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In vitro evaluation of fungicides and bio-controls against *Macrophomina phaseolina* causing *Macrophomina* stem blight in pigeonpea

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Abstract
An attempt was made to test the efficacy of fungicides and bio-control agents against *Macrophomina phaseolina* causing stem blight in pigeonpea under laboratory conditions for identifying suitable fungicides for field application. Experiment was carried using poison food technique employing contact, systemic and combination of both formulation fungicides. Among the contact fungicides tested, copper sulphate pentahydrate 23.99% SC and mancozeb 75% WP recorded significantly higher mycelial inhibition of 100 percent at all the concentration of tested (0.1, 0.2 and 0.3%). In case of systemic fungicides carbendazim 50% WP, tebuconazole 25% EC, propiconazole 25% EC thiophanate methyl 70% WP, hexaconazole 5% SC, difenoconazole 25% EC, Kresoxim-methyl 44.3% W/W recorded 100 percent mycelial inhibition at all the concentrations tested. With respect to combination of both contact and systemic mode of action fungicides all the tested chemicals showed cent percent inhibition at all the concentrations tested except in Thiophonatemethyl 45%+Pyraclostrobin 5% FS which showed 83.70 percent inhibition at 0.1 and 0.2 concentrations and 89.26 percent inhibition at 0.3 percent concentration. In case of Bio-controls tested *Trichoderma asperellum* showed maximum mycelial inhibition (81.06%) while *Pseudomonas fluorescence* and *Bacillus subtilis* showed zero percent inhibition. These results are basis and indicators for selecting the best performing fungicides in formulating the strategies for management of Macrohomina stem blight disease due to their promising effect and efficacy against the causal organism.

Keywords: *Macrophomina phaseolina*, fungicides, *Macrophomina*, pigeonpea

Introduction
Macrophomina phaseolina (Tassi) Goid. causing stem blight and cankers is becoming very severe in many parts of pigeonpea growing regions in the country. This is a soil-borne pathogen spread worldwide infecting more than 500 plant species across 100 families causing seedling blight, stem blight/stem canker, charcoal rot, and root rot (Ghosh *et al*., 2018) [9]. In India, the first report of *Macrophomina* stem canker occurrence was reported by Kannaiyan *et al*. (1979) [10] in part of Eastern Uttar Pradesh (Varanasi and Mirzapur districts). The disease is reported in the states of Bihar, Madhya Pradesh, Maharashtra, Orissa, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Gujarat, and Rajasthan (Kannaiyan *et al*., 1980) [11]. The fungus incites necrotic lesions on the stem and girdles the plant at the base leading to premature flower drop that eventually results in total witling and the death of the plant. It causes huge economic losses ranging from 10-100 percent (Smita *et al*., 2015) [12].
The decline in pigeonpea production was recorded in Karnataka and Maharashtra during 2021 and 2022 due to high incidences of *Phytophthora* stem blight followed by *Macrophomina* stem blight (stem canker) and dry root rot due to *Rhizoctonia bataticola* (Taub.) Butler (Annual Report 2022 AICRP on Kharif Pulses). None of the cultivated cultivars could withstand the disease overcome. The climate change has been influencing this lesser-known *Macrophomina* stem canker and root rot disease into major epidemic. The dry and warm climate favourable to the disease development are frequently witnessed in the past three years. In view of the severe outbreak of this disease, new insights into the management became necessary to curtail the disease and its spread. The current study was undertaken to know the efficacy of different types of fungicides against the pathogen for its effective control under *in vitro*. The results of the study were anticipated to draw the strategies for the field management of the disease.

Materials and Methods

Different contact, systemic, and combined fungicides were tested against *Macrophomina phaseolina* under *in vitro* conditions using poison food technique on PDA medium. Systemic fungicides were assessed at concentrations of 0.05, 0.10, and 0.15 percent. Contact fungicides were tested at concentrations of 0.1,0.2, and 0.3 percent and fungicides of both contact and systemic at concentrations of 0.1,0.2, and 0.3 percent against the pathogen.

The PDA media (100 ml) was prepared in 250ml flasks for each fungicide and its individual concentrations. After autoclaving, the molten media was cooled to semisolid. Required concentration of fungicide was added separately to each flask using sterilised micropipette. The fungicide mixed (poisoned) media was poured in sterilised Petri plates aseptically under laminar air flow cabinet and allowed to solidify. Pathogen mycelial discs of 5 mm size were prepared from an actively growing 7 days old pathogen culture. One disc of the pathogen culture was placed separately for each plate in the middle and three replications were maintained for each concentration of the test fungicides. A control plates was maintained without any fungicides to PDA. The culture plates were maintained at 30 °C in an incubator. Pathogen growth was monitored daily and after 3days of incubation, colony growth was recorded in all the plates.

The percent inhibition of mycelial growth in poison plates was calculated following the formula given by Vincent (1947) [8].

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

Where,
I= Percent mycelial inhibition of test pathogen (%)
C= Mycelial growth of the pathogen in control
T= Mycelial growth of the pathogen in treatment

Table 1: List of contact fungicides evaluated

Sr. No	Contact fungicides	Trade name	Concentrations
1	Copper oxychloride 50% WP	Blitox 50	0.1,0.2 and 0.3 percent
2	Chlorothalonil 75% WP	Kavach	0.1,0.2 and 0.3 percent
3	Mancozeb 75% WP	M 45	0.1,0.2 and 0.3 percent
4	Copper hydroxide 53.8% DF	Kocide	0.1,0.2 and 0.3 percent
5	Copper sulphate pentahydrate 23.99% SC	Mastercop	0.1,0.2 and 0.3 percent

Table 2: List of systemic fungicides evaluated

Sr. No	Systemic fungicides	Trade name	Concentrations
1	Carbendazim 50%WP	Bavistin	0.05,0.10 and 0.20 percent
2	Tebuconazole 25%EC	Folicure	0.05,0.10 and 0.20 percent
3	Propiconazole 25%EC	Tilt	0.05,0.10 and 0.20 percent
4	Thiophanate methyl 70%WP	Topsin-M	0.05,0.10 and 0.20 percent
5	Hexaconazole 5%SC	Contaf plus	0.05,0.10 and 0.20 percent
6	Difenoconazole 25%EC	Score	0.05,0.10 and 0.20 percent
7	Kresoxim-methyl 44.3% W/W	Ergon	0.05,0.10 and 0.20 percent

Table 3: List of systemic fungicides evaluated

Sl. No.	Combination fungicides	Trade name	Concentrations
1	Thiophanate methyl 45% + Pyraclostrobin 5%FS	Xelora	0.1,0.2 and 0.3 percent
2	Carbendazim 12% +Mancozeb 63%WP	Saaf	0.1,0.2 and 0.3 percent
3	Mancozeb50% + Carbendazim 25%WS	Sprint	0.1,0.2 and 0.3 percent
4	Carboxin 37.5%+Thiram 37.5%WP	Vitavax	0.1,0.2 and 0.3 percent
5	Penflufen13. 28% + Trifloxystrobin 13.28%SC	Evergol extend	0.1,0.2 and 0.3 percent
6	Azoxystrobin 8.3%+Mancozeb 66.7%WG	Avancer glow	0.1,0.2 and 0.3 percent
7	Hexaconazole 4%+Zineb 68%WP	Avatar	0.1,0.2 and 0.3 percent
8	Metalaxyl 4%+Mancozeb 64%WP	Ridomil gold	0.1,0.2 and 0.3 percent
9	Prochloraz 5.7%+Tebuconazole 1.4%ES	Shoresh	0.1,0.2 and 0.3 percent

Bioagents like *Trichoderma* spps, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their efficacy through dual culture technique against *M. phaseolina*. The fungal bio-agent and the fungal test pathogen were inoculated side by side on a single Petri plate containing PDA medium. Whereas, the bacterial bioagents were streaked one day earlier to the test pathogen. Three replications were maintained for each bio-control agent with control having only pathogen in the centre of the plate. The plates were incubated until the pathogen reaches the periphery of the Petri plates in control plates. The colony diameter of both bio-agents and the fungus was measured in both directions and average was recorded. The percent inhibition on growth of the test pathogen was calculated by using the formula given below by (Vincent, 1974) [8].

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

Where,
C = Growth of mycelium in control (mm)
T = Growth of mycelium in treatment (mm)

Results and Discussion

The fungicides of different mode of action both sole and combined were subjected to *in vitro* testing at different concentrations to know their efficacy against *Macrophomin phaseolina*. Among the five different contact fungicides tested

mancozeb 75 WP and copper sulphate pentahydrate 23.99 SC recorded 100 percent inhibition of pathogen at all concentrations (*i.e*., 0.1, 0.2, and 0.3%) tested followed by chlorothalonil at concentrations of 0.1, 0.2, and 0.3 percent with mycelial inhibition of 60.00, 71.85, and 78.52 percent respectively, the results are on par with copper hydroxide also. No mycelial inhibition was noticed by copper oxychloride against the target pathogen at any of the doses tested.

Among the different contact fungicides tested, mancozeb was found superior at lowest concentrations also. In an earlier study by Ravichandran and Hedge (2017) [6] al so similar findings were observed. They found complete inhibition of the test pathogen at 0.2 percent concentration and could inhibit the growth completely. The other three fungicides *i.e*., chlorothalonil, copper oxychloride and copper hydroxide had minimal impact on the pathogen. The pathogen could grow on these poisoned media from 60-100 percent in the Petri plates. Bhanusri *et al*. (2022) [1] also observed minimal growth inhibition of *Macrophomina phaseolina* by copper oxychloride infecting chickpea.

All the seven different systemic fungicides (tebuconazole 25EC, propiconazole 25EC, hexaconazole 5SC, difenoconazole 25EC, Kresoxim-methyl 44.3 W/W, carbendazim 50WP and thiophanate methyl 70WP) tested for their efficacy in mycelial inhibition of test pathogen recorded 100 percent pathogen growth inhibition (Table 5 and Fig.1) at all the concentrations (0.05, 0.1 and 0.2%) with non-significant correlation between the treatments. In an earlier study by Kumari and Katoch (2020) [4], carbendazim (0.1%) was found most effective in completely reducing the mycelial growth of *M. phaseolina in vitro* causing dry root rot in pulses. This fungicide inhibited the germination, growth, and multiplication of the pathogen. Savaliya *et al*. (2020) [7] also recorded cent percent mycelial inhibition of *M. phaseolina* infecting sesame by tebuconazole and propiconazole (0.005, 0.001, 0.025 and 0.050%) at different concentrations.

The study had assessment of nine different combination fungicides against *M. phaseolina*. All the fungicides *viz*., carbendazim 12 + mancozeb 63WP, mancozeb 50 +carbendazim 25 WS, carboxin 37.5 + thiram 37.5 WP, penflufen 13.28 + trifloxystrobin 13.28 SC, azoxystrobin 8.3 + mancozeb 66.7 WG, hexaconazole 4 + zineb 68 WP, metalaxyl 4 + mancozeb 64 WP and prochloraz 5.7 + tebuconazole 1.4 ES showed 100 percent mycelial inhibition at all the concentrations of 0.1, 0.2 and 0.3 percent tested, except thiophanate methyl45 + pyraclostrobin 5 FS (Table 6 and Fig.2).

Different scientists have noticed similar promising action by contact and systemic combination mode of action fungicides against *M. phaseolina*. Maruti *et al*. (2017) [5] in their study found carbendazim + mancozeb and carboxin + thiram most effective against *M. phaseolina* infecting pigeonpea with cent percent mycelial growth inhibition at different concentrations tested (0.10%, 0.20% and 0.30%). Similarly, Karibasappa *et al*. (2020) [3] also observed maximum mycelial growth inhibition (100%) of *M. phaseolina* (sesame root rot) by carboxin + thiram at 2000 ppm

Among the bio-agents tested, *Trichoderma asperellum* was found most effective compared to other bio-control agents and showed maximum fungal growth inhibition of *M. phaseolina* (81.06%), followed by *Trichoderma harzianum*-31(54.4%) and *Trichoderma harzianum*-33 (50.30%). The bacterial bio-agents *Pseudomonas fluorescens* and *Bacillus subtilis* could not inhibit the growth of *M. phaseolina*, instead the pathogen over grown both the biocontrol agents (Table. 7 and Fig.4). Similar findings were observed by Dudhe *et al*. (2023) [2] who reported that *Trichoderma* spp's were more effective against *M. phaseolina* causing dry root rot of pigeonpea with a mycelial inhibition of 92 percent followed by *Pseudomonas fluorescence* (28.70%) and *Bacillus subtilis* (18.67%). also found that *Trichoderma* (74.26%) was superior over *Pseudomonas* (50%) and *Bacillus* (38.71%) in inhibiting the mycelia growth of *M. phaseolina* infecting fenugreek.

Table 4: *In vitro* efficacy of contact fungicides against *Macrophomina phaseolina* causing stem blight of pigeonpea

Sl. No.	Name of the fungicide	Growth of myceliam (mm)			
	Concentration (%)	**0.1**	**0.2**	**0.3**	**Mean**
1	Copper oxychloride 50% WP	0.00* (00.00)**	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)
2	Chlorothalonil 75% WP	60.00 (50.77)	71.85 (57.96)	78.52 (62.39)	70.12 (56.86)
3	Mancozeb 75% WP	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
4	Copper hydroxide 53.8% DF	61.48 (51.64)	69.26 (56.33)	80.00 (63.43)	70.24 (56.94)
5	Copper sulphate pentahydrate 23.99% SC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
	Mean	64.29 (53.30)	50.22 (45.13)	71.70 (57.86)	
		S. Em±		C. D. at 1%	
	Fungicides (F)	0.35		1.06	
	Concentration (C)	0.27		0.82	
	F×C	0.61		1.83	

*Original value **Arc sine transformed value

Table 5: *In vitro* efficacy of systemic fungicides against *Macrophomina phaseolina* causing stem blight of pigeonpea

Sl. No.	Name of the fungicide	Growth of mycelium (mm)			
	Concentration (%)	**0.05**	**0.1**	**0.2**	**Mean**
1	Carbendazim 50% WP	100* (90.00)**	100 (90.00)	100 (90.00)	100 (90.00)
2	Tebuconazole 25% EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3	Propiconazole 25% EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
4	Thiophonate methyl 70% WP	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5	Hexaconazole 5% SC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6	Difenoconazole 25% EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
7	Kresoxim-methyl 44.3% W/W	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
	Mean	100 (90.00)	100 (90.00)	100 (90.00)	
		S. Em±		C. D. at 1%	
	Fungicides (F)	NS		NS	
	Concentration (C)	NS		NS	
	F×C	NS		NS	

*Original value **Arc sine transformed value

Table 6: *In vitro* efficacy of combination fungicides against *Macrophomina phaseolina* causing stem blight of pigeonpea

Sl. No.	Name of the fungicide	Growth of mycelium (mm)			
	Concentration (%)	**0.1**	**0.2**	**0.3**	**Mean**
1	Thiophonatemethyl45%+Pyraclostrobin5% FS	83.70* (66.19)**	83.70 (66.19)	89.26 (70.87)	85.55 (67.75)
2	Carbendazim12%+Mancozeb63% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
3	Mancozeb50%+Carbendazim 25% WS	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
4	Carboxin37.5%+Thiram37.5% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
5	Penflufen13.28%+ Trifloxystrobin 13.28% SC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
6	Azoxystrobin 8.3%+Mancozeb 66.7% WG	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
7	Hexaconazole 4%+Zineb 68% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
8	Metalaxyl 4%+Mancozeb 64% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
9	Prochloraz 5.7%+Tebuconazole 1.4% ES	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	Mean	98.18 (82.25)	98.81 (83.74)	98.18 (82.25)	
		S. Em±		C. D. at 1%	
	Fungicides (F)	0.32		1.19	
	Concentration (C)	0.18		0.69	
	F×C	0.55		2.06	

*Original value **Arc sine transformed value

Table 7: *In vitro* antagonistic activity of bio controls against *Macrophomina phaseolina* causing stem blight of pigeonpea

Sl. No	Bio control	Growth of mycelium (mm)	Mycelial inhibition (%) *
1	*Trichoderma asperellum*	17.0	81.06* (64.20) **
2	*Trichoderma harzianum-31*	43.6	54.4 (47.52)
3	*Trichoderma harzianum-33*	44.6	50.3 (45.17)
4	*Pseudomonas fluorescens*	90.0	0.00
5	*Bacillus subtilis*	90.0	0.00
	Control	90.0	0.00
	S.Em. ±	-	0.45
	C. D. at 1%	-	2.1

*Original value **Arc sine transformed value

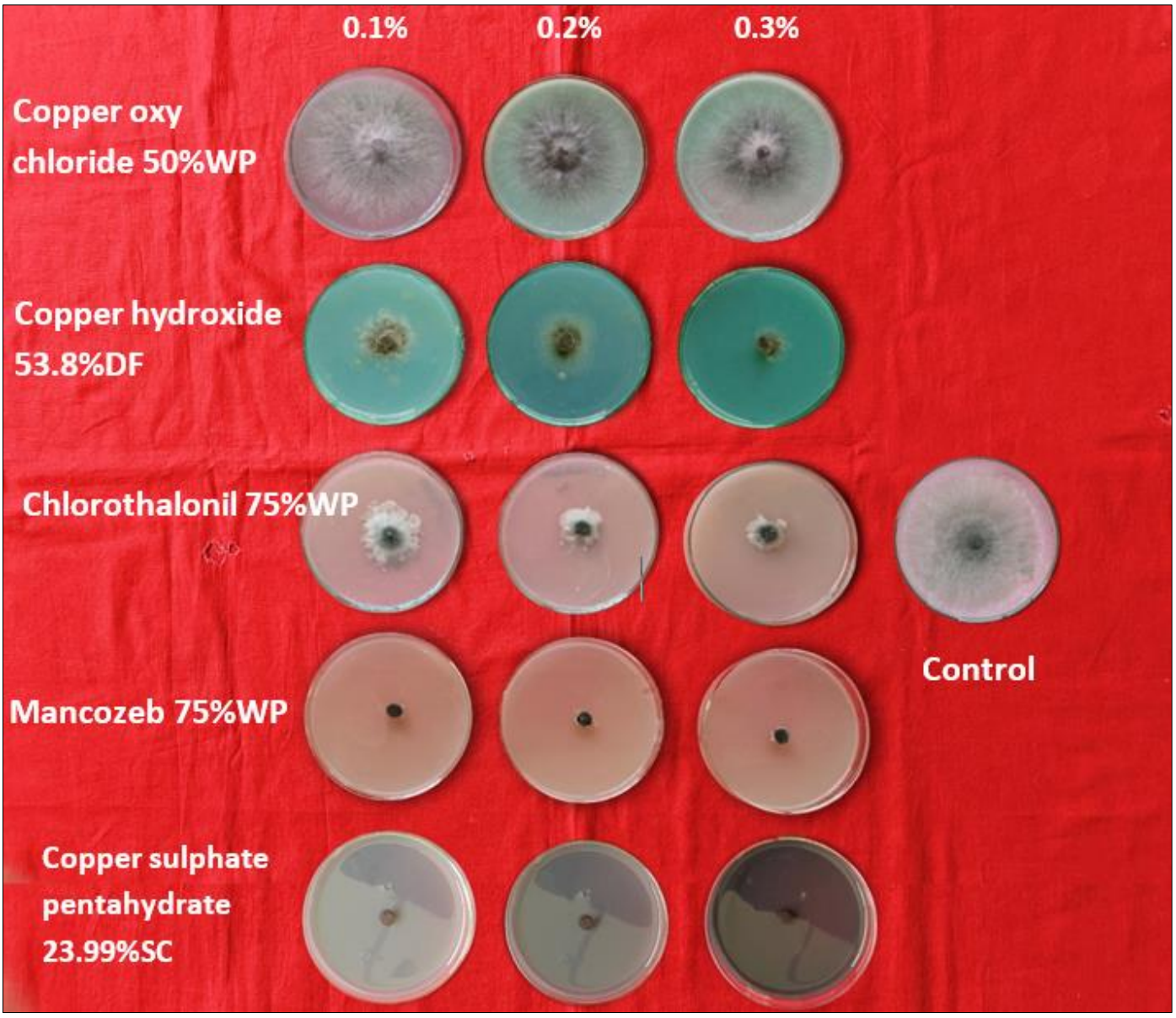

Plate 1: Efficacy of contact fungicides against *Macrophomina phaseolina*

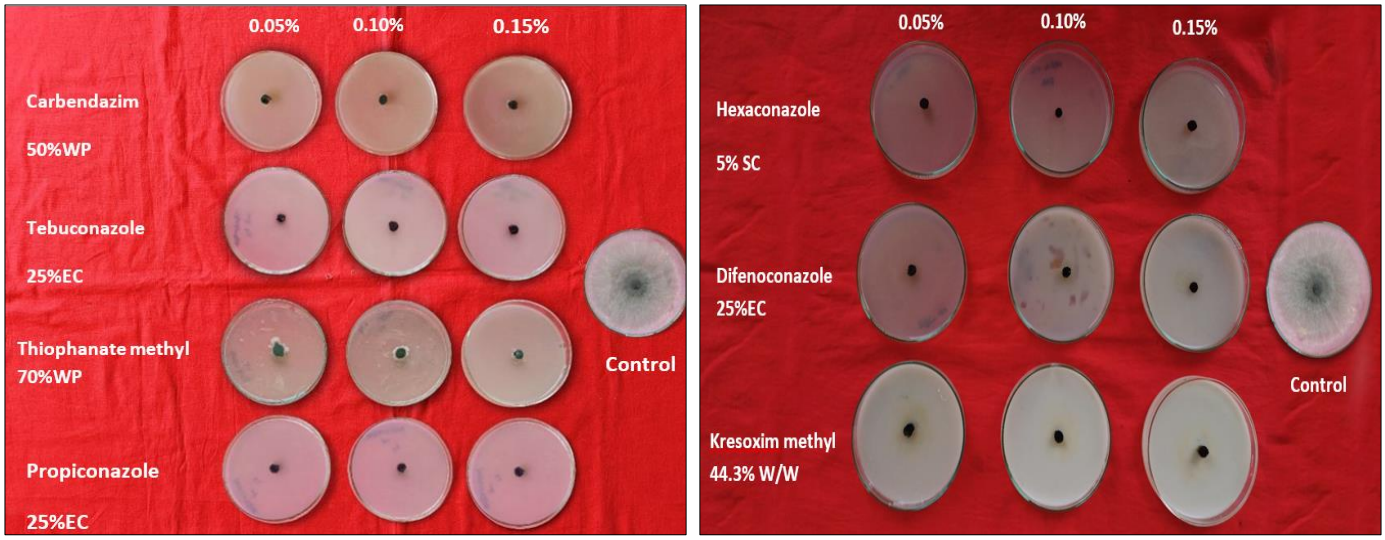

Plate 2: Efficacy of systemic Fungicides against *Macrophomina phaseolina*

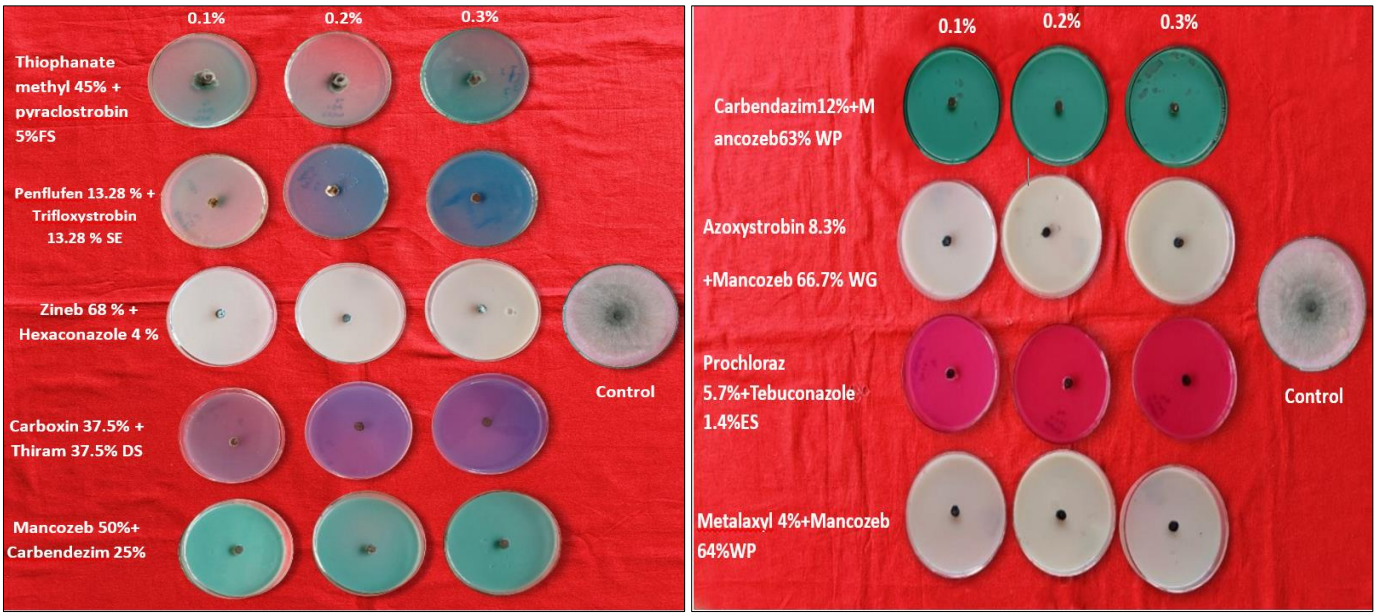

Plate 3: Efficacy of combination fungicides against *Macrophomina phaseolina*

Plate 4: Antagonistic activity of bio-controls against *Macrophomina phaseolina*

Conclusion

The present study revealed that among the contact fungicides mancozeb and copper sulphate pentahydrate among systemic tebuconazole, propiconazole, carbendazim, hexaconazole, difenoconazole, Kresoxim-methyl and thiophanate methyl and among the combi, carbendazim + mancozeb, Carboxin + thiram, Penflufen + trifloxystrobin, azoxystrobin + mancozeb, hexaconazole + Zineb, metalaxyl + mancozeb and prochloraz + tebuconazole were most effective in inhibiting the mycelial growth of *M. phaseolina* under *in vitro*. In biocontrol agents, *Trichoderma asperellum* was found effective. These best performing fungicides and biocontrol agents shall be employed for further field efficacy study and best performing shall be recommended for field application in the efforts of management of the disease.

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