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Testing the efficacy of different fungicides against *Colletotrichum capsici* under in vitro Conditions

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Abstract

An key crop for the global economy, the chilli (Capsicum annuum L.), is badly affected by fruit rot, which can reduce yields by up to 50%. The most popular method for controlling anthracnose is the use of chemical fungicides. Based on pathogenicity studies, isolate Cc-3 was recorded as the highest virulent with early and late symptoms on chilli fruit among 19 different isolates. Isolate Cc -3 was used for the study of the management of Colletotrichum capsici by evaluating different fungicides using poisoned food technique. The efficacy of different fungicides viz., carbendazim 50 % WP, captan 50% WP, copper oxy chloride 50 % WP, difeniconazole 25 % EC, tebuconazole 25.9 % EC, azoxystrobin 23 % SC, azoxystrobin 11 % + tebuconazole 18.3 % w/w SC, carbendazim 12 % + mancozeb 63 % WP, carbendazim 12 % + flusilazole 12.5 % SE, prochloraz 24.4 % + tebuconazole 12.15 % w/w EW, tebuconazole 50 % + trifoxystrobin 25 % WG, metiram 55 % + pyraclostrobin 5 % WG, picoxystrobin + tricyclazole 20.33 % w/w SC, hexaconazole 5 % + captan 70 % WP were tested against Colletotrichum capsici pathogen collected from pinapaka village of Khammam area under in vitro conditions. The results from in vitro studies on the different fungicides tested, the EC50/ ED50 (µg ml-1) values shown less in Tebuconazole 25.9 % EC (18) followed by difenoconazole 25 % EC (115), carbendazim 12 % + mancozeb 63 % WP (316), hexaconazole 5 % + captan 70 % WP (406), carbendazim 25 % + flusilazole 12.5 % SE (549), azoxystrobin 11 % + tebuconazole 18.3 % w/w SC (689) and prochloraz 24.4 % + tebuconazole 12.1 % w/w EW (762). Minimum Inhibitory Concentration (MIC) was recorded lowest in tebuconazole 25.9 % EC (100 µg ml-1) followed by difenoconazole 25 % EC (250 µg ml-1) and highest was recorded in azoxystrobin 23 % SC (3500 µg ml-1) among all the six individual fungicides. Minimum Inhibitory Concentration (MIC) was recorded lowest in carbendazim 12 % + mancozeb 63 % WP (1000 µg ml-1) followed by hexaconazole 5 % + captan 70 % WP (1250 µg ml-1) and highest was recorded in tebuconazole 50 % + trifloxystobin 25 % WG (4000 µg ml-1) among all eight combination fungicides.

Keywords: *Colletotrichum capsici, fungicides, in vitro*

Introduction

India is known as The Home of Spices, and Indian spices are prized for their therapeutic properties. India is third in the world for the production of chillies, one of the main crops used to make spices [10]. In India, pre and post-harvest losses of chilli are found to be morethan 50 per cent [9]. Fruit rot caused by *C. capsici* was reported to reduce the marketable yield from 2.5 to 11.6 per cent depending on the variety [7]. Fruit rot alone reduces the fruit yield by more than 50 per cent in different parts of India [3]; [8] and substantial reduction in quality characters among different parts of India [1]; [4].

A wide range from 10 per cent to 80 per cent reduction

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in fruit yield has also been reported [11]. The disease incidence varies from 44 to 51 per cent [13]. Recently, [14] have reported a decreased fruit yield from 50.3 to 58.6 per cent due to fruit rot incidence plots in untreated control as compared to the treated plots. The yield loss extends even up to 100 per cent and reduces the marketability. Numerous systemic and contact fungicides have been suggested for the management of the fruit rot fungus [6]. Hence, the chemical management of *C. capsici* is very important in chilli cultivation.

Material and Methods

Poisoned food technique was adopted as per the procedure given by [2] to determine the bio-efficacy of fungicides against *C. capsici in vitro* by taking one highly pathogenic isolate from the collected isolates. The fungicides used for evaluation of bio-efficacy in different concentrations against *C. capsici* to know the effective fungicide for management of the pathogen are listed below in table 1.

Poisoned food technique

For each treatment, 60 ml of potato dextrose sugar (PDA) medium was taken in 100 ml conical flask and sterilized. To this, the required quantity of fungicide was added at luke warm state to get desired concentration of each fungicide (1, 10, 25, 50, 100, 150, 200, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3250, 3500, 3750 and 4000 µg ml^{-1}). Three replications were maintained for each treatment. Five mm discs of the test fungal culture of *C. capsici* was obtained with sterilized cork borer and transferred to the centre of the poisoned medium in each of the Petri plates. Similarly controls were maintained by placing 5 mm disk of test fungal culture in the centre of un poisoned medium in the plates. All the petriplates were incubated at 28 ± 1^0 C in biological oxygen demand (BOD) incubator. The diameter of fungal colony was measured in each of the treatment when the fungal colony growth in the control plate was full. Per cent mycelial inhibition was calculated in each treatment by comparison with control plates by the following formula [12].

I = 100 (C-T)/C

Where, I = Percent inhibition

C= Growth in control,

T= Growth in treatment

Based on the above procedure, the observations were recorded and the efficacy of the fungicide was studied. The individual EC 50/ED 50 values were calculated by using probit analysis (Finney, 1952). Minimum Inhibitory Concentration (MIC) values were calculated from the per cent mycelial inhibition data observed at different concentrations of the fungicides studied.

Statistical analysis

The data was taken to determine the percentage mycelial inhibition of the fungus in treatment and control against the isolated pathogens causing fruit rot of chilli. Thus, the data obtained from the results were subjected to statistical analysis by using probit analysis. All of the statistical analysis were evaluated using SPSS/INDO Stat software. The data were subjected to square root and angular transformation values wherever necessary and analysed by adopting **a** completely randomized design (CRD) as suggested by [5].

Results and Discussion

Efficacy of different fungicides against *Colletotrichum capsici* under *in vitro* conditions.

Based on pathogenicity studies, isolate *Cc*-3 was recorded as the highest virulent with early and late symptoms on chilli fruit. Based on molecular identification, isolate *Cc* -3 was found to be *Colletotrichum capsici*. Therefore, isolate *Cc* -3 was used for the study of the management of *Colletotrichum capsici* by evaluating different fungicides using poisoned food technique.The efficacy of different fungicides *viz*., carbendazim 50 % WP, captan 50 % WP, copper oxychloride 50 % WP, difeniconazole 25 % EC, tebuconazole 25.9 % EC, azoxystrobin 23 % SC, azoxystrobin 11 % + tebuconazole 18.3 % w/w SC, carbendazim 12 % + mancozeb 63 % WP, carbendazim 12 % + flusilazole 12.5 % SE, prochloraz 24.4 % + tebuconazole 12.15 w/w EW, tebuconazole 50 % + trifoxystrobin 25 % WG, metiram 55 % + pyraclostrobin 5 % WG, picoxystrobin + tricyclazole 20.33 % w/w SC, hexaconazole 5 % + captan 70 % WP were tested against *Colletotrichum capsici* pathogen collected from pinapaka village of Khammam area under *in vitro* conditions.

The per cent mycelial inhibition of all the fungicides recorded after 18 days after inoculation (DAI) evaluated at different concentrations as 1µg ml^{-1}, 10 µg ml^{-1}, 25 µg ml^{-1}, 50 µg ml^{-1}, 100 µg ml^{-1}, 150 µg ml^{-1}, 200 µg ml^{-1}, 250 µg ml^{-1}, 500 µg ml^{-1}, 750 µg ml^{-1}, 1000 µg ml^{-1}, 1250 µg ml^{-1}, 1500µg ml^{-1}, 1750 µg ml^{-1},

Table 1 List of fungicides tested against Colletotrichum capsici

S. No	Name of the fungicide	Chemical name	Trade name	Manufacturing company
1	Carbendazim 50%WP	Methyl (1H-1,3-benzimidazol-2-yl)carbamate	Bavistin 50 WP	Crystal Crop Protection
2	Captan 50% WP	(3aR,7aS)-2-[(Trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione	Captan 50 W	Syngenta Agrochemicals, Mumbai
3	Copper Oxy Chloride 50%WP	Copper oxychloride	Blitox 50 WP	Rallis India Ltd. Mumbai
4	Difenoconazole 25% EC	1-((2-(2-Chloro-4-(4-chlorophenoxy) phenyl)-4-methyl-1,3-dioxolan-2-yl) methyl)-1H-1,2,4-Triazole	Score 25 EC	Syngenta GroupCompany
5	Tebuconazole 25.9%EC	5-(4-chlorophenyl)-1,1,1- trideuterio-3-(1,2,4 triazol-1-ylmethyl)-2,2- bis(trideuteriomethyl) pentan-3-ol	Folicur 250 EC	Bayer Crop Science Ltd. Mumbai
6	Azoxystrobin 23% SC	Methyl (2*E*)-2-(2-{[6-2cyanophenoxy) pyrimidin-4y1]oxy}phenyl)-3-methoxyprop-2-enoate	Amistar 23 SC	Syngenta Agrochemicals, Mumbai
7	Azoxystrobin 11% + Tebuconazole 18.3% w/w SC	Methyl (2*E*)-2-(2-{[6-2cyanophenoxy) pyrimidin-4- y1] oxy} phenyl)-3- methoxyprop-2-enoate + 5- (4-chlorophenyl)-1,1,1-trideuterio-3-(1,2,4triazol-1-ylmethyl)-2,2-bis(trideuteriomethyl) pentan-3-Ol	Custodia SC	ADAMA Agricultural-solutions
8	Carbendazim12%+ Mancozeb 63%WP	Methyl 1H benzimidazol-2-ylcarbamate + Manganese ethylenebis (dithiocarbamate)(Polymeric) complex with zinc salt	Bendaco	Hindustan Crop Science
9	Carbendazim 25 %+ Flusilazole 12.5% SE	2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol	Saaf	Dhanuka Agritech Limited
10	Prochloraz 24.4% + Tebuconazole 12.1% w/w EW	1H-imidazole-1-carboxamide substituted by a propyl and a 2-(2,4,6- trichlorophenoxy)ethyl 1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol	Zamir	Mahakal Agro Trading
11	Tebuconazole 50% + Trifloxystrobin 25% WG	1-(4-Chlorophenyl)- 4,4-dimethyl-3-(1H, 1,2,4-triazol-1- yl methyl) pentan- 3-ol +methyl (E)- methoxyimino-{(E)-α- [1-(α,α,α- trifluoro-m- tolyl)ethylideneaminooxy]-o-tolyl} acetate	Nativo 75 WG	Bayer Crop Science, AG,Germany
12	Metiram 55% + Pyraclostrobin 5% WG	MethylN-{2-[1-(4-chlorophenyl)- 1H-pyrazol-3-yl]oxymethyl]phenyl}(N-methoxy) carbamate	Cabrio Top5WG	BASF SE Production-Crop Protection
13	Picoxystrobin + Tricyclazole 20.33%w/wSC	Methyl (E)-3-methoxy-2-{2-[6-(trifluoromethyl)-2- pyridyl oxymethyl] phenyl} acrylate+12-methyl-7-thia-2,4,5- triazatricyclo[6.4.0.0^{2,6}]dodeca-1(12),3,5,8,10-pentaene	Fantom EC	BASF SE Production-Crop Protection
14	Hexaconazole 5%+Captan 70%WP	2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexan-2-ol (3aR,7aS)-2-[(Trichloromethyl) sulfanyl]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione	Kick	Rallis India Limited

2000 µg ml^{-1}, 2250 µg ml^{-1}, 2500 µg ml^{-1}, 2750 µg ml^{-1}, 3000 µg ml^{-1}, 3250 µgml^{-1}, 3500 µg ml^{-1}, 3750 µg ml^{-1} and 4000 µg ml^{-1}.

The results revealed that, EC50/ ED50, EC90/ ED90 and Minimum Inhibitory Concentration (MIC) values of *Colletotrichum capsici* significantly

differed in all the fungicides evaluated at different concentrations as 1µg ml^{-1}, 10 µg ml^{-1}, 25 µg ml^{-1}, 50 µg ml^{-1}, 100 µg ml^{-1}, 150 µg ml^{-1}, 200 µg ml^{-1},250 µg ml^{-1}, 500 µg ml^{-1}, 750 µg ml^{-1}, 1000 µg ml^{-1}, 1250 µg ml^{-1}, 1500 µg ml^{-1}, 1750 µg ml^{-1}, 2000 µg ml^{-1}, 2250 µg ml^{-1}, 2500 µg ml^{-1}, 2750 µg ml^{-1}, 3000 µg ml^{-1}, 3250 µg ml^{-1}, 3500 µg ml^{-1}, 3750 µgml^{-1} and 4000 µg ml^{-1}.

Per cent mycelial inhibition of *Colletotrichum capsici* under individual fungicides

Per cent mycelial inhibition of carbendazim 50 % WP, captan 50 % WP, copper oxychloride 50% WP, difeniconazole 25 % EC, tebuconazole 25.9 % EC, azoxystrobin 23 % SC are given in table 2 and presented in plate 1, 2, 3, 4, 5, 6, 7 and 8.

Among the various treatments tested, the per cent mycelial inhibition for carbendazim 50 % WP fungicide ranged from 10.69 to 100. The least per cent mycelial inhibition was recorded 10.69 (1 µg ml^{-1}) followed by 16.46 (10 µg ml^{-1}), 17.69 (25 µg ml^{-1}), 18.10 (50 µg ml^{-1}), 18.51 (100 µg ml^{-1}),19.34 (150 µg ml^{-1}), 20.98 (200 µg ml^{-1}), 20.57 (250 µg ml^{-1}), 20.81 (500 µg ml^{-1}), 17.69 (750 µgml $^{-1}$), 35.39 (1000 µg ml^{-1}), 50.20 (1250 µg ml^{-1}), 58.84 (1500 µg ml^{-1}), 71.19 (1750 µg ml^{-1}), 81.89(2000 µg ml^{-1}), 70.37 (2250 µg ml^{-1}), 72.83 (2500 µg ml^{-1}), 75.30 (2750 µg ml^{-1}), 76.54 (3000 µgml $^{-1}$) and 100 per cent mycelial inhibition was observed at 3250 µg ml^{-1}.

The per cent mycelial inhibition for captan 50 % WP fungicide ranged from 14. 81 to 100. The least per cent mycelial inhibition was recorded 14.81 (1 µg ml^{-1}) followed by 18.51 (10 µg ml^{-1}),19.34 (25 µg ml^{-1}), 19.34 (50 µg ml^{-1}), 20.57 (100 µg ml^{-1}), 20.16 (150 µg ml^{-1}), 22.63 (200 µg ml^{-1}), 23.86 (250 µg ml^{-1}), 24.27 (500 µg ml^{-1}), 50.20 (750 µg ml^{-1}), 51.85 (1000 µg ml^{-1}), 53.49 (1250µg ml^{-1}), 52.67 (1500 µg ml^{-1}), 77.36 (1750 µg ml^{-1}), 83.53 (2000 µg ml^{-1}), 85.18 (2250 µg ml^{-1})and 100 per cent mycelial inhibition was observed at 2500 µg ml^{-1}. The per cent mycelial inhibition for copper oxychloride 50 % WP fungicide ranged from 12.34 to 100. The least per cent mycelial inhibition was recorded 12.34 (1 µg ml^{-1}) followed by 14.81 (10 µg ml^{-1}), 16.04 (25 µg ml^{-1}), 16.46(50 µg ml^{-1}), 16.87 (100 µg ml^{-1}), 19.34 (150 µg ml^{-1}), 23.86 (200 µg ml^{-1}), 18.10 (250 µg ml^{-1}),19.34 (500 µg ml^{-1}), 28.80 (750 µg ml^{-1}), 78.18 (1000 µg ml^{-1}), 79.42 (1250 µg ml^{-1}), 81.89 (1500µg ml^{-1}), 83.12 (1750 µg ml^{-1}), 38.68 (2000 µg ml^{-1}), 74.07 (2250 µg ml^{-1}) and 100 per cent mycelialinhibition was observed at 2500 µg ml^{-1}. The per cent mycelial inhibition for difeniconazole 25 % ECfungicide ranged from 16.87 to 100. The least per cent mycelial inhibition was recorded 16.87 (1 µg ml^{-1}) followed by 18.51 (10 µg ml^{-1}), 19.34 (25 µg ml^{-1}), 22.22 (50 µg ml^{-1}), 23.86 (100 µg ml^{-1}),54.32 (150 µg ml^{-1}), 90.12 (200 µg ml^{-1}) and 100 per cent mycelial inhibition were observed at 250 µg ml^{-1}.

The per cent mycelial inhibition for tebuconazole 25.9 % EC fungicide ranged from 18.93 to 100. The least per cent mycelial inhibition was recorded 18.93 (1 µg ml^{-1}) followed by 27.57 (10 µg ml^{-1}), 31.68 (25 µg ml^{-1}) and 100 per cent mycelial inhibition was observed at 50 µg ml^{-1}. The per cent mycelial inhibition for azoxystrobin 23 % SC fungicide ranged from 12.34 to 100. The least per cent mycelial inhibition was recorded 12.34 (1 µg ml^{-1}) followed by 13.58 (10 µg ml^{-1}), 16.87 (25 µg ml^{-1}), 17.69 (50 µg ml^{-1}), 18.51 (100 µg ml^{-1}), 21.39 (150 µg ml^{-1}), 22.22 (200 µg ml^{-1}), 23.04 (250 µgml $^{-1}$), 23.45 (500 µg ml^{-1}), 31.68 (750 µg ml^{-1}), 33.74 (1000 µg ml^{-1}), 41.56 (1250 µg ml^{-1}), 29.62(1500 µg ml^{-1}), 23.45 (1750 µg ml^{-1}), 32.09 (2000 µg ml^{-1}), 72.83 (2250 µg ml^{-1}), 80.24 (2500 µg ml^{-1}), 77.78 (2750 µg ml^{-1}), 85.18 (3000 µg ml^{-1}), 87.65 (3250 µg ml^{-1}) and 100 per cent mycelial inhibition was observed at 3500 µg ml^{-1}.

Per cent mycelial inhibition of *Colletotrichum capsici* under combination of fungicides

Per cent mycelial inhibition of carbendazim 12 % + mancozeb 63 % WP, carbendazim 12 % + flusilazole 12.5 % SE, prochloraz 24.4 % + tebuconazole 12.15 w/w, hexaconazole 5 % + captan 70 % WP, azoxystrobin 11 % + tebuconazole 18.3 % w/w SC, tebuconazole 50 % + trifoxystrobin 25 %WG, metiram 55 % + pyraclostrobin 5 % WG and picoxystrobin + tricyclazole 20.33 % w/w SC are given in table 3 and presented in plate 8, 9, 10, 11, 12, 13 and 14.

Among the various treatments tested, per cent mycelial inhibition azoxystrobin 11 % + tebuconazole 18.3 % w/w SC fungicide ranged from 16.46 to 100. The least per cent mycelial inhibition was recorded 16.46 (1 µg ml^{-1}) followed by 19.34 (10 µg ml^{-1}), 30.04 (25 µg ml^{-1}), 33.74 (50 µg ml^{-1}), 37.03 (100 µg ml^{-1}), 37.86 (150 µg ml^{-1}), 37.03 (200 µg ml^{-1}), 54.32 (250 µg ml^{-1}), 56.79 (500 µg ml^{-1}), 54.32 (750 µg ml^{-1}), 52.26 (1000 µg ml^{-1}), 52.67 (1250 µg ml^{-1}), 65.02 (1500 µg ml^{-1}), 81.89 (1750 µg ml^{-1}), 83.53 (2000 µg ml^{-1}), 72.83 (2250 µg ml^{-1}) and 100 per cent mycelial inhibition was observed at 2500 µg ml^{-1}.

Table 2 Efficacy of individual fungicides on per cent mycelial inhibition of Colletotrichum capsici under in vitro.

Concentration (µg ml $^{-1}$)	Per cent mycelial inhibition					
	Carbendazim 50% WP	**Captan 50% WP**	**Copper Oxy Chloride 50% WP**	**Difeniconazole 25% EC**	**Tebuconazole 25.9% EC**	**Azoxystrobin 23% SC**
1	10.69 (18.42**)	14.81 (22.17**)	12.34 (20.24**)	16.87 (23.56**)	18.93 (25.08**)	12.34 (19.96**)
10	16.46 (23.56)	18.51 (24.33)	14.81 (21.95)	18.51 (24.33)	27.57 (31.50)	13.58 (21.11)
25	17.69 (24.33)	19.34 (25.82)	16.04 (22.77)	19.34 (25.82)	31.68 (33.81)	16.87 (22.77)
50	18.10 (24.33)	19.34 (25.83)	16.46 (24.33)	22.22 (27.26)	23.45 (33.19)	17.69 (23.56)
100	18.51 (25.09)	20.57 (26.55)	16.87 (24.33)	23.86 (28.64)	100 (90.00)	18.51 (25.08)
150	19.34 (25.83)	20.16 (26.55)	19.34 (25.08)	54.32 (47.27)	100 (90.00)	21.39 (27.95)
200	20.98 (26.55)	22.63 (27.96)	23.86 (28.64)	90.12 (72.53)	100 (90.00)	22.22 (28.64)
250	20.57 (26.55)	23.86 (27.9)	18.10 (24.33)	100 (90.00)	100 (90.00)	23.04 (28.64)
500	21.81 (26.55)	24.27 (29.30)	19.34 (25.82)	100 (90.00)	100 (90.00)	23.45 (33.19)
750	17.69 (24.33)	50.20 (44.98)	28.80 (31.93)	100 (90.00)	100 (90.00)	31.68 (35.04)
1000	35.39 (36.25)	51.85 (45.55)	78.18 (62.00)	100 (90.00)	100 (90.00)	33.74 (35.04)
1250	50.20 (44.98)	53.49 (46.70)	79.42 (62.00)	100 (90.00)	100 (90.00)	41.56 (40.38)
1500	58.84 (49.58)	52.67 (46.12)	81.89 (64.13)	100 (90.00)	100 (90.00)	29.62 (32.56)
1750	71.19 (57.39)	77.36 (61.32)	83.12 (65.62)	100 (90.00)	100 (90.00)	23.45 (28.64)
2000	81.89 (63.41)	83.53 (65.62)	38.68 (38.04)	100 (90.00)	100 (90.00)	32.09 (33.81)
2250	70.37 (56.77)	85.18 (67.19)	38.56 (38.04)	-	-	72.83 (58.03)
2500	72.83 (58.02)	100 (90.00)	100 (90.00)	-	-	80.24 (63.41)
2750	75.30 (59.97)	100 (90.00)	100 (90.00)	-	-	77.78 (61.32)
3000	76.54 (59.97)	100 (90.00)	100 (90.00)	-	-	85.18 (67.19)
3250	100 (90.00)	100 (90.00)	100 (90.00)	-	-	87.65 (68.84)
3500	100 (90.00)	100 (90.00)	100 (90.00)	-	-	100 (90.00)
3750	100 (90.00)	100 (90.00)	100 (90.00)	-	-	100 (90.00)
4000	100 (90.00)	100 (90.00)	100 (90.00)	-	-	100 (90.00)
C.D.	0.225	0.220	0.184	0.137	0.294	0.175
SE(m)	0.077	0.070	0.063	0.047	0.101	0.061

Continued....

SE(d)	0.110	0.110	0.089	100 (90.00)	100 (90.00)	32.09 (33.81)
C.V.	2.451	2.450	2.148	-	-	72.83 (58.03)

*Mean of three replications

**Figures in parenthesis are angular transformed values

The per cent mycelial inhibition for carbendazim 12 % + Mancozeb 63 % WP fungicide ranged from 8.64 to 100. The least per cent mycelial inhibition was recorded 8.64 (1 µg ml $^{-1}$) followed by 11.11 (10 µg ml $^{-1}$), 14.81 (25 µg ml $^{-1}$), 16.04 (50 µg ml $^{-1}$), 40.74 (100 µg ml $^{-1}$), 39.50 (150 µg ml $^{-1}$), 45.67 (200 µg ml $^{-1}$), 54.32 (250 µg ml $^{-1}$), 67.90 (500 µg ml $^{-1}$), 77.77 (750µg ml $^{-1}$) and 100 percent mycelial inhibition was observed at 1000 µg ml $^{-1}$.

The per cent mycelial inhibition for carbendazim 12 % + flusilazole 12.5 % SE fungicide ranged from 13.16 to 100. The least per cent mycelial inhibition was recorded 13.16 (1 µg ml $^{-1}$) followed by14.40 (10 µg ml $^{-1}$), 17.69 (25 µg ml $^{-1}$), 19.75 (50 µg ml $^{-1}$), 23.45 (100 µg ml $^{-1}$), 34.97 (150 µg ml $^{-1}$), 44.85 (200 µg ml $^{-1}$), 58.84 (250 µg ml $^{-1}$), 60.08 (500 µg ml $^{-1}$), 65.02 (750 µg ml $^{-1}$), 71.19 (1000µg ml $^{-1}$), 65.02 (1250 µg ml $^{-1}$), 79.42 (1500 µg ml $^{-1}$) and 100 per cent mycelial inhibition was observed at 1750 µg ml $^{-1}$.

The per cent mycelial inhibition for prochloraz 24.4 % + tebuconazole 12.15 w/w EW fungicide ranged from 11.11 to 100. The least per cent mycelial inhibition was recorded 11.11 (1 µg ml $^{-1}$) followed by 12.75 (10 µg ml $^{-1}$), 14.40 (25 µg ml $^{-1}$), 18.51 (50 µg ml $^{-1}$), 21.81 (100 µg ml $^{-1}$), 29.21(150 µg ml $^{-1}$), 30.04 (200 µg ml $^{-1}$), 34.56 (250 µg ml $^{-1}$), 40.74 (500 µg ml $^{-1}$), 45.67 (750 µg ml $^{-1}$),62.55 (1000 µg ml $^{-1}$), 70.37 (1250 µg ml $^{-1}$), 77.77 (1500 µg ml $^{-1}$), 82.71 (1750 µg ml $^{-1}$) and 100 percent mycelial inhibition was observed at 2000 µg ml $^{-1}$.

The per cent mycelial inhibition for tebuconazole 50 % + trifoxystrobin 25 % WG fungicide ranged from 16.46 to 100. The least per cent mycelial inhibition was recorded 13.99 (1 µg ml $^{-1}$) followed by 18.10 (10 µg ml $^{-1}$), 20.16 (25 µg ml $^{-1}$), 21.39 (50 µg ml $^{-1}$), 22.22 (100 µg ml $^{-1}$), 23.04(150 µg ml $^{-1}$), 23.45 (200 µg ml $^{-1}$), 26.33 (250 µg ml $^{-1}$), 28.80 (500 µg ml $^{-1}$), 32.51(750 µg ml $^{-1}$),41.15 (1000 µg ml $^{-1}$), 41.15 (1250 µg ml $^{-1}$), 51.02 (1500 µg ml $^{-1}$), 72.83 (1750 µg ml $^{-1}$), 88.06 (2000µg ml $^{-1}$), 58.02 (2250 µg ml $^{-1}$), 70.37 (2500 µg ml $^{-1}$), 85.18 (2750 µg ml $^{-1}$), 71.60 (3000 µg ml $^{-1}$),75.30 (3250 µg ml $^{-1}$), 77.77 (3500 µg ml $^{-1}$), 85.18 (3750 µg ml $^{-1}$) and 100 per cent mycelial inhibition was observed at 4000 µg ml $^{-1}$.

The per cent mycelial inhibition for metiram 55 % + pyraclostrobin 5 % WG fungicide ranged from 14.81 to 100. The least per cent mycelial inhibition was recorded 14.81 (1 µg ml $^{-1}$) followed by15.63 (10 µg ml $^{-1}$), 16.46 (25 µg ml $^{-1}$), 16.46 (50 µg ml $^{-1}$), 16.04 (100 µg ml $^{-1}$), 15.22 (150 µg ml $^{-1}$), 20.16 (200 µg ml $^{-1}$), 20.57 (250 µg ml $^{-1}$), 20.98 (500 µg ml $^{-1}$), 23.86 (750 µg ml $^{-1}$), 25.92 (1000µg ml $^{-1}$), 27.98 (1250 µg ml $^{-1}$), 28.80 (1500 µg ml $^{-1}$), 32.92 (1750 µg ml $^{-1}$), 48.14 (2000 µg ml $^{-1}$),72.83 (2250 µg ml $^{-1}$), 76.54 (2500 µg ml $^{-1}$), 80.24 (2750 µg ml $^{-1}$), 97.53 (3000 µg ml $^{-1}$) and 100 percent mycelial inhibition was observed at 3250 µg ml $^{-1}$.

The per cent mycelial inhibition for picoxystrobin + tricyclazole 20.33 % w/w SC fungicide ranged from 11.11 to 100. The least per cent mycelial inhibition was recorded 11.11 (1 µg ml $^{-1}$) followed by 11.93 (10 µg ml $^{-1}$), 12.34 (25 µg ml $^{-1}$), 22.22 (50 µg ml $^{-1}$), 28.80 (100 µg ml $^{-1}$), 29.21(150 µg ml $^{-1}$), 28.80 (200 µg ml $^{-1}$), 36.62 (250 µg ml $^{-1}$), 38.68 (500 µg ml $^{-1}$), 59.67 (750 µg ml $^{-1}$),77.36 (1000 µg ml $^{-1}$), 78.60 (1250 µg ml $^{-1}$), 79.01 (1500 µg ml $^{-1}$), 67.90 (1750 µg ml $^{-1}$), 60.49 (2000µg ml $^{-1}$), 70.37 (2250 µg ml $^{-1}$) and 100 per cent mycelial inhibition was observed at 2500 µg ml $^{-1}$.

The per cent mycelial inhibition for hexaconazole 5 % + captan 70 % WP fungicide ranged from 13.58 to 100. The least per cent mycelial inhibition was recorded 13.58 (1 µg ml $^{-1}$) followed by 16.46(10 µg ml $^{-1}$), 17.69 (25 µg ml $^{-1}$), 20.57 (50 µg ml $^{-1}$), 26.33 (100 µg ml $^{-1}$), 29.62 (150 µg ml $^{-1}$), 29.62(200 µg ml $^{-1}$), 41.15 (250 µg ml $^{-1}$), 72.01 (500 µg ml $^{-1}$), 76.54 (750 µg ml $^{-1}$), 79.83 (1000 µg ml $^{-1}$)and 100 per cent mycelial inhibition was observed at 1250 µg ml $^{-1}$.

EC50/ED50 (Half maximal effective concentration / effective dose)

Among the 14 different fungicicdes tested under probit analysis, EC50/ED50 was recorded lowest

in tebuconazole 25.9 % EC (18) followed by difenoconazole 25 % EC (113), carbendazim 12% + mancozeb 63 % WP (316), hexaconazole 5 % + captan 70 % WP (406), carbendazim 25 % +flusilazole 12.5 % SE (549), azoxystrobin 11 % + tebuconazole 18.3 % w/w SC (689), prochloraz24.4 % + tebuconazole 12.1 % w/w EW (762), picoxystrobin + tricyclazole 20.33 % w/w SC (853),captan 50% WP (978), copper oxychloride 50 % WP (1040), carbendazim 50 % WP (1350), tebuconazole 50 % + trifloxystrobin 25 % WG (1465), metiram 55 % + pyraclostrobin 5 % WG (1549) and highest was recorded in azoxystrobin 23 % SC (1598).

EC50/ED50 (90 % maximal effective concentration / effective dose)

EC90/ED90 was recorded lowest in tebuconazole 25.9 % EC (50) followed by difenoconazole 25 % EC (229), carbendazim 12 % + mancozeb 63 % WP (763), hexaconazole 5 % + captan 70 %WP (1246), carbendazim 25 % + flusilazole 12.5 % SE (1542), prochloraz 24.4 % + tebuconazole12.1 % w/w EW (1812), captan 50 % WP (2223), picoxystrobin + tricyclazole 20.33 % w/w SC (2262), azoxystrobin 11 % + tebuconazole 18.3 % w/w SC (2263), copper oxychloride 50 % WP (2352), carbendazim 50 % WP (3001), metiram 55 % + pyraclostrobin 5 % WG (3144), azoxystrobin23 % SC (3429) and highest was recorded in tebuconazole 50 % + trifloxystrobin 25 % WG (3720).

MIC (Minimum Inhibitory Concentration)

MIC (µg $ml^{-1)}$ was recorded lowest in tebuconazole 25.9 % EC (100) followed by difeniconazole 25 % EC (250), carbendazim 12 % + mancozeb 63 % WP (1000), hexaconazole 5 % + captan 70 %WP (1250), carbendazim 25 % + flusilazole 12.5 % SE (1750), prochloraz 24.4 % + tebuconazole 12.1% w/w EW (2000), captan 50 % WP (2250), picoxystrobin + tricyclazole 20.33 % w/w SC (2500),azoxystrobin 11 % + tebuconazole 18.3 % w/w SC (2500), copper oxychloride 50 % WP (2500),carbendazim 50 % WP (3250), metiram 55 % + pyraclostrobin 5 % WG (3250), azoxystrobin 23 %SC (3500) and highest was recorded in tebuconazole 50 % + trifloxystrobin 25 % WG (4000).

The per cent mycelial inhibition for carbendazim 50 % WP was ranged from 10.69 (1µg ml^{-1}) to 100 (3250 µg ml^{-1}), for captan 50 % WP 14.81 (1µg ml^{-1}) to 100 (2500 µg ml^{-1}), copper oxy chloride50 % WP 12.34 (1µg ml^{-1}) to 100 (2500 µg ml^{-1}), difeniconazole 25 % EC 16.87 (1µg ml^{-1}) to 100(3250 µg ml^{-1}), tebuconazole 25.9 % EC 18.93 (1µg ml^{-1}) to 100 (100 µg ml^{-1}), azoxystrobin 23 % SC 12.34 (1µg ml^{-1}) to 100 (3500 µg ml^{-1}), azoxystrobin 11 % + tebuconazole18.3 % w/w SC 16.46 (1µg ml^{-1}) to 100 (3250 µg ml^{-1}), carbendazim 12 % + mancozeb 63 % WP 8.64 (1µg ml^{-1}) to 100 (3250 µg ml^{-1}), carbendazim 12 % + flusilazole 12.5 % SE 13.16 (1µg ml^{-1}) to 100 (3250 µg ml^{-1}), prochloraz 24.4 % + tebuconazole 12.15 w/w EW 11.11 (1µg ml^{-1}) to 100 (3250 µg ml^{-1}), tebuconazole 50 % + trifoxystrobin 25 % WG 16.46 (1µg ml^{-1}) to 100 (3250 µg ml^{-1}), metiram 55 % + pyraclostrobin 5 % WG 14.81 (1µg ml^{-1}) to 100 (3250 µg ml^{-1}), picoxystrobin + tricyclazole 20.33 % w/w SC 11.11(1µgml^{-1}) to 100 (3250 µg ml^{-1}), hexaconazole 5 % + captan 70 % WP 13.58 (1µg ml^{-1}) to 100 (3250 µg ml^{-1}).

Summary And Conclusions

Based on pathogenicity studies, isolate *Cc*-3 was found as the highest virulent isolate. Hence, it was used for fungicide screening under *in vitro* conditions. Efficacy of six individual and eight combination fungicides was screened against *Colletotrichum capsici* pathogen.

The results showed all the 14 fungicides were effective in inhibiting the radial growth of *Colletotrichum capsici* compared to the control. The results revealed that, per cent mycelial inhibition, EC50/ ED50 and MIC values of *Colletotrichum capsici* significantly differed in all the fungicides evaluated at different concentrations as 1µg ml^{-1}, 10 µg ml^{-1}, 25 µg ml^{-1}, 50 µg ml^{-1}, 100 µg ml^{-1}, 150µg ml^{-1}, 200 µg ml^{-1}, 250 µg ml^{-1}, 500 µg ml^{-1}, 750 µg ml^{-1}, 1000 µg ml^{-1}, 1250 µg ml^{-1}, 1500 µg ml^{-1}, 1750 µg ml^{-1}, 2000 µg ml^{-1}, 2250 µg ml^{-1}, 2500 µg ml^{-1}, 2750 µg ml^{-1}, 3000 µg ml^{-1}, 3250 µg ml^{-1},3500 µg ml^{-1}, 3750 µg ml^{-1} and 4000 µg ml^{-1}.

Among the different individual fungicides tested under probit analysis, EC50/ED50 values were recorded lowest in tebuconazole 25.9 % EC (18 µg ml^{-1}) followed by difenoconazole 25 % EC (113 µg ml^{-1}) and highest EC50/ED50 was recorded in azoxystrobin 23 % SC (3429 µg ml^{-1}).

Among all the eight combination fungicides, EC50/ ED50 values was recorded lowest in carbendazim 12 %+ mancozeb 63 % WP (316 µg ml^{-1}) followed by hexaconazole 5% + captan 70 %WP (406 µg ml^{-1}) and highest EC50/ED50 were recorded in tebuconazole 50 % + trifloxystrobin 25 % WG (3720 µg ml^{-1}).

Table -3 Efficacy of combination of fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

Concentration(µg ml $^{-1}$)	Per cent mycelial inhibition							
	Azoxystrobin 11 % + Tebuconazole18.3 % w/w SC	**Carbendazim 12% + Mancozeb 63% WP**	**Carbendazim 12% + Flusilazole 12.5% SE**	**Prochloraz 24.4 % + Tebuconazole 12.15 w/w EW**	**Tebuconazole 50 % + Trifoxystrobin 25 % WG**	**Metiram 55 % + Pyraclostrobin 5 % WG**	**Picoxystrobin + Tricyclazole 20.33 % w/w SC**	**Hexaconazole 5% + Captan70% WP**
1	16.46 (23.54**)	8.64 (17.43**)	13.16 (21.11**)	11.11 (20.24**)	13.99 (20.81**)	14.81 (21.95**)	11.11 (19.35**)	13.58 (21.95**)
10	19.34 (25.82)	11.11 (19.35)	14.40 (21.95)	12.74 (21.11)	18.10 (25.08)	15.63 (22.77)	11.93 (20.24)	16.46 (23.56)
25	30.04 (33.19)	14.81 (21.11)	17.69 (25.08)	14.40 (22.77)	20.16 (26.55)	16.46 (23.56)	12.34 (20.26)	17.69 (25.08)
50	33.74 (35.04)	16.04 (22.77)	19.75 (25.82)	18.51 (25.08)	21.39 (27.26)	16.46 (23.56)	22.22 (27.95)	20.57 (27.26)
100	37.03 (37.44)	40.74 (38.62)	23.45 (28.64)	21.81 (27.26)	22.22 (27.96)	16.04 (23.56)	28.80 (31.93)	26.33 (29.98)
150	37.86 (37.44)	39.50 (38.62)	34.97 (35.65)	29.21 (32.56)	23.04 (28.64)	15.22 (22.77)	29.21 (32.56)	29.62 (32.56)
200	37.03 (37.45)	45.67 (41.53)	44.85 (42.11)	30.04 (33.81)	23.45 (28.64)	20.16 (26.55)	28.80 (31.49)	29.62 (32.56)
250	54.32 (47.27)	54.32 (47.27)	58.84 (49.58)	34.56 (35.04)	26.33 (30.64)	20.57 (26.55)	36.62 (37.44)	41.15 (40.38)
500	56.79 (48.42)	67.90 (54.91)	60.08 (50.74)	40.74 (39.79)	28.80 (31.93)	20.98 (27.26)	38.68 (38.04)	72.01 (58.03)
750	54.32 (47.27)	77.77 (60.64)	65.02 (53.70)	45.67 (41.53)	32.51 (34.43)	23.86 (28.64)	59.67 (50.16)	76.54 (60.67)
1000	52.26 (46.12)	100 (90.00)	71.19 (56.76)	62.55 (51.92)	41.15 (39.78)	25.92 (29.98)	77.36 (61.32)	79.83 (62.70)
1250	52.67 (46.12)	100 (90.00)	65.02 (53.70)	70.37 (58.03)	41.15 (39.78)	27.98 (31.93)	78.60 (62.01)	100 (90.00)
1500	65.02 (53.70)	100 (90.00)	79.42 (62.70)	77.77 (61.32)	51.02 (45.55)	28.80 (32.57)	79.01 (62.70)	100 (90.00)
1750	81.89 (64.13)	100 (90.00)	100 (90.00)	82.71 (66.42)	72.83 (58.03)	32.92 (35.04)	67.90 (54.91)	100 (90.00)
2000	83.53 (66.40)	100 (90.00)	100 (90.00)	100 (90.00)	88.06 (69.71)	48.14 (43.83)	60.49 (50.74)	100 (90.00)
2250	72.83 (58.03)	-	-	-	58.02 (49.58)	72.83 (58.67)	70.37 (56.76)	-
2500	100 (90.00)	-	-	-	70.37 (56.76)	76.54 (60.64)	100 (90.00)	-
2750	100 (90.00)	-	-	-	85.18 (67.19)	80.24 (63.41)	100 (90.00)	-
3000	100 (90.00)	-	-	-	71.60 (57.39)	97.53 (80.08)	100 (90.00)	-
3250	100 (90.00)	-	-	-	75.30 (59.97)	100 (90.00)	100 (90.00)	-
3500	100 (90.00)	-	-	-	77.77 (61.32)	100 (90.00)	100 (90.00)	-
3750	100 (90.00)	-	-	-	85.18 (67.19)	100 (90.00)	100 (90.00)	-
4000	100 (90.00)	-	-	-	100 (90.00)	100 (90.00)	100 (90.00)	-
C.D.	0.182	0.349	0.184	0.565	0.233	0.205	0.195	0.358

SE(m)	0.064	0.120	0.063	0.195	0.082	0.072	0.068	0.123
SE(d)	0.090	0.170	0.089	0.275	0.115	0.102	0.097	0.174
C.V.	3.874	7.035	2.419	6.874	3.509	2.896	3.821	5.309

*Mean of three replications.
**Figures in parenthesis are angular transformed values

Table 4 List of fungicides tested against Colletotrichum capsici using Probit analysis.

Name of the fungicide	**EC 50/ ED 50 -1 (µg ml)**	**EC90/ ED90 -1 (µg ml)**	**-1 MIC (µg ml)**
Carbendazim 50%WP	1350	3001	3250
Captan 50% WP	978	2223	2500
Copper Oxy Chloride 50%WP	1040	2352	2500
Difenoconazole 25% EC	113	229	250
Tebuconazole 25.9%EC	18	50	100
Azoxystrobin 23% SC	1598	3429	3500
Azoxystrobin 11% + Tebuconazole 18.3% w/w SC	689	2263	2500
Carbendazim 12 %+ Mancozeb 63 % WP	316	763	1000
Carbendazim 25 %+ Flusilazole 12.5 % SE	549	1542	1750
Prochloraz 24.4 % + Tebuconazole 12.1 % w/w EW	762	1812	2000
Tebuconazole 50 % + Trifloxystrobin 25 % WG	1465	3720	4000
Metiram 55 % + Pyraclostrobin 5 % WG	1549	3144	3250
Picoxystrobin + Tricyclazole 20.33 % w/w SC	853	2262	2500
Hexaconazole 5 %+Captan 70 %WP	406	1246	1250

EC90/ED90 was recorded lowest in Tebuconazole 25.9 % EC (50 µg ml^{-1}) followed by difenoconazole 25 % EC (229 µg ml^{-1}) and highest was recorded in azoxystrobin 23 % SC (3429 µg ml^{-1}) among all six individual fungicides, EC90/ED90 was recorded lowest in carbendazim 12 % + mancozeb 63 % WP (763 µg ml^{-1}) followed by hexaconazole 5 % + captan 70 % WP (1246 µg ml^{-1}) and highest was recorded in tebuconazole 50 % + trifloxystrobin 25 % WG (3720 µg ml^{-1}) among alleight combination fungicides.

Minimum Inhibitory Concentration (MIC) was recorded lowest in tebuconazole 25.9 % EC (100 µg ml^{-1}) followed by difenoconazole 25 % EC (250 µg ml^{-1}) and highest was recorded in azoxystrobin23 % SC (3500 µg ml^{-1}) among all the six individual fungicides.

MIC was recorded lowest in carbendazim 12 % + mancozeb 63 % WP (1000 µg ml^{-1}) followed by hexaconazole 5 % + captan 70 % WP (1250 µg ml^{-1}) and highest was recorded in tebuconazole 50 % + trifloxystrobin 25 % WG (4000 µg ml^{-1}) among all eight combination fungicides.

From *in vitro s*tudies on the different fungicides tested against *Colletotrichum capsici* pathogen, among the individual fungicides tebuconazole 25.9 % EC was found to be effective in reducing 100 per cent mycelial inhibition of *Colletotrichum capsici* pathogen at a concentration of 100 µg ml^{-1} followed by difenoconazole 25 % EC at a concentration of 250 µg ml^{-1} and in case of combination fungicides, carbendazim 12 % + mancozeb 63 % WP was found to be effective at a concentration of 1000 µg ml^{-1}.

From the study, it was finally concluded that from the observations of **a** survey that the per cent fruit rot disease incidence was recorded in all the areas

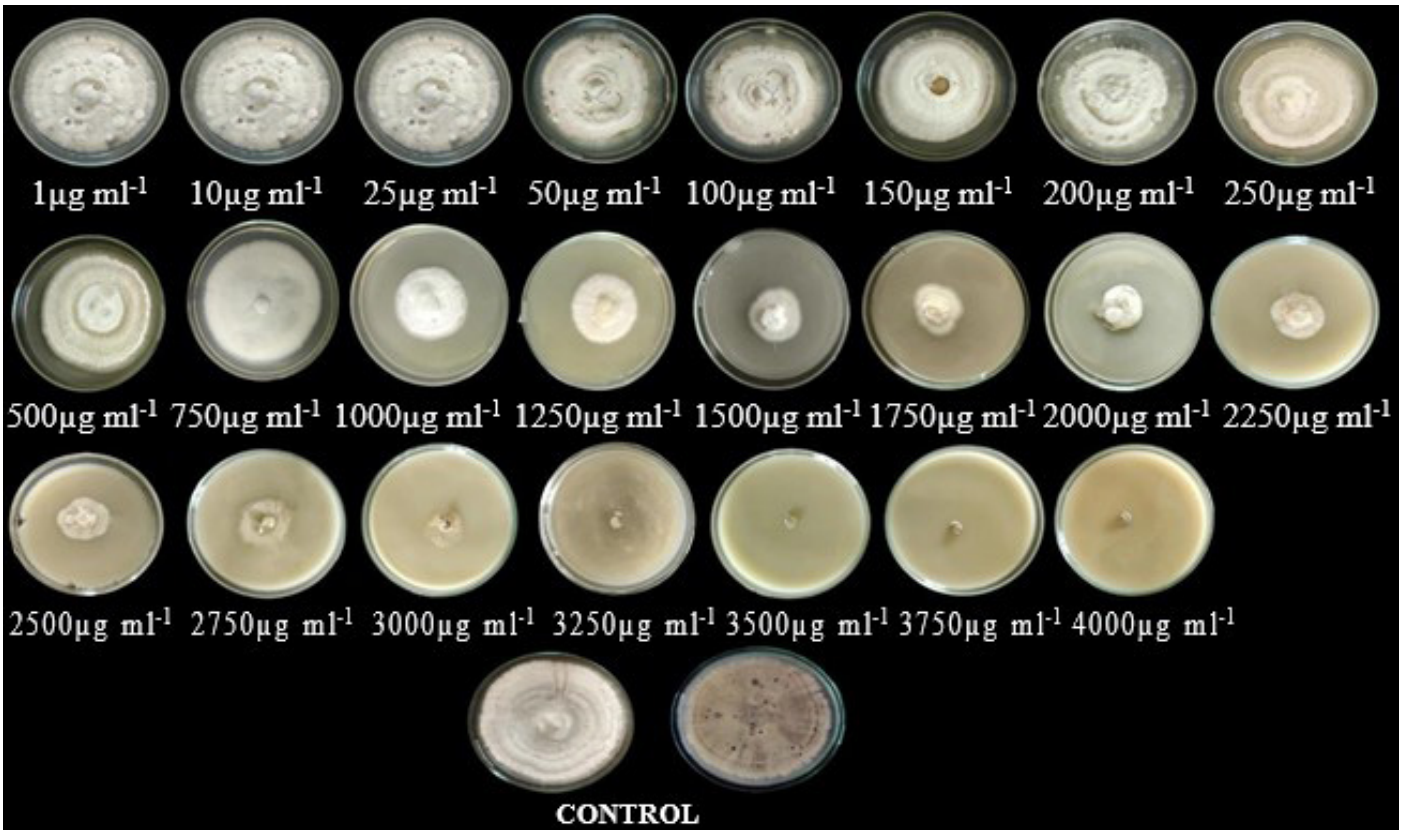

Plate 1 Efficacy of carbendazim 50 % WP fungicide on mycelial inhibition of Colletotrichum capsici under in vitro.

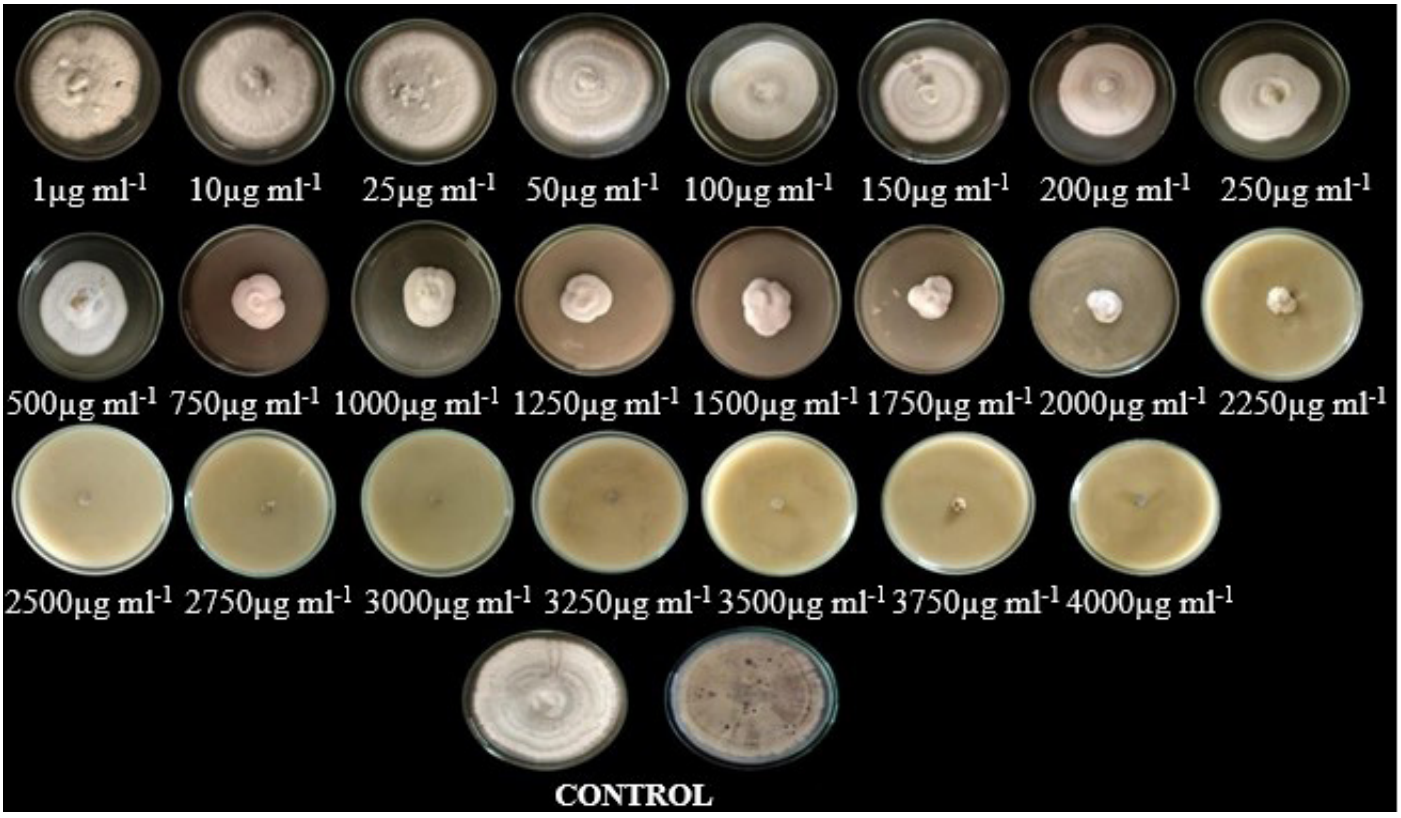

Plate 2 Efficacy of captan 50 % WP fungicide on mycelial inhibition of Colletotrichum capsici under in vitro.

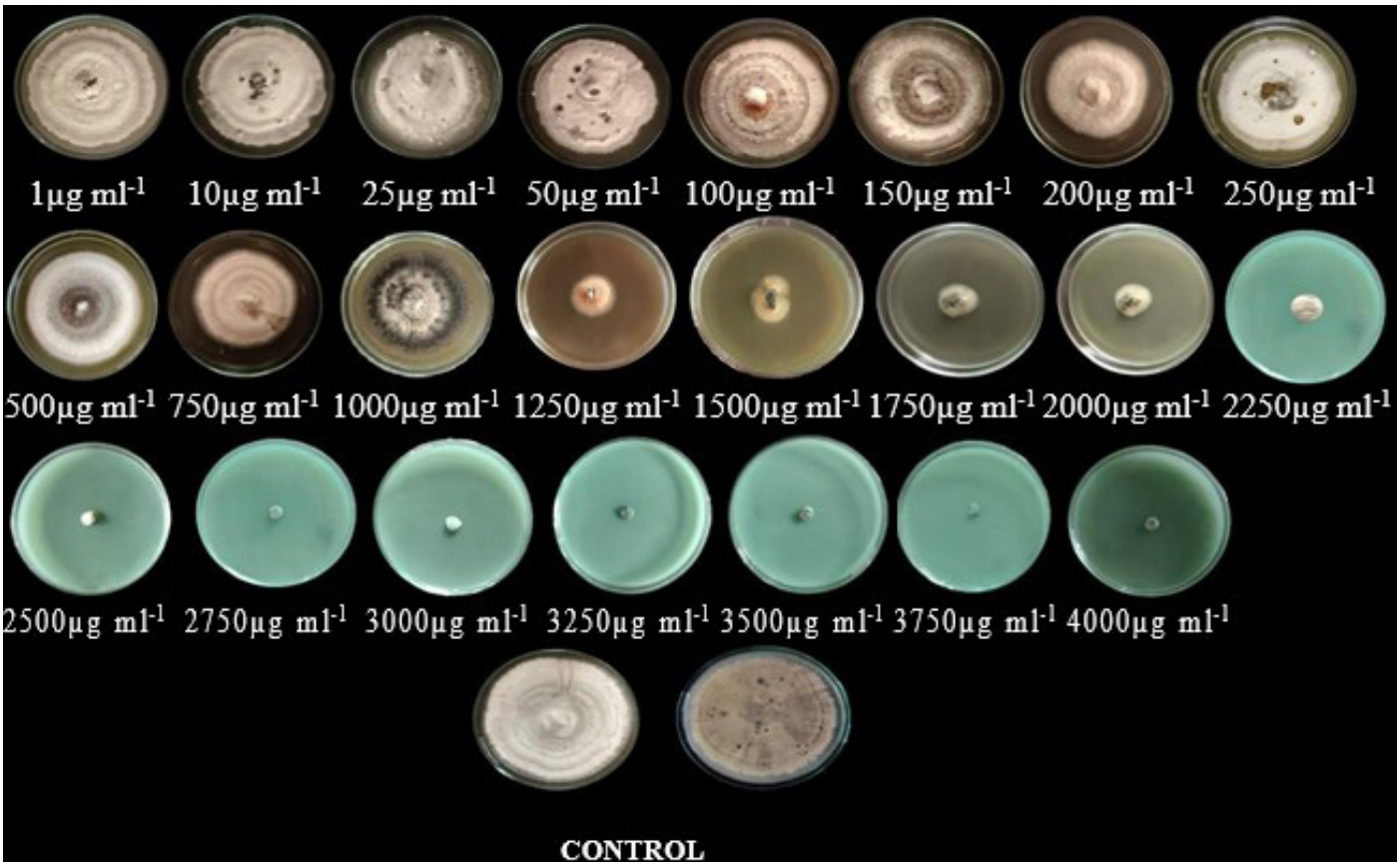

Plate 3 Efficacy of copper oxychloride 50 % WP fungicide on mycelial inhibition of Colletotrichum capsici under in vitro.

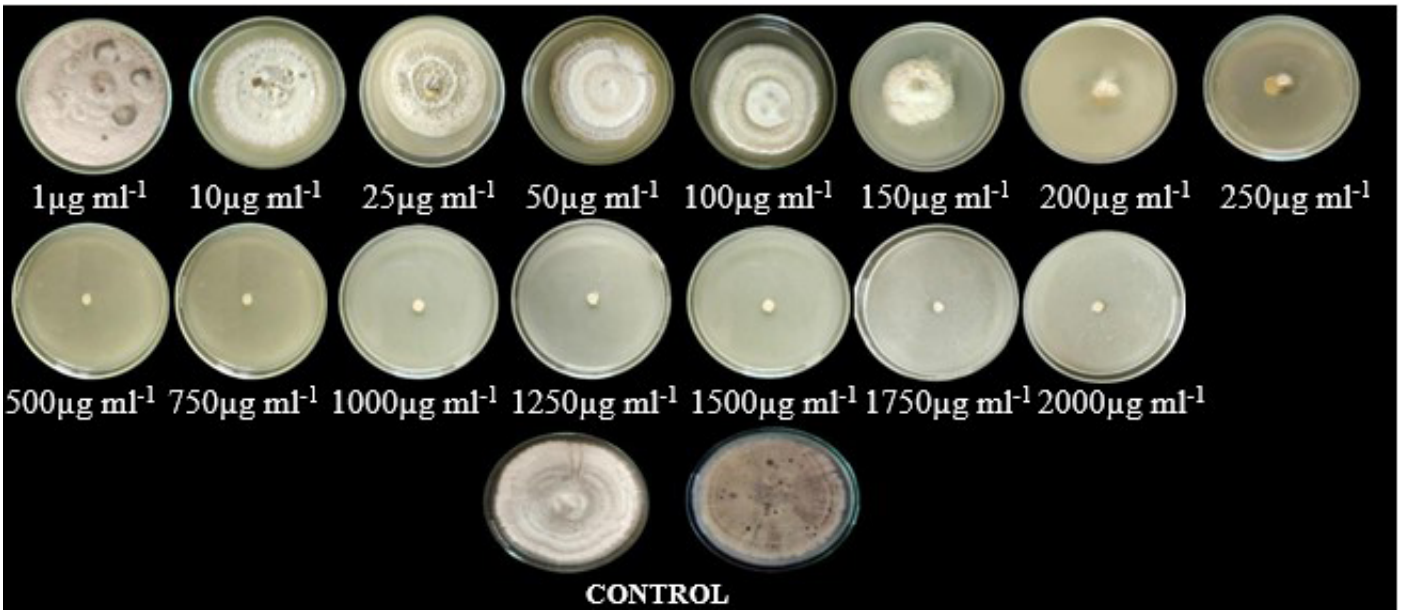

Plate 4 Efficacy of difeniconazole 25 % EC fungicide on mycelial inhibition of Colletotrichum capsici under in vitro.

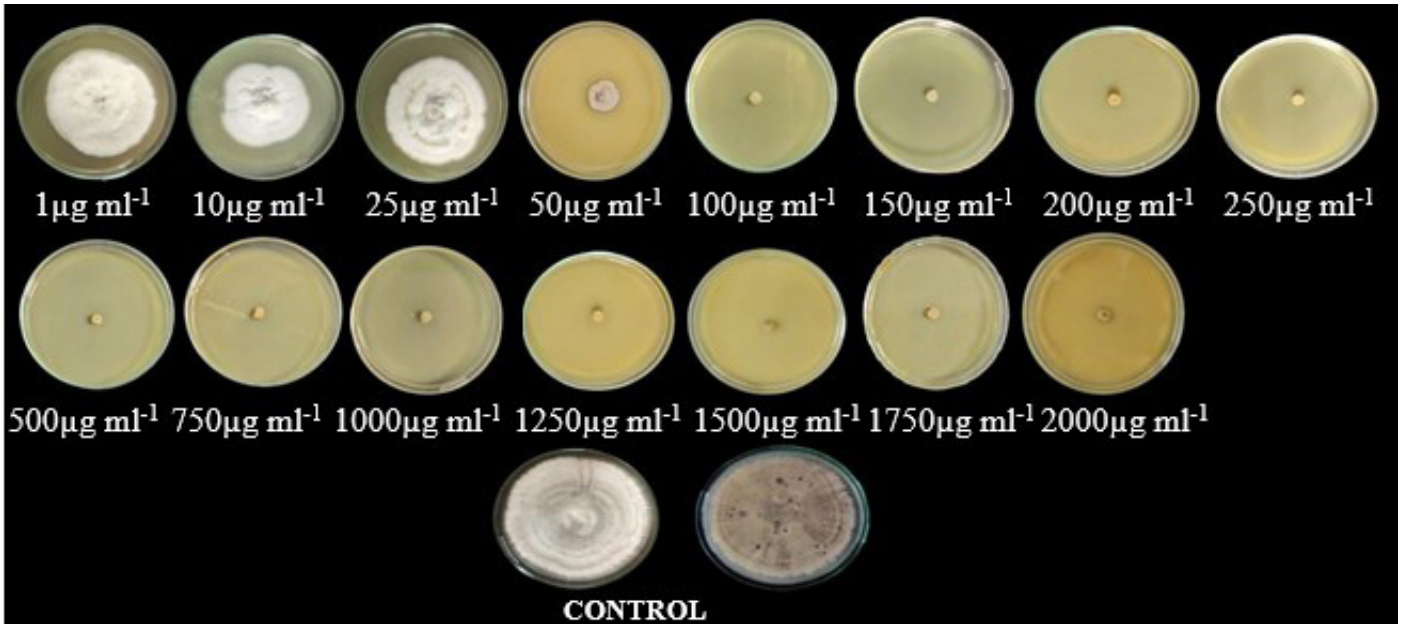

Plate 5 Efficacy of tebuconazole 25.9 % EC fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

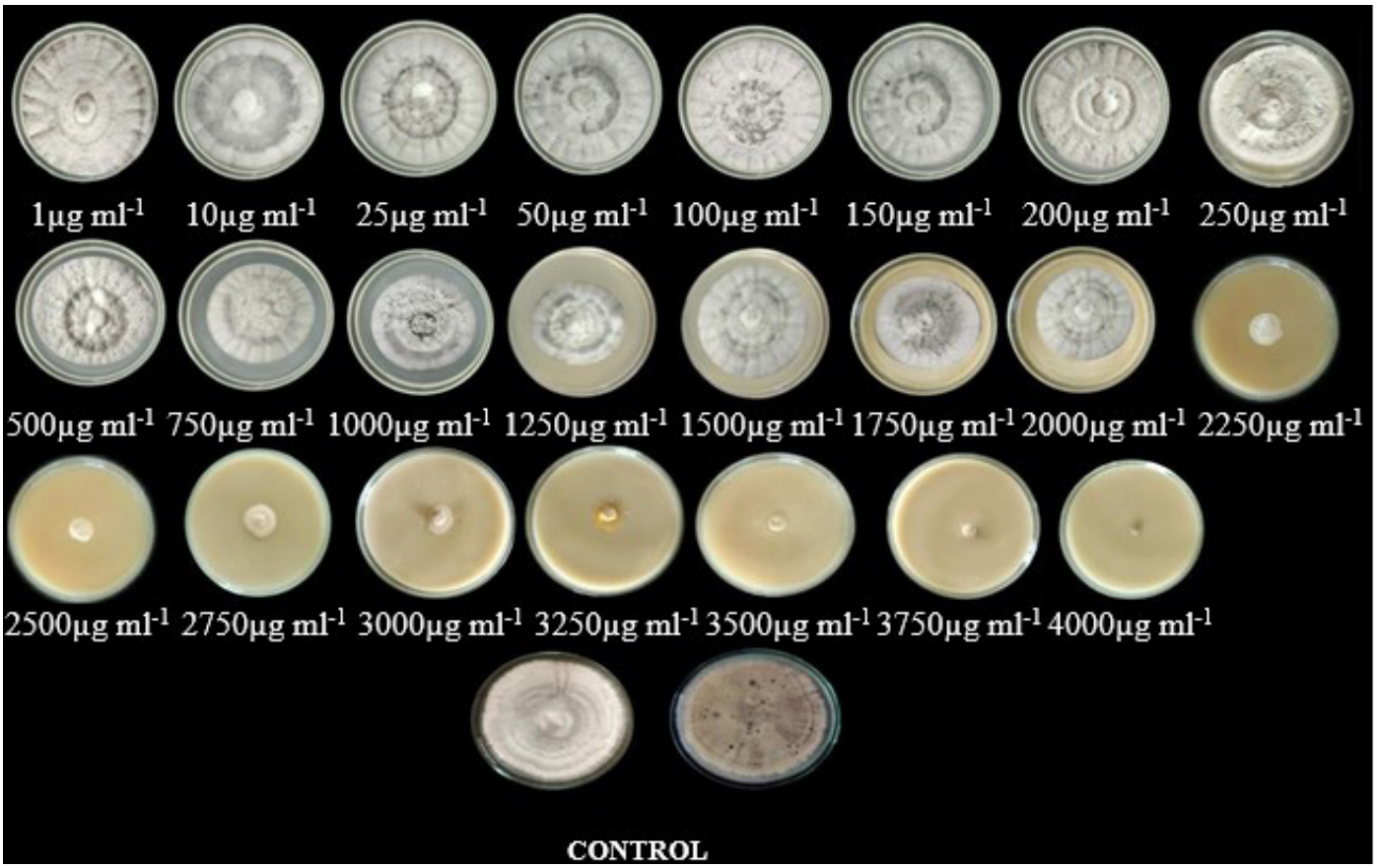

Plate 6 Efficacy of azoxystrobin 23 % SC fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

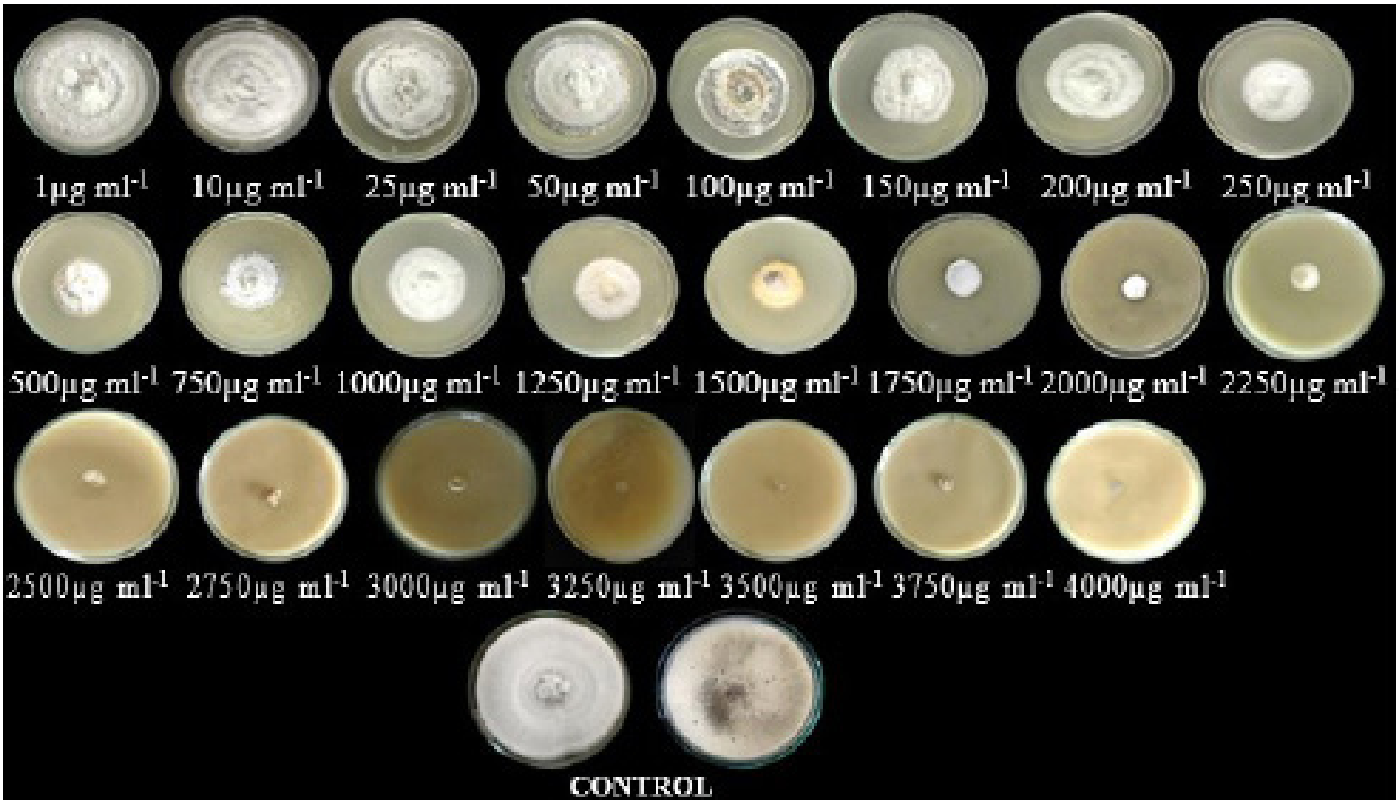

Plate 7 Efficacy of azoxystrobin 11 % + tebuconazole 18.3 % w/w SC fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

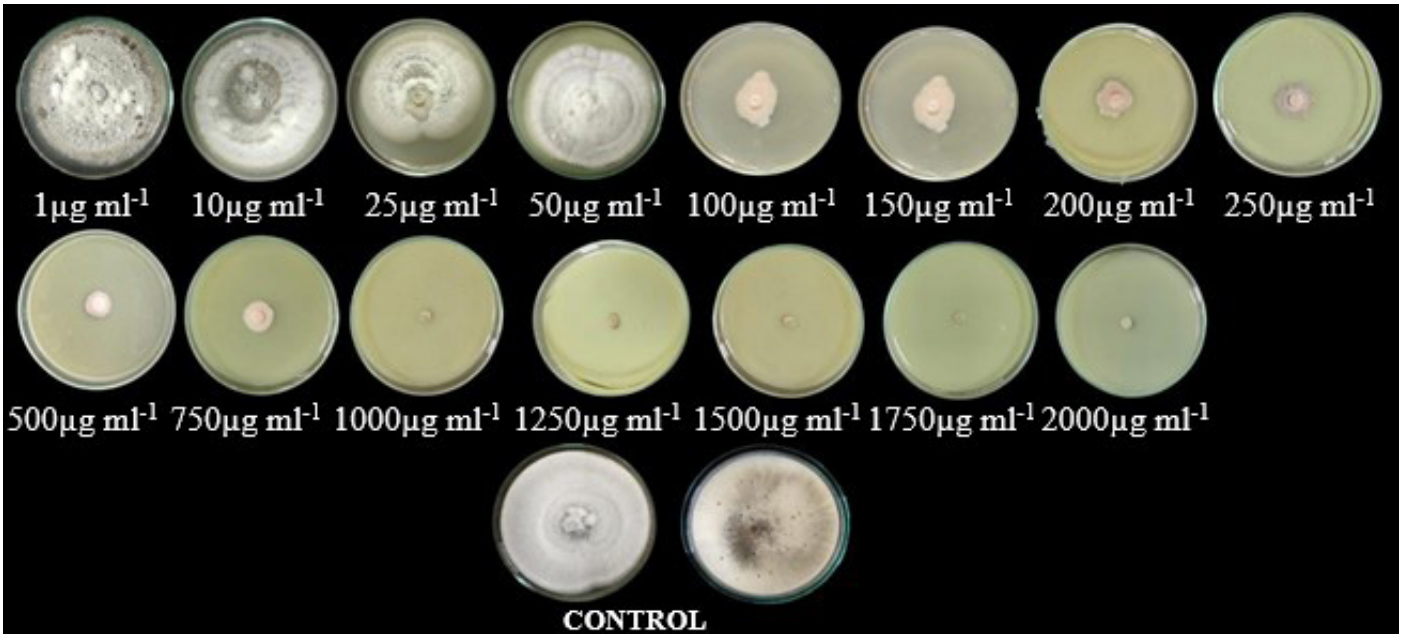

Plate 8 Efficacy of carbendazim 12 % + mancozeb 63 % WP fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

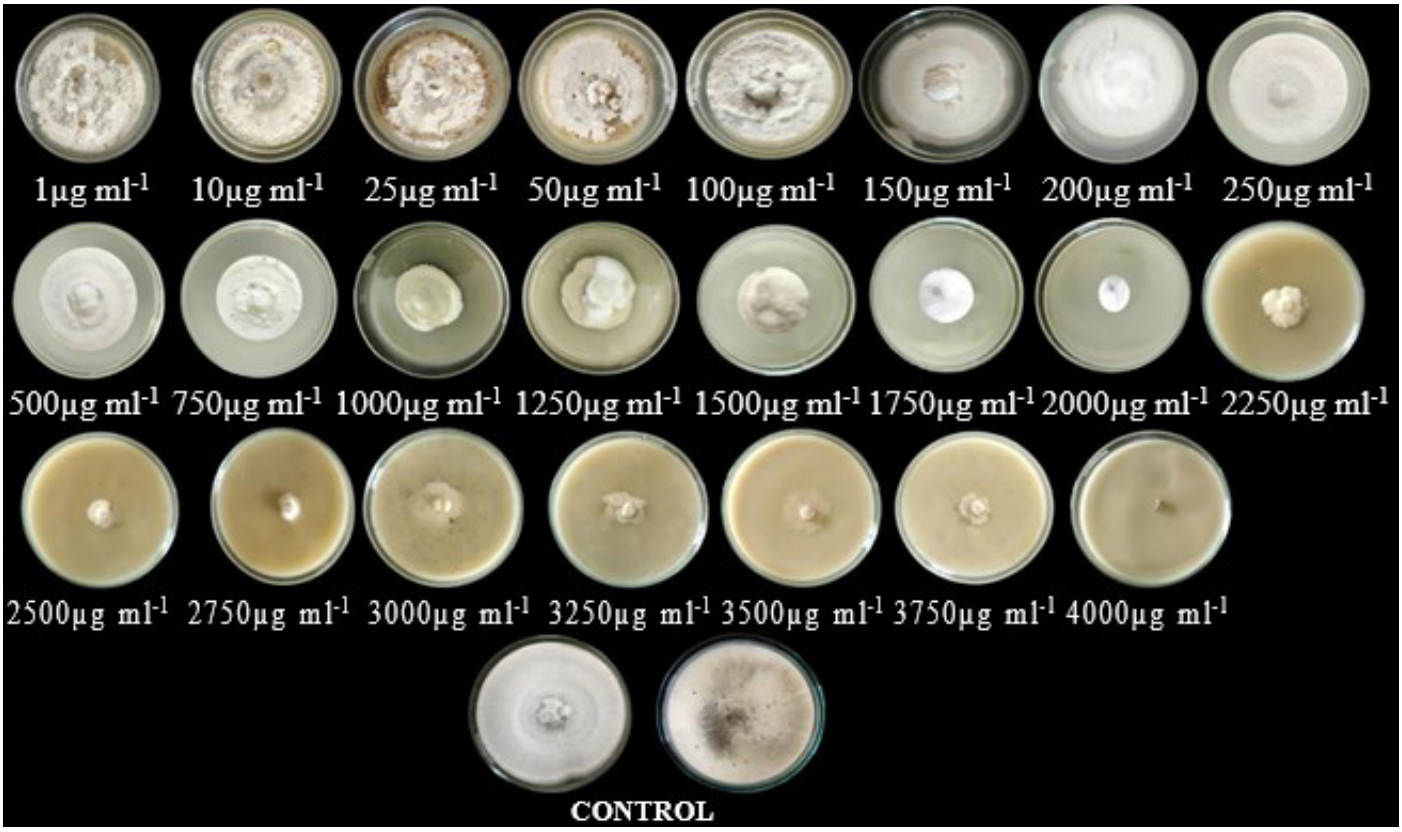

Plate 11 Efficacy of tebuconazole 50 % + trifoxystrobin 25 % WG fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

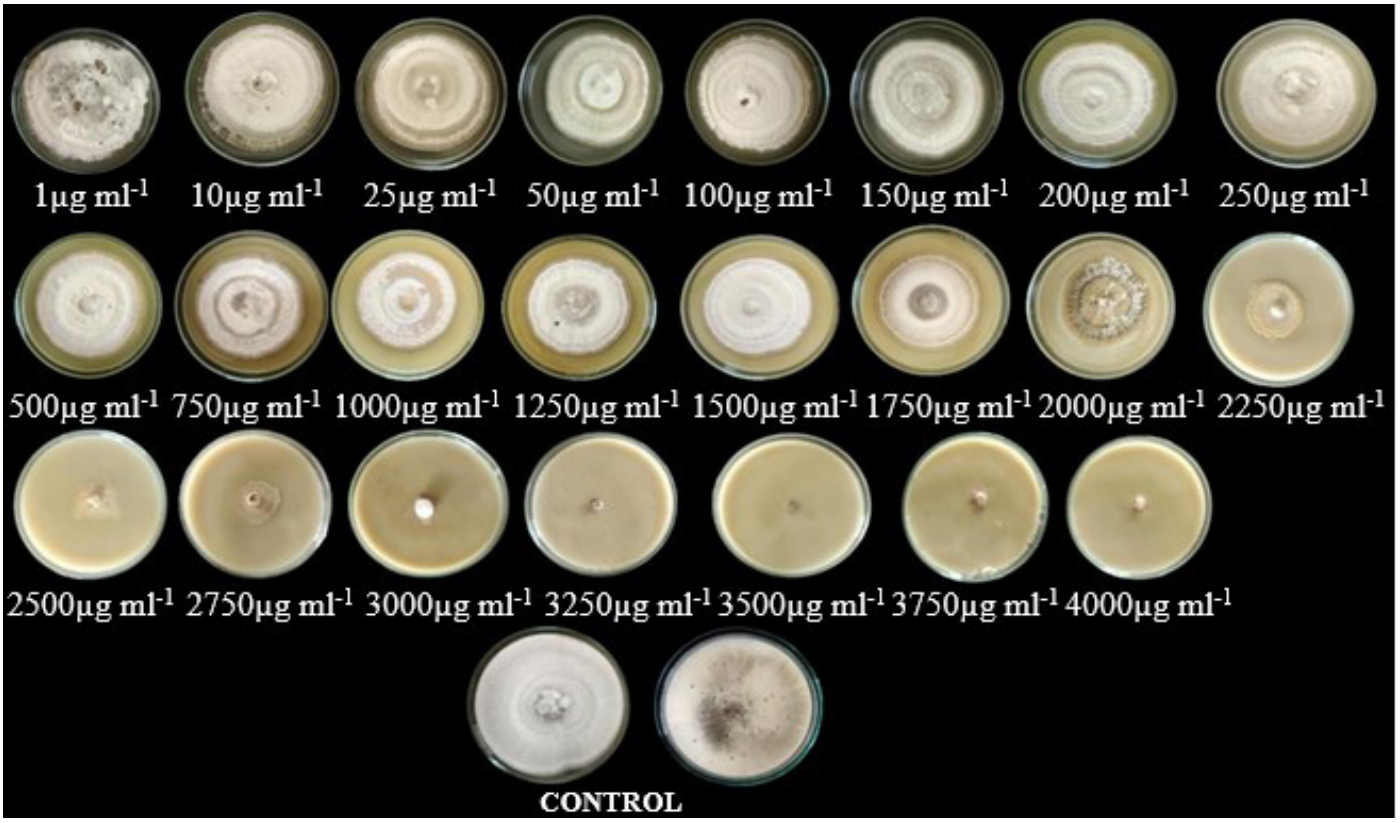

Plate 12 Efficacy of metiram 55 % + pyraclostrobin 5 % WG fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

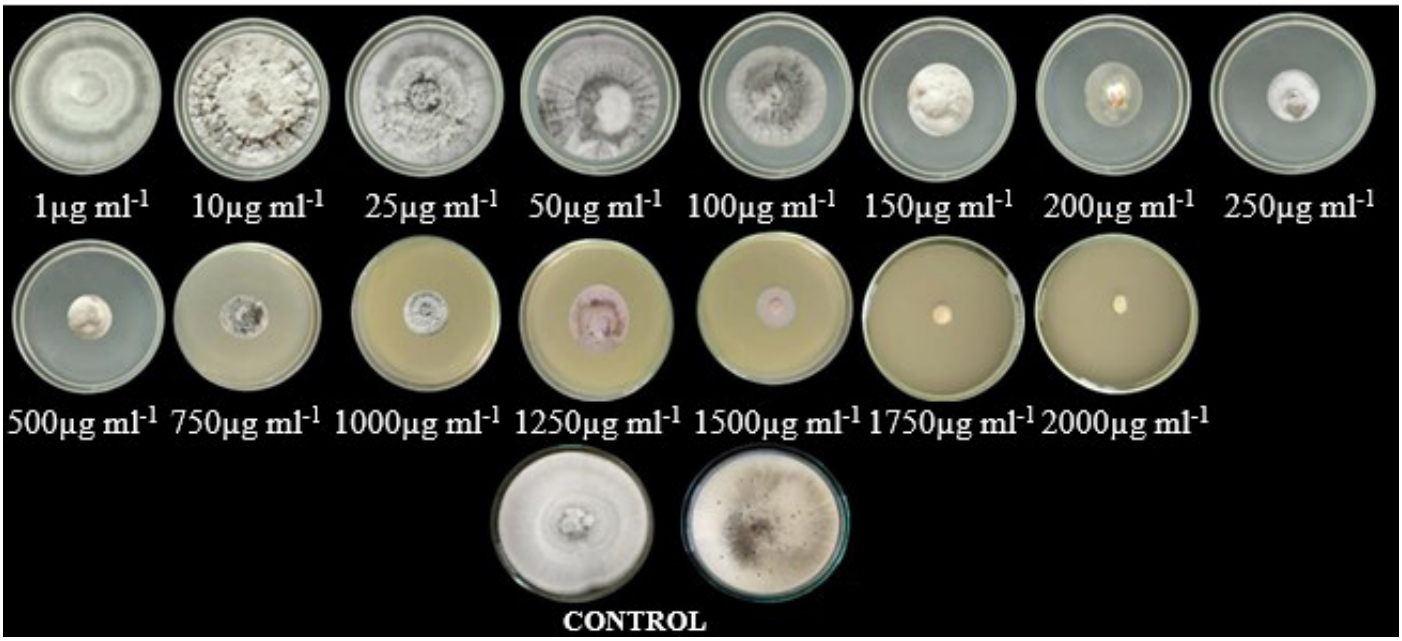

Plate 9 Efficacy of carbendazim 12 % + flusilazole 12.5 % SE fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

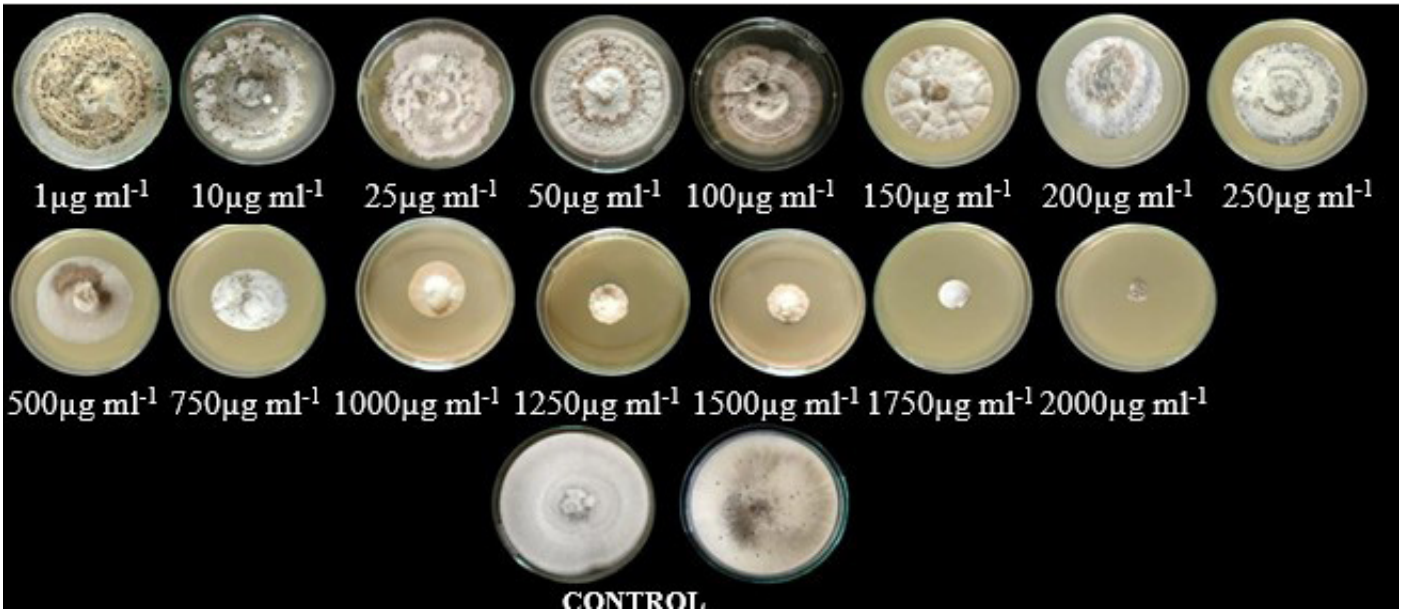

Plate 10 Efficacy of prochloraz 24.4 % + tebuconazole 12.15 w/w EW fungicides on mycelial inhibition of Colletotrichum capsici under in vitro

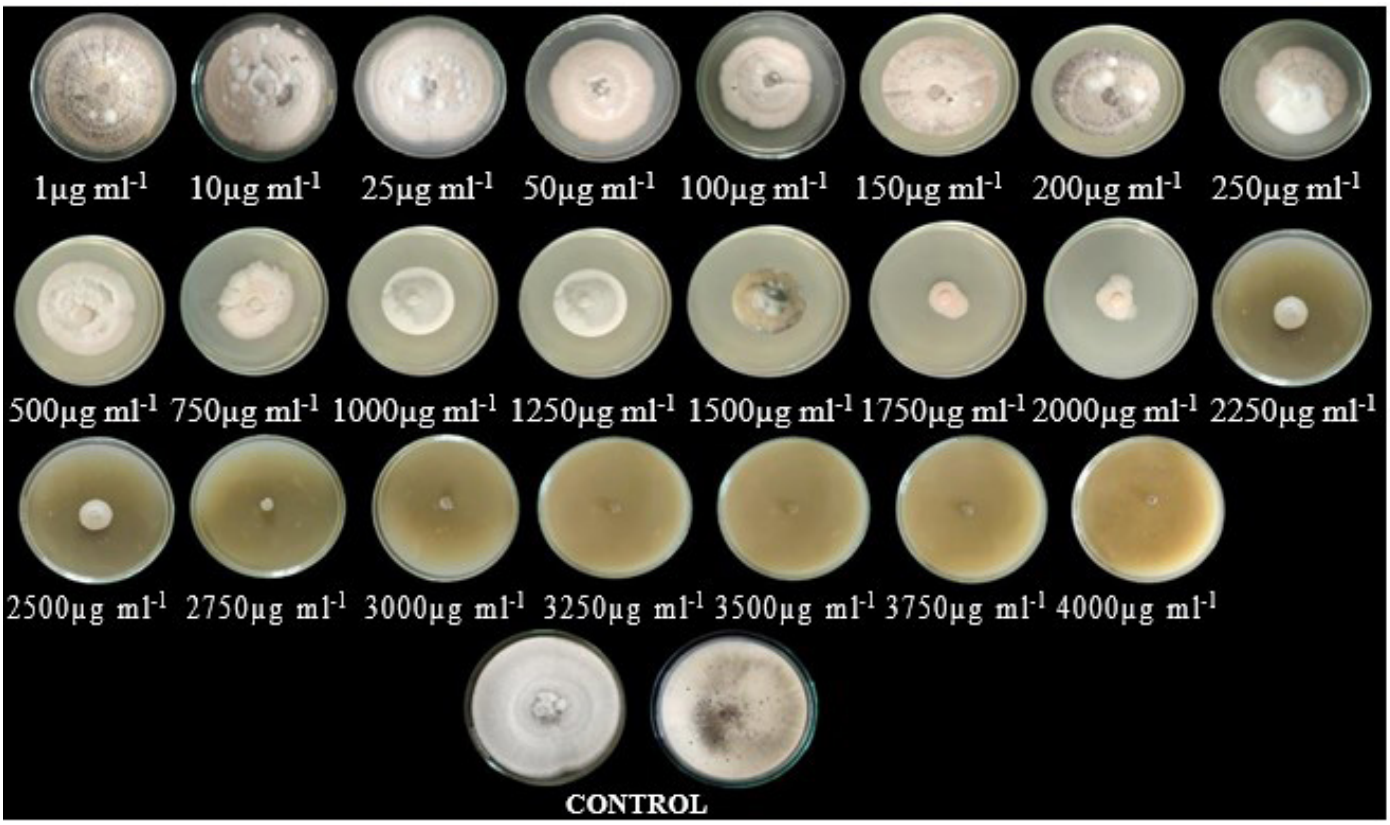

Plate 13 Efficacy of picoxystrobin + tricyclazole 20.33 % w/w SC fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

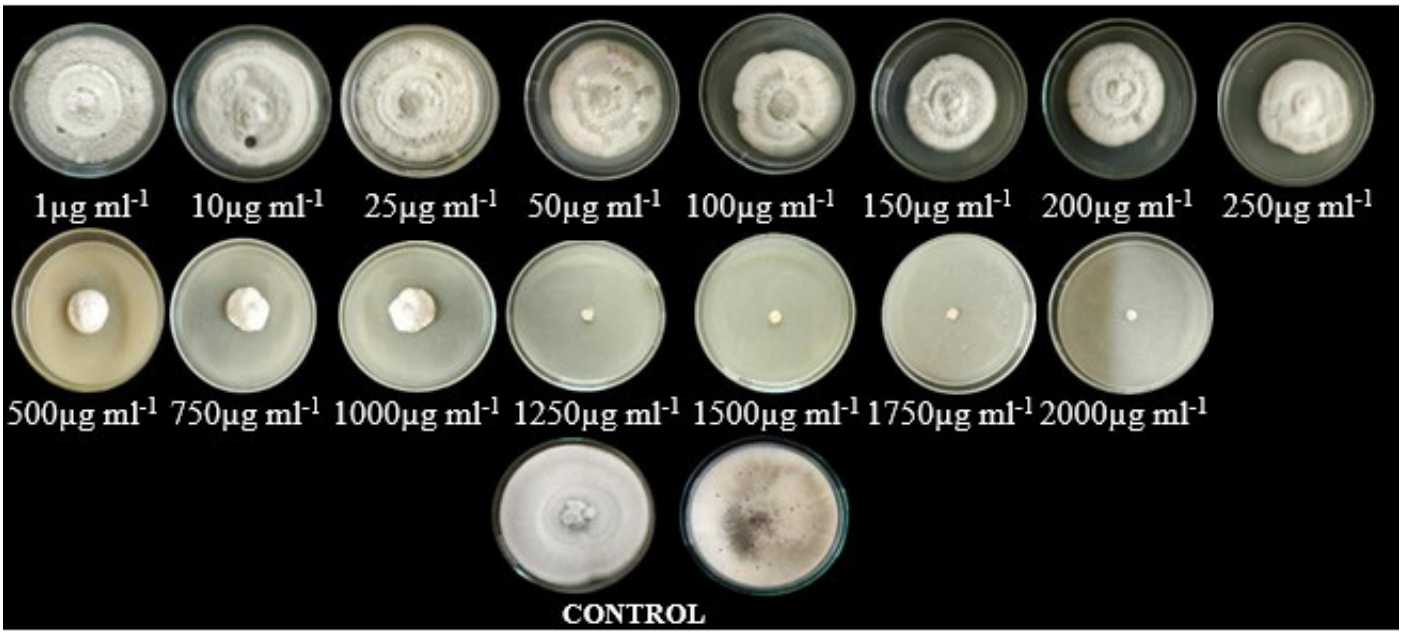

Plate 14 Efficacy of hexaconazole 5 % + captan 70 % WP fungicides on mycelial inhibition of Colletotrichum capsici under in vitro.

surveyed and cultivars. The per cent fruit rot disease incidence varied irrespective of the areas and cultivars which suggests that there is no relation between fruit rot incidence with respect to the areas and cultivars. This gives an idea on the importance of fruit rot disease incidence in chilli in all areas and cultivars for taking the appropriate management practices for getting good yields. Further, from the *in vitro* studies on the efficacy of fungicides tested against fruit rot pathogen, it was found that among the individual fungicides tested tebuconazole 25 %EC was found to be effectively followed by difeniconazole 25 % EC, where as in case of combination fungicides tested, carbendazim + mancozeb 65 % WP followed by hexaconazole + captan 70 % WP were found to be effective against the pathogen which paves the way to think of selecting the appropriate effective fungicides to control the most destructive disease of chilli fruit rot and save the chilli which is the high-value crop grown in *erstwhile* district of Khammam, Telangana state.

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Conflict of interest

The authors declare that they have no conflict of interest

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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