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In vitro aggression of *Trichoderma* species against *Fusarium* induced wilt disease in cotton

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Abstract

A soil-borne fungus called *Fusarium* species assaults plants through their roots at all stages of growth and results in significant economic losses by causing signs of necrosis and wilting. *Trichoderma* species tested against *Fusarium* species under *in vitro* conditions. In this research, isolate of *Trichoderma* species was found in rhizosphere soils of cotton crops using the dual culture approach. It was discovered that various *Fusarium* species, including F1, F2, F3 and F4 were negatively affected by local isolates of *Trichoderma* sp. The studied *Fusarium* species growth was inhibited by the isolates of *Trichoderma* spp. it was also examined how abiotic stresses including pH, temperature and NaCl affected the development of *Trichoderma* isolates. The isolates T1, T4, and T6 were most resilient to abiotic stress. Chitinase generated by local isolates was examined for its particular activity. In the culture supernatant, the antagonist T4 isolate produced more chitinase activity.

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Introduction

Cotton (*Gossypium* spp.) is a valuable cash crop since it produces grain, fibre, and oil, according to Sunilkumar *et al.* (2006). Cotton is the world's most important source of natural fibre, accounting for around 35% of total fibre production Billah *et al.* (2021). It is a renewable resource, and cotton farms employ more people in planting, processing, and textile production than synthetic fibre. Cotton has significant environmental and social benefits, and following oil extraction, cotton seed may be used as animal feed by Rathore *et al.* (2015). However, cotton has been plagued by a variety of pests and diseases, the most serious of which are *Fusarium* and *Verticillium* wilt, which cause significant global cotton economic losses Davis *et al.*, 2006; Cun *et al.* (2002).

Fusarium species is a soil-borne fungal disease that affects plants through their roots at all stages of growth, causing significant economic losses by generating necrosis and wilting symptoms in many agricultural plants and having a significant overall influence on production. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield. *Fusarium* sp. found in its many pathogenic forms, is the most damaging species of the genus where in plants are concerned. A number of new disease reports on *Fusarium* have been submitted to the literature pool on agricultural research Anajat and Kahkashan (2012).

Many pathogenic microorganisms have developed resistance against chemical fungicides. This seriously hinders the management of diseases of crops and agricultural plants. Considering the deleterious effects of synthetic fungicides on life supporting systems, there is an urgent need for alternative agents for the management of pathogenic microorganisms. Biological control is still in its research phase with few studies reported for bacterial wilt Messiha *et al.* (2007). Therefore, it is necessary to explore new measures for controlling *Fusarium* wilt. Microbial fungicides have been confirmed as effective and environmentally friendly measures to control crop soil borne diseases. *Pseudomonas* species demonstrated a significant reduction in disease incidence and an increase in chickpea growth Khalifa *et al.* (2022); Mozumder *et al.* (2022). Meanwhile, *Trichoderma* sp. effectively suppressed *Fusarium* wilt through competition for nutrients and space, mycoparasitism, antibiosis, and improved plant growth, leading to showing well-controlled efficiency Awad-Allah *et al.* (2022); Do Amaral *et al.* (2022); Rao *et al.* 2022).

Trichoderma species are potential fungal bioagents for the management of various economically important plant pathogens. This species is usually preferred due to rapid growth, antagonistic effect, ability to increase nutrient availability, and uptake, secretion of cell wall-degrading enzymes, ability to produce antibiotics and volatile compounds responsible for antibiosis properties Harman *et al.*, (2004); Sarrocco *et al.* (2006). In this study, we tested selected *Trichoderma* species for their biocontrol ability against the *Fusarium* wilt of cotton under *in vitro* conditions.

Materials and methods

Isolation of pathogenic fungi

Evaluation of infected parts of the cotton plant resulted in isolation and identification of *Fusarium* species based on the examination under microscope. Parts of plants with symptoms of *Fusarium* wilt infection were surface sterilised by immersion in 0.3% sodium hypochlorite for 10 minutes, and then in 70% ethanol and later rinsed thoroughly with sterile distilled water. They were transferred to potato dextrose agar (PDA) medium in petriplates and incubated at 26 ± 2°C for seven days (Aneja, 2001). The characteristic growth of the fungus with morphological characters of micro-conidia and macro-conidia and chlamydospores were observed by (Agrios, 2005). Pure cultures were maintained on PDA slants and stored at 4°C in the refrigerator.

Isolation of antagonist

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).

Dual culture experiments

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°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] ×100

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).

Determination of effects on growth of Trichoderma sp. of abiotic stress factors

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 release of 1 mol GLcNAc from chitin Elad *et al.* (1982).

Statistical analysis

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≤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

Isolates of *Trichoderma* sp. were used in this study. *Trichoderma* species were isolated from rhizosphere soils of healthy cotton during cropping season collected from the fields. The soil was sieved (< 2 mm). The some physico-chemical analysis of soil samples are given in Table 1. The mycelial growth of isolates of *Trichoderma* sp. was examined in media containing different pH and temperature (Table 2).

Table 1. Physical and chemical properties of the soils isolated of *Trichoderma* spp.

Isolates	Some soil properties					
	Organic matter (%)	N (%)	CaCO3 (%)	EC (dS m-1 at 25 °C)	pH	Texture grade
T1	1.33	0.086	29	0.37	8.35	clay
T2	1.46	0.069	34	0.32	8.46	clay
T3	1.99	0.096	26	0.28	8.21	clay
T4	1.57	0.070	19	0.26	8.34	clay
T5	1.37	0.097	39	0.34	8.35	clay
T6	1.99	0.084	30	0.27	7.8	clay

Table 2. The medium mycelial growth (mm) of isolates at different pH and temperature levels.

Isolates	pH			Temperature (°C)		
	5	6	7	15	20	30
T1	130.3	88.4	70.3	192.3	176.1	168.5
T2	109.2	79.0	67.1	170.4	167.1	150.6
T3	102.2	79.2	65.8	172.8	161.5	151.6
T4	132.9	86.5	70.1	181.1	180.2	170.2
T5	111.4	82.1	60.3	164.0	160.7	155.1
T6	129.7	84.7	69.4	180.5	174.3	166.1

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

Isolates	F1	F2	F3	F4
T1	77.8±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]	80.8±2.42[b]
T4	73.3±2.19[c]	82.8±2.48[b]	93.2±2.79[a]	79.4±2.38[c]
T5	86.4±2.59[a]	73.3±2.19[c]	83.8±2.51[b]	77.9±2.33[c]
T6	82.2±2.46[a]	84.6±2.53[a]	79.4±2.34[c]	82.3±2.46[a]
Variance	26.45067	52.23467	44.129	93.23767
Mean	78.833	77.566	84.88	76.183

Table 4. Inhibition (%) of mycelial growth of *Trichoderma* sp. isolates in NaCl levels.

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

T1, T4 and T6 isolates were formed quickly the mycelia at 15°C. Also, the mycelial formation of isolates with the increase of temperature was reduced at 30°C, respectively and the growths of isolates were decreased at increasing different pH levels. T1, T4 and T6 isolates respectively have been observed as the most acidic pH-resistant isolates. At neutral pH isolate T1 produced significantly higher biomass and radial diameter as compared to others. The isolates were showed different tolerance at different temperature and pH treatments. Poosapati *et al.* (2014) observations on *T. asperellum* survived and germinated properly at 28°C and underwent harsh temperature stress conditions. Also, Petrisor *et al.* (2016) reported that *T. viride* strain Td50 grew faster at 25-30°C and very slowly at 15°C.

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. These discrepancies, which might be caused by varied pathogen resistance, are thought to be obtained from the isolates production of various antifungal chemicals. Similarly, Hwang *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.

The mycelial growth of local isolates of *Trichoderma* sp. was examined in media containing different concentrations of NaCl (Table 4). At 50 mM NaCl growths of T1, T3, T4, T5 and T6 isolates were not affected as seen in Table 4. In 250 mM NaCl, the mycelial growths of T2 and T5 were inhibited at the highest rate (72.6 % and 61.2 %, respectively). The growth of isolates affected at different rate in increased salt levels. The growth of T4 isolate was not affected in 100 mM NaCl. The most resistant isolates to abiotic stress were T6, respectively and the most sensitive isolates were examined as T1, and T4. Similar results were noted by salinity was identified as one of the environmental parameters limiting *Trichoderma* species antagonistic activity Rawat *et al.* (2013); Poosapati *et al.* (2014). It has been explained that the isolates antifungal metabolites protect against salt Mohamed and Haggag (2005); Rawat *et al.* (2013). Leo Daniel *et al.* (2011) also reported similar results while *T. viride* characterization for abiotic stress. Aside from a decrease in growth rate, salt content in medium (salinity stress) caused

noticeable alterations in morphology and a steady reduction in sporulation with increasing concentration.

Table 5. Chitinase activities by *Trichoderma* sp. isolates and analysis of variance

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

We have compared the activity of chitinase of *Trichoderma* isolates are seen in Table 5. 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}). Similar findings have been obtained in several *Trichoderma* isolates El-Katatny *et al.* (2006); Kucuk and Kıvanc (2004).

Conclusion

In conclusion, biocontrol agents could play an important role in the protection of cotton (*Gossypium* spp.). Among the 6 *Trichoderma* spp. isolate antagonist which produced the highest inhibitory power against *Fusarium* species, a pathogen on cotton, *in vitro* with an inhibition power has been 86.4 and 93.2 %. Further research needs to be carried out field tests (*in vivo*) to determine the antagonism ability of *Trichoderma* spp. isolates in vitro in inhibiting *Fusarium* fungus that causes yellow disease in cotton plants.

References

Sunilkumar G, Campbell LM, Puckhaber L, Stipanovic RD, Rathore KS. 2006. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. Proc. Natl. Acad. Sci. USA. **103**, 18054-18059.

Billah M, Li F, Yang Z. 2021. Regulatory Network of Cotton Genes in Response to Salt, Drought and Wilt Diseases (*Verticillium* and *Fusarium*): Progress and Perspective. Front. Plant Sci. **12**, 759245.

Rathore KS, Campbell LM, Sherwood S, Nunes E. 2015. Cotton (*Gossypium hirsutum* L.). Methods Mol. Biol. **1224**, 11–23.

Davis RM, Colyer PD, Rothrock CS, Kochman JK. 2006. *Fusarium* Wilt of Cotton: Population Diversity and Implications for Management. Plant Dis., **90**, 692–703.

Cun M, Guiliang J, Chuanlin Z. 2002. The advances in cotton breeding resistance to *Fusarium* and *Verticillium* wilts in China during past fifty years. Zhongguo Nongye Kexue **35**, 508-513.

Anajat B, Kahkashan P. 2012. In vitro inhibition potential of *Phoenix dactylifera* extracts on the growth of pathogenic fungi. Journal of Medicine Plants Research **6**(6)114-123.

Messiha NAS, Van Diepeningen AD, Farag NS, Abdallah SA, Janse Van JD, Bruggen AHC. 2007. *Stenotrophomonas maltophilia* a new potential biocontrol agent of *Ralstonia solanacearum*, causal agent of potato brown rot. European Journal of Plant Pathology **118**, 211-235.

Khalifa MW, Rouag N, Bouhadida M. 2022. Evaluation of the antagonistic effect of *Pseudomonas* rhizobacteria on *Fusarium* wilt of chickpea. Agriculture **12**, 429.

Mozumder AB, Chanda K, Chorei R, Prasad HK. 2022. An evaluation of aluminum tolerant *Pseudomonas aeruginosa* A7 for in vivo suppression of *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* and growth promotion of chickpea. Microorganisms **10**, 568.

Awad-Allah EF, Shams AH, Helaly AA, Ragheb EI. 2022. Effective applications of *Trichoderma* spp. as biofertilizers and biocontrol agents mitigate tomato *Fusarium* wilt disease. Agriculture **12**, 1950.

Do Amaral ACT, De Holanda Cavalcanti Maciel M, Machado AR, De Oliveira LG, Lima CS, Da Costa AF, De Oliveira NT. 2022. *Trichoderma* as a biological agent of *Fusarium oxysporum* species complex and *Vigna unguiculata* growth promoter. Eur. J. Plant Pathol. **163**, 875-890.

Rao Y, Zeng L, Jiang H, Mei L, Wang Y. 2022. *Trichoderma atroviride* LZ42 releases volatile organic compounds promoting plant growth and suppressing *Fusarium* wilt disease in tomato seedlings. BMC Microbiol. **22**, 88.

Harman G, Howell C, Viterbo A, Chet I, Lorito M. 2004. *Trichoderma* species opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. **2**, 43-56.

Sarrocco S, Mikkelsen L, Vergara M, Jensen DF, Lubeck M, Vannacci G. 2006. Histopathological studies of sclerotia of phytopathogenic fungi parasitized by a GFP transformed *Trichoderma virens* antagonistic strain. Mycol. Res. **110**, 179-187.

Aneja KR. 2001. "Experiments in Microbiology, Plant Pathology Tissue Culture and Mushroom Production Technology," New Age International, New Delhi.

Agrios GN. 2005. "Plant Pathology," Elsevier Academic Press.

Elad Y, Chet I, Henis Y. 1982. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica **9**, 59-67.

Gams W, Anderson TH, Domsch W. 1980. Compendium of soil fungi. Academic Press (London) Ltd., London, UK. p 860.

Rifaii MA. 1969. Revision of the genus *Trichoderma*. Mycological Papers **116**, 1-56.

Dennis C, Webster J. 1971. Antagonism properties of species groups of *Trichoderma*, III hypal intreactions. Transactions of the British Mycological Society **57**, 363-369.

Poosapati S, Ravulapalli PD, Tippirishetty N, Vishwanathaswamy DK, Chunduri S. 2014. Selection of high temperature and salinity tolerant *Trichoderma* isolates with antagonistic activity against *Sclerotium rolfsii* Springerplus **3**, 641. DOI: 10.1186/2193- 1801-3-641.

Mohamed HAA, Haggag WM. 2005. Biocontrol Potential of Salinity Tolerant Mutants of *Trichoderma harzianum* against *Fusarium oxysporum* Causing Tomato Wilt Disease. Arab Journal of Biotechnology **8**, 35-48.

Gomez KA, Gomez AA. 1984. Statistical procedures for Agricultural Research (2nd edn). John Wiley and sons, 680pp.

Cochran GW, Cox GM. 1992. Experimental Designs (2nd edn). John willey and Sons Inc, 611pp.

Petrisor C, Paica A, Florica, Constantinescu F. 2016. Influence of abiotic factors on in vitro growth of *Trichoderma* strains. Proc. Rom. Acad. Series., B **18**(1), 11-14.

Li YT, Hwang SG, Huang Y, Huang CH. 2017. Effects of *Trichoderma asperellum* on nutrient uptake and *Fusarium* wilt of tomato. Crop Protection, 2:1-8.

Rawat L, Singh Y, Shukla N, Kumar J. 2013. Salinity tolerant *Trichoderma harzianum* reinforces NaCl tolerance and reduces population dynamics of *Fusarium oxysporum* f.sp. ciceri in chickpea (*Cicer arietinum* L.) under salt stress conditions. Archives of Phytopathology and Plant Protection **46**, 1442-1467.

Leo Daniel AE, Praveen Kumar G, Desai S, Mir Hassan ASK. 2011. In vitro characterization of *Trichoderma viride* for abiotic stress tolerance and field evaluation against root rot disease in *Vigna mungo* L. J. Biofertil Biopestici. **2,** 111.

El-Katatny MH, Abdelzaher MA, Shoulkamy MA. 2006. Antagonistic actions *of Pythium oligandrum* and *Trichoderma harzianum* against phytopathogenic fungi (*Fusarium oxysporum* and *Pythium uitimum* var. ultimum). Archives of Phytopathology and Plant Protection **39**, 289-301.

Kucuk C, Kıvanc M. 2004. *In vitro* Antifungal Activity of Strains of *Trichoderma harzianum*. Turkish Journal of Biology **28**, 111-115.