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BIOCONTROL MECHANISMS OF *TRICHODERMA* STRAINS ISOLATED FROM SOILS OF KAVANGARAI CHENNAI

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ABSTRACT

An attempt was made to isolate Trichoderma strains from the red soils of Kavangarai and study their biocontrol mechanisms using different substrates to identifying its potential on the production of many antimicrobial substances. The isolated Trichoderma strains exhibited good antagonistic activities against the phytopathogens by producing siderophores, chitinase and volatile compounds. This characteristics of the Trichoderma strains has proven that it could be a best option for controlling many phytopathogens since it is readily available in soils.

KEYWORDS: *Siderophores, Trichoderma, Rhizoctoniasolani, Chitinase, Antagonistic Biocontrol*

Article History

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INTRODUCTION

The use of chemical fungicide has been replaced by biocontrol agents because of the increase in emergence of fungicide resistant fungal strains and public concerns about the impact of these fungicides on human health and environment. Biological control is presented as an alternative tool to reduce the yield loss caused the diseases in plants. The use of microorganisms as biocontrol agents is risk-free as these organisms come from the same ecosystem (Melina Sartori et al, 2015)

The availability of food resources on the earth continue to reduce by the diseases caused by the phytopathogens and the economic potential of the nursery and greenhouse industries is also affected by the phytopathogens (Pinstrup-Andersen, 2000). Fungal phytopathogens cause various diseases in crop plantsand our modern agriculture practice still highly depends on the use of fungicides to control the diseases in plants (Zadoks and Waibel, 2000). The decrease in effectiveness of fungicides as well as risks associated with the fungicides on the fruits and leaves, have highlighted the need for more potent and safer alternative control measures for plant diseases (Haggag and Mohamed, 2007).The adverseeffects to the soil and environment due to indiscriminate use of chemicals has been a great public concern and hence a tremendous opportunity exists to develop new biocontrol agents (BCAs) as direct substitutes for chemicals or as key components in integrated diseases management systems that are more biointensive and eco-friendly (Gerhardson, 2002; Noling, 2002).

Today, a number of BCAs have been reported and available as commercial products. The common BCAs from bacterial genera include *Bacillus, Agrobacterium,* and *Pseudomonas*. The fungal genera include *Trichoderma, Gliocladium, Ampelomyces*and *Coniothyrium* and the genus *Streptomyces* from actinomycetes not only control the

phytopathogens but also promote the growth and development of plants (Vinale *et al., 2008*). Fungal based BCAs have gained wide acceptance next to *Bacillus thuringiensis*, primarily because of their broader spectrum in terms of increase of crop yield and disease control(Copping and Menn, 2000).

The *Trichoderma* strains exhibit biological control against fungal plant pathogens either indirectly by competing for space and nutrients, modifying the environmental conditions, or promoting plant growth and plant defense mechanisms and by antibiosis, or directly, by actions such as mycoparasitism (Benítez *et al*., 2004)**.** Therefore, the present paper aims to isolate *Trichoderma* spp. from the soils and study their different biological control mechanisms and growth characteristics of the strains isolated.

MATERIALS AND METHODS

Isolation of Trichoderma

The soil samples were collected from Kavangarai, Chennai. Ten gram of soil sample was suspended in 95 ml of sterile distilled water. One mL of sample solution was serially diluted in 9 mL of sterile distilled water up to 10 dilutions. Each 1 ml of samples from the dilutions 10^{-3} and 10^{-5} were taken and poured into sterile petridishes. To this, 20 ml of molten *Trichoderma* selective medium was poured and swirled well and the plates were incubated at room temperature. After three to five days, the appearance of colonies with formation of green-coloured spores was observed. The spores were subcultured on PDA medium to get pure culture and the pure cultures were maintained in the same medium. The identification of the fungal strains was carried out using the Key suggested by Rifai (1969).

Antagonistic Action of T. Viride Strains Against Fungal PATHOGENS **:**The antagonistic activity of all the *T. viride* isolates against *Macrophominaphaseolina, Rhizoctoniasolani, Fusariumudum* and *Fusariumoxysporum* were determined by dual culture technique on PDA medium (Huang and Hoes, 1976). Mycelial discs (9 mm in diameter) were cut out from the culture of *T. viride* strains and also from the fungal phytopathogens and were placed at the periphery of 90 mm Petriplates under aseptic condition. The two mycelial discs of the antagonists and the pathogens were placed at opposite poles and the plates were incubated at room temperature for 3 days and observed for the antagonistic activity. The growth inhibition of the phytopathogen in terms of radial mycelial growth with reference to control pathogen plates was measured and the mycelial growth inhibition was calculated using the below formula

$$I = \frac{C-T}{C} \times 100$$

where, I = percent inhibition C = radial growth in control and T = radial growth of fungus in dual plate.

Tolerance to Copper Oxychloride

All *Trichoderma viride* strains were tested for the copper oxychloridetolerance using different concentrations (1, 2, 3, 4 and 5 mM) of fungicide amended Potato Dextrose Agar medium. Mycelial disc of 9 mm was placed in the center of the fungicide amended Potato Dextrose Agar medium and in every 24 hour the mycelial growth of fungus was measured.

Toleranceto Carbendazim

All *Trichoderma viride* strains were tested for their tolerance to different concentration of fungicide - carbendazim (1, 5, 10, 15 and 20 µm) amended in Potato Dextrose Agar medium. The mycelial disc of 9 mm from the fungal culture was cut and placed in the center of the fungicide amended PDA medium and in every 24 hour the mycelial growth of fungus was measured.

Quantitative Assay of Chitinase

Reagents: 50mM sodium acetate buffer was prepared by dissolving sodium acetate in the required volume of water and the pH adjusted to 5.2 using glacial acetic acid. About 10 g of *p*-dimethyl amino benzaldehyde (p-DMAB) was dissolved in 100 ml of glacial acetic acid, which contain 12.5 % (v/v) 10 N HCl. It was diluted with 9 volumes of glacial acetic acid before use. Potassium tetraborate (0.08 M, pH 9.2) was prepared by adding calculated amount of KOH and Boric acid.

Procedure

A mixture of 1 ml of enzyme solution and 1 ml of suspension of colloidal chitin (0.1 % in 50 mM sodium acetate buffer, pH 5.2) was incubated at 37^{o}C in a water bath for 2 h with constant shaking. The release of N-acetyl glucosamine in reaction mixture was estimated by the method of Reissig *et al.,* (1955). Controls without enzymes, substrate and with boiled enzyme were maintained. The reaction was terminated by adding 0.1 ml of 0.08 M potassium tetraborate (pH 9.2) to 0.5 ml of reaction mixture. The content of the test tubes was boiled in a water bath for 3 min. After terminating enzyme reaction, 3 ml of diluted DMAB (1 ml DMAB + 9 ml glacial acetic acid) reagent was added and incubated at 37^{o}C for 15 min. The released product was read at 585 nm in spectrophotometer. One unit of the enzyme was defined on the amount of N-acetylglucosamine was released in the reaction mixture by the action of enzyme in 1 mL per min. The specific enzyme activity was expressed as unit/mg of protein / mL /min.

Siderophores Production Assay

The production of siderophores was detected by absorption spectra, bioassay, or the universal Chrome-Azurol-S (CAS) assay and antagonistic studies in Potato Dextrose agar medium amended with iron amended and without iron.

CAS Assay for Siderophore Production

The CAS (60.5 mg) was dissolved in 50 mL of Glass distilled water and mixed with 10 mL of ferric chloride solution (1 mM $FeCl_3.6H_2O$in 10 mM HCl). This solution was added to 72.9 mg of Hexadecyl trimethyl ammonium bromide (HDTMA) which is dissolved in 40 ml of distilled water. This blue coloured medium was sterilized by autoclave and added to the PIPES agar medium (at warm molten state) before pouring into the petriplates.

The PIPES agar was prepared by dissolving 30.24 g of PIPES buffer in 750 mL of distilled water containing 15 g of agar. The pH of the PIPES medium was adjusted to 6.8 by adding 1.06 g of NaOH pellets. The PDA medium was poured into the sterile petriplates and once it solidified, half of it was cut and removed. This half space was filled with sterile CAS agar medium. Mycelial disc was inoculated as far as possible from the centre of the plate on the PDA medium. The color change of the CAS medium from blue to brown was observed after incubation.

Role of Iron on the Antagonistic Activity

The role of iron competition for the inhibition of phytopathogen *Rhizoctoniasolani* was assessed by dual plate assay on PDA plates amended with 1 mM $FeCl_3$. The medium used for this experiment was prepared with double distilled deionized water. Plate assay conducted using iron-free PDA plates served as the control. After incubation the plates were observed for the reversal of inhibition in iron amended PDA plates, as there can be no siderophore production under iron abundant conditions.

Mycoparasitism by Trichoderma Viride

The mycoparasitism study was carried out by microscopic study of *Trichodermaviride* on *Rhizoctoniasolani*. The two fungi were grown in dual plate and after 48 h the mycelia of the two fungi were gently scraped from the zone of interaction and transferred to a clean glass slide, stained with cotton blue dye and observed under light microscope for fungal interactions.

Volatile Compounds Production

A simple plate assay was performed to find out the effect and production of volatile compounds by *T. viride* strains on the mycelial growth of the pathogen *R. solani*. The mycelial discs of the *Trichoderma viride* and *Rhizoctoniasolani* were inoculated on the sterile PDA medium. Only the bottom part of plates of both the fungi were combined together and sealed with parafilm such that the phytopathogen inoculated plate as upper lid and antagonist inoculated plates served as bottom under aseptic condition. In order to prevent the escape of volatile compounds produced, if any by *Trichoderma viride,* the plates were sealed with parafilm. The principle of this assay is, if the fungi in the bottom lid produces any volatile compounds that would diffuse in the set up and bring its antagonist effect on the phytopathogen which was present in the upper lid. The *R. solani* and the antagonists individually on the PDA media served as controls.

RESULTS

Morphological Characteristics

Trichoderma isolates R1 and R2 showed some variations as compared with the other isolates, which had almost the same characteristics on PDA medium (Table 1) Based on the morphological characteristics as observed under light microscope all the isolates (R1 to R6) were identified as *Trichoderma viride.* (Fig 1)

Antagonistic Activity of T. Viride Strains against Fungal Pathogens

All the six *T. viride* strains inhibited the mycelial growth of the four fungal pathogens, *Rhizoctoniasolani, Macrophominaphaseolina*, *Fusariumoxysporum*, and *Fusariumudum* (Fig. 2-5) Maximum percent inhibition of pathogens growth was observed when the pathogens were cultured with *T. viride* R1 (Table 2).

Fungicide Tolerance of T. Viride Strains

All the *Trichoderma* strains grew on PDA amended with the fungicides - Copper oxychloride at 1 - 5 mM/ml concentrations and Carbendazim at 1 - 20 µm/ml concentrations. *T. viride* R3 showed more tolerance to copper oxychloride and it had the highest tolerance to all the concentration as compared to other strains and it had completed its growth on Petriplates by third day itself at 1 µm/mL concentration (Fig.6). All the isolates had a little growth ranging from 1.2 – 1.4 cm at 20 µm/ml concentration (Table 3).Similarly the *T. viride* R3 showed more tolerance to carbenadzim and it had the highest tolerance up to 15 µm/ml concentration as compared to other strains (Fig.7). All the strains had a little growth ranging from 1.2 – 1.4 cm in diameter at 20 µm/ml concentration (Table 4).

Quantitative assay of Chitinase Production by T. Viride Strains in CDBCC Broth

All the strains produced chitinase with the specific activity ranging from 0.73 to 1.78 units/mg of protein. However *T. viride* R3 produced the highest amount of 1.78 units/mg protein (Table 5).

SIDEROPHORE PRODUCTION

Spectrophotometric Assay

The culture filtrates of all the six strains of *T. viride* in iron amended and as well as iron deficient broth showed an increase in OD value after the addition of 2 M $FeCl_3$(Table 6) The iron deficient culture filtrates of isolates *T. viride* R1, *T. viride* R3 and *T. viride* R6 showed a drastic increase in OD after the addition of 2M $FeCl_3$.

Plate Assay (CAS assay)

T. viride strain R3 showed the production of siderophores by changing the colour of the CAS medium from blue to pink even beyond its colony boundary (Fig.10).

Role of Iron on the Antagonistic Activity of T. Viride

T. viride R1, R3 and R6 inhibited the growth of *R. solani* more in PDA medium without the amendment of 1 mM of $FeCl_3$ as compared to the one with the amendment (Fig. 11 and Table 7).

Mycoparasitism Study

When the zone of hyphal interaction between *T. viride* sp. and *R. solani* was observed under the light microscope, the mycelium of *T. viride* was seen coiling around the pathogen's mycelium.

Volatile Compounds Production

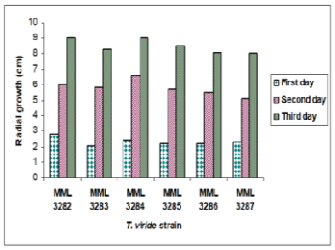
The volatile compounds produced by *T. viride* strain inhibited the substrate and aerial mycelial growth of *R. solani*after second day (Fig.10). The degree of effect on the pathogen differed among the *T. viride* strains. Maximum percent inhibition of pathogen's mycelium was by *T. viride* R6. More aerial growth of pathogen was observed with *T. viride* R1 although it had arrested the substrate mycelium.

Also it was observed that growth of *T. viride* strains was affected by their own volatiles. Mycelial growth of *T. viride* and the pathogen resumed after the release of the volatiles. Further the strains of *Trichoderma* sporulated by the next day of release of the volatiles. It was observed that the sclerotial formation in *R. solani*did not occur on the control plate also.

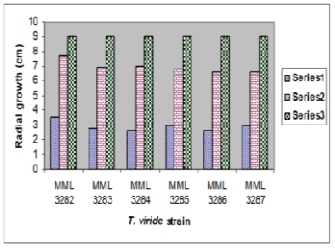
Effect of Ethyl Acetate Extract of T. Virider1 Culture Filtrate On the Mycelial Growth of R. Solani

No mycelial inhibition of *R. solani* was observed even with a crude extract of *T. viride* R1 at 1 mg/ml concentration.

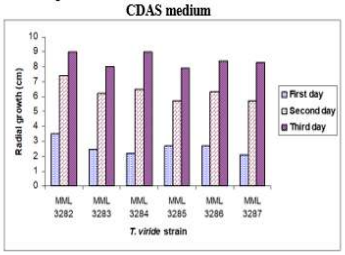
Graph 1. Growth of *T. viride* strains on PDA medium



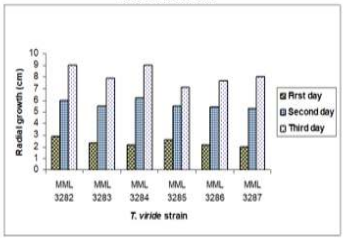
Graph 2. Growth of *T. viride* strains on PDYEA medium



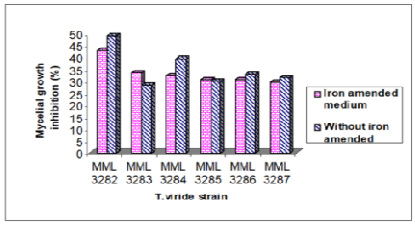
Graph. 3. Growth kinetics of *T. viride* strains in CDAS medium



Graph 4. Growth of *T. viride* strains on CDACC medium



Graph 5. Role of iron competition on the antagonistic activity of *T. viride* strains against *R. solani*



Graph 6. Spectrometric detection of siderophore production in iron free medium

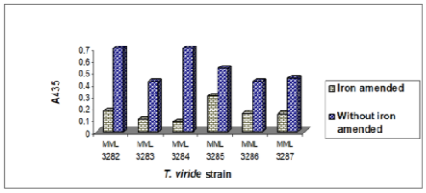
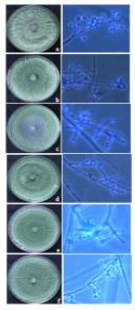


Fig. 1. Structure of morphology, mycelium, conidiophores and conidia of *Trichoderma* isolates



a: MML3282 d: MML3282
b: MML3283 e: MML3282
c: MML3284 f: MML3282

Fig. 2. Morphology of *T. viride* strains on PDA medium

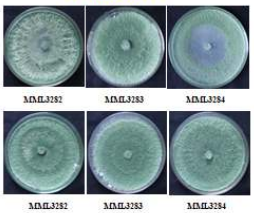


Fig. 3. Morphology of *T. viride* strains on PDYEA medium

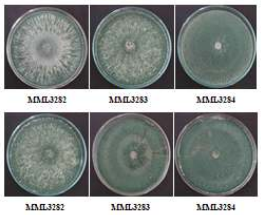


Fig. 4. Morphology of *T. viride* strains on CDAS medium

Fig. 5. Morphology of *T. viride* strains on CDACC medium

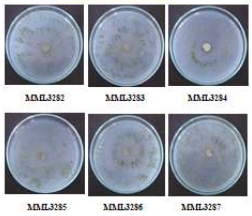


Fig. 8. Carbendazim tolerance of *T. viride* strains

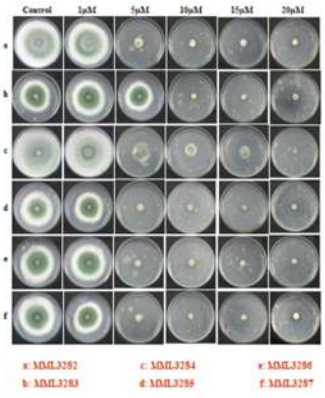


Fig. 09. Copper oxychloride tolerance of *T. viride* strains

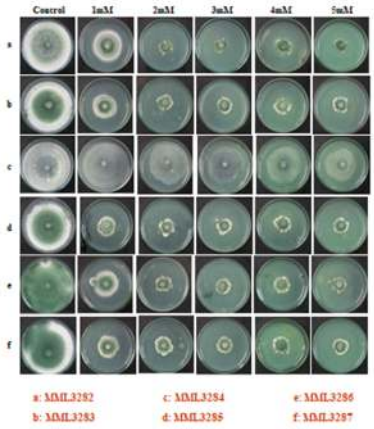


Fig. 6. Antagonistic activity of *T. viride* strains against *R. solani*

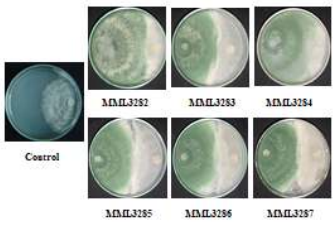


Fig. 7. Antagonistic activity of *T. viride* strains against *M. phaseolina*

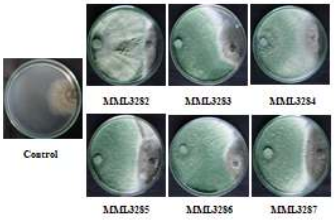


Fig. 10. CAS Assay for siderophore production by *T. viride* MML3284

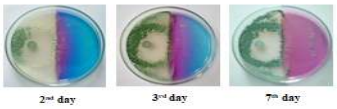


Fig. 11. Role of iron in antagonistic activity of *T. viride* strains

Fig. 12. HCN production by *T. viride* strains

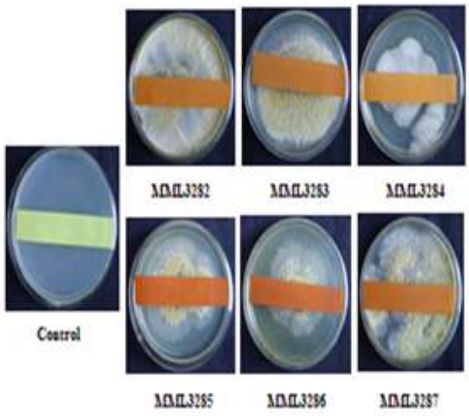


Fig. 13. Effect of volatile compounds of *T. viride* strains on *R. solani* mycelial growth

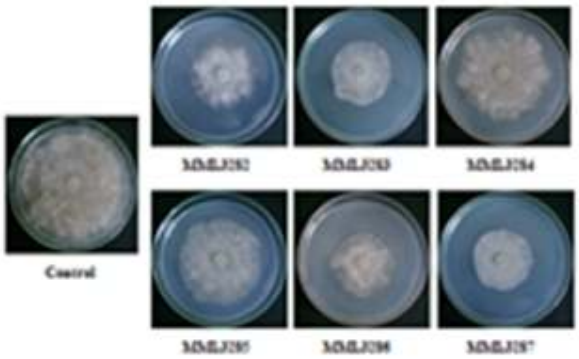


Table 1: Morphological Characteristics of *Trichoderma*isolates

Isolate	Morphology	Sporulation	Pigmentation
MML 3282	Very dense mycelial growth	Green and white	Green and white
MML 3283	Dense mycelium	Dark green	Green
MML 3284	Sparse in the centre of the colony	No pigmentation in the center of the colony	Green
MML 3285	Dense mycelium	Dark green	Green
MML 3286	Dense mycelium	Dark green	Green
MML 3287	Dense mycelium	Dark green	Green

Table 2: Mycelial Growth of T.Viride Strains on Different Media

Strain	PDA			PDYEA			CDACC			CDAS		
	Culture age (Days)			Culture age (Days)			Culture age (Days)			Culture age (Days)		
	1	2	3	1	2	3	1	2	3	1	2	3
MML 3282	2.8	6.0	9.0	3.5	7.7	9.0	2.9	6.0	9.0	3.5	7.4	9.0
MML 3283	2.1	5.8	8.3	2.7	6.9	9.0	2.3	5.5	7.9	2.5	6.2	8.0
MML 3284	2.4	6.6	9.0	2.6	7.0	9.0	2.2	6.2	9.0	2.2	6.5	9.0
MML 3285	2.2	5.7	8.5	2.9	6.8	9.0	2.6	5.5	7.1	2.7	5.7	7.9
MML 3286	2.2	5.5	8.1	2.6	6.6	9.0	2.2	5.4	7.7	2.7	6.3	8.4
MML 3287	2.3	5.1	8.0	2.9	6.6	9.0	2.0	5.3	8.0	2.1	5.7	8.3

Table 3: *In Vitro* Antagonistic Activity of *T. Viride* Strains Against Fungal Phytopathogens Expressed As Percent Inhibition

Strain	*R. solani*	*M. phaseolina*	*F. oxysporum*	*F. udum*
MML3282	61.5	45.2	36.1	56.5
MML3283	38.5	16.1	14.3	18.7
MML3284	69.2	38.7	30.4	69.6
MML3285	41.0	16.5	21.7	17.4
MML3286	30.8	29.0	18.7	13.0
MML3287	20.5	16.5	18.7	26.1

Table 4: Tolerance Spectrum of *T. Viride* Strains To Different Concentrations of Carbendazim

Strain	Carbendazim concentration (µM)/Radial growth (cm)				
	1 µM	5 µM	10 µM	15 µM	20 µM
MML3282	8.2	3.8	1.9	1.4	1.3
MML3283	7.2	6.9	1.4	1.4	1.3
MML3284	9	4.1	2.8	2.6	1.4
MML3285	7.2	1.6	1.4	1.4	1.4
MML3286	8.4	1.6	1.6	1.3	1.2
MML3287	7.4	1.8	1.4	1.3	1.2

Table 5: Tolerance Spectrum of *T. Viride* Strains to Different Concentrations of Copper Oxychloride

Strain	Copperoxychloride Concentration (mM)/Radial growth (cm)				
	1 mM	2 mM	3 mM	4 mM	5 mM
MML3282	6.0	3.6	3.3	3.1	2.7
MML3283	5.3	3.3	3.2	3.2	3.1
MML3284	8.6	7.7	6.9	6.5	5.5
MML3285	4.7	3.3	3.0	2.9	2.4
MML3286	5.4	4.9	4.2	3.9	3.1
MML3287	4.8	3.3	3.1	2.7	2.5

Table 6: Total Protein Content and Chitinase Activity of *T. Viride* Strains in Colloidal Chitin Amended Medium

Strain	Protein Content (µg/ml)	Specific Activity (units/mg of protein)
MML3282	25.5	1.17
MML3283	15.3	1.45
MML3284	18.4	1.78
MML3285	24.3	1.66
MML3286	27.1	0.73
MML3287	18.8	1.09

Table 7: Spectrophotmetric Assay for Siderophores Production By *T. Viride* Strains

Strain	Iron amended broth		Iron deficient broth	
	Without addition of 2M $FeCl_3$	With addition of 2M $FeCl_3$	Without addition of 2M $FeCl_3$	With addition of 2M $FeCl_3$
MML3282	0.298	0.416	0.176	0.698
MML3283	0.213	0.539	0.107	0.420
MML3284	0.098	0.488	0.082	0.699
MML3285	0.272	0.871	0.303	0.538
MML3286	0.156	0.640	0.159	0.420
MML3287	0.182	0.675	0.155	0.450

Table 8: Role of Iron Competition on the Antagonistic Activity of *Trichoderma Viride* Strains Against *R. Solan*

Strain	Percent Inhibition of *R. solani* mycelial Growth	
	Iron Amended Medium	Iron Free Medium
MML3282	43.0	49.2
MML3283	33.8	28.5
MML3284	32.4	39.4
MML3285	30.7	30.1
MML3286	30.7	33.3
MML3287	29.5	31.7

DISCUSSION

The genus *Trichoderma* comprises fungal species highly focused for their mycoparasitic activities against various phytopathogens. The major trophical diseases such as sheath blight disease of rice are caused by pathogens namely *Rhizoctoniasolani*, *Fusariumoxysporum*, *Alternaria alternate* (Gveroska and Ziberski, 2012) and *Sclerotiumrolfsii* were reported to be controlled by the *Trichoderma* species (Adeline Su Yien Ting and Jing Yun Chai, 2015).*Trichoderma* strains can grow in polluted soil also, because they are naturally resistant to many toxic compounds, including herbicides, fungicides and pesticides such as DDT, and phenolic compounds. *Trichoderma* strains recover very rapidly after the addition of sub-lethal doses of some of these compounds (Lorito, 2004). In the present study, *Trichodermaviride* strains could easily grow in lower concentrations of fungicide amended medium and growth was also seen in higher concentrations. According to the reports of Vinale*et al.* (2004), *Trichoderma* strains (*T. harzianum* T22 and *T. atroviride* P1) could show tolerance to up to 5 mM concentration of copper oxychloride and similarly, all the *T. viride* strains could grow in PDA amended with 5 mM of copper oxychloride. The minimum growth was observed in third day was 2.4 cm for *T. viride* R4

Many beneficial fungi possess mechanisms that allow them to efficiently cure or prevent both foliar and root diseases caused by pathogenic fungi. Several species of *Trichoderma* are used as biocontrol agent against several phytopathogens fungi, including *Rhizoctoniasolani* (Howell, 2003). Its successful use as a biocontrol agent is its efficient coiling process followed by a substantial production of hydrolytic enzymes, act synergistically (Almeida *et al.,* 2007). the production of several lytic enzyme systems and the role of extracellular enzymes in mycoparasitism has been well documented by several researchers *e.g.*, proteolytic enzymes (Pozo *et al.,* 2004; Kredics *et al*., 2005); β 1,3- glucanolytic system; chitinase (de la Cruz and Llobell, 1999; Kubicek *etal*., 2001). This complex group of extracellular enzymes have been reported to be a key factor in pathogen cell wall lysis during mycoparasitism (Verma *et al*., 2007).

In the present study, microscopic observation of the mycelia from the zone of interaction revealed that the hyphae of *Trichoderma* sp. had coiled around the larger hyphae of *R. solani.* In *Trichoderma* spp. antibiosis is considered the next important mechanism in biocontrol of the pathogens but in the present study, the secondary metabolites (crude extract) did not inhibit the growth of *R. solani* in contrast to the the reports that the different metabolites of *Trichoderma*spp like gliotoxin, viridin, gliovirin and 6-pentyl-alpha-pyrone (6PAP) have antifungal activity (Howell and Stipanovic, 1983).

The adverse effect of volatiles could be clearly seen in the form of mycelial growth suppression in *R. solani.* However, it was also observed that, these volatiles produced by *Trichoderma* had self-inhibitory on mycelial growth and sporulation. The fast growing nature of *Trichoderma* makes it a better competitor in the plant rhizosphere. During the

process of competition, the antagonist may suppress the growth of the pathogen population in the rhizosphere and thus reduce disease development (Ranasinghe*t al.,* 2006). Sivan and Chet (1989) demonstrated that competition for nutrients is the major mechanism used by *T. harzianum* to control *F. oxysporum* f. sp. *melonis.* Moreover, *Trichoderma* has a strong capacity to mobilize and take up soil nutrients, thus making it more efficient and competitive than many other soil microbes (Benı'te*zet al.,* 2004). It was seen that the fast growing strain R1 and R3 showed the highest inhibition of mycelial growth of fungal phytopathogens.

Several biocontrol agents are reported to produce low molecular weight iron chelating ligands called siderophores that suppress the pathogens (Spadaro and Gullino, 2005) can be an advantage to the antagonists and can help to enhance the biocontrol efficiency. Mehalawy *et al*., 2007 reported that *T. viride* isolate produced siderophore and helped to reduce the diseases incidence of damping-off caused by *R. solani* in cotton seedlings. In the present study, four strains of *T. viride* produced siderophore, which was seen in the reversal of myeclail growth inhibition of *R. solani*in iron amended PDA medium. Also *T. viride* R3 brought about the colour change of in CAS medium from blue to pink beyond the growth of the colony, indicating the production of siderophores.

The microbial siderophores can often be utilized by plant systems through the action of enzymes like ferric reductase (Ma and Nomoto, 1996) and this can lead to higher accumulation of iron in plants (Sharma *et al.,* 2003). In the present study, the formulations of *T. viride* applied plants showed higher root and shoot lengths and overall good growth of green gram and tomato.

CONCLUSIONS

The six *Trichoderma*isolates were identified as *T. viride.* All the strains showed good antagonistic activity against fungal phytopathogens. Also, the formulations of effective strain *T. viride* R1 improved the plant growth in green gram and tomato. Based on the results it is concluded that further field experimental research on the *Trichoderma* strain isolated from the soils are efficient biocontrol agents and have phytopathogenic effects suggesting that it could be a potential organism for the control of diseases in plants and provide nutritional supplements to the plants growth and wellbeing.

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