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***In-vitro* study of fungicides, botanicals and bioagents against *Sclerotium rolfii* Sacc. causing collar rot disease in tomato (*Solanum lycopersicum* L.)**

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Abstract

Tomato (*Solanum lycopersicum* L.) is an important and extensively cultivated vegetable crop in both the tropics and subtropics globally, India accounts for about 11.40% of the world's tomato production and is the 2nd largest producer after China. The share of major vegetable crops in India is potato (25.5%), onion (11.9%), followed by tomato (11.5%) (Anon, 2024). It is an important crop from both a production and industry point of view; demand for fresh fruits is increasing day by day. Tomato fruits are eaten raw in salad or cooked. Large quantities of tomatoes are used to prepare juice, ketchup, soup, pickles, and powder and add a variety of colours and flavours to food. Collar rot caused by *Sclerotium rolfii* is a destructive disease of tomato and other Solanaceous crops as well as a wide range of ornamentals, legumes and cucurbits (Bhagat, 2022). In this context, fungicides, botanicals and bioagents were evaluated against *Sclerotium rolfii* Sacc. Among systemic fungicides Tebuconazole and Propiconazole 25% EC at 0.025, 0.05 and 0.1 per cent concentrations, contact fungicides viz Mancozeb 75% WP and Chlorothalonil 75% WP, where S combi fungicides viz., Captan 70% + hexaconazole 5% WP, Carboxin 37.5% + Thiram 37.5% WP, Carbendazim 12% + Mancozeb 63% WP, and Tebuconazole 50% + Trifloxystrobin 13.28% WG at 0.1, 0.15 and 0.2 per cent concentration were shown complete (100%) inhibition of mycelia. In the case of botanicals Tulsi (*Ocimum sanctum*) was recorded highest per cent inhibition at 10% concentration (91.98)% and in bioagents *Trichoderma atroviride* -5 (Ta- 5) was found effective which shows 72.12 per cent of mycelial inhibition. These results indicate that fungicides, botanicals and bioagents can be combined for sustainable tomato collar rot management, although greenhouse and field trials are needed before large-scale adoption.

Keywords: Tomato, fungicides, botanicals, bioagents, *Sclerotium rolfii*

Introduction

Tomato (*Solanum lycopersicum* L.) is a widely cultivated vegetable crop in tropical and subtropical regions, belonging to the family Solanaceae. Tomato cultivation is severely affected by collar rot caused by *Sclerotium rolfii* Sacc, a major soil-borne disease responsible for 10–45% yield losses in tropical and subtropical regions. It is one of the most destructive diseases of tomato in Karnataka, causing collar rot, wilting, and plant death, leading to significant yield losses and reduced market value. It is pertinent to generate information on the efficacy of available and new fungicides, botanicals and bioagents for managing the disease. Hence, the present study was undertaken to screen various fungicides, botanicals and bioagents under *in vitro* conditions against *Sclerotium rolfii*.

Materials and Methods

Isolation and purification of *S. rolfii*

The fungus was isolated from collar rot infected tomato plants by direct plating method and were aseptically placed on Potato Dextrose Agar (PDA) medium. The plates were incubated at 27 ± 1 °C for 3–5 days. The emerging fungal growth was purified by the hyphal tip technique by transferring a 2–3 mm hyphal tip from the actively growing colony margin onto fresh PDA medium and incubating at 27 ± 1 °C for 5–7 days. The fungal colonies, on development were identified using simple microscope and recorded the mycelial characters. The purified culture was

examined for contamination to obtain a pure culture. The pure culture was maintained on PDA slants at 4°C and sub cultured periodically for further studies. The fungal colonies development was identified and proved pathogenicity.

***In vitro* evaluation of fungicides**

Four systemic fungicides (Difenoconazole 25% EC, Carbendazim 50% WP, Propiconazole 25% EC and Tebuconazole 5% EC). Contact fungicides (Captan 50% WP, Chlorothalonil 75% WP, Copper oxychloride 50% WP and Mancozeb 75% WP) combi product fungicides (Tebuconazole 50% + Trifloxystrobin 13.28%) 75% WG, (Carbendazim 12% + Mancozeb 63%) 75% WP, (Carboxin 37.5% + Thiram 37.5%) 75% WP and (Captan 70% + Hexaconazole 5%) 75% WP were tested against *S. rolfii* for inhibition of the fungal radial growth on potato dextrose agar by using poisoned food technique against *S. rolfii* for inhibition of the fungal radial growth on potato dextrose agar by using poisoned food technique Wedajo (2015) ^[15]. *In vitro* condition, Systemic fungicides were tested at 0.025, 0.05 and 0.1 per cent concentrations. For both contact and combi product were tested in 0.1, 0.15, 0.2 per cent concentrations.

***In vitro* evaluation of botanicals**

Nine botanicals viz wild senna (Leaf extract), Datura (Leaf extract), Mint (Leaf extract), Parthenium (Leaf extract), Mulberry (Leaf extract), Garlic (Bulb extract), Tulsi (Leaf extract), Ginger (Rhizome extract) and Kalmegh (Leaf extract) were tested against the growth of *S. rolfii* *in vitro* by poisoned food technique Wedajo (2015) ^[15]. Fresh healthy plant parts of 50 g (leaves/bulb) collected from field were washed with distilled water and air dried and crushed in 50 ml of sterile water. The crushed product was tied in muslin cloth and the filtrate was extracted. The stock solution was further diluted into desired concentrations of 5.0, 7.5 and 10.0 per cent. The results were expressed in terms of per cent inhibition of mycelial growth over control. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947) ^[13].

***In vitro* evaluation of bioagents**

The twenty fungal antagonists viz., *T. yunnanense* (12 isolates), *T. atrobunneum* (5 isolates), *T. crassum* (2 isolates) and *T. lixii* (1 isolate) were collected from department of plant pathology, Kittur rani chennamma college of horticulture, Arabhavi. Were evaluate their bioefficacy through dual culture technique. (Mc Kinney, 1923) The bioagents and the *S. rolfii* were inoculated side by side on a single Petri dish containing solidified PDA medium. Three replications were maintained for each treatment with one control by maintaining only pathogen and bioagent separately. They were incubated at 27±1 °C. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947) ^[13].

Results and Discussion

***In vitro* evaluation Fungicides**

Among the systemic fungicides, Tebuconazole 5% EC and Propiconazole 25% EC exhibited 100% inhibition at all concentrations (0.025, 0.05 and 0.1%) respectively and were significantly superior to others. (Table 1) Difenoconazole 25% EC showed that next best effective fungicide (up to 92.42%), while Carbendazim 50% WP was least effective (13.17%). Interaction effects revealed complete inhibition with

Tebuconazole 5% EC and Propiconazole 25% EC at all concentrations and with Difenoconazole 25% EC at 0.1% were shown cent percent inhibition. The Carbendazim 50% WP remained significantly inferior to inhibiting fungus.

Where as all the tested combi product fungicides showed complete inhibition (100%) of mycelial growth of *S. rolfii* at all concentrations. The fungicides Captan 70% + hexaconazole 5% WP, Carboxin 37.5% + Thiram 37.5% WP, Carbendazim 12% + Mancozeb 63% WP, and

Tebuconazole 50% + Trifloxystrobin 13.28% WG recorded cent per cent mycelial inhibition, indicating that all fungicides were equally effective against the pathogen under *vitro* conditions (Table 1). This may be because by these fungicidal combinations inhibit mycelial growth disrupting essential fungal processes such as ergosterol biosynthesis, cell division, enzyme activity, and mitochondrial respiration, providing both protective and systemic action. These findings agree with earlier reports by Chowdhury *et al.* (1998) ^[4], Virupaksha Prabhu and Hiremath (2003) ^[14], Kulkarni (2007) ^[9] and Arunasri *et al.* (2011) ^[12]. Among contact fungicides evaluated, captan 50% WP, chlorothalonil 75% WP and mancozeb 75% WP completely inhibited the mycelial growth of *Sclerotium rolfii* (100%) at all tested concentrations (0.1, 0.15, and 0.2%) and were significantly superior to copper oxychloride. Copper oxychloride 50% WP was the least effective, recording mycelial inhibition of 2.96%, 3.95%, and 8.64% at 0.1, 0.15, and 0.2% concentrations, respectively, with a mean inhibition of 5.18%. The overall mean mycelial inhibition increased slightly with increasing concentration, from 75.74% at 0.1% to 75.99% at 0.15% and 77.16% at 0.2%. This fungicides shows strong inhibition may be attributed to their multi-site mode of action. Karthikeyan *et al.* (2006) and Reddy *et al.* (2010) ^[11].

***In vitro* evaluation of botanicals**

The nine botanicals tested against *Sclerotium rolfii*. Tulsi (Leaf extract) recorded the highest mean mycelial inhibition of 65.81%, followed by Garlic (Bulb extract) with 54.77% and Ginger (Rhizome extract) with 40.75%. Moderate inhibition was observed in Mulberry (Leaf extract) (25.11%), Kalmegh (Leaf extract) (20.69%), Parthenium (Leaf extract) (19.14%) and Datura (Leaf extract) (18.95%). The least inhibition was recorded in Mint (Leaf extract) (15.83%) and Wild senna (12.12%). The high antifungal activity of Tulsi may be attributed to bioactive compounds such as eugenol and phenolics, which inhibit fungal growth (Kaur *et al.*, 2024) ^[8]. In which all tested botanicals are statically significant with each other. Similar observations were reported by Sahana *et al.* (2017) ^[12], who found that aqueous extracts of medicinal plants effectively suppressed the growth of *Sclerotium rolfii* under *in-vitro* conditions (Table 4).

***In vitro* evaluation of bioagents**

The antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfii* varied significantly. Among the twenty isolates tested, *T. atrobunneum* (Ta-5) recorded the highest mycelial inhibition (72.12%), followed by *T. yunnanense* (Ty-4) (68.88%). These found more effective than remaining isolates (Table 3) In contrast, *T. yunnanense* Ty-3 and Ty-9 showed the lowest inhibition (7.47% and 5.74%, respectively). The superior performance of Ta-5 and Ty-4 may be attributed to their rapid growth, may be attributed to their rapid mycelial growth, production of antifungal metabolites, and competitive ability to colonize the substrate (Harman *et al* 2004) ^[6] antagonistic ability. These results agree with earlier reports on the effectiveness of *Trichoderma* spp. against *S. rolfii* (Dubey and Patel, 2001) ^[5].

Table 1: *In vitro* evaluation of fungicides against *S. rolfsii*. Sacc

Systemic fungicides		Per cent inhibition			
Common name	Trade name	0.025 conc. %	0.05 conc. %	0.1 conc. %	Mean
Difenoconazole 25% EC	Score	81.11 (75.14) *	96.16 (76.18)	100 (89.65)	92.42 (80.32)
Carbendazium 50% WP	Bavistin	4.20 (11.68)	10.00 (18.29)	25.31 (30.11)	13.17 (20.03)
Propiconazole 25%EC	Tilt	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Tebuconazole 5% EC	Folicur	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Mean		71.31(66.53)	76.54 (68.44)	81.33(74.76)	
		Fungicides (F)	Concentration (C)	F × C	
S Em ±		0.50	0.43	0.87	
C D at 1%		2.00	1.73	3.47	
Contact fungicides		Per cent inhibition			
Common name	Trade name	0.10 conc. %	0.15 conc. %	0.2 conc. %	Mean
Captan 50% WP	Captaf	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Chlorothalonil 75% WP	Kavach	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Copper oxychloride 50%WP	Blue copper	2.96 (9.85)	3.95 (11.38)	8.64 (17.06)	5.18 (12.76)
Mncozeb 75%WP	Dithane M-45	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Mean		75.74 (69.70)	75.99 (70.08)	77.16 (71.50)	
		Fungicides (F)	Concentration (C)	F × C	
S Em ±		0.22	0.19	0.39	
C D at 1%		0.89	7.7	1.55	
Combi product fungicide		Per cent inhibition			
Common name	Trade name	0.1 conc. %	0.15 conc. %	0.2 conc. %	Mean
Tebuconazole 50% + Trifloxystrobin 13.28% 75%WG	Nativo	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Carbendazium 12% +Mancozeb 63% 75%WP	SAAF	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Carboxin 37.5% + thiram 37.5% 75% WP	Vitavax power	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Captan 70% + hexaconazole 5% 75% WP	Taqat	100 (89.65)	100 (89.65)	100 (89.65)	100 (89.65)
Mean		100 (89.65)	100 (89.65)	100 (89.65)	
		Fungicides (F)	Concentration (C)	F × C	
S Em±		NS			
C D at 1%		NS			

*Arc sine transformed values; conc. = concentration

Table 2: *In vitro* evaluation of botanicals against *S. rolfsii*. Sacc

Botanicals	Per cent inhibition			
	5% conc.	7.5% conc.	10% conc.	Mean
Wild senna (Leaf extract)	3.64 (19.36) *	4.07 (19.68)	5.80 (21.90)	12.12 (20.31)
Datura (Leaf extract)	4.26 (20.17)	9.44 (25.01)	20.74 (31.35)	18.95 (25.51)
Mint (Leaf extract)	5.68 (21.66)	8.09 (23.97)	8.89 (24.56)	15.83 (23.39)
Parthenium (Leaf extract)	5.93 (21.97)	9.07 (24.72)	19.07 (30.56)	19.14 (25.75)
Mulberry (Leaf extract)	4.94 (20.19)	18.27 (30.15)	36.73 (37.63)	25.11 (29.56)
Garlic (Bulb extract)	26.23 (33.69)	79.26 (52.46)	89.01 (57.17)	54.77 (47.78)
Tulsi (Leaf extract)	64.01 (46.78)	89.20 (57.27)	91.98 (59.03)	65.81 (54.36)
Ginger (Rhizome extract)	9.14 (24.76)	52.47 (42.92)	72.41 (49.77)	40.75 (39.15)
Kalmegh (Leaf extract)	3.64 (19.36)	5.43 (21.37)	37.47 (37.89)	20.69 (26.21)
Mean	19.54 (25.41)	31.33 (33.06)	40.18 (38.87)	
	Botanicals (B)	Concentration (C)	B × C	
SEm ±	0.31	0.18	0.53	
C D at 1%	0.88	0.51	1.53	

*Arc sine transformed values; conc. = concentration

Table 3: *In vitro* evaluation of *Trichoderma* isolates against *S. rolfsii*. Sacc

SI. No.	<i>Trichoderma</i> isolates	Per cent mycelial inhibition
1	<i>Trichoderma yunnanense</i> (Ty- 1)	65.25 (53.88) ^{*c}
2	<i>Trichoderma yunnanense</i> (Ty- 2)	16.23 (23.75) ^k
3	<i>Trichoderma yunnanense</i> (Ty- 3)	7.47(15.85) ^l
4	<i>Trichoderma atrobrunneum</i> (Ta- 1)	58.83 (50.09) ^{fgh}
5	<i>Trichoderma lixii</i> (Tl- 1)	17.92 (25.04) ^k
6	<i>Trichoderma yunnanense</i> (Ty- 4)	68.88 (56.09) ^b
7	<i>Trichoderma yunnanense</i> (Ty- 5)	62.84 (52.45) ^d
8	<i>Trichoderma yunnanense</i> (Ty- 6)	58.87 (50.11) ^{fgh}
9	<i>Trichoderma yunnanense</i> (Ty- 7)	58.15 (49.69) ^{gh}
10	<i>Trichoderma yunnanense</i> (Ty- 8)	59.75 (50.63) ^{efg}
11	<i>Trichoderma yunnanense</i> (Ty- 9)	5.74 (13.81) ^m
12	<i>Trichoderma yunnanense</i> (Ty- 10)	56.57 (48.78) ^h
13	<i>Trichoderma atrobrunneum</i> (Ta-2)	59.81 (50.66) ^{efg}
14	<i>Trichoderma crassum</i> (Tc-1)	61.67 (51.75) ^{de}
15	<i>Trichoderma atrobrunneum</i> (Ta-3)	49.22 (44.55) ⁱ
16	<i>Trichoderma yunnanense</i> (Ty- 12)	22.69 (28.44) ^j
17	<i>Trichoderma yunnanense</i> (Ty- 15)	22.66 (28.42) ^j
18	<i>Trichoderma atrobrunneum</i> (Ta- 5)	72.12(58.13) ^a
19	<i>Trichoderma crassum</i> (Tc-3)	60.22 (50.90) ^{efg}
20	<i>Trichoderma atrobrunneum</i> (Ta-6)	60.75 (51.21) ^{def}
	S Em±	0.45
	CD at 1%	1.74
	CV (1%)	1.84

*Arc sine transformed values

Conclusion

The present study evaluated the efficacy of fungicides, *Trichoderma* isolates, and botanicals against *Sclerotium rolfsii* Sacc causing collar rot of tomato. Among the fungicides, tebuconazole 5% EC, propiconazole 25% EC, and all combi-product fungicides completely inhibited mycelial growth (100%), while mancozeb 75% WP, chlorothalonil 75% WP, and captan 50%WP were the most effective contact fungicides. Tulsi leaf extract exhibited the highest antifungal activity (91.98%) among the botanicals, and *Trichoderma atrobrunneum* Ta-5 proved to be the most effective bioagent against *S. rolfsii*. These findings indicate that effective fungicides, along with promising bioagents and botanicals can be integrated for the eco-friendly and sustainable management of tomato collar rot. However, further evaluation under greenhouse and field conditions is required to validate their efficacy before recommending them for large-scale cultivation.

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