Trichoderma spp. antagonistic relationship with Fusarium is linked to cotton Wilting

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ABSTRACT

The experiment was carried out at Jamal Mohamed College (A), Triuchirappalli, Tamilnadu, during the Kharif season of 2022–2023, to assess the effectiveness of biological control methods based on *Trichoderma* species in the in vitro management of *Fusarium* species. Using a dual plate experiment, six rhizospheric *Trichoderma* species were isolates from six distinct cotton crop fields were examined in vitro for antagonistic activity against Fusarium wilt infections. *Trichoderma* species, isolate T4, was shown to successfully suppress the radial mycelial growth of the *Fusarium* species by 76.92% under in vitro conditions. The examined *Fusarium* species growth was hindered by the isolates of *Trichoderma* spp. The effects of abiotic stressors as pH, temperature, and NaCl on the growth of *Trichoderma* isolates were also investigated. The isolates with the highest resistance to abiotic stress were T1 and T4. The specific activity of chitinase produced by local isolates was investigated. The NIST and WILEY libraries have provided the results of the T4's ethyl acetate extracted product based on the compounds' retention time, molecular weight, molecular formula, and one chemical structure, which is demonstrated by GCMS analysis. Overall, isolate Tv4 showed the most promise in regulating the development of all *Fusarium* species isolates and demonstrating tolerance to abiotic stressors.

Introduction

According to Cusser *et al*., (2016), cotton is one of the most important crops that provide ecologically sustainable fibre to the world's expanding population. India is the world's largest producer of cotton, with an estimated production of 28.5 million 480 lb. bales in 2020–2021, while the United States is the biggest exporter, with an estimated export of 15 million 480 lb. bales. Nepal produces approximately 92 mt of cotton cropped in an area of 90 ha (Krishi diary, 2019/20). At the moment, cotton is grown in the districts of Dang, Banke, and Bardiya in Nepal on an area of 106, 27, 10 ha, producing 74, 41, and 12 mt of cotton, respectively (MOAD, 2016/17). A variety of biotic and abiotic variables contribute to the decline in cotton fibre production's viability and output. Fusarium wilt (FW, caused by *Fusarium oxysporum* f.sp. *vasinfectum* Atk. Sny & Hans, FOV) and Verticillium wilt (VW, produced by Verticillium dahliae Kleb.) are two soil-borne diseases that pose a significant threat to global cotton output throughout growth (Halpren *et al*., 2018).

By inducing necrosis and wilting signs in many agricultural plants and having a major overall impact on productivity, *Fusarium* species is a soil-borne fungal disease that damages plants

through their roots at all phases of growth, resulting in severe economic losses. Wilted plants, yellowed leaves, and root rot are the hallmarks of the illness brought on by this fungus, which results in little or no crop output. When it comes to plants, *Fusarium* sp. is the most harmful species in the genus due to its numerous pathogenic forms. The literature pool on agricultural research has received several new reports of Fusarium diseases (Anjat and Kahkashan, 2012).

For over 70 years, researchers have studied the biological control mechanisms of *Trichoderma* spp. (Martinez *et al*., 2013). Its antagonistic ability to act as biocontrol agents of several diseases that damage economically and agriculturally significant crops has been well documented (Mayo *et al*., 2015). In order to ascertain the antagonistic impact of *Trichoderma* spp. as a biocontrol technique for the complex of phytopathogens that cause chilli wilting in in vitro assessments utilising dual cultures, this study was conducted..

Materials and Methods

Based on the microscopic analysis, *Fusarium* species were isolated and identified after infected cotton plant parts were evaluated. In order to surface sterilise plant parts exhibiting signs of Fusarium wilt infection, they were submerged in 0.3% sodium hypochlorite for ten minutes, followed by 70% ethanol and a thorough rinsing with sterile distilled water. They were placed on petriplates on potato dextrose agar (PDA) medium and cultured for seven days at 26 ± 2oC (Aneja, 2001). Agrios (2005) noted the fungus's distinctive development, which included chlamydospores and the physical characteristics of micro- and macro-conidia. PDA slants were used to maintain pure cultures, which were then refrigerated at 4^{o}C.

Trichoderma species were isolated from rhizosphere soils of healthy cotton fields. It was using a *Trichoderma* selective agar (TSA) medium by Elad *et al*., (1982). The isolates were identified to primarily on the macroscopic (pigmentation, growth rate, colour etc.) and microscopic morphology (spore morphology, formation etc.) according to the method by Gams *et al*., (1980); (Rifaii, 1969).

Competitive interactions between antagonistic *Trichoderma* sp. local isolates and plant pathogenic fungi were evaluated in dual culture experiments on petridishes (90 mm diameter) containing 20 ml of potato dextrose agar (PDA). Two 5 mm diameter mycelial discs cut from 5 day old cultures of pathogenic fungi and Trichoderma sp. were placed at opposite sides, 30 mm apart in petridishes and incubated in darkness at 30^{o}C. Three replicates were prepared for each pairing.

Radial growth reduction was calculated in relation to growth of the control as follows;

% inhibition of mycelial growth = [(C-T)/C]x100

Where,

C is the radial growth of pathogenic fungi in control plates; T is the radial growth of pathogen in presence of Trichoderma Dennis and Webster, (1971).

The influence of temperature on the growth of *Trichoderma* sp. isolates was determined by Poosapati *et al*., (2014). The influence of different NaCl concentrations (0, 50, 100, 150, 200 and 250 mM) on the growth of Trichoderma sp. isolates was determined on PDA for 5 days Mohamed

and Haggag, (2005). Chitinase activities of isolates were determined by following the released of 1 mol GLcNAc from chitin Elad *et al*., (1982).

The volatile metabolites were analysed by gas chromatography with a single quadrupole mass spectrometer detector (GC-MS) analysis Siddiquee *et al*., (2012). Trichoderma species-derived dry methanolic extracts were diluted in ethyl acetate and put in a 1 mL glass vial before analysis. An HP-5MS (30 m × 0.25 mm and 0.25 µm 5% diphenyl/95% dimethylpolysiloxane) capillary column (J and W Scientific, Folsom, CA, USA) was included with the GC system. The device was set up to begin at 40˚C (held for two minutes), rise to 160˚C by 6˚C/min, and then rise to 260˚C by 10˚C/min (held for four minutes). The carrier gas, which had a flow rate of 1 mL/min, was 99.9% helium. In full-scan acquisition mode, the mass range was 40–450 Da, the ion source temperature was set at 230˚C, and the ionising electron energy was 70 eV Khan *et al*., (2021). The GC-MS spectra of known chemicals from the National Institute of Standards and Technology (NIST) database were compared to the spectra of the volatile metabolites that were discovered. 90% similarity was the identification threshold.

The experimental design was Completely Randomized Design (CRD) with three replicates as described by Gomez and Gomez, (1984). Test of variance was calculated using Analysis of variance (ANOVA) and statistical F-tests were evaluated at $P \leq 0.05$. Differences among treatment means for each measured parameter were further separated using fishers Least Significance Difference (LSD) to determine levels of significance according to Cochran and Cox, (1992).

Results and discussion

Trichoderma sp. isolates were employed in this investigation. During the cropping season, *Trichoderma* species were isolated from the rhizosphere soils of healthy cotton that were gathered from the fields. *Trichoderma* sp. isolates' mycelial development was investigated in medium with varying pH and temperature (Table 1).

Isolates	**pH**			**Temperature (ºC)**		
	5	6	7	15	20	30
T1	130.3	88.5	70.1	192.4	176.1	168.4
T2	109.1	79.1	67.2	170.3	167.1	150.5
T3	102.2	79.2	65.7	172.8	161.4	151.6
T4	133.2	86.5	70.2	181.1	180.0	170.2
T5	111.3	82.3	60.3	164.0	160.7	155.1
T6	129.6	84.7	69.4	180.5	174.3	166.1

Table 1. The medium mycelial growth (mm) of isolates at different pH and temperature levels.

Isolates of *Trichoderma* sp. were tested for mycelial development in medium with varying NaCl concentrations (Table 2). Table 4 shows that the T1, T3, T4, T5, and T6 isolates' growths were unaffected by 50 mM NaCl. The greatest rate of inhibition of T2 and T5 mycelial growths (72.6 % and 61.2 %, respectively) was seen at 250 mM NaCl. Increased salt levels had varying effects on isolates' development. In 100 mM NaCl, the T4 isolate's growth was unaffected. T1 and T4 were shown to be the most vulnerable isolates to abiotic stress, whereas T6 was the most resistant. Based on Poosapati *et al*., (2014), salt was shown to be one of the environmental factors limiting the antagonistic activity of *Trichoderma* species. The antifungal metabolites of the isolates

have been described as providing protection against salt. Rawat *et al*., (2013); Mohamed and Haggag, (2005).

Isolates	NaCl (mM)					
	0	50	100	150	200	250
T1	-	-	3.3	8.8	13.6	21.5
T2	-	1.6	10	13.7	26.7	72.6
T3	-	-	11.1	15.6	25.8	52.7
T4	-	-	-	8.9	15.3	33.8
T5	-	-	8.9	14.2	21.7	61.2
T6	-	-	3.3	11.1	11.5	37.8

Table 2. Inhibition (%) of mycelial growth of *Trichoderma* sp. isolates in NaCl levels.

Results showed that the antagonistic activity of *Trichoderma* sp. isolates against the tested *Fusarium* species varied, with the highest percentage of inhibition in F3 (93.2%) with T2 and T4 isolates (Table 3). Under culture conditions, *Trichoderma* isolates grew considerably quicker on PDA than the tested *Fusarium* species. T1 and T6 isolates were 84.6 percent effective against F2. T5 isolate reduced F1 growth at an 86.4% rate. T3 isolate was 80.8% effective against F4. Similarly, 72 hours after inoculation, *T. harzianum* has a mean diameter of 42.5–56.5 mm at 25–30°C, according Matrood *et al*., (2020). Mycelia from the isolates covered the Petri dishes' surface after 96 hours of inoculation, growing at a pace of 20.44 mm day-1 with negligible differences from one another. After four to five days after inoculation, the hyphae coloured bright or dark green, and the mycelia expanded and covered the surfaces of the dishes. Another one, Li *et al*., (2017) reported *T. asperellum*, *T. harzianum* as most effective growth inhibitors of *Fusarium* species under in vitro culture. *Trichoderma* isolates grew much faster on PDA than the tested *Fusarium* species under culture conditions.

Isolates	F1	F2	F3	F4
T1	77.9 ± 2.33^{b}	84.6 ± 2.53^{a}	79.4 ± 2.38^{c}	80 ± 2.4^{b}
T2	73.3 ± 2.19^{c}	68.7 ± 2.06^{d}	93.2 ± 2.79^{a}	56.7 ± 1.70^{d}
T3	80 ± 2.4^{b}	71.4 ± 2.14^{c}	80.3 ± 2.40^{b}	80.6 ± 2.42^{b}
T4	73.3 ± 2.19^{c}	82.7 ± 2.48^{b}	93.2 ± 2.79^{a}	79.4 ± 2.38^{c}
T5	84.4 ± 2.59^{a}	73.3 ± 2.19^{c}	83.7 ± 2.51^{b}	77.9 ± 2.33^{c}
T6	82.2 ± 2.46^{a}	84.7 ± 2.53^{a}	79.3 ± 2.34^{c}	82.3 ± 2.46^{a}

Table 3. Inhibition rate (%) of growth of pathogenic fungi by *Trichoderma* spp. local isolates in dual culture.

We have compared the activity of chitinase of *Trichoderma* isolates are seen in Table 4. When the results of each chitinase activity were compared, the highest enzyme production was observed in T4 (43.6 mU mg protein-1). The significance of the difference in values was determined through ANOVA at a significance level of 0.01. The lowest chitinase activity was obtained in T3 (9.2 mU mg protein-1). The ability of chitinase to convert oligomer-chitin into monomer chitin may be the cause of the antagonistic process, which allows *Trichoderma* spp. to suppress harmful fungus (Silva *et al*., 2019). Furthermore, *Trichoderma* enhances regressive efficiency by breaking down cell walls and deforming pathogenic hyphae and cells. It does this by

combining chitinase, endo-β-1,3-glucanase, and exo-β-1,3-glucanase to disintegrate the cell walls of pathogenic fungus (Kaur *et al*., 2021).

Isolates	Specific activity (mU mg protein^{-1})
T1	31±0.93^{b}
T2	27±0.81^{c}
T3	9.2±0.27^{f}
T4	43.6±1.30^{a}
T5	17±0.51^{e}
T6	24±0.72^{d}
Variance	140.012
Mean	25.3

Table 4. Chitinase activities by *Trichoderma* sp. isolates and analysis of variance

The ethyl acetate extract of antagonistic *T. viride* contained thirty components, according to GC-MS analysis, of which fifteen were found. By comparing the retention time, molecular formula, and molecular weight with the Wiley spectral library search tool, the identification of the chemical ingredients was verified. Fig 1 displays the active principles together with their molecular weight, molecular formula, and retention time (RT). Table 5 contains fifteen molecule from the isolate. Additionally, the volatile secondary metabolites found in methanolic extracts of the two *Trichoderma* species under investigation (*T. asperellum* AU131 and *T. longibrachiatum* AU158) concurred with earlier findings in the literature by Siddiquee *et al*. (2012).

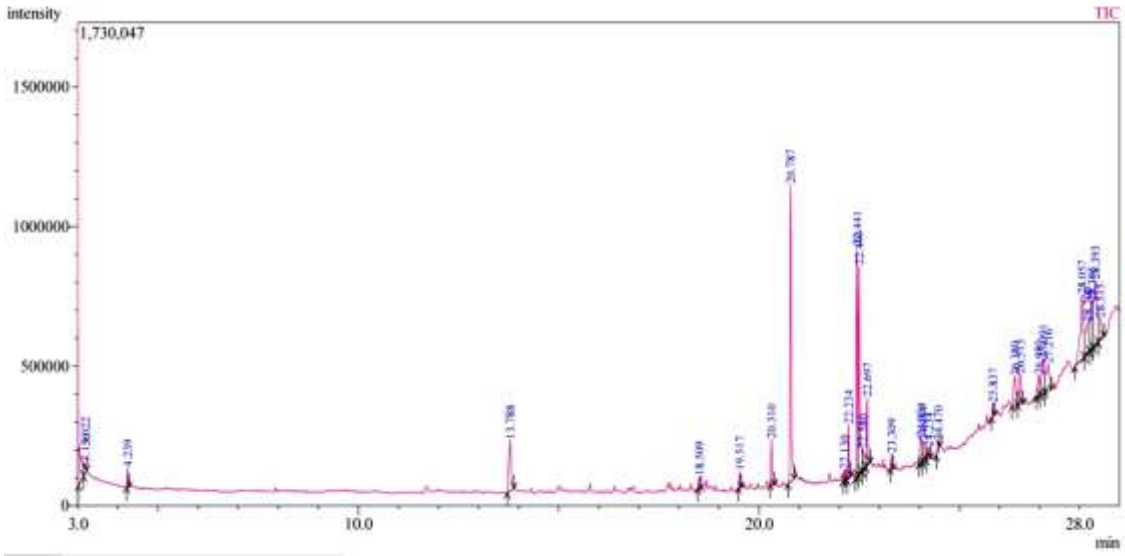

Fig 1. GC-MS chromatogram of ethyl acetate extract of *T. viride*

Peak#	R.Time	Area	Area%	Height	Height%	Secondary Metabolites
1	13.788	357095	0.51	127865	1.15	Benzene, 4-ethenyl-1,2-dimethoxy
2	18.508	160702	0.23	74114	0.67	,4-bis(1,1-dimethylethyl)-Phenol
3	19.518	117751	0.17	72289	0.65	Tetradecanoic acid

4	20.309	375093	0.53	234081	2.11	Pentadecanoic acid, ethyl ester
5	20.588	181391	0.26	71661	0.64	Palmitoleic acid
6	20.680	176874	0.25	93697	0.84	n-Hexadecanoic acid
7	20.781	1768102	2.51	802142	7.22	Linoleic acid ethyl ester
8	22.235	360174	0.51	205488	1.85	Ethyl Oleate
9	22.442	523905	0.74	297255	2.67	Beta-Sitosterol
10	22.491	184542	0.26	91095	0.82	Oleic acid
11	22.654	3188575	4.52	1416801	12.75	Ethyl 15-methyl-hexadecanoate
12	23.309	119076	0.17	69631	0.63	Ergosterol
13	25.480	612859	0.87	65375	0.59	Heptadecanoic acid

Table 5. Volatile organic compounds of *T. viride* identified by GC-MS

Conclusion

In conclusion, cotton (*Gossypium* spp.) may benefit greatly from the use of biocontrol agents. Eighty-four and ninety-two percent of the six *Trichoderma* spp. isolate antagonists shown the strongest inhibitory effect against *Fusarium* species, a cotton-borne disease, in vitro. To ascertain the antagonistic capacity of *Trichoderma* spp. isolates in vitro in suppressing the Fusarium fungus that causes yellow disease in cotton plants, further field testing (in vivo) must be conducted.

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