: Protoplast aggregation in mycelium; E: Anastomosis in mycelium; F: Chlamydospore formation; G: *Trichoderma* conidia on *Corynespora* conidia; H: *Trichoderma* conidia germination on *Corynespora* conidia

by 84.68% in dual cultures and 41.29% in volatile antifungal bioassays. *Trichoderma asperellum* T_1 isolate inhibited the mycelial growth of *C. cassiicola* by 83.79% in dual cultures and 50.38% in cell-free culture filtrates (Burhanah et al. 2019b). Choudhury and Sumanbala (2020) observed that T_1 and T_2 isolates of *Trichoderma* were moderately antagonistic and P_1 isolate of *Pseudomonas* was highly antagonistic against *Corynespora* sp. affecting Sarpagandha.

3.6. Effect of bacterial biocontrol agents on the growth of C. cassiicola

It was observed that all the nine bacterial biocontrol agents (BBCAs: Fluorescent *Pseudomonas*, *Bacillus*, PPMB and Actinomycetes) reduced the growth of *C. cassiicola*. The isolates, PF1, PF3, BS2, ACT1 and ACT2 were found to be effective with 2.37, 2.47, 2.33, 2.37 and 2.30 cm radial growth and 39.23, 36.67, 40.26, 39.23 and 41.03 % inhibition, respectively, as against control (3.90 cm) (Table 7, Plate 6). Interaction of BBCA against Corynespora revealed shrinkage of Corynespora mycelia, reduction of interseptal length, demelanization in mycelia and excessive production of chlamydospores. Finally, hyphal lysis was observed at zone of interaction (Plate 7).

Table 7: Effect of phyllosphere bacterial isolates on the radial growth of *Corynespora cassiicola* in dual culture

Sl. No.	Treat-ment	Colony diameter (cm)	Per cent inhi-bition	Zone of Inhi-bition (cm)	Over growth by the pathogen
1.	PF1	2.37 (1.54)^{ab}	39.23	0.6	-
2.	PF2	3.50 (1.87)^{d}	10.26	-	+
3.	PF3	2.47 (1.57)^{b}	36.67	0.5	-
4.	BS1	3.33 (1.83)^{c}	14.62	-	+
5.	BS2	2.33 (1.53)^{a}	40.26	0.8	-
6.	MB1	3.40 (1.84)^{cd}	12.82	-	+
7.	MB2	3.30 (1.82)^{c}	15.38	-	+
8.	ACT1	2.37 (1.54)^{ab}	39.23	1.0	-
9.	ACT2	2.30 (1.52)^{a}	41.03	0.8	-
10.	Control	3.90 (1.97)^{e}			
	SEm±	0.01			
	CV (%)	1.18			
	CD (p=0.05)	0.03			

Figures in parentheses are arc sine transformed values; Figures indicated with the same alphabets are statistically not significant

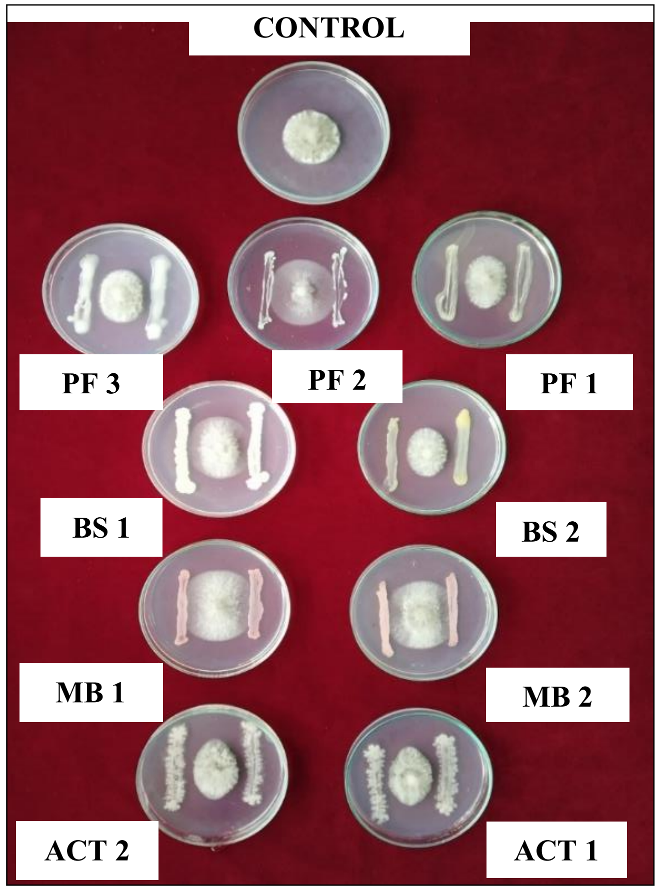

Plate 6: Efficacy of different phyllospheric bacteria against *C. cassiicola*

Mathiyazhagan et al. (2004) reported the bioefficacy of *Bacillus subtilis*, *Pseudomonas fluorescens* and *P. chlororaphis* against Corynespora target spot both *in vitro* and *in vivo*. Manju et al. (2014) reported bio efficacy of *Trichoderma*, *B. subtilis* and *P. fluorescens* against *Corynespora* with 65.80, 60.58 and 26.0% inhibition *in vitro*. Avozani et al. (2022) observed that *P. fluorescens*, *P. agglomerans* and *Bacillus* sp. reduced mycelial growth of *C. cassiicola* from soybean by 74%.

3.7. Field evaluation against Corynespora leaf spot

Pooled data (2019 and 2020) revealed significant differences among the subplots whereas main plots viz., ST with fungicide, ST with BCA and ST with F+BCA did not differ significantly. After the third foliar spray, propiconazole at 0.1% (12.4 PDI) was found on par with carbendazim+mancozeb at 0.1% (13.9 PDI) and significantly superior to other treatments as against control (27.3 PDI) (Table 8). The yield data showed that foliar spray with propiconazole at 0.1% (2931.7 kg ha^{-1}) was on par with carbendazim+mancozeb at 0.1% (2851.1 kg ha^{-1}) and significantly superior to other treatments over control (2265.6 kg ha^{-1}. BC ratio varied between 1.13–1.46 (Table 8). Highest mean BC ratio was observed with propiconazole

Table 8: Efficacy of selected fungicides, biocontrol agents and salicylic acid on cotton yield (Pooled data of 2019–20 and 2020–21)

Treatments	M_1		M_2		M_3		
	PDI	PDC	PDI	PDC	PDI	PDC	Mean
S_1	12.3(20.5)	56.8	12.2(20.3)	54.4	12.8(20.9)	52.5	12.4(20.6)^{a}
S_2	14.3(22.2)	49.4	13.8(21.8)	48.1	13.6(21.6)	49.7	13.9(21.9)^{a}
S_3	19.3(26.0)	32.1	19.2(26.0)	28.1	19.4(26.1)	28.1	19.3(26.0)^{bc}
S_4	21.1(27.2)	25.6	21.0(27.3)	21.3	20.2(26.7)	25.2	20.8(27.0)^{bcde}
S_5	21.2(27.4)	25.3	21.1(27.3)	20.9	20.4(26.8)	24.4	20.9(27.2)^{cde}
S_6	18.5(25.4)	34.7	18.2(25.2)	31.9	18.4(25.4)	31.8	18.4(25.4)^{b}
S_7	20.0(26.6)	29.4	19.3(26.1)	27.5	18.7(25.6)	30.9	19.3(26.1)^{bcd}
S_8	28.3(32.1)		26.7(31.0)		27.0(31.1)		27.3(31.4)^{f}
Mean	19.4		18.9		18.8		
	Main plots (M)	Sub plots (S)	M × S				
SEm±	0.49	0.58	1.01				
CV (%)	9.37	6.73	-				
CD (*p*=0.05)	NS	1.64	NS				

Treatments	Yield (kg ha^{-1}) in pooled data				% increase in yield over control			BC ratio			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	M_1	M_2	M_3	Mean
S_1	2898.3	2900.0	2996.7	2931.7^{a}	27.9	30.2	29.4	1.44	1.44	1.49	1.46
S_2	2815.0	2846.7	2891.7	2851.1^{a}	24.2	27.8	25.8	1.40	1.42	1.44	1.42
S_3	2593.3	2646.7	2650.0	2630.0^{b}	14.4	18.9	16.1	1.29	1.31	1.31	1.30
S_4	2675.0	2625.0	2651.7	2650.6^{b}	18.0	17.9	17.0	1.33	1.31	1.32	1.32
S_5	2598.3	2490.0	2548.3	2545.6^{b}	14.6	11.8	12.4	1.28	1.23	1.25	1.25
S_6	2581.7	2595.0	2553.3	2576.7^{b}	13.9	16.5	13.7	1.17	1.18	1.16	1.17
S_7	2618.3	2583.3	2616.7	2606.1^{b}	15.5	16.0	15.0	1.30	1.28	1.30	1.29
S_8	2266.7	2226.7	2303.3	2265.6^{c}	-	-	-	1.14	1.11	1.15	1.13
Mean											

M_1: Seed treatment with carboxin 37.5%+thiram 37.5% (F); M_2: Seed treatment with *Trichoderma* (BCA); M_3: Seed treatment with F+BCA; S_1: Propiconazole at 0.1%; S_2: Carbendazim 12%+mancozeb 63% at 0.1%; S_3: *Trichoderma* sp.; S_4: *Pseudomonas* at 108cfu ml^{-1}; S_5: *Bacillus* at 10^8 cfu ml^{-1}; S_6: Actinomycetes at 108cfu ml^{-1}; S_7: Salicylic acid at 5 mM; S_8: Control; PDI: Per cent disease index; PDC: Per cent disease control

at 0.1% (1.46) followed by carbendazim+mancozeb at 0.1% (1.42) whereas lowest mean BC ratio was realized in unsprayed control (1.13).

Molina et al. (2019) reported 49.6% disease control on application of mancozeb and 32.4% disease control on application of carbendazim against the soybean target spot. Manju et al. (2019) reported that among the fungal, bacterial biocontrol agents and fungicides tested against target spot of rubber, carbendazim+mancozeb treated plots exhibited significantly reduced the disease severity when compared to control.

Field efficacy of propiconazole, *P. fluorescens* and *T. viride* against Alternaria leaf spot and rust of cotton was reported earlier (Bhattiprolu, 2017). Fluxapyroxad and prothioconazole gave best results in soybean fields (Teramoto et al., 2017). Zhu et al. (2020) reported that protective and curative effects of metaconazole was higher than the difenconazole in detached leaf studies and pot culture experiments and observed that application of metaconazole at 150 g a.i ha^{-1} reduced the disease severity of Corynespora. Roshan Baba et al. (2022) obtained highest reduction of Corynespora leaf spot with propiconazole at

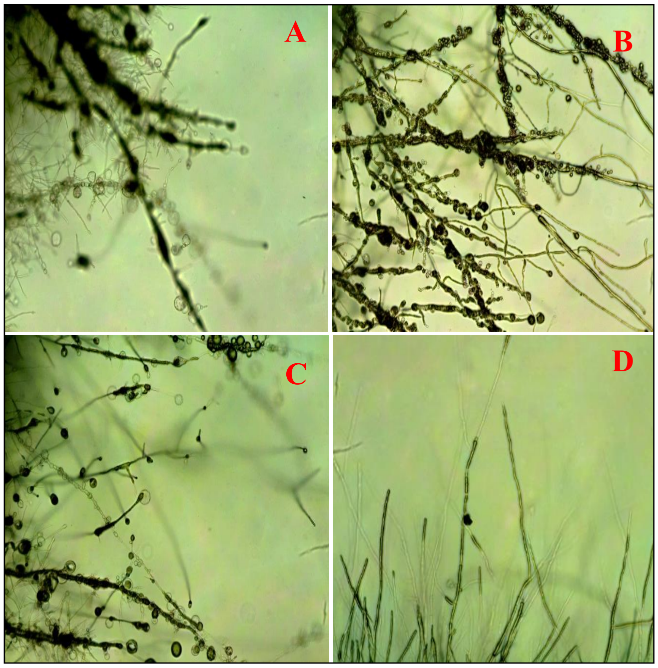

Plate 7: Microscopic observations of effect of antagonistic bacterial isolates on *C. cassiicola* in dual culture (400x); A, B: Chlamydospore formation at ZI; C: Chlamydospore formation away from ZI and D: Hyphal tip lysis at ZI

0.1% and zineb+hexaconazole at 0.2%. Propiconazole at 0.1% was reported effective against Corynespora leaf spot with lowest per cent disease index of 4.67 and 87.61% decrease in disease highest cotton seed yield (2493 kg ha^{-1}), benefit cost ratio (1.68) and maximum incremental benefit cost ratio (15.25) were obtained with propiconazole at 0.1% with 37.50% increase in yield over unsprayed control (Mahesh et al., 2022). Seed treatment with pyraclostrobin 5%+thiophanate methy 45% at 2 ml kg^{-1} followed by spraying tebuconazole 50%+trifloxystrobin 25% at 0.05% at 55 and 75DAS was found effective against target leaf spot of soybean caused by *C. cassiicola* (Hiremath et al., 2023).

4. CONCLUSION

Seed treatment with carboxin 37.5%+thiram 37.5% 2 g kg^{-1} and/or+Trichoderma at 10^8 CFU ml^{-1} followed by foliar sprays with propiconazole at 0.1% and carbendazim+mancozeb at 0.1% were significantly superior and cost effective with BC ratio of 1.46 and 1.42, respectively, against Corynespora leaf spot in cotton. Hence farmers were advised to take up preventive and/or protective measures.

5. FURTHER RESEARCH

Since the pathogen, *C. cassiicola* has recently developed as major disease of cotton, pathogen variability studies along with developing forecasting models on long term is necessary. Factors leading to increased incidence and severity need to be studied critically. For strengthening IDM, artificial screening system in field and poly-house should be developed to identify resistant genotypes.

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