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Bio-Control Efficiency of *Trichoderma viride* against Stem Rot of Tuberose Caused by *Sclerotium rolfsii*

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A B S T R A C T

Tuberose is an important flower crop for cut flower and decorations which is a chief raw material for the production of high-grade perfumes. Recent days stem rot of tuberos is an important disease that affects the quality of flowers. The objective of this paper is to evaluate the efficiency of *T. viride* isolates against *S. rolfsii* and improves growth parameters of tuberose. Among the different antagonists tested, *Trichoderma* is found be very effective against *S. rolfsii*. The isolates *T. viride* are named as Tv_1 – Tv_{10}. The efficient isolate have been identified as Tv_5 by dual culture technique, which shows maximum mycelial inhibition (77.15), this was followed by the isolates Tv_4 (75.94) and Tv_1 (72.96) in the decreasing order, the least growth inhibition of pathogen was exhibited by the isolate Tv_{10} (55.72 %). The poison food technique @ 40 % concentration completely inhibits the mycelial growth on solid media.

Introduction

Tuberose (*Polianthes tuberosa* L.) is a commercially important ornamental bulbous plant cultivated in the world as well as in India for cut and loose flower trade. The losses due to stem rot disease may go up to 50-60 per cent (Kakade, 2007). In Tamil Nadu the losses due to stem rot ranges from 25-40 per cent (Theradimani *et al.*, 2018). The area under tuberose cultivation in India is about 14.92 '000 ha with a production of 106.49 '000 MT of loose flowers and 89.83 lakh nos. of cut flowers in 2016-17 (www.indiastat.com). Total export of floriculture products during the year 2015-16 was 22,518 MT with value of Rs. 479 crores (www.apeda.gov.in).

Different species of bacteria *Bacillus subtilis*, *Pseudomonas fluorescens* and fungi *Trichoderma viride* are reported to be effective. The effectiveness of several bio-

control agents against several soil borne pathogens were recorded (Sivasakthi *et al.*, 2014; Zape *et al.*, 2014; Gowdra Nagamma, 2015). Among that *T. viride* is one of the best to control soil borne plant pathogens. Bio-control agents are gaining importance due to their plant growth promoting and diseases reduction capabilities (Thahir Basha *et al.*, 2012).

Several workers reported that successful application of antagonists can control *S. rolfsii* in various crops (Parmar *et al.*, 2015; Dwivedi *et al.*, 2016; Ramzan *et al.*, 2016). Using compatible strains of plant growth promoting and bio-control microorganisms such as *Trichoderma* spp to maximize plant growth has been globally demonstrated (Srivastava *et al.*, 2010; Singh and Singh, 2014).

Materials and Methods

Isolation and purification of *T. viride*

Soil samples were collected from rhizosphere soil of tuberose for the isolation of *Trichoderma* spp. Samples were brought to laboratory and stored at 4°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 *Trichoderma* specific medium (TSM) (Elad and Chet, 1983). Plates were incubated at 28±2°C for 48-72 hrs. Isolated colonies were further purified by single hyphal tip and plating on PDA medium. Purified cultures are stored in agar slants for further use.

Morphological identification was done based on cultural characterization and microscopic observation (Savitha and Sriram, 2015). For morphological characterization of these isolates, characters describing colony morphology, conidial size and breadth (µm). Microscopic observations were followed by 3% KOH mount and slide culture technique stained with lacto phenol cotton blue and examined with fluorescent microscope.

Dual culture technique

The antagonistic activity of bio control agents (Tv_1-Tv_{10}) against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile Petri dish containing 15 ml of solidified PDA medium a seven mm mycelial disc obtained from five days old culture of *Trichoderma* spp. was placed under aseptic conditions. Similarly, at the opposite end approximately 75 mm away from the *Trichoderma* culture disc, a seven mm mycelial disc obtained from seven days old culture of *S. rolfsii* was placed and incubated. A control was maintained by inoculating *S. rolfsii* alone at one end of the Petri dish. The plates were incubated at room temperature (28 ± 2°C) for seven days.

Per cent inhibition

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

Where, C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual plate

I - inhibition Percent

Based on the dual culture technique the effective *T. viride* were identified and used for further studies.

Poison food technique

PDA medium was prepared in 100 ml conical flask and autoclaved. Filtered antagonistic poison of *Trichoderma* of 5, 10, 15 and 20 ml were added to 45, 40, 35 and 30 ml aliquots respectively in flasks so as to get the final concentration of 10, 20, 30 and 40 per cent.

The incorporation of fungicide carbendazim @ 0.1% in the medium was used for comparison. PDA medium without culture filtrate served as control. Each plate was inoculated at the center with a seven old culture disc (6 mm) of pathogen and incubated at room temperature (28±2°C).

Results and Discussion

Cultural characteristics of *S. rolfsii* isolates

Growth and cultural characters of native *T. viride* isolates

A total of ten isolates of *T. viride* were able to be isolated from tuberose rhizosphere soil in different localities. The colony characters of ten native isolates of *T. viride* were observed visually on third and seventh day after inoculation and the results are presented in table 2. The colony morphology of the isolates (Tv_1, Tv_4, Tv_8 and Tv_9) was almost similar and showed profuse white to green mycelium. The isolates (Tv_3, Tv_7, Tv_8 and Tv_{10}) showed moderate white mycelial growth. The colonies reached 80-90 mm diameter within five days at room temperature (28±2°C). Sporulation started after 48h of incubation for all the isolates. The conidiophores showed typical pyramidal branching *viz.,* short branches near the tip and longer branches with frequent branching at the bottom. The conidia of Tv_5 were almost globose and measured 2.80 - 3.75µm length and 2.50 – 5.10 µm breath was recorded as large compared to other isolates. The cultural and morphological characteristics of *Trichoderma* native isolates agreed with those described by Domsch *et al.,* (1980) and therefore ten isolates were identified as *T. viride* and designated as Tv_1 to Tv_{10}. *Trichoderma* is worldwide in occurrence and can be easily isolated from soil. The capability of *Trichoderma* as bio control agent against plant diseases has been reported by several workers (Wells *et al.,* 1972; Sharon *et al.,* 2001). Antagonistic effects of *Trichoderma* against *S. rolfsii* have already been reported by various other researchers on different hosts (Chet, 1987; Elad *et al.,* 1983; Harman *et al.,* 1980; Papavizas, 1985; Prasun, 1997).

In the present study Tv_5 has highly efficient in reduction of stem rot of tuberose under *in vitro* and *in vivo* conditions. The inhibitor effect is due to different mechanisms viz., competition, parasitism, antibiosis and lysis.

In vitro efficacy of *T. viride* against *S. rolfsii* (Sr_1) by Dual culture method

In general, all the native *T. viride* isolates tested significantly inhibited the mycelial growth of *S. rolfsii* (Table 2). However, among the isolate Tv_5 showed the maximum growth inhibition of *S. rolfsii* up to 77.15 per cent respectively. This was followed by the isolates Tv_4 (75.94) and Tv_1 (72.96) in the decreasing order, the least growth inhibition of pathogen was exhibited by the isolate Tv_{10} (55.72 %). These results are in agreement with earlier workers (Karthikeyan *et al.,* 2006; Darvin *et al.,* 2013; Padmaja *et al.,* 2013; Pan *et al.,* 2013; Swathi *et al.,* 2015; Dwivedi and Ganesh Prasad, 2016; Hirpara *et al.,* 2017) (Plate 1).

Sala *et al.,* (2007) studied on the effectiveness of *Trichoderma* as bio-control agent against a number of soil-borne pathogens (*Pythium* sp., *Rhizoctonia solani* and *Sclerotium rolfsii*) and reported that the inhibition is due to several mechanisms, such as antibiosis, mycoparasitism, induction of defense responses and other adjunct mechanisms, such as growth promotion.

Table.1 Isolation and cultural characters of native isolate *Trichoderma viride*

S.No	Isolates	Locality	Colony morphology	Conidia size	
				Length (µ)	Breadth (µ)
1	Tv_1	Palwadi	Profuse mycelium with dark green sporulation	2.35-3.30	2.20-4.50
2	Tv_2	Varagur	Whitish green mycelium with dull sporulation	2.10-3.10	2.05-4.00
3	Tv_3	Baisuhalli	Moderate white mycelium with bright green sporulation	2.20-3.20	2.40-4.30
4	Tv_4	Pulikurai	Initial white mycelium later becomes fluffy green	2.55-3.30	2.40-4.70
5	Tv_5	Karagathahalli	Bright green sporulation	2.80-3.75	2.50-5.10
6	Tv_6	Kadampatti	Thin white mycelium with complete green sporulation	2.00-3.15	2.25-4.10
7	Tv_7	Perungulathur	Initially dull white mycelium later becomes dark green	2.30-3.00	2.00-4.00
8	Tv_8	Vanapurm	profuse mycelium later become cottony and green sporulation	2.35-2.90	2.40-3.85
9	Tv_9	Royandpuram	Profuse mycelium with green sporulation	2.20-3.00	2.30-3.90
10	Tv_{10}	Thandarampatti	Moderate white mycelium later become deep green sporulation	2.30-2.85	1.90-3.75

Table.2 *In vitro* efficiency of *Trichoderma viride* against *Sclerotium rolfsii* (SR_1) by dual culture method

Isolates number	Mycelial growth (mm)	Inhibition zone (mm)	Per cent inhibition over control
Tv_1	24.33	10.38	72.96
Tv_2	29.84	9.31	66.84
Tv_3	26.62	9.65	70.42
Tv_4	21.65	11.30	75.94
Tv_5	20.56	12.58	77.15
Tv_6	27.74	9.46	69.17
Tv_7	32.43	8.81	63.96
Tv_8	37.33	7.05	58.52
Tv_9	34.15	7.71	62.05
Tv_{10}	39.85	6.80	55.72
Control	**90.00**	**0.0**	**100**
	S.Ed CD (0.05)	0.01 0.05	

Data in parantheses indicate angular transformed values

Table.3 Effect of culture filtrate of *Trichoderma viride* on the mycelia growth of *Sclerotium rolfsii* (SR_1) by Poison food technique

T.no	Concentration of cultural filtrate (%)	Solid medium		Liquid medium	
		Mycelial growth (mm)	Per cent inhibition over control (%)	Mycelial dry weight (mg)	Per cent inhibition over control (%)
T_1	10	38.65	57.05	320.83	28.84
T_2	20	26.11	70.98	112.45	75.02
T_3	30	10.38	88.46	69.72	84.51
T_4	40	0.00	100	4.21	99.06
T_5	Carbendazim (0.1%)	0.13	99.85	2.87	99.36
T_6	Control	90	-	450.25	-
	S.Ed CD (0.05)	0.07 0.15		1.21 2.58	

Data in parantheses indicate angular transformed values

Plate.1 In vitro efficiency of *Trichoderma viride* against *S. rolfsii* (dual culture method)

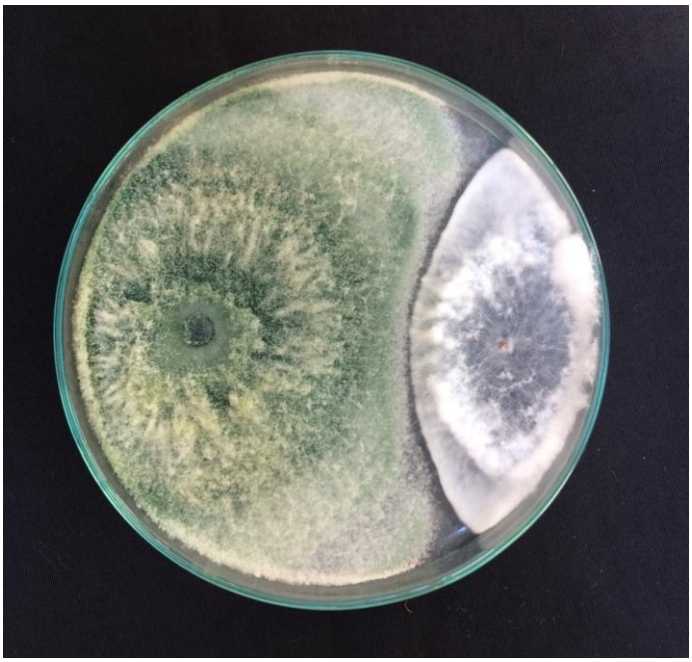
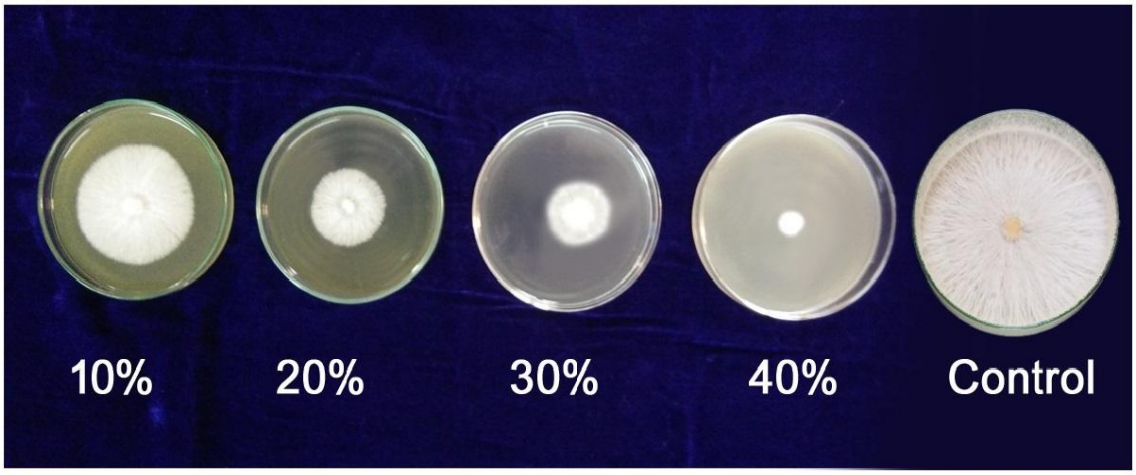


Plate.2 Evaluation of *Trichoderma viride* against *S. rolfsii* (Poison food technique)



T. viride was found to inhibit *in vitro* growth *S. rolfsii* by coiling around mycelium of *S. rolfsii* resulting in lysis of hyphae (Fouzia and Seleem, 2005). Babu and Kumar (2008) also reported that *T. harzianum*-3 (Th-3) inhibited mycelial growth of *S. rolfsii* by 83 per cent in dual culture; the sclerotial population was also reduced by coiling around the aerial hyphae of *S. rolfsii* and produced haustoria like structure and penetrated the hyphae *S. rolfsii* and dis-organized the protoplasm and controlled *S. rolfsii*. *T. viride* may also affect the growth of pathogen either through antibiosis (or) mycoparasitism. Besides they may also produce antifungal phenolic compounds (viridian, gliotoxin and Trichodermins) (Sababanday *et al.,* 2008; Rahel Ratnakumari *et al.,* 2011) which might be responsible for the inhibition of pathogen.

Effect of culture filtrate of *T. viride* on the mycelial growth and mycelial dry weight of *S. rolfsii* (Sr_1) by Poison food technique

The mycelial growth of *S. rolfsii* was found to be reduced with an increase in the conc. of culture filtrates of *T. viride* and the reduction was significantly the maximum in the case of *T. viride* with 38.65, 26.11, 10.38 and 0.00 mm at 10, 20, 30 and 40 per cent conc. of the culture filtrate respectively against the maximum growth of 90 mm in the control. The same trend was maintained in the case of liquid medium assay. The flasks inoculated with pathogen and amended with culture filtrate of *T. viride* recorded significant reduction in the mycelial dry weight whereas, the flasks inoculated with *S. rolfsii* alone (control) recorded the maximum. The culture filtrate of all the *T. viride* isolates significantly inhibited the growth of *S. rolfsii*. Generally, an increase in the concentration of the culture filtrate reduced the growth of the pathogen. (Table 3) (Plate 2).

Siddanagour (2005) reported reduction in mycelial growth of *S. rolfsii* when PDA amended with culture filtrates *Trichoderma* spp. culture filtrates of *Trichoderma* spp inhibited the mycelial growth and sclerotial germination of *S. sclerotiorum* (Kapil and Kapoor 2005). Vengatesh (2013) reported that culture filtrate of isolates -I_2 (THA) recorded complete inhibition of *S. rolfsii* at 15% concentration. The cell free culture filtrate of *T. viride* and *T. harzianum* showed 100 per cent mycelial growth inhibition at 60 and 80 per cent conc. against *S. rolfsii* (Swathi *et al.,* 2015). These earlier reports are in line with the present findings. It was clearly stated that

T. viride isolated from rhizosphere soil of tuberose have strong and virulent antagonist against stem rot of tuberose caused by *S. rolfsii*. The combination of bulb treatment and soil application is found to be very effective.

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