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In vitro evaluation of bio agents against *Sclerotium rolfsii* Sacc. Causing southern blight of tomato

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Abstract
A survey was conducted for the collection of *Trichoderma* spp in different districts of major tomato growing areas of Tamil Nadu *viz*., Allanganallur, Checkanurani, Natham, Poonjuthi, Sirumalai, Andipatti and Pavoorchathiram. Fiveteen isolates of *Trichoderma* spp were isolated from the rhizosphere soil of tomato. These isolates were screened against southern blight pathogens. Among the fourteen isolates tested, the isolate TV22B had maximum inhibition of 83.33 per cent inhibition over control and with mean mycelial growth of *Sclerotium rolfsii* of 1.50 cm under *in vitro*. Minimum per cent inhibition over control was recorded by TV1313M with 36.44 per cent and mean mycelial growth of 5.72 cm.

Keywords: Tomato, *Sclerotium rolfsii*, *Trichoderma* spp, *In vitro*

Introduction
Tomato (*Lycopersicon esculentum* L.) is an important nutritive rich and warm season vegetable crop grown throughout the world. In the world, tomato is cultivated in an area of 4.8 million hectare with an annual production of 161.8 million tonnes (Anonymous 2012) [1]. The phytopathogenic fungi, disease caused by *Sclerotium rolfsii*, a soil borne fungi which causes foot rot or collar rot of tomato is gaining a serious status. It is known to be pathogenic on nearly 500 plant species. The disease is also referred as *Sclerotium* blight, *Sclerotium* wilt, southern blight, southern stem rot and white mold which cause 55-95% mortality of the crop at seedling stage under condusive conditions (Gurha and Dubey, 1982) [3]. *S. rolfsii* Sacc. is widely distributed in tropics, subtropics and also in warmer parts of temperate zone of the world. *Trichoderma* species are generally known as biological control agents (BCAs) against several plant diseases. Several *Trichoderma* species have multifaceted actions including parasitism (Monteiro *et al.*, 2010) [6]. *Trichoderma* species are known to produce several metabolites of agricultural significance. The metabolites exhibit antimicrobial activities, as well as induction of resistance to plant pathogens. For instance, harzianolide produced by *Trichoderma koningii* and *T. harzianum* exhibits antifungal activity and is a plant growth regulator. The trichokonin produced by *T. koningii* has the same attributes, i.e. antifungal and plant defense inducer (Xiao-Yan *et al.*, 2006) [11].

Materials and Methods
Isolation and identification of the pathogens
The pathogens were isolated from the infected collar region of tomato plants showing typical collar rot symptoms by tissue segment method on Potato Dextrose Agar (PDA) medium Rangaswami (2005) [8]. The infected portions were surface sterilized with 0.1% mercuric chloride for 30 seconds, and subsequently washed thrice in sterile distilled water to remove the traces of mercuric chloride. Then, the infected portions were placed in sterilized petri dishes containing potato dextrose agar (PDA) medium and incubated at the laboratory conditions at 25 ± 2°C for seven days.

Survey and isolation of *Trichoderma* spp
A survey was conducted in various districts of Tamil Nadu *viz*., Allanganallur, Checkanurani, Natham, Poonjuthi, Sirumalai, Andipatti and Pavoorchathiram for the collection of rhizosphere soil of tomato from agricultural ecosystems.

The biocontrol agents were isolated from rhizosphere soil by serial dilution (Pramer and Schmidt, 1956) [7] *Trichoderma* selective medium for *Trichoderma* spp.

In vitro* evaluation of bioagents against *S. rolfsii

For *in vitro* evaluation of antagonists, twenty ml of sterilized and cooled Potato dextrose agar was poured into sterilized Petri plates. The fungal antagonists were evaluated by inoculating the pathogen at the one side of the Petri plates and antagonist at exactly opposite side of the same plate by leaving 3-4 cm gap. For this purpose freshly growing cultures were used. In case of bacterial antagonist evaluation, two mycelial discs of pathogen were inoculated and bacterial antagonist was streaked in the centre of the Petri plate. After required period of incubation i.e. when the growth in control plate reached 90 mm diameter the radial growth of the pathogen was measured. The percentage inhibition over control was worked out according to the equation given Vincent (1927) [9].

I = C-T/C x 100

Where,
C illustrate the radial growth of *S. rolfsii* in control, and
'T' represents the radial growth of *S. rolfsii* with treatment.

Results and Discussion

Collection and isolation of *Trichoderma* spp.

Fourteen strains of *Trichoderma* spp were isolated from rhizosphere soils of tomato and these strains were collected from different district of Tamil Nadu *viz.*, Allanganallur, Checkanurani, Natham, Poonjuthi, Sirumalai, Andipatti and Pavoorchathiram. Among the fourteen isolates, five isolates were screened effectively against the *S. rolfsii* (Table1). (Kale *et al.*, 2018) [4] reported that Sixteen samples were collected from rhizospheric soil of tomato crop of eight districts of marathwada region. From these soil samples 16 isolates were isolated on PDA medium.

This rhizospheric soil was isolated by using 10^{-4} to 10^{-5} dilution by dilution plate technique. Out of 16, only 8 rhizospheric soil samples had the population of *Trichoderma* spp. By visual observation *Trichoderma* spp. were identified as *Trichoderma viride* isolates, *T. harzianum* and *T. hamatum* isolates.

In vitro* antagonism of *Trichoderma* spp isolates against radial mycelial growth of *S. rolfsii

The fourteen 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 TV22B had maximum inhibition of 83.33 per cent inhibition over control and with mean mycelial growth of 1.50 cm under *in vitro*. It was followed by TV33C and TV55E with per cent inhibition of 68.88 and 65.55 per cent inhibition over control and with the mycelia growth of 2.80 cm and 3.10 cm respectively. Minimum per cent inhibition over control was recorded by TV1313M with 36.44 per cent and mean mycelial growth of 5.72 cm (Table 2; Plate 1,Fig 1) 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 Wright (1956) [10]. (Kotasthane *et al.*, 2015) [5] reported that *T. viride* isolate TV2 significantly exerted the maximum inhibition of 72.00 per cent on the mycelial growth (25.00 mm) of the pathogen as against 90.00 mm colony diameter in control (Table 3). Lowest inhibition was recorded by isolate TV3 with mycelia growth of 36.00 mm and 60.00 per cent inhibition over control. (Bhuiyan *et al.*, 2012) [2] also reported that 20 isolates of *T. harzianum* were tested against *S. rolfsii* on PDA and the results are presented in Table 5. All the tested isolates of Trichoderma showed more than 60% inhibition of the radial growth of the test pathogens *S. rolfsii* over control. Among the tested isolates, TH-18 showed the highest (83.06%) reduction of the radial growth (Figure 1) followed by TH-2 (74.19%). Among the tested isolates, TH-2, TH-3, TH-8, TH-10, TH-20 and TH 76 l showed significantly similar results in inhibiting *S. rolfsii*. The lowest radial growth inhibition of *S. rolfsii* was observed in isolate TH-12 C (65.01%) and TH-16 (65.81%), respectively.

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

S.NO	Isolate	Location	District
1.	TV11A	Allanganallur	Madurai
2.	TV22B	Checkanurani,	Madurai
3.	TV33C	Natham	Dindigul
4.	TV44D	Poonjuthi	Madurai
5.	TV55E	Sirumalai	Dindigul
6.	TV66F	Andipatti	Theni
7.	TV77G	Pavoorchathiram.	Tirunelvelli
8.	TV88H	Thirumangalam	Madurai
9.	TV99I	Palamedu	Madurai
10.	TV1010J	Kovilpatti	Tuticorin
11.	TV1111K	Srivilliputhur	Virudunagar
12.	TV1212L	Sathur	Virudunagar
13.	TV1313M	Rajapalayam	Virudunagar
14.	TV1414N	Aruppukotai	Virudunagar

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

S.NO	Isolate	Mycelial growth (mm)	Per cent inhibition over control (%)
1.	TV11A	3.40^{g}	62.22 (52.07)
2.	TV22B	1.50^{l}	83.33 (65.90)
3.	TV33C	2.80^{k}	68.88 (56.09)
4.	TV44D	2.95^{j}	67.00 (54.94)
5.	TV55E	3.10^{ij}	65.55 (54.06)
6.	TV66F	4.10^{c}	54.44 (47.55)
7.	TV77G	3.20^{hi}	64.44 (53.39)
8.	TV88H	3.32^{gh}	63.00 (52.54)
9.	TV99I	3.60^{f}	60.00 (50.77)
10.	TV1010J	3.73^{ef}	58.55 (49.92)
11.	TV1111K	3.90^{d}	56.66 (48.83)
12.	TV1212L	3.81^{de}	57.66 (49.41)
13.	TV1313M	5.72^{a}	36.44 (37.13)
14.	TV1414N	4.80^{b}	46.66 (43.08)
15.	Control	9.0	00.00
		CD	0.14

*Mean of three replications. Means with the same letter do not have significant difference according to Duncan's multiple range test at $p< 0.05$.
**Values in the parentheses are arc sine transformed values

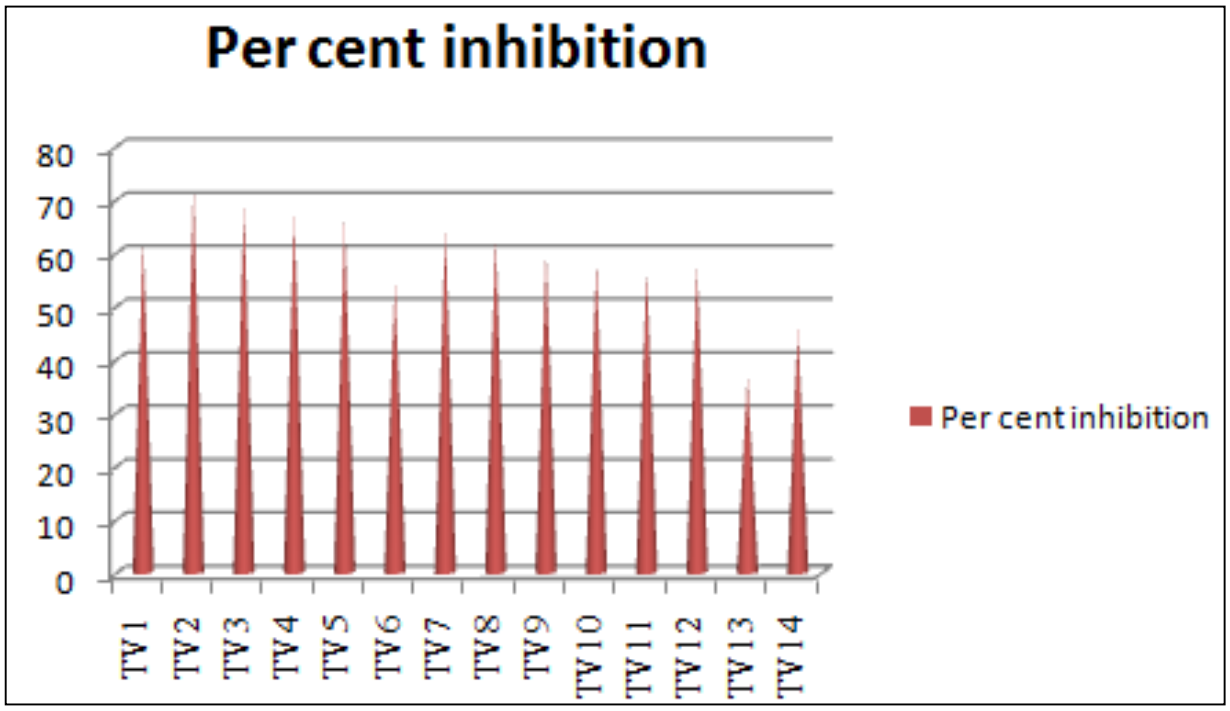

Fig 1: *In vitro* screening of *Trichoderma* spp against *S. rolfsii*

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 collar rot pathogens.

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