

Original Research Article

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Evaluation of Different Fungicides against *Macrophomina phaseolina* (Tassi) Goid. Causing Dry Root Rot of Chickpea (*Cicer arietinum* L.) *in vitro*

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ABSTRACT

Keywords

Chickpea, dry root rot, *in vitro*, *Macrophomina phaseolina*, per cent growth inhibition, fungicides

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Chickpea (*Cicer arietinum* L.) is one of the major legume pulse crops and it is majorly grown in India and other semi-arid regions of the world. Dry root rot of chickpea is the most destructive disease of chickpea. Therefore, in present studies fungicides (six systemic, seven non systemic and six ready mix fungicides) were tested *in vitro* at three different concentrations by poisoned food technique for evaluating their efficacy against *M. phaseolina*. Among systemic fungicides, significantly highest average mycelial growth inhibition was with carbendazim (85.88%), thiophanate methyl (84.32%) followed by hexaconazole (75.29%) was also considerably effective fungicide but tebuconazole (47.06%), azoxystrobin (36.08%), propiconazole (36.86%) proved comparatively less in their efficacy against *M. phaseolina*. Among non-systemic fungicides, significantly highest average mycelial growth inhibition over control was recorded in mancozeb (90.20%), followed by chlorothalonil (88.24%). The remaining fungicides viz., zineb (78.04%), thiram (76.47%), and propineb (49.41%), were moderately effective. While sulphur (23.53%) recorded least effective in growth inhibition as compared to other fungicides against *M. phaseolina*. Among ready mix fungicides, significantly highest per cent growth inhibition over control was recorded (88.24%) in carbendazim 12% + mancozeb 63% followed by carboxin 37.5% + thiram 37.5%, (83.14%). The remaining fungicides viz., pyraclostrobin 5% + mitiram 55% (70.59%), captan 70% + hexaconazole 5% (76.47%) and pyraclostrobin 5% + mitiram 55% (59.21%) were moderately effective. While pyraclostrobin 12.5% + epoxiconazole 4.7%, (38.82%) and hexaconazole 4% + zineb 68% (27.45%), recorded least effective in growth inhibition as compared to other fungicides against *M. phaseolina*.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the major legume pulse crops and it is majorly grown in India and other semi-arid regions of the world. It belongs to the family Fabaceae, subfamily Faboideae. Chickpea is a self-pollinating diploid crop with chromosome number (2n=16) and a genome consisting of 740 Mbp. It is documented to have originated in south-eastern Turkey from where it has

spread to other countries of the world. Among the major pulse crops, chickpea contributes nearly 32.6 per cent and 40.5 per cent of total pulse area and total pulse production, respectively.

In India, chickpea is cultivated in an area of about 8.32 million ha with a production of 9.8 metric tone and 925 kg/ha productivity (Anonymous, 2018). The chickpea crop was reported to be attacked by nearly 172

pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes) from all over the world (Nene *et al.*, 1996). Some of the serious diseases in chickpea are of dry and wet root rot [*Rhizoctonia bataticola*, (Taub.) Butler], wilt [*Fusarium oxysporum* f. sp. *ciceri*, (Padwick) Snyd. & Hans.] ascochyta blight [*Ascochyta rabiei*, (Pass.) Labr.] and collar rot (*Sclerotium rolfsii* Sacc.). Among the diseases of chickpea, dry root rot has an emerging the most destructive and constraint to chickpea productivity and production, as the disease is more prevalent during hot temperature of 30 to 35°C and low soil moisture conditions (Taya *et al.*, 1988; Pande and Sharma, 2010). *R. bataticola* is a soil-inhabiting pathogen and capable of infecting chickpea at any crop stage, but most commonly infects chickpea at post-reproductive stage in dry and warm regions (Sharma and Pande, 2013).

Materials and Methods

Isolation of pathogens

Diseased specimens were to be brought to laboratory and examined under microscope for preliminary examination. Isolation of pathogen, small pieces of infected samples were cut from the diseased portion along with some healthy tissues and surface sterilized with 0.1 per cent mercuric chloride solution for 1 minute followed by three washing with sterilized distilled water. The surface sterilized pieces were transferred to 20ml poured potato dextrose agar (PDA) plates and incubated at 27 ± 2 °C. After seven days of incubation, the fungal growth was transferred aseptically on PDA slants and purified following hyphal tip method.

In vitro evaluation of fungicides

Efficacy of seven non-systemic fungicides and six systemic and ready-mix fungicides was evaluated *in vitro* at various

concentrations against *M. phaseolina*, applying Poisoned food technique (Dhingra and Sinclair, 1995) and using PDA as basal culture medium. Systemic fungicides at the rate 50, 250 and 500 ppm while non-systemic at the rate 1000, 2000 and 2500 and ready-mix fungicides at the rate 500, 1500 and 2000 ppm. Based on active ingredient, requisite quantity of the fungicide was mixed in 100 ml potato dextrose agar medium in 250 ml flask and well shaken to facilitate uniform mixture of fungicides and 20 ml was poured in each sterilized plate (90 mm diameter). After 24h a disc of five mm was placed in the centre of each poured plate. The discs were cut with the help of a sterilized cork borer from 10 days old culture of *M. phaseolina*. Inoculated plates were incubated at 27 ± 1 °C. The colony growth was measured after 24 hrs interval till the entire plate of control treatment was completely covered with mycelium. Suitable check was maintained without fungicide and inoculated with *M. phaseolina*. The per cent growth inhibition (PGI) over control was calculated using the following formula.

$$\text{PGI (\%)} = \frac{C-T}{C} \times 100$$

Where, PGI (%) = Per cent growth inhibition
C = Average diameter (mm) of mycelial colony of control plate
T = Average diameter (mm) of mycelial colony of treated plate in treated plates

Results and Discussion

Effect of different systemic fungicides on growth inhibition of *M. phaseolina*

Effect of these fungicides on radial growth and inhibition of test pathogen were recorded. All the treatments were replicated thrice and a suitable untreated control (without fungicide) was also maintained.

Results (Plate 1 and Table 1) revealed that the fungicides tested significantly inhibited growth of the test fungus over untreated control. Further, it was found that per cent inhibition of the test pathogen was increased with the increase in concentration of the fungicides tested. At 50 ppm concentration, significantly highest per cent growth inhibition over control was recorded in carbendazim (82.35%) followed by thiophanate methyl (78.82%). At 250 ppm concentration, propiconazole recorded significantly highest per cent growth inhibition (74.44%) followed by carbendazim (85.88%). At 500 ppm concentration, cent per cent growth inhibition of the pathogen recorded by carbendazim (90.59%) and followed by thiophanate methyl (84.71%). Thus, both the fungicides proved the most effective for *M. Phaseolina*. Next best fungicide in order of merit was hexaconazole. While, the rest of the fungicides were comparatively medium or less effective against *M. Phaseolina*. Azoxystrobin was found least effective at all concentration as compared to other fungicides. The similar results were found and recorded by Chattopadhyay and Kalpana (2002), Khan and Gangopadhyay (2008) Kumari and Shekhawat (2012) and Ebenezar and Wesely (2000) found that carbendazim at 0.1% completely inhibited the growth of *M. phaseolina*.

Effects of non-systemic fungicides on the growth of *M. phaseolina*

Results (Plate 2 and Table 2) revealed that all non-systemic tested significantly inhibited mycelial growth of *M. phaseolina*, over untreated control. At 1000 ppm concentration, significantly highest per cent growth inhibition over control was recorded in mancozeb (82.74%) followed by chlorothalonil (78.82%), zineb (70.59%), thiram (64.71%) and propineb (41.56%) were

moderately effective. While sulphur (12.15%) recorded least effective in growth inhibition. At 2000 ppm concentration, mancozeb (85.88%) followed by chlorothalonil (83.92%), the remaining fungicides viz., zineb (72.55%), thiram (70.20%). At 2500 ppm concentration mancozeb (90.20%), followed by chlorothalonil (88.24%). The remaining fungicides viz., zineb (78.04%), thiram (76.47%), and propineb (49.41%) were moderately effective. While sulphur (23.53%) recorded least effective in growth inhibition as compared to other fungicides against *M. phaseolina*.

Thus, both the fungicides proved the most effective for *M. phaseolina*. The present findings are confirmed with the results Ravichandran and Hedge (2017) observed that the fungicides chlorothalonil and mancozeb at 0.2 per cent were effective. Maruti *et al.*, (2017) reported that mancozeb and thiram showed 100 per cent inhibition at 0.3 per cent concentration. Sangappa and Malleesh (2016) recorded that the fungicides thiophanate methyl and triadimefon showed 100 per cent mycelia inhibition at 0.1, 0.2 and 0.3%.

Effect of ready-mix fungicides on growth inhibition of *M. phaseolina*

Results (Plate 3 and Table 3) revealed that all ready-mix fungicides tested significantly inhibited mycelial growth of *M. phaseolina*, over untreated control. Further, per cent mycelial inhibition was increased with increase in concentrations of the fungicides tested. At 500 ppm concentration, highest per cent growth inhibition over control was recorded (82.35%) in carbendazim 12% + mancozeb 63% followed by carboxin 37.5% + thiram 37.5% (78.82%). The remaining fungicides viz., pyraclostrobin 5% + mitiram 55% (65.09%), captan 70% + hexaconazole 5% (62.74%) against *M. phaseolina*.

Table.1 Evaluation of systemic fungicides against *M. phaseolina* in vitro

Tr. No	Technical name of fungicides	Concentration (ppm)	Average colony diameter (mm) @	Per cent growth inhibition
T₁	Carbendazim (50 WP)	50	3.92 (15.00)	82.35
		250	3.52 (12.00)	85.88
		500	2.88 (8.00)	90.59
T₂	Tebuconazole (25.9EC)	50	6.96 (48.00)	43.53
		250	6.74 (45.00)	47.06
		500	6.59 (43.00)	49.41
T₃	Propiconazole (25 EC)	50	7.67 (58.33)	31.38
		250	7.36 (53.67)	36.86
		500	7.06 (49.33)	41.96
T₄	Azoxystrobin (23 SC)	50	8.27 (68.00)	20.00
		250	7.40 (54.33)	36.08
		500	7.24 (52.00)	38.82
T₅	Hexaconazole (5 EC)	50	4.98 (24.33)	71.38
		250	4.63 (21.00)	75.29
		500	4.17 (17.00)	80.00
T₆	Thiophanate methyl (70 WP)	50	4.29 (18.00)	78.82
		250	3.71 (13.33)	84.32
		500	3.67 (13.00)	84.71
T₇	Control		9.25 (85.00)	
S. Em. ±			0.17	
C. D. at 5%			0.50	
C. V. (%)			4.71	

Table.2 Evaluation of non-systemic fungicides against *M. phaseolina* *in vitro*

Tr. no.	Technical name of fungicides	Concentration (ppm)	Average colony diameter (mm) @	Per cent growth inhibition
T ₁	Copperoxychloride (50WP)	1000	7.40 (54.33)	36.08
		2000	6.51 (42.00)	50.59
		2500	6.11 (37.00)	56.47
T ₂	Mancozeb (75WP)	1000	3.89 (14.67)	82.74
		2000	3.53 (12.00)	85.88
		2500	2.96 (8.33)	90.20
T ₃	Chlorothalonil (75WP)	1000	4.30 (18.00)	78.82
		2000	3.76 (13.67)	83.92
		2500	3.23 (10.00)	88.24
T ₄	Propineb (70WP)	1000	7.08 (49.67)	41.56
		2000	7.01 (48.67)	42.74
		2500	6.59 (43.00)	49.41
T ₅	Thiram (75WP)	1000	5.52 (30.00)	64.71
		2000	5.08 (25.33)	70.20
		2500	4.52 (20.00)	76.47
T ₆	Zineb (75WP)	1000	5.04 (25.00)	70.59
		2000	4.87 (23.33)	72.55
		2500	4.38 (18.67)	78.04
T ₇	Sulphur (80 WP)	1000	8.67 (74.67)	12.15
		2000	8.38 (69.67)	18.04
		2500	8.09 (65.00)	23.53
T ₈	Control		9.25 (85.00)	
S. Em. ±			0.15	
C. D. at 5 %			0.44	
C. V. %			4.23	

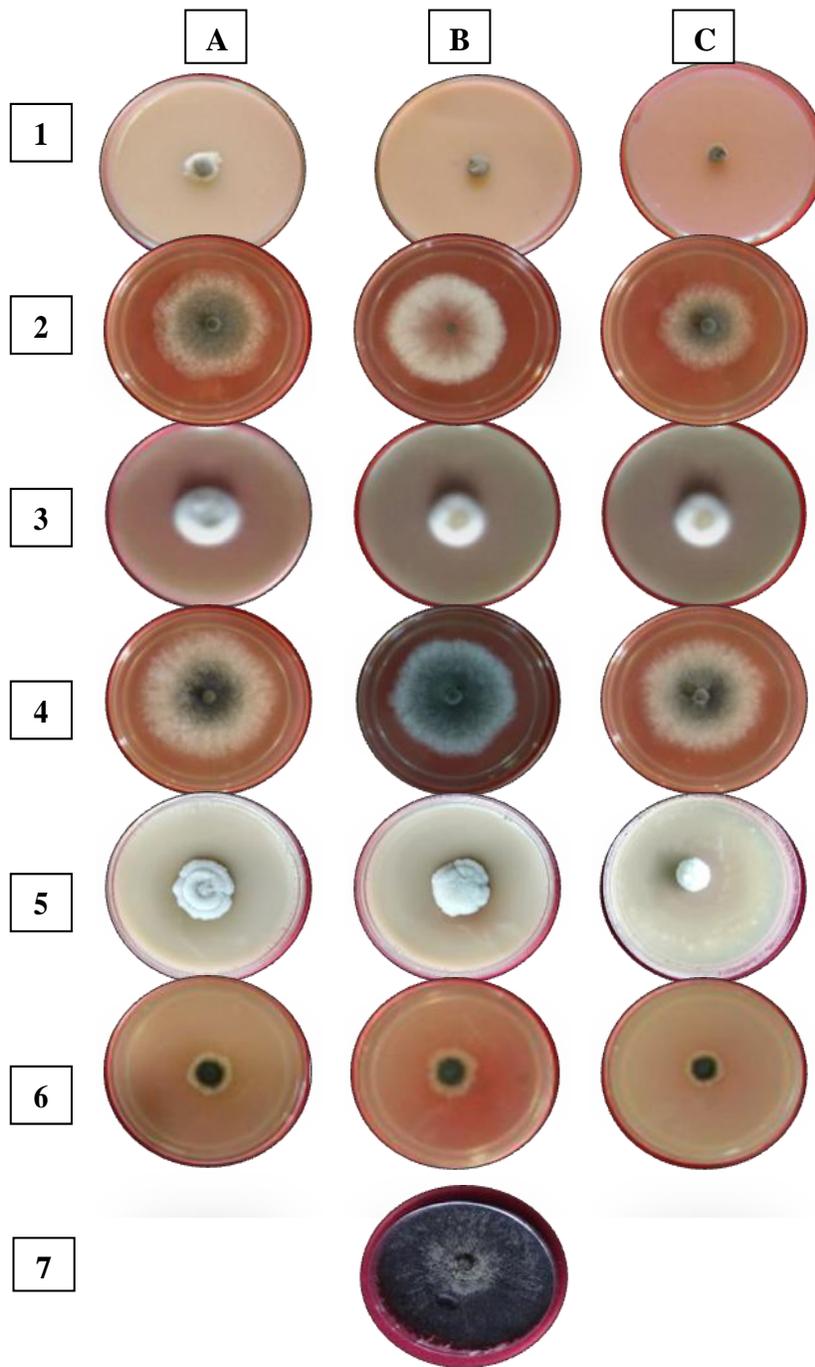
Table.3 Evaluation of ready-mix fungicides against *M. phaseolina* in vitro

Tr. no.	Technical name of fungicides	Concentration (ppm)	Average colony diameter (mm) @	Per cent growth inhibition
T ₁	Carbendazim (12%) + Mancozeb (63%)	500	3.93 (15.00)	82.35
		1500	3.53 (12.00)	85.88
		2000	3.23 (10.00)	88.24
T ₂	Carboxin (37.5%) + Thiram (37.5%)	500	4.28 (18.00)	78.82
		1500	3.84 (14.33)	83.14
		2000	3.34 (10.67)	87.45
T ₃	Captan (50%) + Hexaconazole (5%)	500	5.67 (31.67)	62.74
		1500	5.01 (24.67)	70.98
		2000	4.52 (20.00)	76.47
T ₄	Pyraclostrobin (12.5%) + Epoxiconazole (4.7%),	500	6.39 (40.33)	52.55
		1500	5.95 (35.00)	58.82
		2000	5.93 (34.67)	59.21
T ₅	Hexaconazole(4%) + Zineb (68%)	500	8.11 (65.33)	23.14
		1500	7.88 (61.67)	27.45
		2000	7.24 (52.00)	38.82
T ₆	Pyraclostrobin (5%) + Mitiram (55%)	500	5.49 (29.67)	65.09
		1500	5.27 (27.33)	67.85
		2000	5.05 (25.00)	70.59
T ₇	Control		9.25 (85.00)	
S. Em. ±			0.14	
C. D. at 5%			0.41	
C. V. (%)			4.03	

@ Mean of three replications

* Figures outside parenthesis are $\sqrt{x+0.5}$ transformed value

** Figures in parenthesis are original values



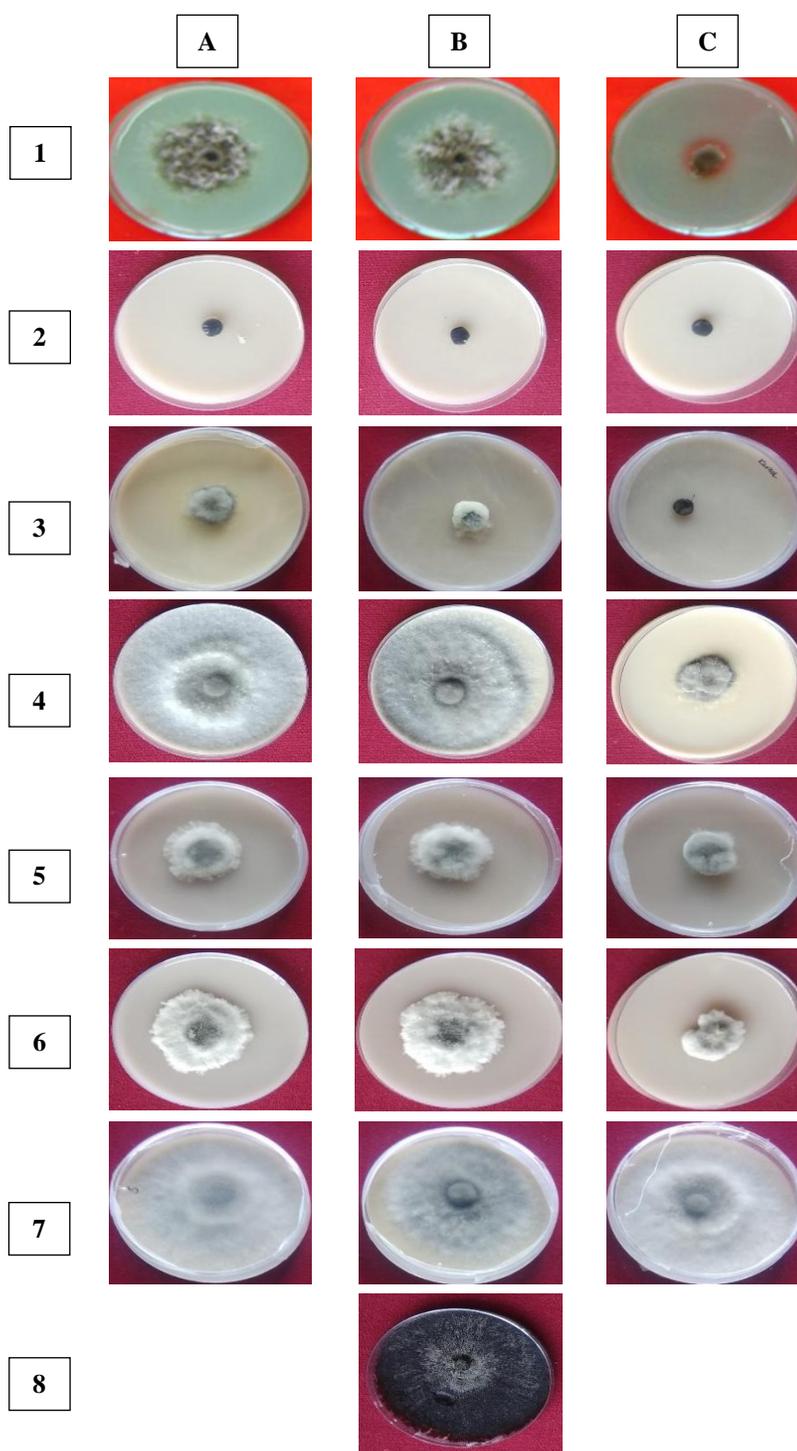
Fungicides

- | | |
|-------------------------|-----------------------------|
| 1. Carbenazim 50 WP | 5. Hexaconazole 5 EC |
| 2. Tebuconazole 25.9 EC | 6. Thiophanate methyl 70 WP |
| 3. Propiconazole 25 EC | 7. Control |
| 4. Azoxystrobin 23 SC | |

Concentrations

- A. 50ppm
 B. 250ppm
 C. 500ppm

Plate.1 Growth inhibition of *M. phaseolina* at different concentrations of systemic fungicides



Fungicides

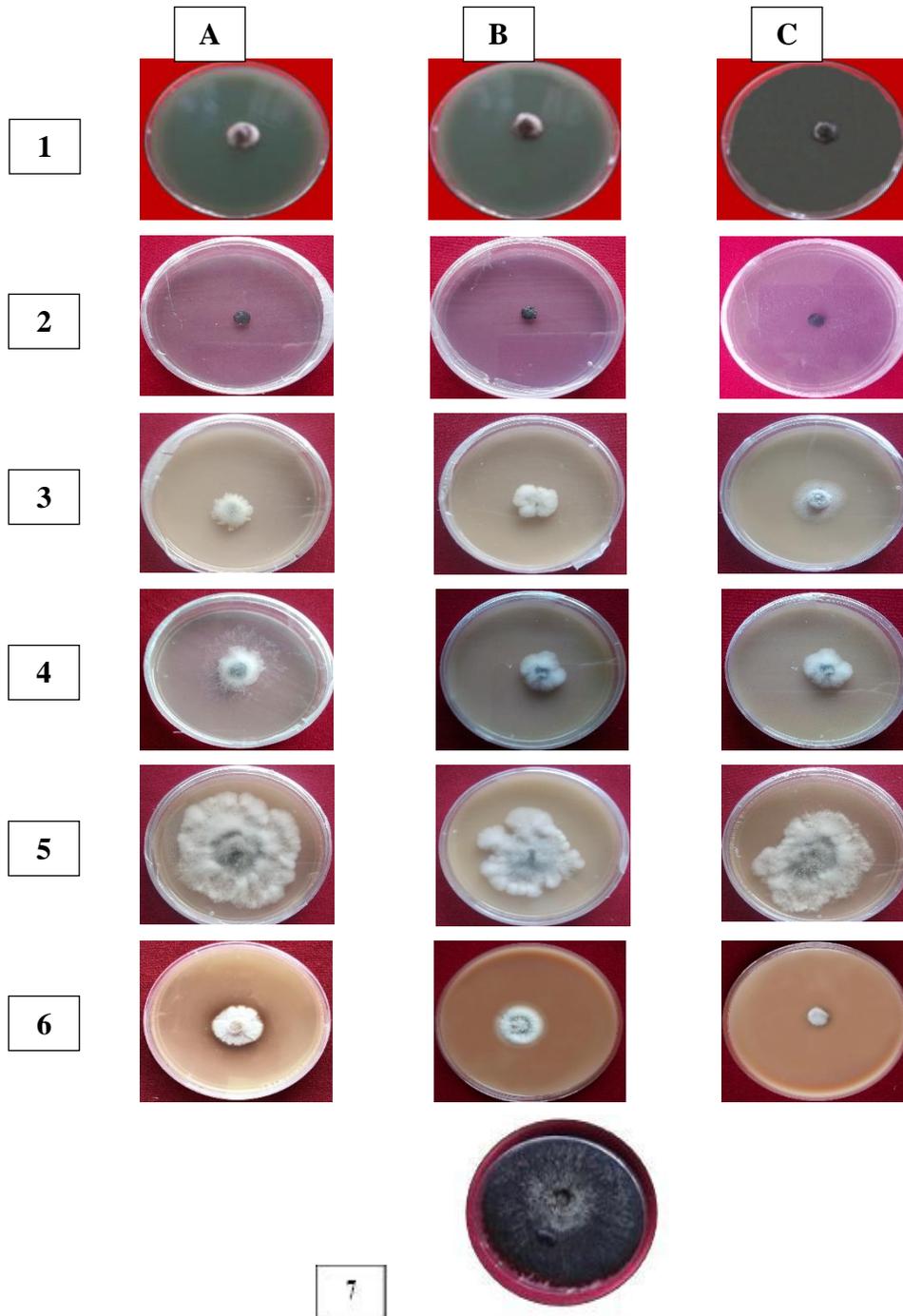
1. Copper oxychloride 50 WP
2. Mancozeb 75 WP
3. Chlorothalonil 75 WP
4. Thiram 75 WP

5. Zineb 75 WP
6. Propineb 70 WP
7. Sulphur 80 WDG
8. Control

Concentrations

- A. 1000ppm
- B. 2000ppm
- C. 2500ppm

Plate.2 Growth inhibition of *M. phaseolina* at different concentrations of non-systemic fungicides



Fungicides

1. Carbendazim 12% + Mancozeb 63% WP
2. Carboxin 37.5% + Thiram 37.5% WP
3. Captan 70 % + Hexaconazole 5 % WP
4. Pyraclostrobin 12.5% + Epoxiconazole 4.7% SE
6. Pyraclostrobin 5% + Mitiram 55%

Concentrations

- A. 500ppm
- B. 1500ppm
- C. 2000ppm
5. Hexaconazole 4% + Zineb 68%
7. Control

Plate.3 Growth inhibition of *M. phaseolina* at different concentrations of ready-mix fungicides

At 1500 ppm concentration, highest per cent growth inhibition over control was recorded (85.88%) in carbendazim 12% + mancozeb 63% followed by carboxin 37.5% + thiram 37.5% (83.14%). The remaining fungicides viz., pyraclostrobin 5% + mitiram 55% (67.85%), captan 70% + hexaconazole 5% (70.98%) and pyraclostrobin 5% + mitiram 55% (67.85%) were moderately effective against *M. phaseolina*.

At 2000 ppm concentration, highest per cent growth inhibition over control was recorded (88.24%) in carbendazim 12% + mancozeb 63% followed by carboxin 37.5% + thiram 37.5%, (83.14%). The remaining fungicides viz., pyraclostrobin 5% + mitiram 55% (70.59%), captan 70% + hexaconazole 5% (76.47%) and pyraclostrobin 5% + mitiram 55% (59.21%) were moderately effective against *M. phaseolina*.

Different fungicides greatly varied in their efficacy to inhibit the growth of fungus under study. The growth inhibition per cent positively correlated with increase in concentration for all the chemicals tested. It is inferred from results that there was less mycelial growth of the pathogen in carbendazim 12% + mancozeb 63% and carboxin 37.5% + thiram 37.5% at 2000 ppm and also significantly lesser growth at 500 and 1500 ppm compared to the Thus, both the fungicides proved the most effective for *M. phaseolina*.

The results are in accords with work of the Maruti *et al.*, (2017) found that carbendazim 12% + mancozeb 63% WP, carboxin 37.5% + thiram 37.5% WP showed cent per cent of inhibition of *R. bataticola* at all the concentrations i.e., 0.10%, 0.20% and 0.30%. Sangappa and Mallesh (2016) observed that carbendazim 12% + mancozeb 63%, showed complete inhibition of mycelial growth at all the concentrations, i.e., 0.05, 0.10 and 0.2%.

Results concluded that among the systemic fungicides the minimum mycelial growth was recorded in carbendazim and thiophanate methyl at 500 ppm and also significantly lesser growth at 50 and 250 ppm compared to rest of concentrations. In case of non-systemic fungicides, mancozeb and chlorothalonil proved maximum growth inhibition of *M. phaseolina* at 2500 ppm and also significantly lesser growth at 1000 and 2000 ppm.

Among ready mix fungicides at various concentrations were screened *in vitro* against *M. phaseolina*, in Carbendazim (12%) + Mancozeb (63%) and Carboxin (37.5%) + Thiram (37.5%) were found highest growth inhibition at 2000 ppm and also significantly lesser growth at 500 and 1500 ppm compare with other fungicides.

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