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Bioefficacy Studies of *Trichoderma viride* on Soil-Borne Pathogens

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ABSTRACT

Trichoderma viride was isolated from the rhizosphere of tomato (*Lycopersicon esculentum* Mill., cultivar 'Pusa Ruby') plants from sick plots in and around Bangalore containing soil-borne fungal pathogens such as *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Fusarium oxysporum* which cause damping off, stem rot and wilt, respectively. The efficacy of *T. viride* was evaluated in *vitro* and *in vivo*. The results of *in vitro* studies showed that *T. viride* inhibited the mycelial growth of *R. solani*, *S. sclerotiorum* and *F. oxysporum* on PDA. *F. oxysporum* was greatly inhibited by *T. viride* in the *in vitro* studies compared to *S. sclerotiorum* and *R. solani.* So, the efficacy of *T. viride* in the management of wilt was carried out in a pot experiment. In the evaluation of the efficacy of *T. viride* in *in vivo*, seed treatment followed by soil application with the *T. viride* effectively increased the shoot and root length and shoot and root weight and reduced the wilt caused by *F. oxysporum* compared to untreated controls in tomato.

Keywords: *Fusarium oxysporum*, *Lycopersicon esculentum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, tomato

INTRODUCTION

It is well accepted that agricultural production must be increased considerably in the foreseeable future to meet the food and feed demands of a rising human population. The damage caused by pathogens is decreasing the yield of crops. Tomato, *Lycopersicon esculentum* Mill., is one of the most popular and widely consumed vegetables all over the world and one of the most important vegetable crops grown in India. It stands fourth among the largest tomato producers in the world with an annual production of 10.3 million Mt. (FAOSTAT Agriculture Data 2008). Its yield is drastically reduced by fungal diseases (Magad *et al*. 2007). The damage evoked by soil-borne pathogens is usually underestimated, since it appears below ground. Plant diseases caused by below-ground pathogens may be considered as limiting factors of plant health and securing yield quantitatively and qualitatively.

The use of agrochemicals affects the sustainability of agriculture in a significant way due to environmental contamination by the indiscriminate use of these products, which have reduced the biodiversity of the ecosystem and have brought about problems to public health. These facts have been reported under 'Food and Environment Protection Act, 1985, Part III. Control of Pesticide regulations 1986' by the Pesticide Safety Directorate (Kings Pool, York Y01 7PX) in 1992.

Besides chemicals, various researchers suggested other control measures in view of the need to replace highly toxic and potentially polluting chemicals used to control plant parasitic nematodes and fungi, with less dangerous chemicals or preferably with biological control agents and botanicals (Oostendrop and Sikora 1989; Jayalakshmi *et al.* 2009; Poornima and Rakesh 2009). Among many bio-control agents the high degree of ecological adaptability shown by strains within the genus *Trichoderma* is reflected in its world-wide distribution, under different environmental conditions, and its survival on various substrates. This considerable variation, coupled with their amenability to be cultivated on inexpensive substrates, makes *Trichoderma* isolates attractive candidates for a variety of biological control applications (Harman 2006). Some species of the genus *Tricoderma* have been used as bio-control agents against soil-borne phytopathogenic fungal pathogens (Chet 1987, 1990; Jensen and Wolffhechel 1995). The antagonistic ability of *Tricoderma harzianum* Rifai against several soil-borne plant pathogens was tested under greenhouse and field conditions (Dennis and Webster 1971; Elad *et al.* 1980a, 1980b, 1981). The use of molasses-enriched clay granules as a food base for *T. harzianum* to control *Sclerotium rolfsii* in peanuts was suggested by Backman and Rodriguez-Kabana (1975). Wheat-bran-grown cultures of *T. harzianum* added to soil in greenhouse plantings reduced damping off caused by *R. solani* in beans (*Phaseolus vulgaris* var.), tomatoes (*Lycopersicon esculentum* Mill.) and eggplants (*Solanum melongena* Linn.).

Several modes of action have been proposed to explain the bio-control of plant pathogens by *Trichoderma*; these include production of antibiotics and cell wall-degrading enzymes, competition for key nutrients, parasitism, stimulation of plant defence mechanisms and plant growth-promoting effect (Inbar *et al.* 1994; Sharon *et al*. 2001; Jayalakshmi *et al.* 2009). Considering all these mechanisms, the present work was aimed to study the effect of *T. viride* on fungal pathogens *in vitro* and *in vivo*.

MATERIALS AND METHODS

Isolation of *T. viride*

T. viride was isolated from rhizosphere soil samples obtained from tomato plantations in and around Bangalore. Tomato plants were pulled out of the soil gently with intact roots and rhizosphere soil, and transferred to the laboratory. Rhizosphere soil (1 g) adhering

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Research Note

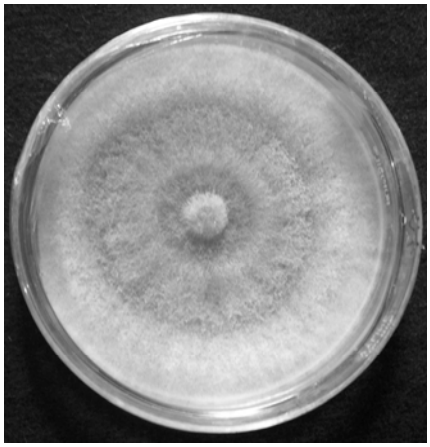

Fig. 1 ***Trichoderma viride*** **isolated on PDA medium.**

to the root surface was collected and was suspended in sterile water and serial dilutions of that were then plated onto *Trichoderma* selective medium (Martin's agar medium)(Martin 1950; Nguyen 1988). Plates were incubated at 25 ± 1°C for 48 h in a BOD incubator. *T. viride* was isolated and maintained on potato dextrose agar (PDA) medium (**Fig. 1**). Identification of *T. viride* was conducted by using a monograph described by Rifai (1969) and was used for further antagonistic activity testing.

Isolation of *R. solani*, *S. sclerotiorum* and *F. oxysporum*

Pure cultures of *R. solani*, *S. sclerotiorum* and *F. oxysporum* isolated from the infested plants from the sick plots were maintained on PDA in Petri dishes at 25 ± 1°C.

Efficacy of *T. viride in vitro* against pathogens

To test the antagonistic potentiality *in vitro,* a 5-mm disc cut from a 5-days-old culture of *T. viride* was placed 5 cm apart from a 5-mm disc of *R. solani*, *S. sclerotiorum* and *F. oxysporum*, respectively on a separate PDA Petri dishes. These inoculated Petri dishes were incubated at 25 ± 1°C in a BOD incubator. The experiments were independently repeated three times. The growth of the advancing colony of both pathogenic fungi was recorded periodically. *F. oxysporum* was greatly inhibited by *T. viride*, so it was selected for the pot experiment to study the efficacy of *T. viride in vivo*.

Efficacy of *T. viride* in *in vivo* against wilt-causing *F. oxysporum*

Fresh inoculum of *T. viride* and *F. oxysporum* on sorghum (*Sorghum bicolour* (L.) Moench) seeds were produced respectively by mass multiplying the pure cultures which were maintained on PDA in Petri dishes at 25 ± 1°C. Healthy sorghum seeds were soaked in 5% (w/v) sucrose solution for 16 h and then strained and placed into 500-ml conical flasks to give 200 cm^3 of sorghum seeds/flask. Flasks with sorghum seeds were plugged with cotton and sterilized by autoclaving at 121°C for 30 min. The conical flasks containing sterilized sorghum seeds were inoculated with 1 cm/diameter PDA discs punched from the periphery of actively growing 5-day-old cultures of *T. viride* and *F. oxysporum*. After inoculation, the flasks were then placed in a BOD incubator at 25 ± 1°C and the fungi were allowed to grow with periodic shaking of the flasks. Thus, the surface of all sorghum seeds was colonized and Colony Forming Unit reached above 10^8 CFU/g culture.

The experiment was conducted in glasshouse using susceptible tomato variety cv. 'Pusa Ruby'. Tomato seedlings raised in sterilized soil to evaluate the performance of *T. viride* against *F. oxysporum*. The earthen pots of 18 cm top diameter were filled with a mixture of autoclaved sandy loam soil (sand 70%, silt 22%, clay 8% and pH 7.5) and compost (4: 1). The temperature and relative

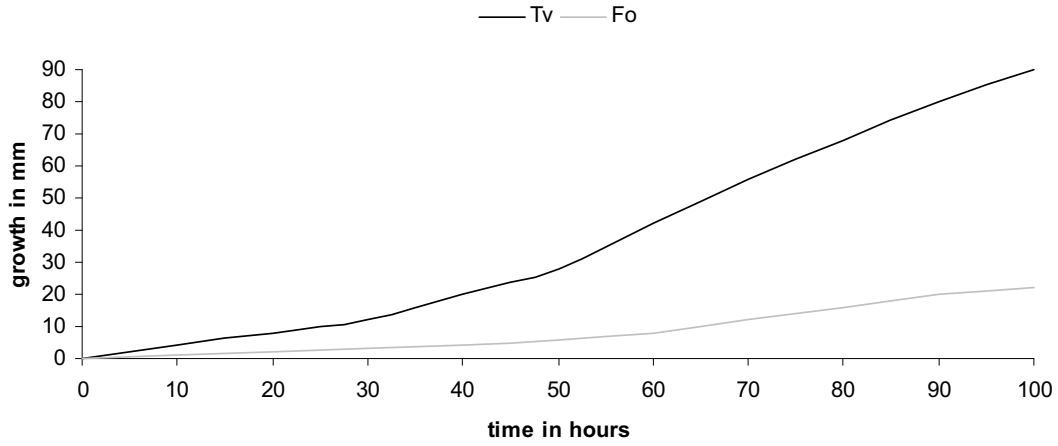

Fig. 2 Comparison of relative growth rates of ***T. viride*** **(Tv) and** ***F. oxysporum*** **(Fo) growth on potato dextrose agar medium.**

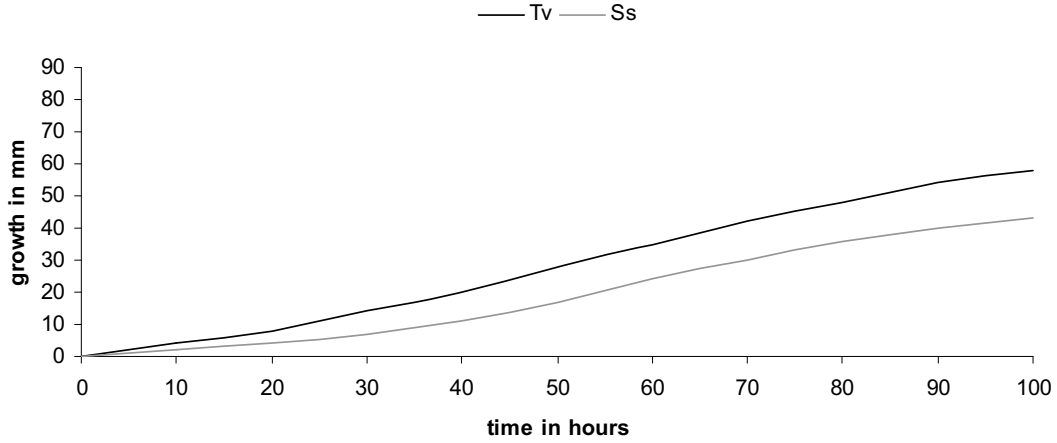

Fig. 3 Comparison of relative growth rates of ***T. viride*** **(Tv) and** ***S. sclerotiorum*** **(Ss) growth on potato dextrose agar medium.**

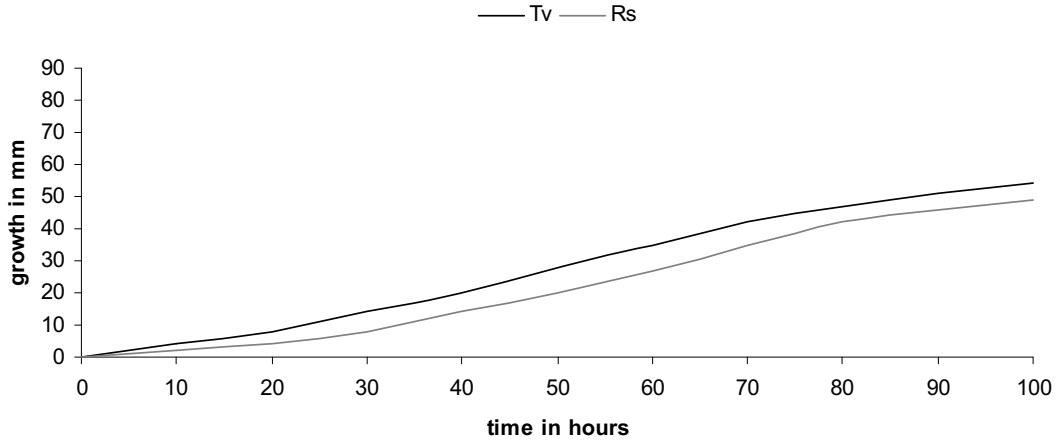

Fig. 4 Comparison of relative growth rates of *T. viride* (Tv) and *R. solani* (Rs) growth on potato dextrose agar medium.

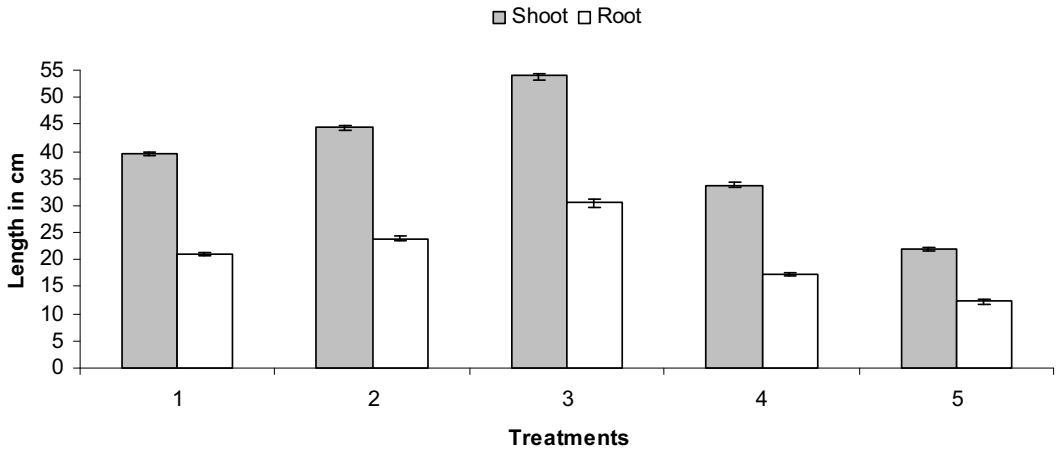

Fig. 5 Effect of *T. viride* on growth parameter of tomato under glasshouse conditions. Each value is the mean of three replicates with five seedlings/replicates. Error bars indicate mean ± S.E. Treatments are: (1) Seedling treatment with *T. viride*; (2) Soil amendment with *T. viride*; (3) Seedling treatment + Soil amendment with *T. viride*; (4) Control; (5) *F. oxysporum.*

humidity of the glasshouse were set at 30°C and 80%, respectively in order to favour disease development. Two-weeks old seedlings of tomato dipped in a suspension of *T. viride* with the CFU (2×10^8) and transplanted in pots containing soil amended with 10g of *T. viride* grown on sorghum seeds (2×10^8 spores/g). This treatment of seedling and soil application with *T. viride* was also carried out individually. Tomato seedlings transplanted in sterilized soil without any bio-agent served as control. Similarly tomato seedlings were transplanted and maintained separately without any bio-agent to be inoculated with *F. oxysporum*, served as control for *F. oxysporum*. Twenty five days after transplanting, each seedling was inoculated with 2ml spore suspension of *F. oxysporum* into 1-cm holes around the base of the plant which were then filled with soil. Spore suspension was made from the colonized sorghum seeds so that final CFU of *F. oxysporum* was maintained at 10^8 CFU/ml. Each treatment was replicated three times and the pots were arranged in a completely randomized design in a glass house. Two months after inoculation of *Fusarium*, the plants were uprooted and observations were made on plant growth parameters, disease incidence and plant mortality.

Statistical analyses

The values were expressed as mean ± S.E.M. The data were evaluated by analysis of variance (ANOVA) followed by Tukey's pairwise comparison tests to assess the statistical significance. $P < 0.0001$ was considered as statistically significant, using software ezANOVA ver. 0.98.

RESULTS AND DISCUSSION

Results obtained from *in vitro* tests proved that *T. viride* has the ability to suppress the growth of fungal pathogens. Under favourable conditions of laboratory test, *T. viride* showed high antagonism against *F. oxysporum* followed by *S. sclerotiorum* and *R. solani* by inhibiting mycelial growth on PDA. The growth of *T. viride* was significantly faster than *F. oxysporium*, *S. sclerotiorum* and *R. Solani* (**Figs. 2-4**). The antagonistic ability of *T. viride* varied with different organism i.e., *F. oxysporium*, *S. sclerotiorum* and *R. solani.* This phenomenon was probably correlated with the differences in levels of hydrolytic enzymes produced by *T. viride*, when it comes in contact with the mycelium of *F. oxysporum*, *S. sclerotiorum* and *R. solani* (Elad *et al.* 1982). *Trichoderma* sp. was assumed to attack the pathogen's mycelium firstly by dissolving cell walls in certain locations followed by hyphae penetration; then producing other extracellular enzymes, lipase, protease (Chet *et al.* 1981) and β-1,3-glucanase (Mitchell and Alexander 1963; Ferreira *et al.* 2007) to continue the lysis process. Comparison of *in vitro* growth rates signifies that competition, perhaps, is playing a substantial role in antagonism and in suppression of the population of *F. oxysporum.* The rapid growth may probably provide an advantage to *T. viride* over *F. oxysporum* in competing for nutrients and space utilisation. The role of competition for nutrient and space in suppressing the populations of *Fusarium* species has been explained by Cugudda and Garibaldi (1987) and Widden and Scattolin (1988).

The results obtained from the pot experiment conducted in glasshouse revealed that both treatment of seedling and soil application with *T. viride* has proved more effective in increasing the shoot length, root length, shoot weight and root weight, when compared to its application individually (**Figs. 5-7**). The effect of different treatments on various growth parameters, were significant as compared to untreated control and *F. oxysporum* inoculated plants. The increase in plant height may be due to the fact that enhanced

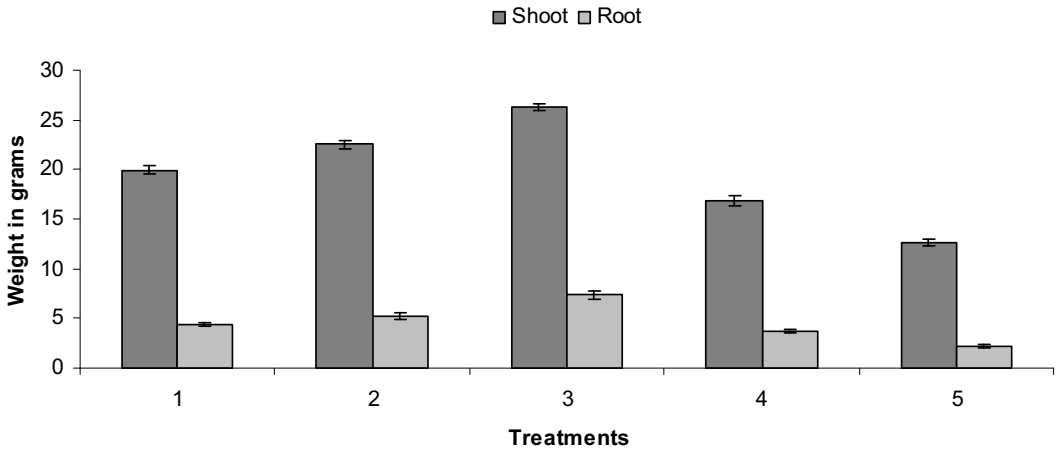

Fig. 6 Effect of *T. viride* on growth parameter (Fresh weight) of tomato under glasshouse conditions. Each value is the mean of three replicates with five seedlings/replicates. Error bars indicate mean ± S.E. Treatments are: (1) Seedling treatment with *T. viride*; (2) Soil amendment with *T. viride*; (3) Seedling treatment + Soil amendment with *T. viride*; (4) Control; (5) *F. oxysporum*.

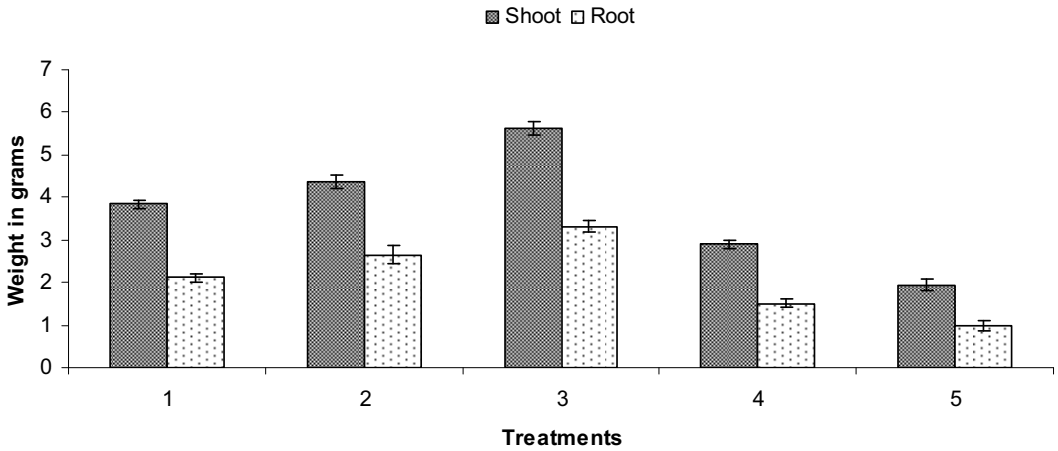

Fig. 7 Effect of *T. viride* on growth parameter (dry weight) of tomato under glasshouse conditions. Each value is the mean of three replicates with five seedlings/replicates. Error bars indicate mean ± S.E. Treatments are: (1) Seedling treatment with *T. viride*; (2) Soil amendment with *T. viride*; (3) Seedling treatment + soil amendment with *T. viride*; (4) Control; (5) *F. oxysporum*.

Table 1 Effect of *T. viride* on disease incidence and plant mortality.

Treatments	Spore density of *T. viride* in rhizosphere (CFU/g)	Disease incidence (%)	Plant mortality (%)
Seedling treatment with *T. viride*	9×10^4	80	60
Soil amendment with *T. viride*	17×10^4	72	50
Seedling treatment + soil amendment with *T. viride*	28×10^5	65	30
Control	----	22	13
F. oxysporum	----	100	90

availability of nutrients through solubilisation of insoluble and sparingly soluble minerals due to the presence of *Trichoderma* sp. in rhizosphere further enhanced the absorption of nutrients therefore resulting in increased plant growth (Altomare *et al*. 1999). Similar increase in plant height of *Capsicum annuum* and *Oryza sativa* has been reported by Cruz and Cisterna (1998) and Mathivanan *et al.* (2005), respectively. Mathivanan *et al.* (2000) also reported that in naturally infested soil, seed treatment followed by soil application of bio-fungicide significantly decreased root diseases in eggplant and sunflower.

Further, maximum spore density of *T. viride* is observed in the treatment where both seedling and soil was treated with *T. viride* (**Table 1**). This could be correlated to the reduced plant mortality and significant suppression of disease by *T. viride*. Adams (1990) indicated that *Trichoderma* requires a minimum of 10^5 CFU/g of soil to achieve effective disease control. Because of the high density of propagules in seed treatments followed by soil application of biofungicide, the establishment and multiplication of *T. viride* in soil could be rapid (Mathivanan *et al.* 2000). This may have prevented the accrual of pathogen populations. Cano and Catedral (1994) also recorded a decrease in wilt disease of cotton caused by *Fusarium oxysporum* f.sp. *vasinfectum* Snyder & Hansen. following seed treatment and soil drenching with spores of *Trichoderma*.

The results obtained from the present study indicated that, application of *T. viride* both as seedling and the soil treatment was more effective against *F. oxysporum* which causes wilt disease in tomato.

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