

Original Research Article

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## *In vitro* Evaluation of Bio-Agents and Fungicides against Foot Rot Pathogen (*Sclerotium rolfii* Sacc.) of Tomato

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### ABSTRACT

#### Keywords

*Sclerotium rolfii*,  
*In vitro*, Fungicides,  
Bioagents, Dual  
culture technique  
and poison food  
technique.

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Foot rot of tomato is an important disease which causes severe loss in tomato production. The present study was carried out to evaluate five bioagents and six fungicides against *Sclerotium rolfii*. Among the five antagonists tested the maximum inhibition was observed in *Trichoderma virens* (67%) and contrarily, no inhibition was observed in *Bacillus subtilis*. Six fungicides viz., carbendazim, captan, tebuconazole, copper oxychloride, hexaconazole and Tebuconazole +Trifloxytrobin at different concentrations (50, 100, 150, 200, 250 and 500 ppm), were tested. Among the tested fungicides, systemic fungicides like hexaconazole, tebuconazole and combi product, Nativo (tebuconazole 50% + trifloxystrobin 25%) showed complete inhibition of *S. rolfii* at all the concentration tested.

### Introduction

Tomato (*Solanum lycopersicum* L.) is an important nutritive rich and warm season vegetable crop grown throughout the world. Tomato suffers from a number of fungal, bacterial, nematode and many viral diseases. The annual loss of vegetable due to the pathogen is 10 per cent, major loss is due to the fungal pathogen. Among the phytopathogenic fungi, disease caused by *Sclerotium rolfii*, a soil borne fungi which causes foot rot or collar rot of tomato is gaining a serious status. *S. rolfii* is polyphagous and most destructive soil borne fungus and was first reported by Rolfs (1892) as a cause of tomato blight in Florida. Later, Saccardo (1911) named the fungus as

*Sclerotium rolfii*. But, in India the root rot caused by this pathogen was first reported by Shaw and Ajrekar (1915) who had isolated the pathogen from rotting potatoes which they identified as *Rhizoctonia destruens* Tassi. Later studies convinced Ajrekar that the fungus involved in rotting of potato was *Sclerotium rolfii* but not *R. destruens* (Ramakrishnan, 1930). 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 conducive conditions (Gurha and Dubey 1982). Among the phytopathogenic fungi, disease caused by *Sclerotium rolfii*, a soil borne fungi which

causes foot rot or collar rot of tomato is gaining a serious status. *S. rolfsii* has become a major limiting factor and the challenging to both farmers and scientists. Many approaches have been exploited for management of this disease, like cultural practices. Owing to soil borne nature of the pathogen and its wide host range management through chemicals as well as bio-agents is important. Keeping this in view present investigation was carried to know the effect of bio-agents and fungicides against *S. rolfsii* *in vitro*.

### Materials and Methods

Tomato plants showing the typical symptoms of foot rot were collected and isolation of the fungus was done by following standard tissue isolation technique. Pure culture was transferred to PDA slants periodically for further studies. *In vitro* evaluation was carried out with five bio-agents *viz.*, *Trichoderma viride*, *T. harzianum*, *T. virens*, *Pseudomonas fluorescens*, *Bacillus subtilis* against *S. rolfsii* through dual culture technique.

**Dual culture technique:** It was followed for the *in vitro* screening of bio-agents. Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal bio-control agents, mycelial disc of test fungus was inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. In case of bacterial antagonist two mycelial discs of pathogen were inoculated at the periphery of the Petri plate and bacterial antagonist was streaked in the center of the same plate. Three replications were maintained for each treatment. The plates were incubated at  $27\pm 1^{\circ}\text{C}$ . After required period of incubation *i.e* when the growth in control plate recorded 90 mm in diameter, the radial growth of the pathogens was measured and zone of inhibition was recorded by measuring the

clear distance between the margin of the test fungus and antagonistic organism. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

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

Where,

I = per cent inhibition

C = growth in control

T = growth in treatment

**Poisoned food technique:** The efficacy of six fungicides *viz.*, carbendazim, captan, tebuconazole, copper oxychloride, hexaconazole and Tebuconazole +Trifloxytrobin at different concentrations (50, 100, 150, 200, 250 and 500 ppm), were assayed against *Sclerotium rolfsii*. These fungicides were evaluated by using "Poison food technique" under laboratory conditions (Grover and Moore, 1962). Required quantity of individual fungicide was added separately into sterilized molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Three replications were maintained for each concentration. Inoculated plates were incubated at room temperature and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the formula given by Vincent (1947).

### Results and Discussion

#### *In vitro* evaluation of bio-agents against *Sclerotium rolfsii*

The results, thus obtained (Table 1 and Plate 1) revealed that, maximum inhibition of

mycelia growth (67%) was recorded in *Trichoderma virens* after four days of incubation. Other bio-agents viz., *Trichoderma viride* and *Trichoderma harzianum* recorded 61% and 44% inhibition respectively. However *B. subtilis* did not show any inhibition of mycelial growth of *S. rolfsii* as the pathogen over grew the bio-agents.

Similarly after eight days of inoculation, *Trichoderma virens* showed highest inhibition (70%) followed by *Trichoderma viride* (63%). Whereas *Pseudomonas fluorescens* and *Trichoderma harzianum* recorded 12% and 44% inhibition respectively. *Trichoderma harzianum* did not show variation in the per cent inhibition as the incubation time was increased.

Biological control is a potential non-chemical, effective and eco friendly means for plant disease management. In the present

investigation the efficacy of bio-agents against *S. rolfsii* was assessed by the dual culture technique. The present study is confirmatory with the observations made by Iqbal *et al.* (1995), Virupaksha Prabhu *et al.* (1997) and Kulkarni (2007). This might be due to the production of antibiotics, which diffused, air filled pores, which are detrimental to the growth of *S. rolfsii*. and also may be due to higher competitive ability of *Trichoderma* spp.

***In vitro* evaluation of fungicides against *Sclerotium rolfsii***

The result revealed that, among the systemic fungicides hexaconazole and tebuconazole were found to be highly effective at all concentrations tested and these inhibited cent per cent mycelia growth of *S. rolfsii* and were significantly superior over control (Table 2 and Plate 2).

**Table.1** *In vitro* evaluation of bio-agents against foot rot fungus *Sclerotium rolfsii*

Treatments	BIOAGENTS	PER CENT INHIBITION (Days after incubation)	
		4	8
T <sub>1</sub>	<i>Trichoderma viride</i>	61 (45.75)*	63 (46.24)
T <sub>2</sub>	<i>Trichoderma harzianum</i>	44 (40.12)	44 (40.12)
T <sub>3</sub>	<i>Trichoderma virens</i>	67 (47.81)	70 (48.88)
T <sub>4</sub>	<i>Pseudomonas fluorescens</i>	11 (26.01)	12 (26.71)
T <sub>5</sub>	<i>Bacillus subtilis</i>	0	0
	<b>SEm±</b>	<b>0.62</b>	<b>0.79</b>
	<b>C.D at 1%</b>	<b>1.92</b>	<b>2.49</b>

\*Figures in parenthesis are arcsine transformed values

**Table.2** *In vitro* evaluation of botanicals against foot rot fungus *Sclerotium rolfsii*

Treatments	Extracts of Botanicals	Mean per cent mycelial inhibition			
		Concentration of botanicals (%)			
		5	10	15	MEAN
T <sub>1</sub>	Neem leaf	75.55 (60.35)*	91.11 (72.64)	93.33 (75.02)	86.66 (68.56)
T <sub>2</sub>	Eucalyptus leaf	38.88 (38.56)	50.00 (44.99)	53.00 (46.71)	47.29 (43.44)
T <sub>3</sub>	Jathropa leaf	22.55 (28.34)	24.44 (29.62)	44.07 (41.58)	30.35 (33.42)
T <sub>4</sub>	Tulsi leaf	38.88 (38.56)	59.67 (50.56)	61.67 (51.73)	53.40 (46.94)
T <sub>5</sub>	Garlic bulb	97.77 (81.40)	98.88 (83.91)	100 (89.98)	98.88 (83.91)
T <sub>6</sub>	Onion bulb	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)
T <sub>7</sub>	Marigold leaf	86.33 (68.28)	89.67 (71.23)	90.78 (72.31)	88.92 (70.54)
		<b>Botanicals</b>	<b>Concentrations</b>	<b>B X C</b>	
	<b>SEm±</b>	<b>0.150</b>	<b>0.139</b>	<b>0.367</b>	
	<b>C.D at 1%</b>	<b>0.569</b>	<b>0.527</b>	<b>1.393</b>	

\*Figures in parenthesis are arcsine transformed value

**Table.3** *In vitro* evaluation of fungicides against foot rot fungus *Sclerotium rolfsii*

Treatments	Fungicides	Mean per cent mycelial inhibition					
		Concentration (ppm)					
		50	100	150	200	250	500
T <sub>1</sub>	Carbendazim (50 % WP)	0	0	0	0	11.10 (19.45)	88.80 (70.43)
T <sub>2</sub>	Captan (50 % WP)	0	0	0	11.11 (19.46)	66.66 (54.72)	100 (89.98)
T <sub>3</sub>	Tebuconazole (25.9 % EC)	100 * (89.98)	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)
T <sub>4</sub>	Copper oxychloride (50 % WP)	0	0	0	0	5.55 (13.62)	33.33 (35.25)
T <sub>5</sub>	Hexaconazole (5 % SC)	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)
T <sub>6</sub>	Tebuconazole + Trifloxystrobin (50%+25% WG)	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)	100 (89.98)
		<b>Fungicide</b>	<b>COC.</b>	<b>F X C</b>			
	<b>SEm±</b>	<b>0.122</b>	<b>0.113</b>	<b>0.30</b>			
	<b>C.D at 1%</b>	<b>0.45</b>	<b>0.422</b>	<b>1.117</b>			

\*Figures in parenthesis are arcsine transformed value

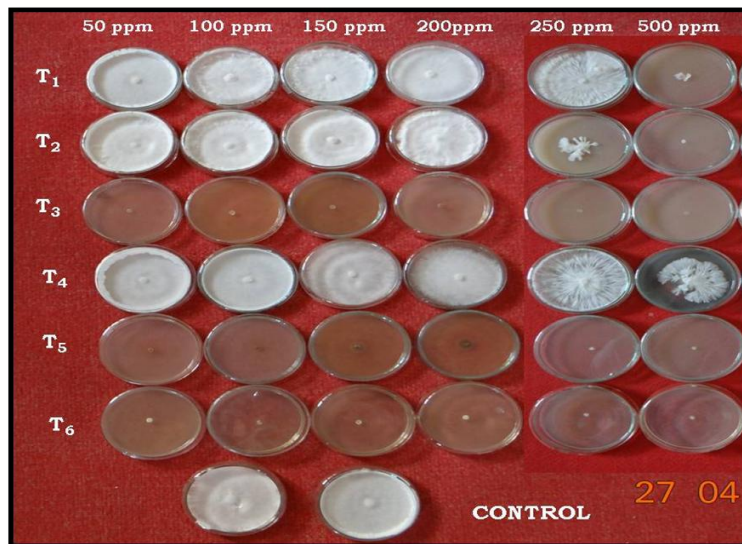
**Plate.1** Effect of bio-agents on the mycelia growth of *Sclerotium rolfsii* on tomato



**Legend:**

- T<sub>1</sub>: *Trichoderma viride*
- T<sub>2</sub>: *Trichoderma harzianum*
- T<sub>3</sub>: *Trichoderma virens*
- T<sub>4</sub>: *Pseudomonas fluorescens*
- T<sub>5</sub>: *Bacillus subtilis*

**Plate.2** Effect of fungicides on the mycelia growth of *Sclerotium rolfsii* on tomato



**Legend:**

- T<sub>1</sub>: Carbendazim
- T<sub>2</sub>: Captan
- T<sub>3</sub>: Tebuconazole
- T<sub>4</sub>: Copperoxichloride
- T<sub>5</sub>: Hexaconazole
- T<sub>6</sub>: Tebuconazole 50% + Trifloxystrobin 25% (Nativo)

Carbendazim does not show any inhibition of the mycelial growth of *Sclerotium rolfsii* at 50, 100, 150 and 200 ppm and it was comparable to control. However at 250 ppm and 500 ppm pathogen was inhibited by 11.10 and 88.80 per cent respectively. Non-systemic fungicide, captan was highly effective at higher concentration as it inhibited the mycelial growth *Sclerotium rolfsii* up to 66.66 and 100 per cent at 250 and 500 ppm respectively. However it does not showed any inhibition at lower concentrations (50, 100 and 150 ppm). Copper oxychloride (COC) was found to be least effective in inhibiting the growth of *Sclerotium rolfsii* as it inhibited only 5.55 and 33.33 per cent at 250 and 500 ppm respectively, where as combi product, tebuconazole 50% + trifloxystrobin 25% (Nativo) showed cent per cent inhibition at all the concentration tested (Table 3).

The results of present investigation are in confirmatory with the findings of earlier workers. Sharma and Verma (1985); Kulkarni (2007), Johnson and Subramanyam (2000) and Arunasri *et al.* (2011) reported that the triazoles (hexaconazole, propiconazole, difenconazole) and combi products containing triazoles *viz.*, Avatar, Merger and Nativo were highly inhibitive to the growth of *Sclerotium rolfsii*. In the present study the effective fungicides *viz.*, triazoles and tebuconazole + trifloxystrobin probably may act as antifungal agents and imparts its poisoning effect on metabolic process of pathogen, therefore, the growth of the *S. rolfsii* might be adversely affected. The present findings suggest that the *Trichoderma spp* and systemic fungicides have direct effect on the foot rot causing fungi *S. rolfsii*.

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