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RESEARCH

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Combined application of effective *Trichoderma, Pseudomonas* and arbuscular mycorrhiza spp. reduced soil-borne diseases and boosted growth in cotton

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Abstract

Background The most common soil-borne diseases in cotton are root rot and wilt, which are caused by *Rhizoctonia solani* (Taub) Butler and *Fusarium oxysporum* f. sp. *vasinfectum*, respectively. These two diseases significantly reduce plant stand and production. Under extreme circumstances, the application of fungicides does not provide satisfactory management of these diseases and also pollutes the environment. The effect of biocontrol agents, their combinations and fungicides on root rot and wilt management and plant growth in *Gossypium hirsutum* and *G. arboreum* cultivars CSH-3129 and CICR-3 were studied during 2017–18 and 2018–19.

Results Out of six isolates of *Trichoderma* spp., *T. asperellum* (Th-11) was the most effective for inhibiting the mycelial growth of *R. solani* and *F. oxysporum* f. sp. *vasinfectum* (64.4–100%). The combined seed treatment of *T. asperellum* (Th-11, c.f.u. 2×10^8/g) + *Pseudomonas fluorescens* (c.f.u. 2×10^8/g) + arbuscular mycorrhizal fungi (AMF; 1200 IP/g) resulted in the highest plant vigour index in CSH-3129 (890.9%) and CICR-3 cultivars (393.5%) at 15 days after treatment. Ninety days after sowing, the combined seed treatments of *T. asperellum* (Th-11) + *P. fluorescens* + AMF followed by *T. asperellum* (Th-11) + *P. fluorescens* showed the lowest area under the disease progress curve in CICR-3 and CSH 3129. Two-year pooled results indicated that the combined seed treatment with *T. asperellum* (Th-11) + *P. fluorescens* + AMF reduced the root rot disease by 51 and 57.5% in CICR-3 and CSH-3129 cultivars, respectively, under field conditions.

Conclusion The present investigation suggested that combined application of the most effective strains of *T. asperellum* (Th-11) @10 g/kg + *P. fluorescens* @10 g/kg and AMF @20 g/kg can effectively manage root rot and wilt diseases up to 60 days after sowing and enhance plant growth under field conditions. However, the application rates of these biocontrol agents *vis-à-vis* load of pathogen inoculum in the field must be further evaluated for improved and long-term effects.

Keywords Biocontrol, Cotton, Combined application, *Gossypium* spp., Root rot, Wilt, *Trichoderma* spp.

Background

Upland cotton (*Gossypium hirsutum*) and tree/desi cotton (*G. arboreum*) are immensely important crops for the sustainable economy and livelihoods of the Indian subcontinent. The two main soil-borne fungal diseases are root rot, which is caused by *Rhizoctonia solani* (Taub) Butler and *R. bataticola* Kuhn (Syn—*Macrophomina phaseolina*), and wilt, which is caused by *Fusarium*

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oxysporum f. sp. *vasinfectum*. Both the *G. hirsutum* and *G. arboreum* species are afflicted by the root rot disease, which is more severe in tree/desi cotton. However, *F. oxysporum* f. sp. *vasinfectum*-induced wilt can strike *G. arboreum* at any stage of development (Monga et al. 2004). Both pathogens are associated with cotton seedling disease complex with varying degrees of virulence. Additionally, *F. oxysporum* f. sp. *vasinfectum*, causing wilt disease in *Gossypium* spp., occurs in most of the cotton production regions of the world (Colyer 2001).

Depending on the cultivars, location and cultural practices, both diseases are detrimental from seeding to harvesting and significantly reduce plant stand and cotton yield. Both *Fusarium* and *Rhizoctonia* are seed-borne and soil-borne fungal pathogens that can persist for a longer time on infected cotton seeds, crop residue in the soil and other host plant detritus, making their management difficult (Sain et al. 2023). Fungicide management of root rot can be accomplished by treating seeds with carboxin 37.5% + thiram 37.5% WS during the early stages, and foliar application of some fungicides is possible later on, but only to a certain extent. In extreme environments and areas with a large amount of pathogen inoculum, fungicide control of these diseases becomes more difficult (Monga et al. 2018). Continuous and indiscriminate use of these chemical fungicide increases the likelihood of adverse effects on the environment. Biological control, often known as one of the important components of integrated management, is a crucial and environmentally friendly management strategy that can dramatically reduce disease in modern agriculture (Cook 1985). Several researchers have shown that *Trichoderma* spp. have the potential to be a biocontrol agent against soil-borne pathogens such as *Sclerotinia*, *Fusarium*, *Pythium* and *Rhizoctonia* species in cotton and other crops (Hassanein 2010; Hassanein et al. 2010; Mukhopadhyay and Kumar 2020). To identify the most virulent isolate of *Trichoderma* spp. against both *Rhizoctonia* and *Fusarium*, various *Trichoderma* spp. isolates were screened. To compare with fungicide treatments, the most virulent strain of *Trichoderma* was also tested to assess how seed treatments applied both alone and in conjunction with other biological agents affected the seed- and soil-borne pathogens of cotton. Laboratory and field studies were conducted in 2017 and 2018 to find out the best treatment combination for the management of root rot and wilt diseases in cotton (*G. hirsutum* and *G. arboreum*).

Methods

Pathogen and biocontrol agents

The fungal isolates and biocontrol agents were collected from the farmers' fields in Punjab, Haryana and Rajasthan states of India during the cotton growing season (April to November). Samples collected from the farmer's field were confirmed for root rot pathogens under a compound microscope. The fungal cultures were purified and maintained on potato dextrose agar (PDA). The fungal isolates of *R. solani*, *F. oxysporum* and *Trichoderma* spp. were identified based on morphological characteristics and microscopic observations. Identification of promising *Trichoderma* spp. was characterized and confirmed at the molecular level using internal transcribed spacer (ITS) region—ITS1/ITS4 primers and sequences were submitted to NCBI (Huang and Madan 1999). Based on location and mycelia growth variability, the six *R. solani* from Haryana, Punjab and Rajasthan, and two *F. oxysporum* f. sp. *vasinfectum* from Haryana and Rajasthan were purified. Six *Trichoderma* spp. isolates from cotton fields in Haryana, Punjab and Rajasthan were also purified. The pathogenicity of the *R. solani* and *F. oxysporum* f. sp. *vasinfectum* isolates was confirmed. All the purified cultures of pathogens and biocontrol were stored on PDA plates/slants in the frost-free refrigerator at 4℃ temperature (Celfrost Middlebay Company) until further study.

Laboratory bioassay study

The dual culture bioassay was conducted to find out the most antagonistic/virulent isolates of *Trichoderma* spp. for their ability to suppress the mycelial growth of root rot pathogens. All six isolates of *Trichoderma* spp. were evaluated against root rot and wilt-causing fungal isolates. In in vitro dual culture assay, an agar disc (6 mm) was taken from 7-day-old PDA culture plates of each *Trichoderma* isolate and placed at the periphery of the PDA plates (9 mm). Another same size agar disc of *Rhizoctonia* sp. or *Fusarium* sp. isolate was also placed at the periphery on the opposite end of the same Petri dish separately for both the pathogen isolates. Petri dishes were placed in the dark and incubated at 25 °C until the PDA medium was completely covered with pathogen mycelia. Negative control was also maintained. Experiments were conducted in a completely randomized design (CRD), and each treatment was replicated thrice. Observations were recorded on the dual culture plates, 7 days after incubation. The radial growth of *Rhizoctonia* sp. and/or *Fusarium* sp. mycelial towards the antagonistic fungus (Ri) and that on a control plate (Rc) were measured, separately. The percentage inhibition of radial growth was calculated using the formula: $(Rc - Ri)/Rc \times 100$.

Seed germination and seedling vigour assay study

An in vitro bioassay was also conducted to evaluate the effect of different treatments on seed germination and seedling vigour index. The blotter test bioassay was used to evaluate the effect of eleven seed treatments alone and in combinations, *i.e. T. asperellum* (Th-11 c.f.u. 2×10^8/g) @10 g/kg seed, *P. fluorescens* (Pf) (IP Ltd commercial formulation c.f.u. 2×10^8/g) @10 g/kg seed, arbuscular mycorrhizal fungi (AMF) (HBTI Ltd commercial formulation, 1200 IP/g) @20 g/kg seed, a combination of Th-11 + Pf, Th-11 + AMF, Pf + AMF, Th-11 + Pf + AMF, carbendazim @2 g/kg seed, thiophanate-methyl@2 g/kg seed, carboxin 75%WP @2 g/kg seed and propiconazole 25%EC @ 2 ml/kg seed. An untreated control was also maintained for the comparison. For each treatment, 50 cotton seeds of each variety CSH-3129 (*G. hirsutum*) and CICR-3 (*G. arboreum*) were placed on the moist blotting sheets (20 × 20 cm). The sheets were then rolled, and rubber bands were used to keep them intact and placed in the glass beaker filled with sterilized distilled water up to 1/3 level. For each treatment, three replications were maintained. The seed germination, root and shoot length of the seedlings were recorded, 15 days after incubation/treatment at 25 ± 2 °C in BOD. The seedling vigour index was calculated by using the formula given by Abdul-Baki and Anderson (1973): germination (%) x (shoot length + root length).

On-station field trails

On-station field trials were conducted in a randomized complete block design (RCBD) in the root rot sick field for two consecutive years (2017–18 and 2018–19). Twelve seed treatments, namely *T. asperellum* (Th-11, c.f.u. 2×10^8/g) @10 g/kg seed, *P. fluorescens* (Pf, c.f.u. 2×10^8/g) @10 g/kg seed, AMF, 1200 IP/g @20 g/kg seed, combined Th + Pf, Th + AMF, Pf + AMF, Th + Pf + AMF, carbendazim @2 g/kg seed, thiophanate-methyl@2 g/kg seed, carboxin 75% WP @2 g/kg seed and propiconazole 25% EC @ 2 ml/kg seed, were evaluated. An untreated control was also maintained for the comparison. Each treatment was replicated thrice. Observations were recorded at 15-day intervals starting from 15 days after sowing (DAS).

Statistical analysis

Laboratory and field study data including the percentage inhibition of fungal pathogen radial growth in Petri plates, seed rotting and vigour index in blotter test, and plant mortality in field conditions were statistically analysed using analysis of variance (ANOVA) of completely randomized design (CRD) and randomized complete block design (RCBD), respectively. The treatment effects were compared to the least significant difference test (LSD) at $P \leq 0.05$ with the help of the computer program OP STAT (Sheoran et al. 1998). To compare the overall development of disease under each treatment during the season, the area under the disease progress curve (AUDPC) was calculated for individual treatment for both the cultivars, separately from the calculated disease severity values of the original root rot severity data collected at 30, 45, 60, 75 and 90 DAS using the formula of Wilcoxson et al. (1975): AUDPC = Σ [(Xi + Xi + 1)/2]ti −t, where "Xi" and "Xi" + 1 are severity in the form of per cent CLCuD incidence on the date "i" and date "I" + 1, respectively, and "ti" is the number of days between date "I" and date "i" + 1.

Results

Laboratory study on mycelial growth inhibition

The confrontational assays revealed that all the *Trichoderma* spp. isolates showed a variable response for inhibition of mycelial growth to the individual isolates of *R. solani* and *F. oxysporum* f. sp. *vasinfectum.* Among six *Trichoderma* spp. isolates, *T. asperellum* (Th-11) was observed to be superior over other *Trichoderma* spp. isolates in terms of in vitro inhibition of mycelial growth of *R. solani* and *F. oxysporum* f. sp. *vasinfectum* ranging from 64.4 to 100% (Table 1, Additional file 1: Table S1). Hence, the best performing, *T. asperellum* (Th-11) isolate had the best scope as a seed treatment for the management of root rot and wilt diseases in both *G. hirsutum* and *G. arboreum* cultivars under field situations.

Laboratory studies on seed germination and seedling vigour index

A blotter test study was conducted to evaluate the effect of different seed treatments on seed germination, seed rotting and plant vigour index. The treatments were tested on *G. hirsutum* variety CSH-3129 and *G. arboreum* variety CICR-3 for up to 15 days. Data indicated that in both CSH-3129 and CICR-3, the lowest seed rotting was recorded with the combined seed treatment of *T. asperellum* (Th-11) + *P. fluorescens* + AMF (8 and 17.6%), followed by propiconazole 25% EC (29.4 and 31.6%) and *T. asperellum* (Th-11) + *P. fluorescens* (37.5 and 40.7%). Overall, the highest plant vigour index in CSH-3129 at 15 DAT was recorded with the combined seed treatment of *T. asperellum* (Th-11) + *P. fluorescens* + AMF (890.9%), followed by *Trichoderma* alone (833.9%) and propiconazole 25%EC (715.1%). The highest plant vigour index in CICR-3 at 15 days after treatment (DAT) was recorded with the combined seed treatment of *T. asperellum* (Th-11) + *P. fluorescens* (393.5%), followed by combined treatment of *T. asperellum* (Th-11) + *P. fluorescens* + AMF (371.6%) and propiconazole 25% EC (345.3%) (Table 2, Additional file 1: Table S2).

Field experiments

Field experiments in root rot sick soil carried out over two seasons (2017–18 and 2018–19) revealed that the untreated control group, 90 days after sowing (DAS)

Table 1 Mycelial growth inhibition of root rot and wilt disease-causing pathogen isolates by *Trichoderma* spp. isolates

Isolates (antagonist/pathogenic)	***Rhizoctonia solani***		***Fusarium oxysporum* f. sp. *vasinfectum***	
	Per cent inhibition range of six isolates $	**Average inhibition of six isolates*#**	**Per cent inhibition range of two isolates $**	**Average inhibition of two isolates*#**
Trichoderma virens Th-1 (S3OP581431)	33.3–77.8	52.4(46.5)	69.0–100.0	84.5(73.1)
T. virens Th-2	11.1–53.3	30.8(32.8)	37.5–58.5	48.0(43.8)
T. asperellum Th-4 (OP581434)	47.9–77.8	62.6(52.5)	71.0- 100.0	85.5(73.7)
T. asperellum Th-11 (OP581435)	64.4–90.0	77.9(62.6)	81.0–100.0	90.5(77.1)
T. asperellum Th-12	0.0–44.4	24.5(25.4)	18.8–51.5	35.1(35.7)
T. asperellum Th-102	12.8–54.7	29.9(32.3)	32.5–70.0	51.2(45.75)
LSD $P \leq 0.05$		4.21		3.24
SE(m)		1.43		1.15

$ Three isolates of *R.solani* from Haryana state, two from Punjab and one from Rajasthan state, and one Fov isolate each from Haryana and Rajasthan

Figures are the average inhibition of six isolates of *Rhizoctonia solani* and two isolates of *Fusarium oxysporum*. f. sp. *vasinfectum*, and detailed data with statistical analysis are in Additional file 1: Table S1

* Figures in parentheses are arcsign values

Table 2 Effect of bioagents and fungicides on seed germination and plant vigour index of *Gossypium hirsutum* and *G. arboreum* cotton cultivars

Treatments	***Gossypium hirsutum* variety CICR 3129***		***G. arboreum* variety CICR-3***	
	Seed rotting %	**Vigour index$**	**Seed rotting %**	**Vigour index$**
Trichoderma asperellum (Th-11)	48.9	833.8	57.1	322.2
Pseudomonas fluorescens (Pf)	41.2	608.0	55.6	312.0
Arbuscular mycorrhizal fungi (AMF)	56.0	421.1	68.2	230.0
Th + Pf	40.7	448.9	37.5	393.5
Th + AMF	72.5	380.0	57.6	319.9
Pf + AMF	60.0	214.4	57.1	293.1
Th + Pf + AMF	8.3	890.9	17.6	371.6
Carbendazim	57.1	466.2	65.5	191.4
Thiophanate-methyl	57.7	293.3	63.6	157.7
Carboxin 75%WP	50.0	363.4	53.8	259.2
Propiconazole 25%EC	29.4	715.1	31.6	345.3
Control	73.1	138.0	71.4	202.2
LSD $P \leq 0.05$	1.375	172.3	2.251	1940.3
SE(m)	0.455	7.631	0.744	5.564

* Observations were recorded 15 days after sowing

$ The vigour index (%) is calculated based on per cent germination multiplied by sum of root and shoot length, detailed data with statistical analysis are in Additional file 1: Table S2

during both seasons, had the highest root rot incidence of 81 and 44.2%, in the *G. arboreum* cultivar CICR-3, respectively (Table 3). Similar to this, in *G. hirsutum* cultivar CSH-3129, the highest root rot incidence in the untreated control was observed at 49.7 and 27.2%, in 2017–18 and 2018–19, respectively, at 90 DAS (Table 4). The area under the disease progress curve (AUDPC) was determined independently for each treatment, and measurements of the percentage mortality were taken every two weeks between 30 and 90 DAS. The combined seed treatment with *T. asperellum* (Th-11) + *P. fluorescens* + AMF and seed treatment with *T. asperellum* (Th-11) alone throughout both years had the lowest AUDPC in CSH-3129 (Table 3, Additional file 1: Table S3). The seed treatment with *T. asperellum* (Th-11) + *P. fluorescens* during both years had the lowest AUDPC in CICR-3 (Table 4, Additional file 1: Table S4). However, at 60 DAS in *G. arboreum* CICR-3 and *G. hirsutum*

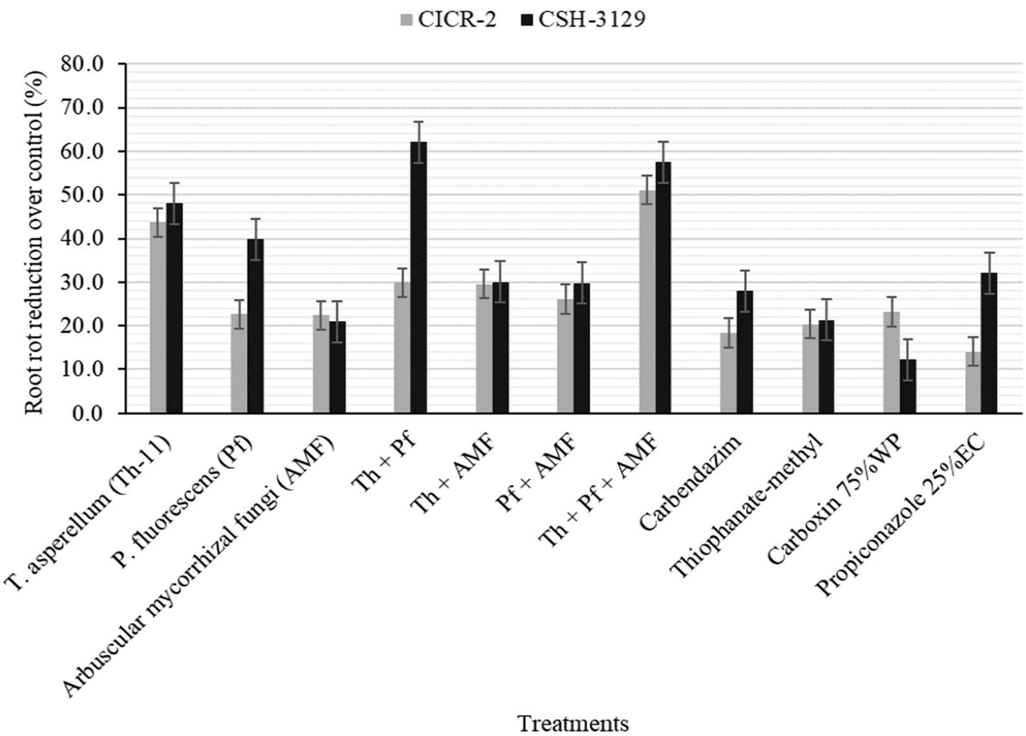

Fig. 1 Effect of seed treatment with biocontrol agents and fungicides on reduction of root rot in *Gossypium arboreum* and *G. hirsutum* cultivars under sick field experiment at 60 days after sowing under sick field conditions (Pooled data of 2017 and 2018)

Table 3 Effect of bioagents and fungicides on root rot incidence in *Gossypium arboreum* cultivar CICR-3

Treatments	Mortality (%) at different DAS[#] 2017–18*			AUDPC[$]	Mortality (%) at different DAS 2018–19			AUDPC
	30	60	90		30	60	90	
Trichoderma asperellum (Th-11)	28.1(31.3)	51.8(46.0)	57.1(49.2)	639.0	2.1(4.9)	10.2(18.6)	19.8(26.3)	201.0
Pseudomonas fluorescens (Pf)	38.1(37.8)	67.1(55.5)	69.9(57.7)	789.8	1.5(5.8)	18.0(25.1	22.4(28.2)	222.0
Arbuscular mycorrhizal fungi (AMF)	32.3(34.2)	52.2(46.1)	71.3(57.9)	777.0	3.0(8.2)	33.2(35.2)	39.8(39.1)	357.8
Th + *Pf*	20.9(27.1)	62.8(52.7)	67.2(56.9)	637.5	3.9(8.9)	14.3(22.1)	23.1(28.7)	264.0
Th + AMF	38.5(37.7)	62.7(52.5)	69.0(57.0)	825.0	1.1(3.5)	14.8(22.5)	27.4(31.5)	248.3
Pf + AMF	25.1(28.8)	58.1(55.0)	67.6(60.5)	695.3	0.0(0.0)	23.2(28.6)	28.6(32.3)	273.0
Th + *Pf* + AMF	24.4(29.3	45.0 (42.0)	56.1(48.5)	603.8	1.5(4.0)	8.8(17.2)	20.3(26.7)	196.5
Carbendazim	35.2(36.1)	68.8(57.2)	80.8(65.0)	870.0	3.8(9.0)	21.0(27.1)	23.7(29.1)	263.3
Thiophanate-methyl	24.8(28.9)	66.7(56.1)	66.7(56.1)	686.3	4.0(9.0)	20.9(27.2)	25.6(30.4)	257.3
Carboxin 75%WP	19.6(25.1)	67.8(56.4)	76.1(59.4)	717.8	2.2(6.9)	16.7(24.1)	23.3(28.8)	239.3
Propiconazole 25%EC	38.4(37.8)	70.4(57.4)	73.2(59.4)	837.0	15.3(22.7)	24.1(29.4)	23.3(28.8)	320.3
Control	42.9(40.8)	71.9(63.1)	81.0(73.6)	929.3	26.8 (31.1)	38.0 (38.1)	44.2 (41.7)	573.8
LSD $P \leq 0.05$	N/A	N/A	N/A	234.4	10.8	4.1	3.8	193.9
SE(m)	4.6	7.5	9.5	6.535	3.7	1.4	1.3	5.634

[*] Figures in parentheses are arcsign values

[$] AUDPC = Area under disease progress curve, detailed data with statistical analysis are in Additional file 1: Table S3

[#] Days after sowing

Table 4 Effect of bioagents and fungicides on root rot incidence in *Gossypium hirsutum* cultivar CSH-3129

Treatments	Mortality (%) at different DAS[#] 2017–18*			AUDPC[$]	Mortality (%) at different DAS 2018–19			AUDPC
	30[#]	60	90		30	60	90	
Trichoderma asperellum (Th-11)	3.9(6.7)	27.4(31.4)	39.0(38.5)	293.3	3.5(10.7)	9.9(18.3)	15.0(22.7)	167.3
Pseudomonas fluorescens (Pf)	2.2(5.0)	30.8(33.3)	32.6(34.7)	261.0	3.3(10.5)	12.4(20.7)	17.9(24.7)	173.3
Arbuscular mycorrhizal fungi (AMF)	19.1(20.9)	39.1(38.1)	46.2(42.8)	463.5	1.0(5.7)	17.7(24.8)	23.8(29.1)	194.3
Th + Pf	5.3(11.0)	21.7(26.7)	32.6(34.7)	202.5	0.7(4.7)	5.5(13.5)	10.2(18.5)	96.8
Th + AMF	18.8(24.7)	34.6(35.8)	48.9(43.8)	433.5	4.1(11.7)	15.6(23.2)	21.2(27.4)	204.8
Pf + AMF	13.6(17.8)	32.6(34.7)	49.3(44.6)	361.5	5.1(13.1)	17.8(24.9)	21.1(27.3)	234.0
Th + Pf + AMF	7.4(12.6)	18.9(24.8)	39.5(38.8)	324.0	3.7(11.1)	11.6(19.7)	12.8(20.7)	159.0
Carbendazim	8.3(10.0)	35.8(35.7)	49.3(44.6)	330.8	1.1(6.1)	15.9(23.8)	17.9(24.9)	183.8
Thiophanate-methyl	7.4(9.4)	39.1(38.1)	48.6(44.0)	379.5	3.4(10.6)	17.4(24.6)	25.0(30.0)	231.8
Carboxin 75%WP	10.7(18.5)	45.2(42.3)	48.4(44.2)	443.3	2.5(9.1)	17.8(24.8)	25.0(29.9)	234.8
Propiconazole 25%EC	15.6(19.1)	36.9(37.3)	49.3(44.6)	476.3	6.2(14.5)	11.9(20.1)	13.6(20.9)	162.0
Control	24.5(23.9)	49.7(44.8)	49.7(44.8)	556.5	7.9(16.3)	22.0(27.7)	27.2(31.3)	309.8
LSD $P \leq 0.05$	N/A	N/A	N/A	181.3	N/A	4.6	5.6	156.3
SE(m)	6.8	6.3	6.3	4.524	3.9	1.6	1.9	5.142

[*] Figures in parentheses are arcsign values[$]AUDPC = Area under disease progress curve, detailed data with statistical analysis are in Additional file 1: Table S4

[#] Days after sowing

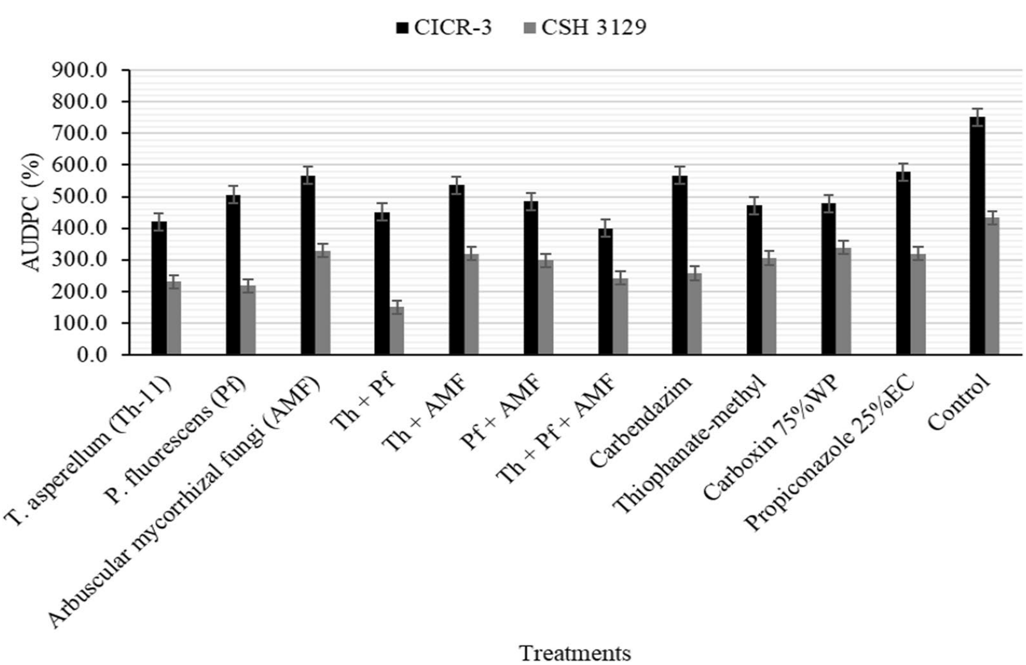

Fig. 2 Effect of seed treatment with biocontrol agents and fungicides on the area under disease progress curve (AUDPC) in *Gossypium arboreum* and *G. hirsutum* cultivars under sick field experiment (Pooled data of 2017 and 2018)

CSH-3129 cultivars, the combined seed treatment with *T. asperellum* (Th-11) + *P. fluorescens* + AMF, followed by *T. asperellum* (Th-11) + *P. fluorescens* and *T. asperellum* (Th-11) alone, demonstrated the lowest plant mortality and AUDPC for both years (Figs. 1, 2).

Overall, the combined seed treatment with *T. asperellum* (Th-11) @ (10 g/kg), *P. fluorescens* (10 g/kg) and AMF (20 g/kg) led to the highest reduction in root rot and wilt disease over control in both the cultivars (57.5

and 51%), respectively, up to 60 days after sowing under the root rot sick field during 2017–18 and 2018–19.

Discussion

Continuous and indiscriminate use of fungicides increases the likelihood of adverse effects on the environment. Bioagents offer a sustainable option in integrated management that can dramatically reduce disease in modern agriculture. However, the use of antagonistic fungi alone may not provide their full disease control and plant growth potential holistically. Six *Trichoderma* spp. isolates were evaluated for their in vitro antagonistic effect on root rot and wilt-causing pathogens, seed germination and plant vigour index. Further, the best-performing *T. asperellum* (Th-11) was used for further laboratory and field evaluation studies and compared to fungicides and in combination with other biocontrol agents. The best performing *T. asperellum* (Th-11) isolate showed the best results as a seed treatment for the management of root rot (64.4%) and wilt (100%) diseases in both *G. hirsutum* and *G. arboreum* cultivars under field situations. Similarly, Elad et al (1982) demonstrated that the seed coating with *T. harzianum* reduced 83 and 43–60% root rot incidence in cotton caused by *R. solani* in greenhouse and field conditions, respectively. The previous study conducted by other researchers observed isolates variability in the *Trichoderma* spp. (Naher et al. 2019). Among 27 isolates of *Trichoderma*, three isolates were found to be promising against *Macrophomina phaseolina* disease in cotton under outdoor pot experiment and they significantly increased survival, and improved plant height and dry weight of the surviving cotton seedlings (Abd-Elsalam 2010). Similarly, out of six different *Trichoderma* species isolated from the rhizosphere of paddy, banana, oil palm, rubber, vegetables and grassland soils, *T. parareesei* showed the highest antagonistic activity (91.1%) against *F. oxysporum* (Naher et al. 2019). *Trichoderma* spp. have been reported to stimulate plant growth, and enhance germination, plant survival, nutritional absorption, and growth of roots and shoots in addition to control of soil-borne disease (Mukhopadhyay and Kumar 2020). On the other hand, the root and shoot length of cotton varieties was also reported to be enhanced with carbendazim followed by thiophanate-methyl (Rajput et al 2006). Treating cotton seeds with *P. fluorescens* or pyrrolnitrin at the time of planting in *R. solani*-infested soil increased seedling survival from 30 to 79% and from 13 to 70%, respectively. Pyrrolnitrin an antibiotic produced by *P. fluorescens* and inhibitory to fungi associated with the cotton seedling disease complex can persist for up to 30 days in moist non-sterile soil without measurable loss in its activity (Howell and Stipanovic 1979). *T. virens* has been found to enhance plant biomass production and lateral root growth through an auxin-dependent mechanism, mitogen-activated protein kinase 6 and ethylene and auxin signalling pathways (Contreras-Cornejo et al. 2015). However, in addition to the ability of *Trichoderma* spp. to attack or inhibit the growth of plant pathogens directly, recent discoveries indicated that they can also induce systemic and localized resistance to a variety of plant pathogens, nematodes and insect pests in cotton and other crops and can be used in integrated pest management programmes (Kumar et al. 2019). Reduction in root rot caused by *R. solani* on cotton seedlings and mycoparasitism of *Pythium* were found to be solely due to induced resistance by *T. harzianum* (Shoresh et al 2010). Better root colonization for a longer duration supported this mechanism in controlling plant diseases successfully (Cai et al. 2015). Moreover, the genus of *Trichoderma* is also recognized as endophytic fungi on roots or leaf tissue and sapwood and provides sorts of additional advantages to their host (Cummings et al. 2016).

According to the results of the evaluation of the 12 biological and fungicide treatments applied as a seed treatment on two cultivars, seed treatment with *T. asperellum* (Th-11) and a combined application of *T. asperellum* (Th-11) and *P. fluorescens* were the best treatments. The field experiment on combined seed treatment with the most virulent isolate, *T. asperellum* (Th-11) @ (10 g/kg), *P. fluorescens* (10 g/kg) and AMF (20 g/kg), led to the highest reduction in root rot and wilt diseases over control in both the cultivars (57.5 and 51%) up to 60 days after sowing under the root rot sick fields during 2017–18 and 2018–19, respectively (Figs. 1, 2). Consequently, it can be inferred that the seed treatment with *T. asperellum* (Th-11) mixed with *P. fluorescens* and arbuscular mycorrhizal fungi followed by seed treatment with *T. asperellum* (Th-11) alone and the combined seed treatment with *T. asperellum* (Th-11) + *P. fluorescens* could be one of the best options for the management of root rot and wilt diseases in *G. arboreum* CICR-3 and *G. hirsutum* CSH-3129 cultivars in an integrated disease management programme up to 60 DAS. These treatments were not performed in the same manner up to 90 days after sowing, and the disease severity also rose at the same time. This might be a result of the pathogens' increased inoculum in the sick field. The long-term effect of *T. harzianum* on the *Fusarium* wilt of cotton has been reported by using *T. harzianum* formulation (2×10^7 and 2×10^8 micro-conidia/kg soil) in successive plantings (Sivan and Chet 1986). However, the existence of pathogenic variability among the isolates of root rot pathogens to cause variable disease severities and increases in inoculum level from 2 to 4 per cent significantly increased seedling mortality (Monga et al. 2004). The antagonists remained in the soil for three successive plantings, decreasing the occurrence of *Fusarium* wilt in each growth cycle with the most preparation needed for the initial sowing (Sivan and Chet 1986). According to reports, the antagonistic mechanism of the *Trichoderma* isolates against the root rot pathogens and root colonization by these fungi has been shown to generate major changes in the plant metabolic machinery and give long-term disease control impact (Zin et al., 2020). *T. virens* may play a key role in the biological control of *R. solani*-induced cotton seedling disease by inducing defence responses, particularly terpenoid production, in cotton roots (Howell et al 2000). The seed treatment in cotton with *T. virens* plus metalaxyl resulted in greater seedling stands compared to those in untreated controls and equal to those of the fungicide control, except where disease pressure was very heavy or light (Howell et al. 1997). In addition, AMF became increasingly recognized to promote plant growth and productivity and protecting plants from the negative effects of both abiotic and biotic stresses (Song et al. 2011). *T. koningii* CCM341 gave the highest disease control percentage (100%) in saline and non-saline soil, and *T. harzianum* CCM340, *T. longibrachiatum* NRRL11236 and *T. viride* DSM63065 gave the same disease control percentage (100%) in non-saline soil in greenhouse test against *F. oxysporum* f. sp. *vasinfectum* and *R. solani*, the causal agents of wilt and root rot of cotton (Hassanein 2012).

Conclusion

The results of the study revealed that the seed treatments with *T. asperellum* (Th-11, c.f.u. 2×10^8/g) @10 g/kg + *P. fluorescens* (c.f.u. 2×10^8/g) @10 g/kg and arbuscular mycorrhizal fungi (1200 IP/g) @20 g/kg could effectively manage root rot and wilt disease for a period of up to 60 DAS and enhanced cotton plant growth in field conditions depending upon the pathogen inoculum load. The present research and other studies carried out in the past indicated that while applying management tactics for root rot management in cotton, it is important to consider the pathogenic variability and inoculum level of the targeted area. The rate of *T. asperellum* (Th-11)/*P. fluorescens* preparation should also be applied at a high rate to achieve better and long-term effects during severe root rot and wilt incidence or in sick field conditions, where inoculum of *R. solani* and *F. oxysporum* f. sp. *vasinfectum* is very high. Therefore, the formulations of *T. asperellum/P. fluorescens* with high c.f.u. and better shelf life should be prepared, and used as seed and soil application during 2–3 successive planting to achieve better root rot, wilt disease control and plant growth in cotton.

Abbreviations

ANOVA	Analysis of variance
AUDPC	Area under the disease progress curve
CICR	Central Institute for Cotton Research
CRD	Completely randomized design
DAS	Days after sowing
DAT	Days after treatment
EC	Emulsifiable concentrates
IP	Inoculum potential
ITS	Internal transcribed spacer
LSD	Least significant difference
AMF	Arbuscular mycorrhizal fungi
NCBI	National Centre for Biotechnology Information
PDA	Potato dextrose agar
Pf	*Pseudomonas fluorescens*
RCBD	Randomized complete block design
SEm	Standard error of means
Th-11	*T. asperellum*
WP	Wettable powder
WS	Water-soluble granules

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-023-00739-3.

Additional file 1. **Supplementary table 1**. Mycelial growth inhibition of root rot and wilt disease-causing pathogen isolates by Trichoderma spp. isolates. **Supplementary table 2**. Effect of bioagents and fungicides on seed germination and plant vigour index of Gossypium hirsutum and G. arboreum cotton cultivars. **Supplementary table 3**. Effect of bioagents and fungicides root rot incidence in Gossypium arboreum cultivar CICR-3. **Supplementary table 4**. Effect of bioagents and fungicides on root rot incidence in Gossypium hirsutum cultivar CSH-3129.

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Author contributions

SKS has a major contribution in designing, conducting the experiments, and in writing and editing the manuscript, HD implemented the experiment, collected and compiled the data, and AS assisted during the survey. All authors read and approved the final manuscript.

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Declarations

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Not applicable.

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