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Bio Efficacy of Novel Fungicides against *Fusarium solani* Inducing Mulberry Root Rot

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mulberry root rot is most severe disease-causing considerable yield loss, caused by fungal pathogen *F. solani,* which were managed by the use of effective synthetic chemicals. In this view non-systemic, systemic and combi fungicides evaluated by poison food technique against the root

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rot pathogen, among the novel fungicides evaluated systemic fungicides tebuconazole 25%EC and propiconozole 25% EC were found significantly superior with cent per cent mean mycelial inhibition. Among non-systemic fungicides copper oxy chloride 50% WP was found significantly superior with 96.66 per cent mycelial inhibition at 1000 ppm concentration. Out of six combi products tested metiram 55%WP + Pyraclostrobin 5% WG was significantly effective with maximum mean mycelial inhibition (82.00 %) followed by carbendazim 12 % + mancozeb 63 % WP with 77.11 per cent mean mycelial inhibition. Among these fungicides tested systemic fungicides tebuconozole 25% EC and propiconozole 25% EC has inhibited cent per cent in all three concentrations. Followed by non-systemic fungicide copper oxy chloride with 96.66 per cent inhibition at 1000 ppm respectively.

Keywords: Mulberry; root rot; fungicides; pathogen; Fusarium solani.

1. INTRODUCTION

Mulberry is grown commercially to feed the silkworm (*Bombyx mori* L). Mulberry is grown under different types of soils and climatic conditions so that the pathogen have favorable conditions for the growth of pathogens and development of disease. Mulberry production is affected by several soil borne diseases due to perennial nature of the mulberry provides nutrients for long-term survival and multiplication of soil-borne pathogens [1]. Mulberry is affected by pathogens like fungi, bacteria, virus and nematodes resulting in considerable leaf yield loss up to 15 – 20 per cent and infected plants produce nutritionally inferior leaves with reduced leaf quality. Among the soil-borne diseases, root rot is epidemic in nature and causes 30% mortality of plants with a 15% decrease in leaf yield, besides deteriorating the leaf quality [2]. In mulberry, different kinds of root rot have been reported, such as dry root rot, charcoal root rot, violet root rot, white root rot, black root rot and bacterial root rot [3-5]. Amongst these, dry root rot is caused by *Fusarium solani, Fusarium proliferatum*, charcoal root rot (*Macrophomina phaseolina*) black root rot *Lasiodiplodia theobromae* (*Botryodiplodia theobromae*) are frequently reported in India [6,7]. Root rot disease is caused majorly by *Fusarium solani,* dry root rot is a fast spreading soil borne disease in mulberry garden and the organism causing rot even in nursery stage, that affects all parts of the plant and it spreads rapidly affecting a large number of plants in a short period leading to the abandonment of mulberry gardens [8]. In the absence of resistant varieties and when there is sudden spread of disease, use of fungicides is the better alternative strategy for fast and effective controlling the root rot disease of mulberry. Hence, evaluation of new advanced novel fungicides under *in vitro* conditions is a convenient tool and this can serve as a guide to test the fungicides in field condition know the residue level and safety period to silkworm.

2. MATERIALS AND METHODS

In vitro evaluation of systemic, non-systemic and combi fungicides against mulberry root rot causing pathogen was carried out in the Department of Plant pathology, College of Sericuture, Chintamani, University of Agricultural Sciences, Bengaluru, Karnataka, India during 2021-22. The materials used and methodology followed during the investigation are described below.

Systemic, non-systemic and combi product fungicides were evaluated at different concentrations under *in vitro* conditions. Six systemic fungicides at the concentration of 100, 250 and 500 ppm, six non-systemic and combi fungicides at the concentration of 250, 500 and 1000 ppm were evaluated against the pathogen under laboratory conditions by poisoned food technique using potato dextrose agar medium.

The poisoned medium was prepared by adding required quantity of fungicides to the melted potato dextrose agar medium to obtain the desired concentration. 15 mL of poisoned medium was poured in each sterilized petri dish and suitable checks were maintained without fungicides. Five mm of ten days old fungal disc taken from the periphery of the culture was placed in the centre of poisoned medium and incubated at 28±1°C. The experiment was conducted by using Completely Randomized Design (CRD) and each treatment was replicated thrice. The observations were recorded when the fungal growth was maximum in the untreated control. The colony diameter was measured in three directions and the average was recorded.

The per cent inhibition of mycelial growth over the control was calculated using the formula (Vincent, 1947).

I = C-T / C * 100

Where,

I = Per cent growth inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Table 1. Fungicides evaluated *in vitro* against mulberry root rot pathogens

a) Systemic fungicides

Sl. No.	Common Name	Trade Name	Chemical Name
1	Azoxystrobin 23% SC	Bandstar	Methyl (2E)-2-(2- {[6-(2-cyanophenoxy) pyrimidin-4-yl] oxy} phenyl)-3-methoxyprop-2-enoate
2	Tebuconozole 25% WP	Folicure	1-(4-chlorophenol) - 4.4diamethyle-3- (1, 2,4triazole-1yl-methyl-pemtene-3-ol
3	Carbendazim 50% WP	Prozim	Methyl-2, Benzimidazole Carbomate
4	Difeconozole 25% EC	Detect	Cis, trans-3-chloro-4(4- methyl-2(1H-1, 2,4- traizole-1-yl, methyl)-1, 3-dioxonlan-2yl) Phenyl 4chlorophenyl ether
5	Hexaconozole 5% EC	Clintaf	(RS)-2-(2,4-Dichlorophenyl)-1-(1H- 1,2,4-triazol-1-yl) hexan-2-ol
6	Propiconozole 25% EC	Tilt	1-{2-(2,4dichlorophenyl) penty} -1 H-1,2,4-triazole

b) Non systemic fungicides

Sl. No.	Common Name	Trade Name	Chemical Name
1	Propineb 70% WP	Antracol	Propylenebis (dithiocarbamato) zinc
2	Mancozeb 75% WP	Utane M-45	Manganese ethylene bisdithiocarbomate plus zinc
3	Copper oxychloride 50% WP	Topgun	dicopper(II) chloride trihydroxide
4	Chlorothalonil 50% WP	Kavach	Tetrachloroisophthalonitrate
5	Zineb 75% WP	Indofil Z- 78	zinc ethylenebis(dithiocarbamate)
6	Captan 50% WP	Kapton - 50	N-(trichloro methyl thio) – 4 – cyclohexene1,2,dicarboximide

c) Combi fungicides

Sl. No.	Common Name	Trade Name	Chemical Name
1	Azoxystrobin11% + Tebuconazole18.3% SC	SHAN	Methyl (2E)-2-(2- {[6-(2-cyanophenoxy) pyrimidin-4- yl]oxy} phenyl)-3-methoxyprop-2-enoate + 1-(4-chlorophenol) - 4.4diamethyle-3- (1,2,4triazole-1yl-methyl-pemtene-3-ol
2	Carbendazim12% + Mancozeb 63%WP	TURFF	Methyl 2 benzimidazole carbomae 1 2 + Manganese ethylene bis dithiocarbonateplus zinc
3	Zineb 68% + Hexaconazole4% WP	AVTAR	(RS)-2-(2,4-Dichlorophenyl)-1- (1H-1,2,4-triazol-1-yl) hexan-2- ol + Zinc Ethylenebis (dithiocarbamate)
4	Cymoxanil 8% + Mancozeb63% WP	CurzateM8 (Dupoint)	[2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino) acetamide]Manganese ethylene bisdithiocarbomate plus zinc
5	Metalaxyl 4 % + Mancozeb64% WP	RidomilGold	methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate + Manganese ethylene bis dithiocarbomate plus zinc
6	Metiram 55% + Pyraclostrobin 5% WG	CabrioTop	zinc ammoniate ethlenebis(dithiocarbamate)-poly (ethylene thiuram disulfide) + methyl {2-[1-(4-chlorophenyl) pyrazol-3- yloxymethyl] phenyl}(methoxy) carbamate

3. RESULTS AND DISCUSSION

3.1 *In vitro* Evaluation of Contact Fungicides against *F. solani*

Six different contact fungicides were evaluated at three concentrations *viz.,* 250, 500 and 1000 ppm in laboratory to check the efficiency against *F. solani* through poison food technique. The data pertaining to the per cent inhibition of mycelial growth of *F. solani* in different contact fungicides presented in Table 2, Fig. 1 and Plate 1.

Table 2. *In vitro* evaluation of contact fungicides against *F. solani*

Sl. No.	Name of the Fungicide	Per cent Inhibition of Mycelial Growth (%)			
		Concentrations (ppm)			Mean mycelial inhibition (%)
		250	500	1000	
1	Captan 50% WP	67.33 (55.13) *	81.33 (64.40)	86.66 (68.64)	74.44 (62.72)
2	Chlorathalonil 50% WP	65.37 (53.94)	74.87 (59.83)	80.00 (63.48)	73.34 (59.08)
3	Copper oxy chloride 50% WP	53.33 (46.90)	81.33 (64.40)	96.66 (83.82)	77.11 (65.04)
4	Mancozeb 75% WP	55.333 (48.05)	62.00 (51.94)	74.00 (59.35)	63.78 (53.11)
5	Propineb 75% WP	64.67 (53.60)	66.67 (54.76)	73.33 (58.94)	68.22 (55.77)
6	Zineb 75 % WP	48.67 (44.22)	56.67 (48.86)	79.33 (63.49)	61.56 (52.19)
7	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	50.67 (43.12)	60.38 (49.17)	68.76 (54.80)	60.25 (49.03)
		Fungicide (F)	**Concentration (C)**		**Interaction (F × C)**
F test		*	*		*
S. Em±		**1.69**	**1.12**		**2.94**
CD @ 1%		**4.74**	**3.18**		**8.42**

** Figures in the parentheses are arcsine transformed values*

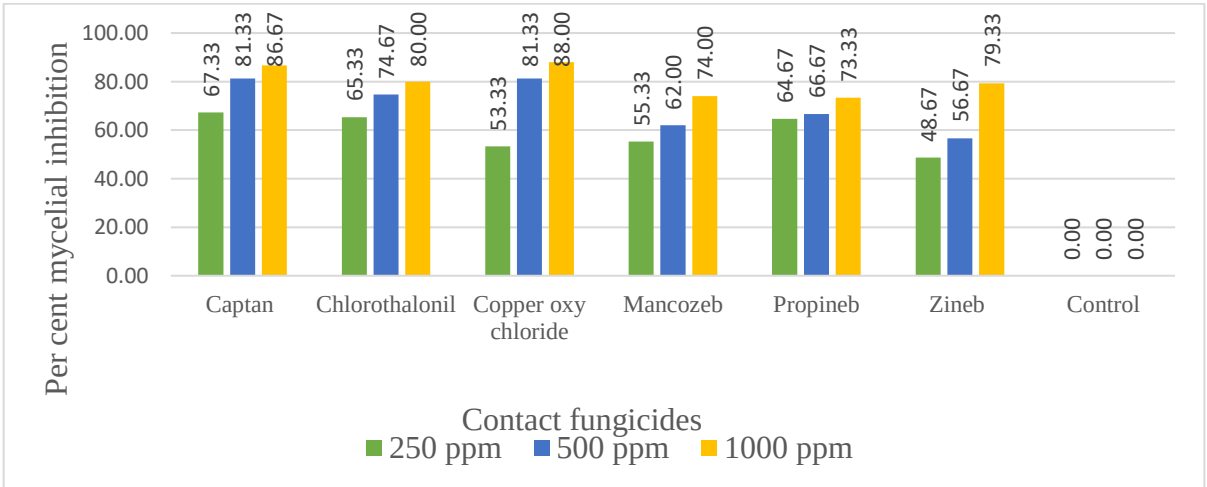

Fig. 1. Effect of contact fungicides on mycelial growth inhibition of *Fusarium solani*

Plate 1. *In vitro* evaluation of different contact fungicides against *Fusarium solani*

The results presented in Table 2 revealed that, there is significant difference between different contact fungicides in per cent inhibition of mycelial growth. Among the contact fungicides tested, Copper oxy chloride was found significantly superior with 77.11 per cent inhibition compared to rest of fungicides. The second most effective fungicide was captan with 74.44 per cent inhibition. Chlorothalonil and propineb recorded the mycelial inhibition of 73.34 and 68.22 per cent respectively. Mancozeb and Zineb were on par with each other and recorded least inhibition of 63.78 and 61.56 per cent respectively. Among different fungicides with three concentrations tested, 1000 ppm was found most effective in inhibiting the mycelial growth of the organism. Copper oxy chloride recorded 88.00 per cent inhibition at 1000 ppm however, propineb recorded least inhibition at this concentration with 73.33 per cent. With respect to the intraction of captan and copper oxy chloride at 1000 ppm concentration recorded 8\6.67 per cent and 88.00 per cent mycelial inhibition respectively and were significantly superior over rest of the fungicides. At 250 and 500 ppm concentration the mycelial inhibition of 67.33, 81.33 per cent and 53.33, 81.33 per cent was observed respectively. Chlorothalonil and propineb showed partial inhibition of the mycelial growth by recording 65.37, 74.66, 80.00 and 64.67, 66.67, 73.33 percent at 250, 500 and 1000 ppm, respectively. Mancozeb showed the varied inhibition of 55.33, 62.00, 74.00 per cent respectively at same concentrations. Zineb was found least effective in inhibiting the growth of *F. solani* even at 250, 500 concentrations and recorded the inhibition of 48.66 and 56.00 respectively.

In the absence of resistant varieties and when there is sudden spread of disease, use of fungicides is the better alternative strategy for controlling the root rot disease of mulberry. Hence, fungicides are the important components of integrated disease management. Evaluation of fungicides under *in vitro* conditions is a convenient tool to screen a large number of fungicides and this can serve as a guide to test the fungicides in field condition know the residue level, safety period to silkworm.

The obtained results were similar to the findings of Bhaliya and Jadeja [9] who evaluated the different contact fungicides *in vitro* against *F. solani*. Among contact (non- systemic) fungicides, maximum inhibition of mycelial growth was observed in mancozeb (100%) and zineb (100%) followed by Chlorothalonil (72.52%). Among the different concentrations of fungicides, Mancozeb and zineb gave 100 per cent inhibition at all concentrations, similarly Gupta et al [10] evaluated different non systemic fungicides *in vitro* by poison food technique against *fusarium solani* root rot. Among different fungicides evaluated copper oxy chloride was found best with 80 per cent of mycelial inhibition.

3.2 *In vitro* Evaluation of Systemic Fungicides against *F. solani*

Six systemic fungicides were tested at three concentrations *viz.,* 100, 250 and 500 ppm against *F. solani* under lab condition by using poison food technique. The per cent inhibition of mycelial growth of *F. solani* in different systemic fungicides presented in (Table 3, Fig. 2 and Plate 2).

Table 3. *In vitro* evaluation of systemic fungicides against *F. solani*

Sl. No.	Name of the Fungicide	Percent Mycelial Inhibition (%)			
		Concentration (ppm)			
		100	250	500	Mean Mycelial Inhibition (%)
1	Azoxystrobin 23% SC	38.33 (38.22) *	57.50 (49.30)	64.17 (53.34)	53.33 (46.95)
2	Tebuconazole25% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
3	Carbendazim50% WP	40.83 (39.68)	46.67 (43.03)	60.83 (51.303)	49.44 (44.67)
4	Difenoconozole 25% EC	56.67 (48.82)	70.00 (56.81)	81.67 (64.77)	69.44 (56.80)
5	Hexaconozole 5% EC	41.67 (40.17)	49.17 (44.50)	65.00 (53.90)	51.94 46.196)
6	Propiconozole25% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
7	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	62.92 (57.80)	70.56 (62.26)	78.61 (67.21)	70.69 (62.42)
		Fungicide (F)		**Concentration (C)**	**Interaction (F×C)**
	F test	*		*	*
	S. Em ±	**1.97**		**1.39**	**3.42**
	CD at 1%	**5.68**		**4.02**	**9.84**

** Figures in the parentheses are arcsine transformed values*

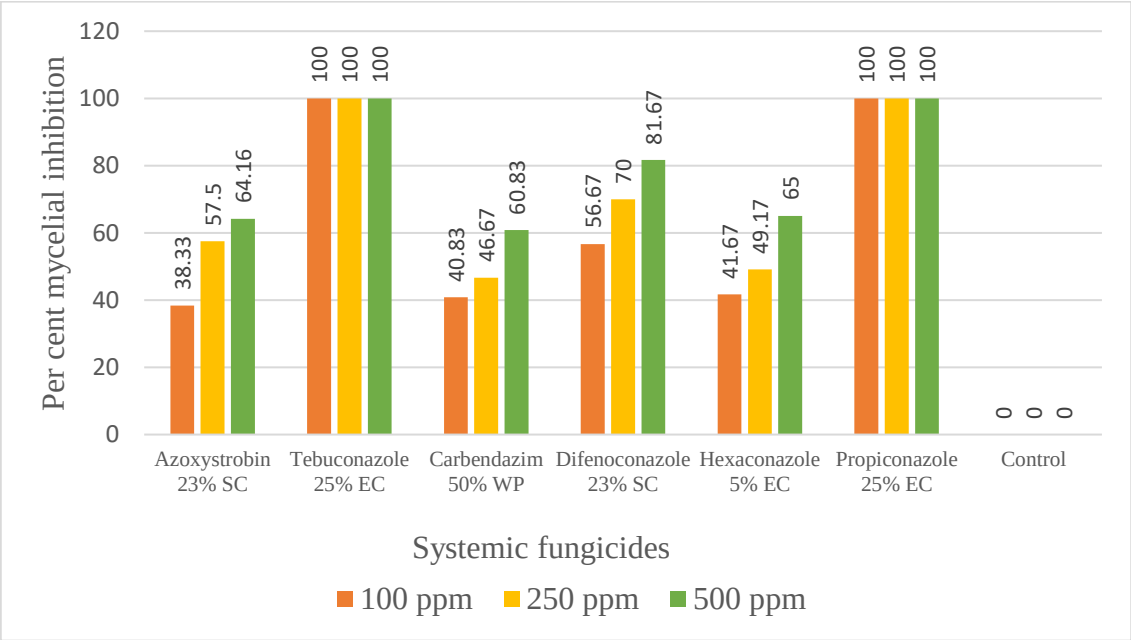

Fig. 2. Effect of systemic fungicides on mycelial growth inhibition of *Fusarium solani*

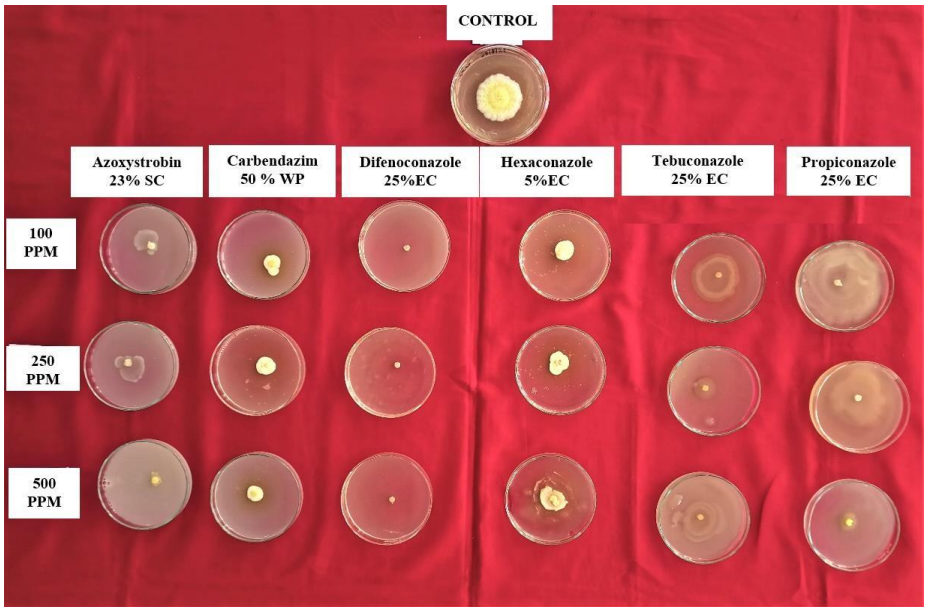

Plate 2. *In vitro* evaluation of different systemic fungicides against *Fusarium solani*

Among the systemic fungicides evaluated, tebuconazole and propiconozole were significantly superior and on par with cent per cent mean mycelial inhibition respectively. Out of three concentrations all concentrations showed the cent per cent mycelial inhibition. Whereas in difenoconazole inhibition of mycelial growth of 69.44 per cent. The mycelial inhibition of 56.68, 70.00 and 81.68 ppm and cent per cent mycelial inhibition was observed in 100, 250 and 500 ppm respectively. Next order was azoxystrobin and hexaconazole with mean mycelial inhibition of 53.33 and 51.94. Out of three concentrations 500 ppm was found most effective in all the fungicides. The mycelial growth of 38.33, 57.50, 64,16 per cent and 41.66, 49.16, 65.00 per cent was observed in 100, 250 and 500 ppm of azoxystrobin and hexaconozole. However least effective of all three concentrations were observed in carbendazim with mean inhibition of 49.99 per cent respectively. With respect to intraction of tebuconozole, propiconozole and difenoconozole at all three concentrations (100, 250 and 500 ppm) recorded cent per cent mycelial inhibition was superior over rest of the fungicides.

The present study is confirmation with the findings of Bhaliya and Jadeja [9] who evaluated the different systemic fungicides *in vitro* against *F. solani*. Among systemic fungicides propiconazole (85.27%) and difenoconazole (75.53%). Similarly, Kapadiya et al. [11] screened the different systemic, under *in vitro* condition against *F. solani*. Among systemic fungicides, tebuconazole 25.9% EC gave cent per cent inhibition of mycelial growth followed by hexoconazole 5% WP (92.69%). Similarly, Gupta et al [10] evaluated different systemic fungicides *in vitro* by poison food technique against *fusarium solani* root rot. Among different fungicides evaluated propiconozole was found best with 90 per cent of mycelial inhibition.

3.3 *In vitro* Evaluation of Combi Fungicides against *F. solani*

Six combi products were tested at three different concentrations *viz.,* 250, 500 and 1000 ppm by using poison food technique under *in vitro* condition. The per cent inhibition of mycelial growth of *M. phasiolina* in different combi fungicides is presented in (Table 4, Fig. 3 and Plate 3).

Out of six combi products tested metiram 55 % + pyraclostrobin 5 % WG was significantly effective with maximum mean mycelial inhibition of 82.00 per cent followed by carbendazim 12 % WP+ mancozeb 63 % WP with (77.11%). Out of three concentrations 79.30, 80.00 and 86.67 per cent inhibition was observed in metiram 55 % + pyraclostrobin 5 % WG, followed by carbendazim 12 % + mancozeb 63 % WP with 65.33, 77.37 and 88.67 per cent mycelial inhibition at 250, 500 and 1000 ppm, respectively. Mycelial inhibition of metiram 55 % + pyraclostrobin 5 % WG is 79.31, 80.00, and 86.76 per cent inhibition at 250, 500 and 1000 ppm, followed by azoxystrobin 11% + tebuconozole 18.3 % SC and zineb 68% +

hexaconozole 4% WP with mean mycelial inhibition of 56.66 and 32.67 per cent inhibition rate was 46.00, 57.33, 66.67 and 29.33, 30.67, 38.00 per cent at 250 and 500 ppm and 1000 ppm concentrations. Whereas in metalaxyl 8 % + mancozeb 64 % WP inhibition of 6.00, 29.33 and 30.66 per cent mycelial inhibition was observed at 250, 500 and 1000 ppm concentrations, respectively. The least inhibition of growth of fungus was recorded in cymoxanil 8% + mancozeb 63% WP with 19.33 per cent of mean mycelial inhibition, 5.33, 22.00, 30.67 per cent Mycelial inhibition was recorded at 250, 500 and 1000 ppm, respectively.

Table 4. *In vitro* evaluation of combi fungicides against *F. solani*

Sl. No.	Fungicide	Per Cent Mycelial Inhibition (%)			
		Concentration (ppm)			Mean Mycelial Inhibition(%)
		250	500	1000	
1	Carbendazim 12 % + Mancozeb 63 % WP	65.33 (53.94) *	77.33 (61.77)	88.67 (70.73)	77.11 (62.15)
2	Azoxystrobin 11% + Tebuconozole 18.3%SC	46.00 (42.67)	57.33 (49.22)	66.67 (54.76)	56.67 (48.88)
3	Metiram 55 % + Pyraclostrobin 5 % WG	79.33 (62.97)	80.00 (63.78)	86.67 (69.32)	82.00 (65.26)
4	Cymoxanil 8 % + Mancozeb 64 % WP	5.33 (12.69)	22.00 (27.94)	30.67 (33.56)	19.33 (24.73)
5	Zineb 68% + Hexaconozole 4% WP	29.33 (32.67)	30.67 (33.54)	38.00 (38.29)	32.67 (34.78)
6	Metalaxyl 8 % + Mancozeb 64 % WP	6.00 (13.84)	29.33 (32.72)	37.33 (37.60)	24.22 (28.00)
7	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	33.05 (31.25)	42.39 (38.38)	49.71 (43.43)	41.71 (37.69)
	Fungicide (F)	**Concentrations (C)**		**Interaction (F × C)**	
F test	*	*		*	
S. Em±	**1.86**	**1.32**		**3.23**	
CD@1%	**5.30**	**3.80**		**9.31**	

** Figures in the parentheses are arcsine transformed values*

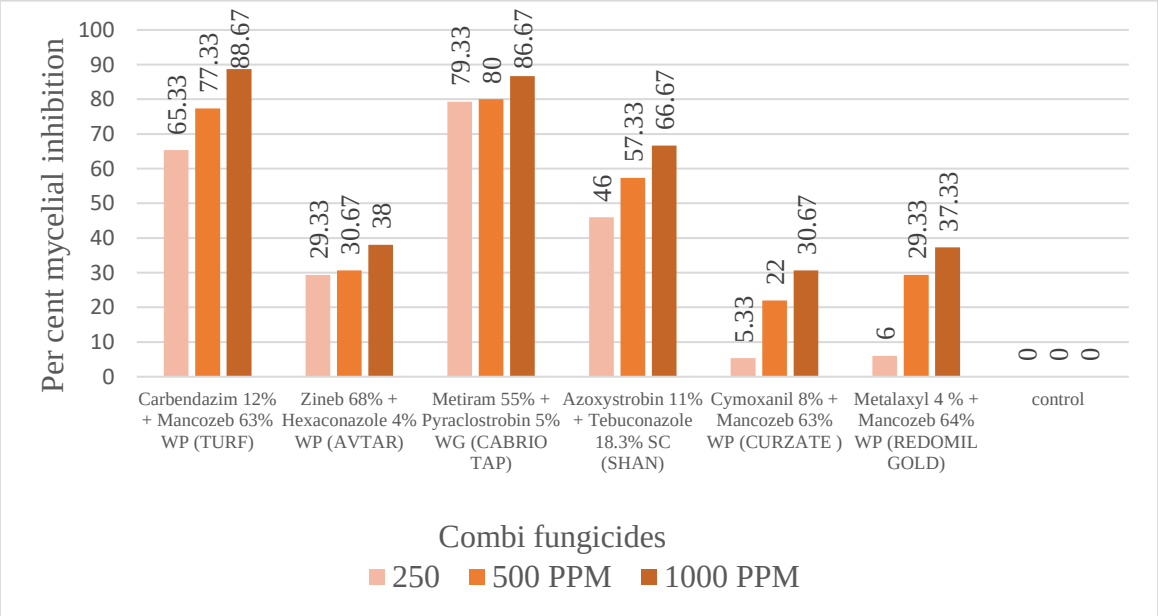

Fig. 3. Effect of combi fungicides on mycelial growth inhibition of *Fusarium solani*

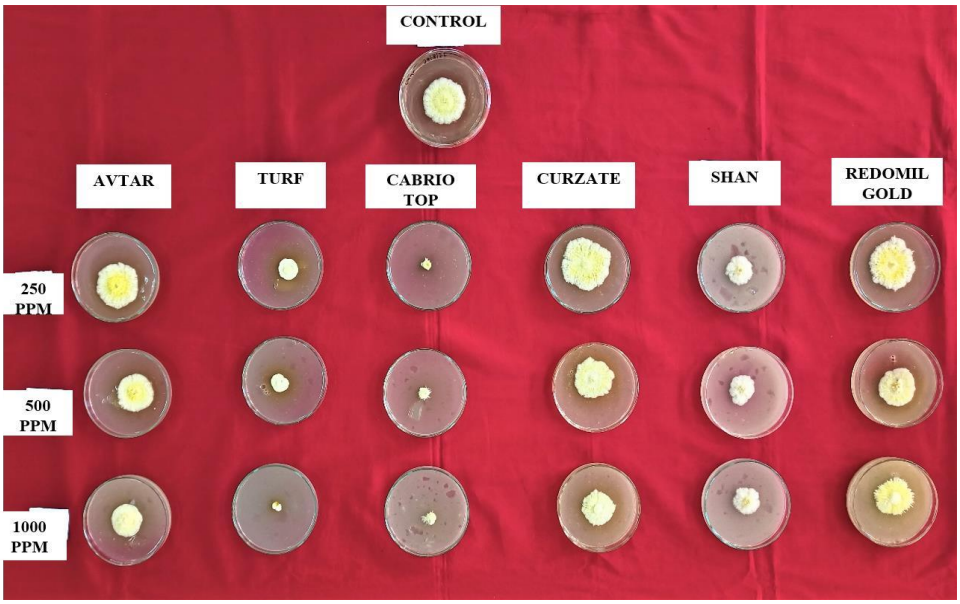

Plate 3. *In vitro* evaluation of different combi fungicides against *Fusarium solani*

These findings are similar to the findings of Narayanan et al. [12] who reported that the fungicides, mixture of Carbendazim + Mancozeb (0.1 %) completely inhibited the mycelial growth of the *F. solani.* Similar observation was made by. Simlarly Bhaliya and Jadeja [9] who evaluated different combination of fungicides *in vitro* against *F. solani* and found that fungicides combination of Cymoxanil + Mancozeb, Carbendazim + Mancozeb and tricyclazole + Mancozeb gave 100 per cent growth inhibition at all concentration followed by carboxin + thiram with 98.79 per cent mean growth inhibition while least (72.19%) inhibition was observed in zineb + hexaconazole.

4. CONCLUSION

Eighteen new generation fungicides were tested against *Fusarium solani* mulberry root rot causing pathogen. Systemic fungicides tebuconozone and propiconozole showed cent per cent mycelial inhibition at all three concentrations of 250, 500, 1000 PPM and copper oxy chloride showed 96.66 per cent mycelial inhibition at 1000 PPM concentration. Thus Combi fungicides, Metiram 55 % + Pyraclostrobin 5 % WG showed 82.00 percent mean mycelial inhibition concentration. followed by and Cymoxanil 8 % + Mancozeb 64 % WP showed 77.11 per cent mean mycelial inhibition at 1000 PPM concentration. Among the different fungicides evaluated systemic and systemic fungicides were found best for the mycelial inhibition of Mulberry root rot caused by *Fusarium solani*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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