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ORIGINAL ARTICLE **OPEN ACCESS**

Efficient *Trichoderma harzianum* were isolated and characterized their Antagonistic efficiency under *In vitro* condition

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ABSTRACT
In order to control soil-borne plant pathogens and improve farming practices, there has been a huge resurgence of interest and research in biological control. Trichoderma is one of the finest bioagents of biological control and has shown to be effective against a spectrum of soil and foliar diseases. To isolate and characterize an efficient antagonistic Trichoderma from sesame rhizosphere soil sample. Isolation of Trichoderma harzianum from sesame rhizosphere soil by serial dilution technique. To screening of antagonistic activity of the different isolates by dual palate technique. To evaluate the efficiency of biomass production and chitinase activity of the different isolates quantitatively. In the present study 12 different Trichoderma spp., were isolated and characterized sesame rhizosphere soil. To evaluate the antagonistic efficiency of the different isolates under in vitro conditions. The maximum biomass and antagonistic activity were recorded at isolate SRT 6. The isolate Trichoderma SRT 6 were shown their maximum compatibility to other isolates against Machrophomina spp. The selected efficient Trichoderma isolate were morphologically and molecular level characterized by 18 S rRNA sequencing. Based on the molecular characterization obtained as Trichoderma harzianum and submit the nucleotide sequence were submitted to NCBI gene bank and get accession number (ON979692). The isolate Trichoderma harzianum shows clear, high competition growth against Machrophomina spp.,
Keywords: *Biocontrol agent, Trichoderma harzianum, Competition, chitinase activity and sesame PGPR*

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INTRODUCTION

Sesame is one of the oldest oil seed crops grown worldwide. There are a number of fungi which can impact sesame yield and output. One of the major diseases causing damage to the sesame crop is charcoal rot, which is caused on by *Machrophomina* spp. A widespread fungus with a well-known name is called *Trichoderma*. Volatile organic compounds (VOCs) generated by Trichoderma have shown [1,2] that Trichoderma species play a key role in preventing and suppressing the growth of a number of plant pathogenic fungi.

The soil-borne necrotrophic fungus *Macrophomina phaseolina* is responsible for infect more than 500 plant species, including cowpea, mung bean, chickpea, sorghum, sunflower, sesame, and others. It is extensively spread in areas with high temperatures and dry conditions. There are no reliable fungicides or other types of controls methods to prevent *M. phaseolina* [3].

Due to their rapid growth, Trichoderma species compete with other soil bacteria for nutrients and space [4]. Trichoderma species can be isolated from soil using all currently known conventional methods due to their rapid rate of growth and quantity of conidia. It has been proven to be connected to endophytes, epiphytes, and the rhizosphere, and it is essential for the health of the soil and the stimulation of plant growth. Trichoderma species have been found in a number of places, however many have not yet been fully investigated [5].

Filamentous fungi of the Trichoderma genera are employed in a variety of fields to improve plant health because their many effects, including stimulating host growth, improving host disease resistance, and enhancing host strength to tolerate abiotic stress [6]. The phytohormone indole-3-acetic acid (IAA) auxin, which is produced by a number of Trichoderma species, including T. virens, T. atroviride, and T. harzianum, has been proposed to be used to stimulate plant root growth [7].

For biological control agents, the strains *T. harzianum, T. viride, and T. virens* have been widely used. According to some study, they suppress a number of phytopathogens, including *Rhizoctonia, Pythium, Sclerotinia, Sclerotium, Fusarium,* and *Macrophomina*, which decreases root rot/wilt complexes and foliar diseases in a variety of crops [8].

The main objective of the present research was to isolate and characterize efficient *Trichoderma harzianum* from sesame rhizosphere soil.

MATERIAL AND METHODS

Soil Samples and Isolation

Rhizosphere soil samples were collected from different ecological habitat of sesame crops of cuddalore district, Tamil Nadu, India for the isolation of Trichoderma spp. Samples were air dried then stored at 4 ^{o}C until used. Five-fold serial dilutions of each soil samples were prepared in sterilized distilled water and 0.5 ml diluted sample was poured on the surface of rose Bengal agar (RBA). Plates were incubated at 28 ± 2 ^{o}C for 96 h. Morphologically different colonies appearing on the plates were purified in the Potato Dextrose Agar (PDA) (HiMedia, India). The purified isolates were preserved at 4 ^{o}C and used during the course of study.

Test cultures

The reference test cultures *Trichoderm viride* NAIMCC-F-03862 were obtained from NBAIM, Mau, Utter Pradesh, India. The pathogen cultures *Macrophomina* spp., *Sclerotia* spp., *Pythium* spp., and *Cercospora* spp., were obtained from Department of plant pathology, Annamalai University, Chidambaram.

Phenotype Characters of the Trichoderma Isolates

Twelve isolates of Trichoderma were evaluated for morphological and cultural traits in two distinct medium RBA and PDA. Mycelial discs (6 mm) of young growing culture of respective isolates of Trichoderma was inoculated in the periphery of the Petri plates containing RBA and PDA media and incubated at 28 ± 2oC for one week. Colony radiuses of 24, 48, and 72 hours were measured. The results of each growth rate experiment were averaged for each isolate after being conducted three times in triplicate. Additional characteristics were also identified, such as the presence of pigments, green conidia, and colony appearance.

Fresh and dry Biomass production

The different *Trichoderma* isolates were inoculated with Potato Dextrose broth and incubate 28 ^{0}C for one week under static condition. After the incubation period to filter the broth with using of filter paper and take fresh and dry biomass of Trichoderma mycelial mat. The fresh and dry biomass were measured by subtract of empty weight of filter paper and with mycelial mat.

Antagonistic Activity of Trichoderma Isolates

Among the different Trichoderma isolates were screened their antagonistic activity by Dual plate technique against *Sclerotia, Macrophomina, pythium* and *cercospora* were used as test organism and grown on Potato Dextrose Agar (PDA) medium. To investigate the biofilms antifungal activity. *Sclerotia, Macrophomina, pythium* and *cercospora* were streaked on the outer periphery of the PDA plates with fungal cultures. After 48 h of incubation at 28±2 °C, the inhibition of radial growth of fungus was measured. phytate degrading strains are being screened. Bacterial strains were evaluated using phytase specific medium with 1.5 percent agars in a plate assay. After 14 days of incubation at 28°C the halo and colony diameters were measured [9].

Chitinase Activity

In this experiment different Trichoderma isolates were grow on PDA broth for one week. The mixture of 0.5 ml culture filtrate, 0.5 ml suspension of colloidal chitin and 1.0 ml of Mcllvaines buffer (pH 4.0) was mixed and incubated at 37^{o}C for 2 h in a water bath with constant shaking for the reaction was stopped by place the tubes under boiling 3 min in heated water bath. After add 3 ml potassium ferricyanide reagent and placed in boiling water for 15 min. The quantity of N-acetyl glucosamine (NAG) released were estimated [10].

The turbidity of mixture was measured in a UV vis spectrophotometer at 420 nm. The quantity of reducing sugar released were estimated from standard curves of NAG and the chitinase enzyme activity was expressed in pkat (pmol/s) per millitre.

Molecular Identification of Trichoderma Species

Sequence analysis of twelve isolates was done to confirm species identity, which initially has been done based solely on morphological parameters. Comparison of oligonucleotide fragments of rDNA sequences, which included the 5.8 S gene and the flanking ITS1 and ITS2 regions, with reference sequences from public databases, showed that they were very similar.

RESULTS AND DISCUSSION

The different 12 isolates were observed their morphological characters under the microscope 40x. The different colony morphology on top of the agar plate were identified as White with dark green colour profused mycelium and initially white with pale green later yellowish color hyphae. The conidia characters of different isolates were identified. The Conidia are hyaline Long, infrequently branched, verticillate and conidia are Frequent branching, verticillate. *Trichoderma* spp. have been studied commonly because of its ability to inhibit soil-borne pathogens and have good plant defense responses [11]. The pure culture and microscopic image of the isolate SRT 6 shows in Fig 1 & 2.

Results shown fresh and dry biomass of different Trichoderma isolates were maximum biomass were recorded at isolate SRT 6 (6.829 & 1.032 g) followed by test culture (6.792 & 0.931 g), SRT 2 (5.638 & 0.850 g) and other isolates. Among the fresh and dry biomass production was one of the most effective characters of antagonism and survivability (Table 1).

Results from the dual culture test showed that all isolates of Trichoderma inhibited mycelial growth of different pathogens. The maximum % of inhibition *Sclerotia* spp., (72.72 %), *Macrophomina* spp., (62.16 %), *Cercospora* spp., (71.42 %) and *Pythium* spp., (73.07 %) were recorded at isolate SRT 6 followed by test culture and other isolates (Table 2) and (Fig 3). Among the results the *Trichoderma harzianum* SRT 6 Show maximum inhibition of fungal pathogens compared to other isolates. recently, *Trichoderma spp.* have been reported to be eco-friendly biological control agents for managing plant diseases, which enable the use of chemical fungicides to be minimized. Trichoderma is especially known for its antagonistic activities against several plant pathogens including *Rhizoctonia solani, Fusarium spp., Alternaria spp., Sclerotinia sclerotiorum, Sclerotium rolfsii*. [12 - 15]. Similar results observed, the antagonistic potential of *T. harzianum* isolates against *Fusarium solani, Fusarium oxysporum,* and *Fusarium incarnatum.*[16].

All isolates showed significantly higher chitinase activities with supplement of different carbon sources as substrates in the basal media. The highest chitinase enzyme activity was recorded *Macrophomina* spp., (44.8) *Sclerotia* spp., (34.6) *Cercospora* spp., (32.2) and *Pythium* spp., (31.5) less when the basal medium was added with mycelial powder of *Macrophomina* spp., *Pythium* spp., and *Cercospora* spp., as compare to *Sclerotia* spp., whereas highest chitinase activity was recorded with *T. viride* (Fig 4). The antagonistic activity of *Trichoderma sp.* against different plant pathogens occurs through different mechanisms of action, including antibiosis, mycoparasitism, and competition for nutrients and space. Production of exo-chitinases by Trichoderma spp. damages cell-walls of the pathogen, thereby discharging oligomers, which play a crucial part in the inhibition of phytopathogens [17]. The presence of many bioactive constituents, the antifungal activities of Trichoderma strains cannot be referred to one bioactive component only, but also to the synergism among several bioactive constituents of Trichoderma extracts [18].

The selected isolate SRT 6 were molecular level characterized by 18 S rRNA sequencing and identified as *Trichoderma harzianum* the sequence data were submitted to the NCBI GenBank, and the allocated accession number is ON979692. A phylogenetic tree was also constructed using the 18S rRNA gene sequence data at Figure 5.

> ON979692 *Trichoderma harzianum* SRT 6

CTGCGAATGGCTCATTATATAAGTTATCGTTTATTTGATAATACTTTACTACTTGGATAACCGTGGTAATTCTAG
AGCTAATACATGCTGAAAATCCCGACTTCGGAAGGGATGTATTTATTAGATTAAAAACCAATGCCCCTCGGGGCT
CTCTGGTGAATCATGATAACTAGTCGAATCGACAGGCCTTGTGCCGGCGATGGCTCATTCAAATTTCTTCCCTAT
CAACTTTCGATGTTTGGGTATTGGCCAAACATGGTGGCAACGGGTAACGGAGGGTTAGGGCTCGACCCCGGAGAA
GGAGCCTGAGAAACGGCTACTACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGT
AGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATCGGAATGAGTACAATTTAAATCCCTTAACGA
GGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTG
TGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCTCACCGCGTGCACTGGTCCGGCCGGGC
CTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGA
GTGCTCAAGGCAGGCCTATGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGTGGTTCTATTTTGTTGG
TTTCTAGGACCGCCGTAATGATTAATAGGGACAGTCGGGGGCATCAGTATTCAATTGTCAGAGGTGAAATTCTTG
GATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAGGAACGAAAGTTAGGGG
ATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGACGATGTTACATTTT
TGACGCGTTCGGCACCTTACGAGAAATCAAA

CONCLUSION

Trichoderma is an important component in integrated pest management strategies. Biocontrol mechanisms of various species of Trichoderma have been isolated and studied. They restrict the growth and proliferation of the pathogens by parasitism and antibiosis as well as molecular approaches are being done. The isolated Trichoderma strains were exhibit different level of antagonistic activity and chitinase activity. In the present study reported the isolate SRT 6 *Trichoderma harzianum* exhibit highest competition

against *Macrophomina* spp and *Sclerotia* spp. The novel report clearly shown the isolate SRT *Trichoderma harzianum* exhibit the maximum competition against *Macrophomina* spp and *Sclerotia* spp.

Table 1 Fresh and Dry biomass production from different *Trichoderma* isolates

Isolate Designation	Fresh biomass (g)	Dry biomass (g)
SRT 1	4.072	0.672
SRT 2	5.638	0.850
SRT 3	4.384	0.693
SRT 4	3.539	0.619
SRT 5	2.793	0.569
SRT 6	6.829	1.032
SRT 7	2.552	0.531
SRT 8	5.295	0.758
SRT 9	3.874	0.630
SRT 10	1.453	0.494
SRT 11	3.169	0.582
SRT 12	4.846	0.616
Test culture	6.792	0.931

Table 2 Antagonistic Activity of different Trichoderma Isolates

Designation of the isolates	Antagonistic activity							
	Sclerotia		*Macrophomina*		*Pythium*		*Cercospora*	
	Growth (mm)	% Inhibition	Growth (mm)	% Inhibition	Growth (mm)	% Inhibition	Growth (mm)	% Inhibition
SRT 1	17	61.36	27	27.02	27	48.07	17	51.42
SRT 2	23	47.72	22	40.54	31	40.38	19	45.71
SRT 3	20	54.54	25	32.43	25	51.92	19	45.71
SRT 4	13	70.45	15	59.45	20	61.53	12	65.71
SRT 5	19	56.81	28	24.32	29	44.23	28	20.00
SRT 6	12	72.72	14	62.16	14	73.07	10	71.42
SRT 7	17	61.36	24	35.13	23	55.76	23	34.28
SRT 8	14	68.18	17	54.05	15	71.15	15	57.14
SRT 9	10	77.27	12	67.56	26	50.00	26	25.71
SRT 10	21	52.27	23	37.83	27	48.07	25	28.57
SRT 11	16	63.63	18	51.35	15	71.15	14	60.00
SRT 12	29	34.09	26	29.72	22	57.69	11	68.57
Test	26	40.90	20	45.94	33	36.53	22	37.14

Figure 1. Pure culture of SRT 6 isolate

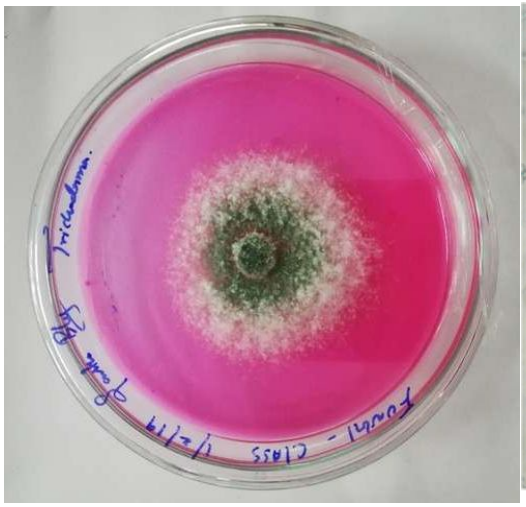

Figure 2 Microscopic image of SRT 6 isolate

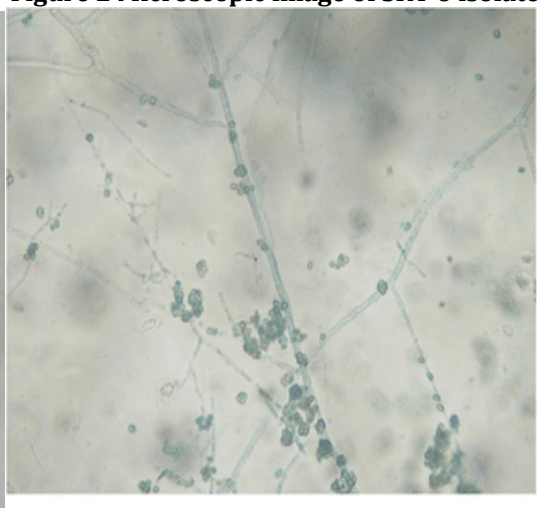

Figure 3 *Trichoderma* SRT 6 competition against *Macrophomina* spp., and *Sclerotia* spp.

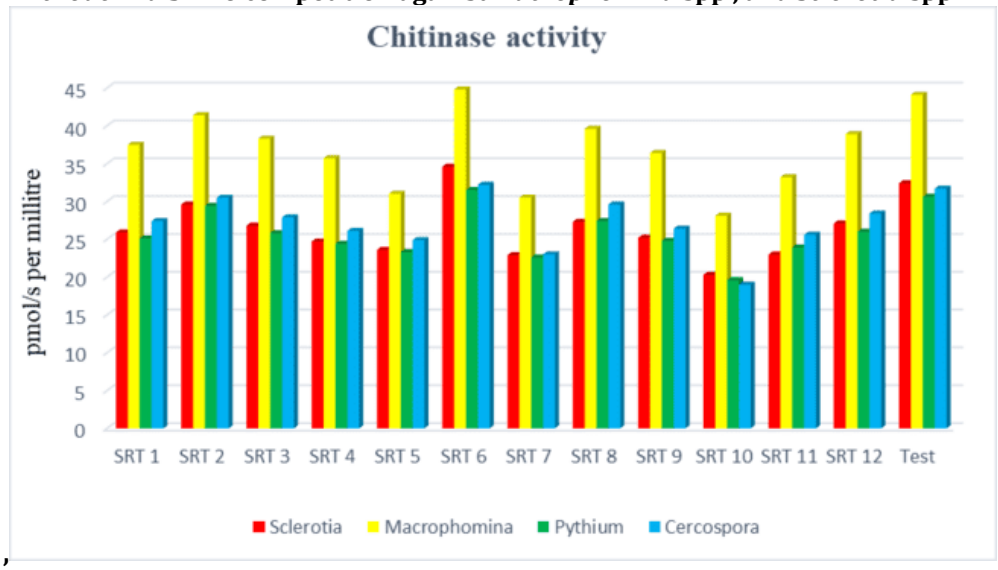

Figure 4. Chitinase activity of the different Trichoderma isolates

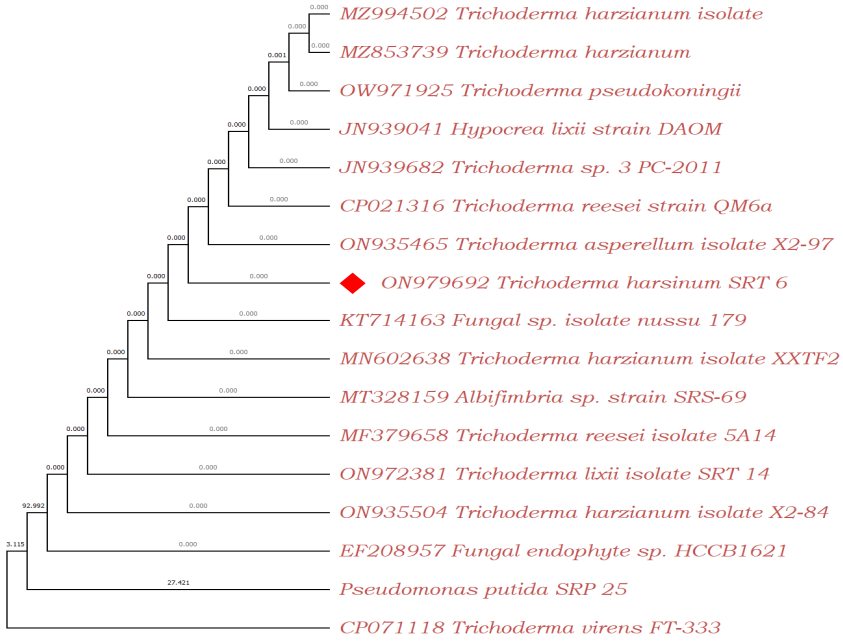

Figure 5. Phylogenetic tree of isolate SRT 6 *Trichoderma harzianum*

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