



***In vitro* Evaluation of Fungicides and Biocontrol Agents against *Rhizoctonia solani* in Tomato**

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Authors' contributions

This work was carried out in collaboration between all authors. Author MR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BV and GUD managed the analyses of the study. Author SRKR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Twenty four fungal biocontrol agents and twelve bacterial biocontrol agents were screened for their efficacy against phytopathogenic fungi *Rhizoctonia solani* through dual culture technique. *Trichoderma harzianum*-1 and *Pseudomonas fluorescense*-2 were found effective in inhibition of mycelium (62.53, 62.20) of *Rhizoctonia solani* under *in vitro* conditions. Ten fungicides were tested for their efficacy against *Rhizoctonia solani* through poisoned food technique and the ten fungicides Tebuconazole + Trifloxystrobin, Propiconazole, Captan + Hexaconazole and Carbendazim were record 100 per cent at recommended and half the recommended dosage under *in vitro* condition.

Keywords: *Rhizoctonia solani*; *Trichoderma harzianum*; *Pseudomonas fluorescense*; dual culture technique; poisoned food technique and tomato.

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1. INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) considered one of the most important economic vegetable crops in India, that 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 damping off and root rot diseases to wide range of vegetable and crop plants including tomato [2]. The incidence of damping off increased from 19 to 90% with increasing the inoculum level of *R. solani*, while the incidence of root rots caused 10 to 80% losses in different vegetables [3]. The tomato cultivars were classified into three groups of resistant, tolerant and susceptible according to their reaction to *Rhizoctonia* infection [4]. In recent years, *R. solani* causes severe damage to tomato cultivars in Egypt. The pathogenic fungi had resistant levels against both bio-control agents and chemicals [5]. 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 *T. harizianum*, *T. viride*, *B. subtilis* and *P. fluorescence* against *R. solani* isolates *in vitro* tests.

2. MATERIALS AND METHODS

The test pathogen *Rhizoctonia solani* was isolated from the disease tomato samples and collected from the farmer's field. Identification of *Rhizoctonia solani* fungus produced light brown mycelium, septate hyphae with 5-14 μm width and over 100 μm . The branching was almost at right angle to hyphal cell. Hyphae exhibited a constriction at the point of branching. The fungus also produced barrel shaped, comparatively smaller cells in groups, which were more than twice as wide as the vegetative cells, ranging from 10-20 μm in length, these structures are often called sclerotia. The individual sclerotium is <2 μm in diameter, round shaped and brown in colour. Fungus was fast growing, colonies dull white in colour initially and later became brown. The pathogen was identified based on the description given by [6].

Pathogenicity test was carried out at seedling stage of tomato by soil inoculation method. Infected seeds (cv. Arka Vikas) were completely rotted and even the rotting of emerging radical was noticed resulting in pre emergence damping

off. The roots of the germinated seedlings exhibited light brown lesions just below the soil level whereas the control plants did not exhibit any of these symptoms. The pathogen was re-isolated from the diseased plants and compared with original isolate and confirmed as *Rhizoctonia solani*.

2.1 Isolation of Fungal and Bacterial Bio control Agents

About 24 isolates of fungal biocontrol agents and 12 bacterial bio control agents were isolated from the rhizosphere samples of tomato collected in Ranga Reddy district. Particularly *Trichoderma* spp, *Pseudomonas* spp and *Bacillus* spp were isolated. Further morphological characteristics of these isolates were studied and identified based on the key provided by [7].

2.2 Identification of Fungal and Bacterial Bio control Agents

For isolation of *Trichoderma* strains, a serial dilution technique was followed. For this purpose one millilitre of each solution was pipetted onto a Rose Bengal Agar (RBA) plate and incubated at 28°C for 1 week. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). After enumeration of cfu, individual colonies were isolated from the same plates and each uncommon colony was reisolated onto a fresh Potato Dextrose Agar (PDA) plate. Distinct morphological characteristics were observed for identification and the plates were stored at 4°C. Two techniques, visual observation on Petri dishes and micro-morphological studies in slide culture, were adopted for identification of *Trichoderma* species. For visual observation, the isolates were grown on PDA agar for 3-5 days. The mode of mycelia growth, colour, odour and changes of medium colour for each isolate were examined every day. For micro-morphological studies, a slide culture technique was used. Examination of the shape, size, arrangement and development of conidiophores, their branching pattern, shape, size, angle to main axis, phialide numbers and conidial shape and colour. Species identification was based on the morphological and taxonomic keys provided by [8]. Rhizosphere soil samples were screened for *Pseudomonas* spp and *Bacillus* spp. using dilution method with King's B Agar as semi selective medium. *Pseudomonas* spp and *Bacillus* isolates were identified by morphological and physiological characteristics

based on Bergeys' Manual of Systematic Bacteriology.

2.3 Evaluation of Fungal and Bacterial Bio control Agents

The rhizosphere microorganisms isolated from tomato plants were screened for their antagonistic activity against the test pathogen *Rhizoctonia solani* by following dual culture technique [9].

2.4 Antagonistic Activity of Fungal Bio-control Agents

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 ($25 \pm 2^\circ\text{C}$) 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.

$$R = \frac{CD - TD}{CD} \times 100$$

Where,

- R = Per cent growth reduction of test pathogen
- CD = Radial growth of test pathogen in check (mm)
- TD = Radial growth of test pathogen in treatment (mm)

2.5 Antagonistic Activity of Bacterial Bio-control Agents

The antagonistic activity of native bacterial isolates were tested against the test pathogen *Rhizoctonia solani*, by following dual culture technique. A gentle superficial streak was made at four ends of the Petri plate on PDA medium by

means of a sterilized inoculation needle. A nine mm PDA culture disc of the pathogen was placed in middle of Petri plate. Three replications along with suitable control were maintained. The plates were incubated in an inverted position at room temperature ($25 \pm 2^\circ\text{C}$) 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.

$$R = \frac{CD - TD}{CD} \times 100$$

Where,

- R = Per cent growth reduction of test pathogen
- CD = Radial growth of test pathogen in check (mm)
- TD = Radial growth of test pathogen in treatment (mm)

2.6 Evaluation of Different Fungicides against *R. solani*

Ten fungicides were evaluated against *Rhizoctonia solani*, by poisoned food technique [10], at two concentrations i.e. at recommended dose and half the recommended dose (Table 1). The required quantities of fungicides were weighed and mixed in the carrot agar medium by thorough shaking for uniform mixing of the fungicide before pouring into Petri dishes so as to get the desired concentration of active ingredient of each fungicide separately i.e. recommended and half the recommended doses. Twenty ml of amended medium was poured in 90 mm sterilized Petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter from 7 day old culture was inoculated at the centre of the Petri plate and then incubated at $18 \pm 2^\circ\text{C}$ for 7 days. Control was maintained without fungicide. Three replications were maintained for each treatment. Per cent inhibition of mycelial growth was calculated using the formula [11].

$$I = (C - T / C) \times 100$$

Where

- I = Per cent inhibition of mycelial growth
- C = Colony diameter in control (mm)
- T = Colony diameter in treatment (mm)

Table 1. List of fungicides tested against *R. solani* by poisoned food technique under *in vitro* conditions

Common name	Trade name	Recommended concentration	Half recommended concentration
Mancozeb	Dithane M-45	0.3 g l ⁻¹	0.15 g l ⁻¹
Pyraclostrobin		0.1 g l ⁻¹	0.05 g l ⁻¹
Tebuconazole + Trifloxystrobin	Nativo	0.4 g l ⁻¹	0.2 g l ⁻¹
Copper Oxy Chloride	COC	0.3 g l ⁻¹	0.15 g l ⁻¹
Metiram + Pyraclostrobin	Cabriotop	0.2 g l ⁻¹	0.1 g l ⁻¹
Cymoxanil + Mancozeb	Curzate	0.08 g l ⁻¹	0.04 g l ⁻¹
Propiconazole	Tilt	0.1 ml l ⁻¹	0.05 ml l ⁻¹
Captan + Hexaconazole	Taqat	0.1 g l ⁻¹	0.05 g l ⁻¹
Thiram	Thiride	0.3 g l ⁻¹	0.15 g l ⁻¹
Carbendazim	Bavistin	0.15 g l ⁻¹	0.75 g l ⁻¹

3. RESULTS AND DISCUSSION

3.1 Evaluation of Native Fungal Bio-control Agents on the Growth of *R. Solani*

The perusal of the data presented in Table 2 and Fig. 1 revealed that the native *T. harzianum* -1 recorded maximum (62.53) per cent inhibition of the pathogen followed by native *T. harzianum* -5 (51.63) and *T. harzianum* -6 (49.50). However most of the *Trichoderma* isolates recorded less than 50 per cent inhibition over control and minimum inhibition was observed in *T. viride*-6 (36.6).

Hyperparasitism between bio-agents and *Rhizoctonia solani* was reported by several workers with various isolates of *T.viride*. Hyperparasitism potential *Trichoderma* spp. was

already been established and documented by [12-14].

In the present study, *T. harzianum* -1 was found superior over control. The results are in agreement with [15-17]. [18], 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 [19] reported the effectiveness of *T. viride* against *R. solani* causing root rot of tomato. [20] Also reported that the rhizosphere isolate Th1 inhibited 87.04 per cent of mycelium of *R. solani*. Our studies indicated that different isolates of *Trichoderma* spp. showed different antagonistic potentiality against *R. solani*. This type of variation in parasitic potential within the different isolates of *Trichoderma* spp was already well established and document by [21].

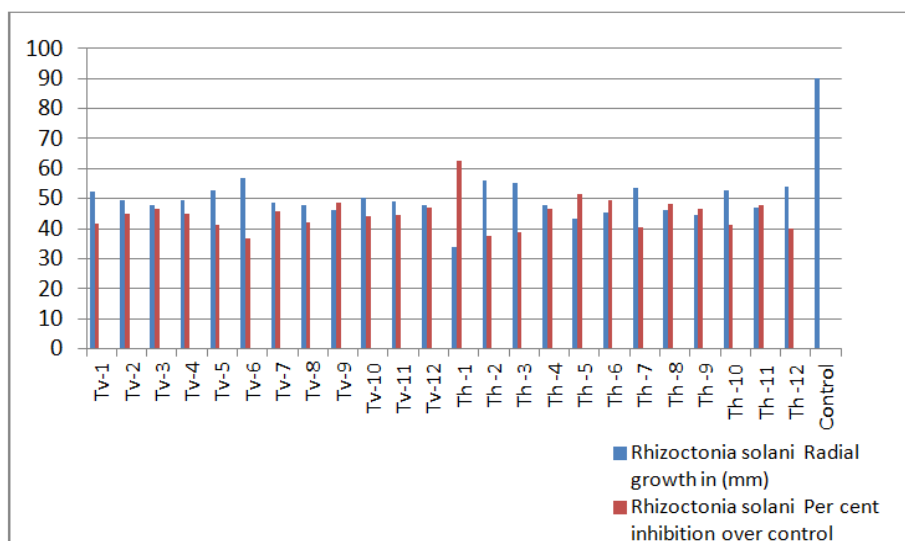


Fig. 1. Antagonistic activity of native *Trichoderma* isolates against *R. solani* (*T.v* -*Trichoderma viride*, *Th*- *Trichoderma harzianum*)

Table 2. Antagonistic activity (per cent inhibition) of native *Trichoderma* isolates against *R. solani*

Treatment	*Radial growth in (mm)	*Per cent inhibition over control (%)
<i>Trichoderma viride</i> -1	52.52	41.56 (40.12)
<i>Trichoderma viride</i> -2	49.36	45.10 (42.12)
<i>Trichoderma viride</i> -3	47.90	46.73 (43.10)
<i>Trichoderma viride</i> -4	49.46	45.00 (42.11)
<i>Trichoderma viride</i> -5	52.76	41.33 (39.98)
<i>Trichoderma viride</i> -6	57.02	36.60 (37.20)
<i>Trichoderma viride</i> -7	48.76	45.76 (42.55)
<i>Trichoderma viride</i> -8	47.62	42.03 (40.39)
<i>Trichoderma viride</i> -9	46.10	48.76 (44.27)
<i>Trichoderma viride</i> -10	50.16	44.20 (41.64)
<i>Trichoderma viride</i> -11	49.8	44.63 (41.90)
<i>Trichoderma viride</i> -12	47.62	47.03 (43.27)
<i>Trichoderma harzianum</i> -1	33.70	62.53 (52.26)
<i>Trichoderma harzianum</i> -2	56.11	37.46 (37.72)
<i>Trichoderma harzianum</i> -3	55.16	38.63 (38.41)
<i>Trichoderma harzianum</i> -4	47.70	46.76 (43.11)
<i>Trichoderma harzianum</i> -5	43.50	51.63 (45.91)
<i>Trichoderma harzianum</i> -6	45.40	49.50 (44.69)
<i>Trichoderma harzianum</i> -7	53.52	40.50 (39.49)
<i>Trichoderma harzianum</i> -8	46.36	48.43 (44.08)
<i>Trichoderma harzianum</i> -9	46.36	48.46 (44.10)
<i>Trichoderma harzianum</i> -10	52.62	41.46 (40.07)
<i>Trichoderma harzianum</i> -11	46.96	47.76 (43.70)
<i>Trichoderma harzianum</i> -12	53.86	40.13 (39.29)
Control	90.00	
CD at 5%	3.75	
SE(d)	1.86	
SE(m)	1.31	

* Mean of three replications * Figures in parentheses are angular transformed value

3.2 Evaluation of Native Bacterial Bio-control Agents on the Growth of *R. solani*

It is evident from the Table 3 and Fig. 2 that out of twelve bacterial isolates tested against the test pathogen *R. solani*, maximum per cent inhibition was recorded with *P. fluorescence*-2 (62.20) followed by *P. fluorescence* -4 (57.73), *B. subtilis* -6 (56.3) and *B. subtilis* -4 (50.7). However, the *B. subtilis* -5 exhibited the minimum per cent inhibition of 31.83 against *R. solani*. The *B. subtilis* bacterial isolates 1, 2, 3 and *P. fluorescence*-1 isolate, *B. subtilis*-5 isolate and *P. fluorescence* -6 isolate, were on par with each other in inhibiting the mycelium of *Rhizoctonia solani*.

In the present investigation out of 12 bacterial isolates, four isolates recorded more than 50 per cent inhibition. Similar observations were made by [22] and found that *Bacillus subtilis* inhibited 57.09 per cent of root rot of french bean caused by *R. solani*.

Sindan et al. [16] reported maximum (65.0 to 95.0) antagonistic activity of *P. fluorescence* by inhibiting mycelial growth and sclerotial production respectively. Similar finding was made by [23] while working on biological control of *R. solani* on potato and lettuce, reported that *Pseudomonas fluorescence* strain B₁ significantly controlled *R. solani*.

Lahlali et al. [24] isolated 220 bacterial strains from healthy potato plants and rhizosphere soils, tested under *in vitro* and found that 25 isolates were effective in inhibiting *R. solani*. The effective strains exhibited the mycelial inhibition of 59.4 to 95.0 per cent. The present study also indicated the effectiveness of four bacterial isolates against *R. solani* with more than 50 per cent growth inhibition.

3.3 *In vitro* Evaluation of Fungicides against *R. solani*

Ten fungicides were evaluated against *R. solani* by poisoned food technique and the results are presented in Table 4, Plate 1 and Fig. 3. The data revealed that there was significant difference between different fungicides in inhibiting the growth of *R. solani*. Fungicides Tebuconazole + Trifloxystrobin, Propiconazole, Captan + Hexaconazole and Carbendazim showed 100 per cent inhibition at recommended

dosage followed by Mancozeb (98.11), COC (95.70), Metiram + Pyraclostrobin (93.93), Cymoxanil + Mancozeb (83.37) and Thiram (83.30). The least inhibition of mycelium growth of *R. solani* was observed on Pyraclostrobin (71.29) at recommended dosage.

Similarly at half recommended dose also the fungicides, Tebuconazole + Trifloxystrobin, Propiconazole, Captan + Hexaconazole and Carbendazim showed 100 per cent inhibition of *R. solani*. The other fungicides COC, Metiram + Pyraclostrobin, Mancozeb, Thiram, Cymoxanil + Mancozeb and Pyraclostrobin exhibited mycelial inhibition of 88.47, 87.77, 80.70, 79.97, 72, 87 and 70.17 per cent respectively. In the present investigation the four fungicides viz.,

Tebuconazole + Trifloxystrobin, Propiconazole, Captan + Hexaconazole at Carbendazim was found superior over other fungicides at both the concentration tested and recorded 100 per cent inhibition of *R. solani*.

Sunderavadana et al. [25] reported Azoxystrobin as best fungicide to control *R. solani* causing sheath blight of rice. [26] suggested six fungicides viz., Propiconazole, hexaconazole, carbendazim, captan, sulphur and mancozeb against web blight of urd bean. Carbendazim and propiconazole were found superior in reducing the mycelia growth of *R. solani*. In the present investigation the fungicides Carbendazim showed its superiority in suppression of *R. solani*.

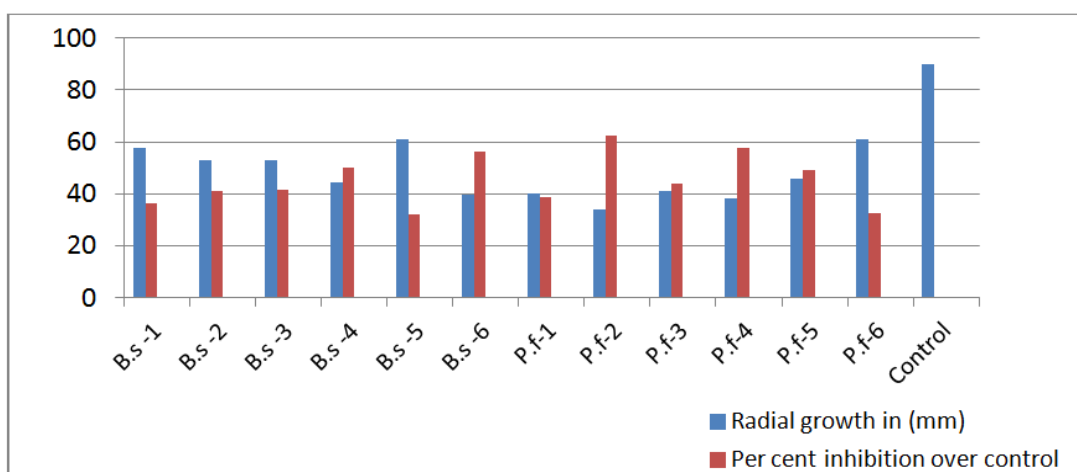


Fig. 2. Effect of native isolates of bacterial bio-agents on radial growth of *R. solani* (B.S- *Bacillus subtilis*) (P.f-*Pseudomonas fluorescence*) isolates

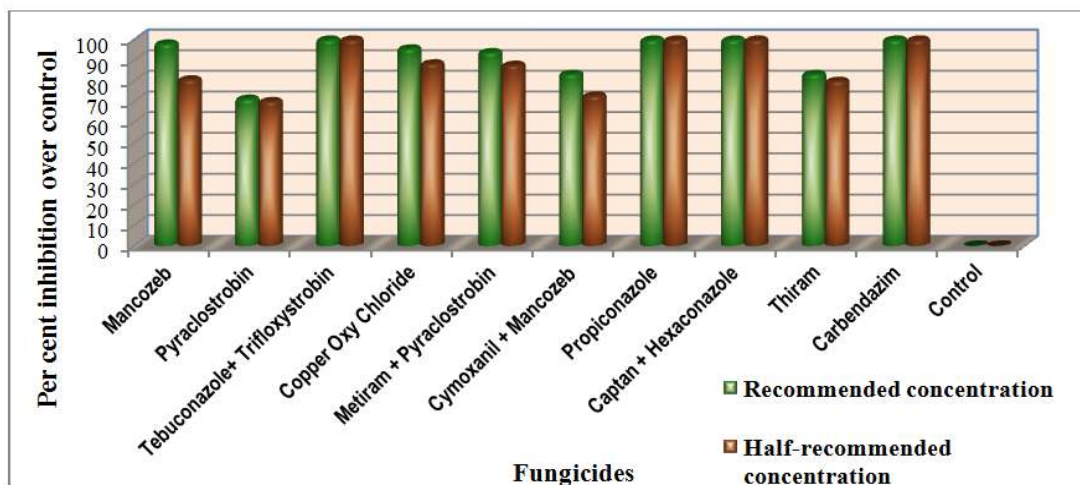


Fig. 3. In vitro evaluation of recommended and half-recommended dosages of fungicides on radial growth *R. solani*

Table 3. Antagonistic activity (per cent inhibition) of native isolates of bacterial bio-agents against *R. solani*

Treatment	*Radial growth in (mm)	*Per cent inhibition over control (%)
<i>Bacillus subtilis</i> -1	57.46	36.13 (36.91)
<i>Bacillus subtilis</i> -2	53.12	40.93 (39.75)
<i>Bacillus subtilis</i> -3	53.00	41.06 (39.83)
<i>Bacillus subtilis</i> -4	44.32	50.7 (45.40)
<i>Bacillus subtilis</i> -5	60.82	31.83 (34.32)
<i>Bacillus subtilis</i> -6	39.63	56.30 (48.64)
<i>Pseudomonas fluorescence</i> -1	40.00	38.83 (38.49)
<i>Pseudomonas fluorescence</i> -2	34.00	62.20 (52.04)
<i>Pseudomonas fluorescence</i> -3	41.04	43.90 (41.49)
<i>Pseudomonas fluorescence</i> -4	38.02	57.73 (49.43)
<i>Pseudomonas fluorescence</i> -5	45.70	49.20 (44.52)
<i>Pseudomonas fluorescence</i> -6	60.82	32.36 (34.64)
Control	90	-
CD at 5%	5.02	
SE(d)	2.41	
SE(m)	1.71	

* Mean of three replications * Figures in parentheses are angular transformed value

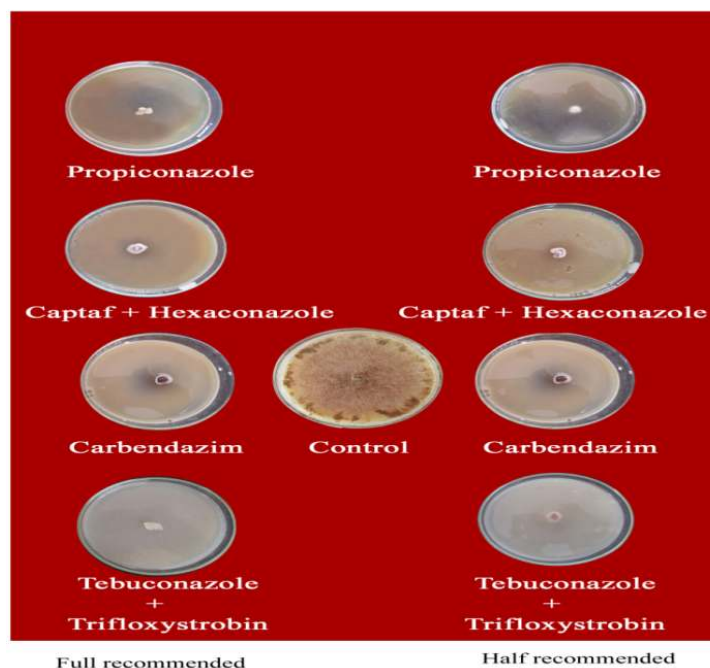


Plate 1. *In vitro* evaluation of fungicides on the radial growth of *Rhizoctonia solani*

Table 4. Per cent inhibition in the *in vitro* evaluation of fungicides on radial growth of *R. solani*

S. No.	Fungicides	Recommended concentration		Half recommended concentration	
		*Radial growth (mm)	*Per cent inhibition over control (%)	*Radial growth (mm)	*Per cent inhibition over control (%)
1	Mancozeb	1.6	98.11 (82.08)	15.6	80.70 (65.38)
2	Pyraclostrobin	25.8	71.29 (57.58)	26.8	70.17 (56.87)
3	Tebuconazole+	0	100.0 (90.00)	0	100.0 (90.00)
	Trifloxystrobin				
4	Copper oxy chloride	3.8	95.70 (78.00)	10.3	88.47 (70.15)
5	Metiram +	5.41	93.93 (75.79)	10.9	87.77 (69.52)
	Pyraclostrobin				
6	Cymoxanil +	14.8	83.37 (75.79)	24.4	72.87 (58.58)
	Mancozeb				
7	Propiconazole	0	100.0 (90.00)	0	100.0 (90.00)
8	Captan +	0	100.0 (90.00)	0	100.0 (90.00)
	Hexaconazole				
9	Thiram	18	83.30 (65.90)	18	79.97 (63.43)
10	Carbendazim	0	100.0 (90.00)	0	100.0 (90.00)
11	Control	90	0.00	90	0.00
	CD		1.761		2.509
	SE(d)		0.838		1.195
	SE(m)		0.593		0.845

* Mean of three replications

Figures in parentheses are angular transformed values

4. CONCLUSION

Among the 24 fungal and 12 bacterial biocontrol agents were tested for their antagonistic activity against *Rhizoctonia solani* and *T. harzianum* -1 (62.53) and *P. fluorescence*- 2 (62.20) percent recorded maximum inhibition of mycelium against *Rhizoctonia solani*.

Among ten fungicides tested Tebuconazole + Trifloxystrobin, Propiconazole, Captan + Hexaconazole and Carbendazim against *R. solani* showed 100.0 per cent inhibition (100.0 per cent) at recommended and half the recommended dosage under *in vitro* condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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