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In vitro evaluation of bio agents against *Rhizoctonia solani* causing root rot of tomato

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Abstract
A survey was conducted for the collection and isolation of *Trichoderma* spp. in different districts of major tomato growing areas viz., Virudhunagar, Nellore, Namakkal, Salem, Theni Ramanathapuram, Sivaganagai, Coimbatore, Madurai, Srikazhi, Villupuram and Chidambaram. Twelve isolates of *Trichoderma* spp. were isolated from the rhizosphere soil of tomato. These isolates were screened against root rot pathogens. Among the twelve isolates tested, the isolate DVT-1 had maximum inhibition of 83.33 percent inhibition over control and with mean mycelial growth of *Rhizoctonia solani* of 1.50 cm under *in vitro*. Minimum percent inhibition over control was recorded by TTM-6 with 36.44 percent and mean mycelial growth of 5.72 cm.

Keywords: Tomato, *Rhizoctonia solani*, *Trichoderma* spp., *in vitro*

Introduction
Tomato (*Lycopersicon esculentum* L.) considered one of the most important vegetable crops in India, crop is attack by several soil borne fungal pathogens [1]. *R. solani* are the most important soil borne fungal pathogen, which develop in both cultured and non-cultured soils, causing the symptoms of root rot disease to wide range of vegetable crops including tomato [2]. The incidence of *R. solani*, caused 10 to 80% losses in different vegetables [3]. Root rot disease has great economic importance where, it infects and destroys the entire root system of tomato plants, limiting their nutritional activity and affecting tomato productivity in the quantity and quality under greenhouse conditions [4, 5]. For controlling *Rhizoctonia* root rot disease of tomato, several management strategies were done to manage such disease. Among the most truly effective and old method for disease control is using of fungicides. However, chemical fungicides are expensive and not environmentally safe besides their hazards to the human health. Recently, *Trichoderma* spp. are considered eco-friendly biocontrol agent against a number of phytopathogens and has been marketed commercially as biopesticides, biofertilizers and soil improvements [6]. This work is aimed to study; the pathogenicity of *R. solani* against some commercial tomato cultivars, fungal pectolytic and cellulolytic enzymes activity fungal toxin production. The inhibition effect of some commercial fungicides and antagonistic effect of common bio-control agents as *Trichoderma* spp. against *R. solani* isolates *in vitro* tests.

Materials and Methods
Isolation and identification of the pathogens
A field survey in several sites (Virudhunagar, Nellore, Namakkal, Salem, Theni, Ramanathapuram, Sivaganagai, Coimbatore, Madurai, Srikazhi, Villupuram and Chidambaram districts was conducted in summer season 2023. Pathogenic fungi were isolated from roots of tomato plants that collected from studied sites. 0.5 to 1cm pieces of roots were cut and washed well then sterilized with 2% sodium hypochlorite for 3 minutes, after that, it washed with sterile water several times to remove traces of sterilization and put in Petri plates on P.D.A medium then incubated for three days at 25± 2 °C, until the colonies of the fungus became clear. Isolates were purified and re-cultured on 9-cm sterile Petri plates containing potato dextrose agar (P.D.A) and kept at 4 °C until used [7].

Survey and isolation of *Trichoderma* spp.
A survey was conducted in various districts *viz*., Virudhunagar, Nellore, Namakkal, Salem, Theni, Ramanathapuram, Sivaganagai, Coimbatore, Madurai, Srikazhi, Villupuram and

Chidambaram districts for the collection of rhizosphere soil of tomato from agricultural ecosystems. The biocontrol agents were isolated from rhizosphere soil by serial dilution [8]. *Trichoderma* selective medium for *Trichoderma* spp.

In vitro evaluation of bioagents against *S. rolfsii*

The test antagonists *Trichoderma* spp. were tested against test pathogen *Rhizoctonia solani* and they were grown on the same plate to test the antagonistic activity. About 15 to 20 ml of melted and cooled PDA medium was poured in to Petri plates and allowed to solidify. Fungal disc of the antagonist of was placed at one end of media on Petri plate. A 9 mm test pathogen PDA culture disc was placed at the opposite end. Four replications along with suitable control were maintained. The plates were incubated in an inverted position at room temperature (25 ± 2 °C) till the mycelial growth in the control plates covered the entire plate. The radial growth of the pathogen was measured and the percentage inhibition was calculated by adopting following formula [9].

$$I = C\text{-}T/C \times 100$$

Where,
C illustrate the radial growth of *R. solani* in control.
'T' represents the radial growth of *R. solani* with treatment.

Results and Discussion

Collection and isolation of *Trichoderma* spp.

Twelve isolates of *Trichoderma* spp. were isolated from rhizosphere soils of tomato and these strains were collected from different district *viz.*, Virudhunagar, Nellore, Namakkal, Salem, Theni Ramanathapuram Sivaganagai, Coimbatore, Madurai, Srikazhi, Villupuram and Chidambaram districts**.** Among the twelve isolates, five isolates were screened effectively against the *R. solani* (Table1) [10]. reported that sixteen samples were collected from rhizospheric soil of tomato crop of eight districts of marathwada region [6], screened 26 isolates of *Trichoderma* against *R. solani* and *F. oxysporum*. The isolates T. *pseudokonigii* TR17 and *T. harzianum*-20 were proved effective against the soil borne pathogens. Similarly [11] reported the effectiveness of *T. viride* against *R. solani* causing root rot of tomato [12]. Also reported that the rhizosphere isolate Th1 inhibited 87.04 percent of mycelium of *R. solani*. Our studies indicated that different isolates of *Trichoderma* spp. showed different antagonistic potentiality against *R. solani*.

In vitro antagonism of *Trichoderma* spp. isolates against radial mycelial growth of *R. solani*

The twelve isolates of *Trichoderma* spp. were tested against the radial mycelial growth of *S. rolfsii* by dual plate method. Among the various isolates of *Trichoderma* spp. were screened, the isolate DVT-1 had maximum inhibition of 68.88 percent inhibition over control and with mean mycelial growth of 2.80 cm under *in vitro*. It was followed by KRN-3 and ATM-2 with percent inhibition of 67.22 and 65.33 percent inhibition over control and with the mycelia growth of 2.95 cm and 3.12 cm respectively. Minimum percent inhibition over control was recorded by TTM-6 with 36.44 percent and mean mycelial growth of 5.70 cm (Table 2) [13]. reported that *Trichoderma* strains inhibited the growth of *Rhizoctonia solani* by 73.3% [14]. reported that the antagonistic activity of *Trichoderma viride* been tested against *Rhizoctonia solani*. *Trichoderma viride* was isolated from *Rhynchostylis retusa* and *Rhizoctonia solani* from *Aerides multifloral* an orchid. Dual culture method was followed and result revealed that *Trichoderma viride* inhibited the mycelial growth of *Rhizoctonia solani* by 79.08%. Antagonistic activity of *Trichoderma viride* against *Rhizoctonia solani* revealed the inhibition of growth of *R. solani* which was 79.08%. Trichodermin, a sesquiterpene antibiotic produced by *Trichoderma* spp. has been reported to be active against fungi. They also produced the antibiotics named as gliotoxin and viridin [15, 16]. Reported that *T. viride* isolate TV2 significantly exerted the maximum inhibition of 72.00 percent on the mycelial growth (25.00 mm) of the pathogen as against 90.00 mm colony diameter in control (Table 3).

Table 1: Isolates of *Trichoderma* spp. collected from different tomato growing areas

S. No.	Isolate	Location	District
1.	DVT-1	Devathanam	Virudhunagar
2.	ATM-2	Athmakur	Nellore
3.	KRN-3	Kathiranallur	Namakkal
4.	ANP-4	Anuppur	Salem
5.	KGP-5	Kamayagoundanpatti	Theni
6.	TTM-6	Thottamangalam	Ramanathapuram
7.	KTM-7	Kottampatti	Sivagangai
8.	SDP-8	Sundarapuram	Coimbatore
9.	PNT-9	Poonjuthi	Madurai
10.	PTR-10	Puthur	Srikazhi
11.	ALT-11	Alathur	Villupuram
12.	AND-12	Adanur	Chidambaram

Table 2: Effect of different isolates of *Trichoderma* spp. on the mycelial growth of *S. rolfsiiu*

S. No.	Isolate	Mycelial growth (mm)	Percent inhibition over control (%)
1.	DVT-1	2.80	68.88
2.	ATM-2	3.12	65.33
3.	KRN-3	2.95	67.22
4.	ANP-4	3.21	64.33
5.	KGP-5	3.32	63.11
6.	TTM-6	5.70	36.00
7.	KTM-7	3.60	60.00
8.	SDP-8	3.80	57.77
9.	PNT-9	3.90	56.66
10.	PTR-10	4.15	53.88
11.	ALT-11	4.85	46.11
12.	AND-12	3.41	62.11

Conclusion

The present study was under taken to find out the effect of *Trichoderma* isolates on the mycelial growth of the pathogens under *in vitro*. Also, these strains showed high antagonistic activity against the root rot pathogens.

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