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In vitro evaluation of fungicides against mycelial growth and sclerotial viability of *Sclerotinia sclerotiorum* (Lib.) *de Bary*, the cause of Sclerotinia rot of Rapeseed-mustard

Kiran Goswami, AK Tewari and Pooja Upadhyay

Abstract
Sclerotinia rot of rapeseed-mustard caused by *Sclerotinia sclerotiorum* (Lib) *de Bary* has become a serious problem in India since last few successive years. Among eleven fungicides tested *in vitro*, Thiophenate methyl and carbendazim + mancozeb (SAAF) proved to be the best as they completely inhibited the mycelial growth of the pathogen at 250 µg a.i./ml. The fungicides *viz.*, iprodione, hexaconazole, triademefon, tebuconazole, carbendazim, benomyl, propiconazole and mancozeb were also effective in inhibiting mycelial growth (92.6 to 97.7%) at 250 µg a.i./ml. The metalaxyl+ mancozeb (ridomil 72 MZ) was found least effective in checking the growth of the pathogen. Benomyl and carbendazim were found most effective in inhibiting sclerotial germination, while others were ineffective. Complete inhibition of sclerotial germination was observed when sclerotia were dipped in benomyl at 100 µg a.i./ml either 5 or 15 minutes, while carbendazim showed complete inhibition at 250 µg a.i./ml. only in 15 minutes dip treatment.

Keywords: fungicides, rapeseed-mustard, sclerotinia sclerotiorum, sclerotial viability

1. Introduction
Among different crops in India, rapeseed-mustard occupies a premier position accounting for 25 and 30 per cent of total oil seed acreage and production respectively. The disease was of minor importance till few years back, but recently it has turned out to be a serious problem in major rapeseed-mustard growing areas in the country and under severe infestation it causes seed yield losses up to 74 per cent (Chauhan *et al*., 1992; Lodha *et al*., 1992; Ghasolia *et al*., 2004; Kang and Chahal, 2000; Krishnia *et al*., 2000; Shivpuri *et al*., 2000, Singh *et al*. 2017a; Singh *et al*. 2017b; Singh *et al*. 2017c; Singh *et al*. 2018; Tiwari *et al*. 2018; Tiwari *et al*. 2019a; Tiwari *et al*. 2019b; Kour *et al*. 2019; Singh *et al*. 2019) [3, 9, 4, 7, 8, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26] or more.

The explosive pathogenicity of the fungus under favourable conditions and ability of sclerotia to withstand under adverse conditions allow it to cause infection on many crops. Use of chemicals has been a practical method of controlling the disease. The fungicides viz, benomyl, carbendazim, thiophenate methyl, iprodione, vinclozolin and mancozeb were found very effective in inhibiting mycelial growth of *S. sclerotiorum, in vitro* (Roy and Saikia, 1979**;** Sharma and Kapoor, 1997; Shivpuri and Gupta, 2001; Chattopadhyay *et al.* 2002) [11, 13, 15, 2]. Sharma and Basandrai (1997) [12] evaluated the effect of fungicides on sclerotial viability of *S. sclerotiorum* and observed that carbendazim and triademefon were most effective in inhibiting sclerotial germination at 100 ppm. The present studies were conducted to screen out the effective fungicides which may completely inhibit the mycelial growth as well as sclerotial germination of *S. sclerotiorum* and could be exploited under natural conditions for the management of the disease.

2. Materials and methods
Eleven systemic and non-systemic fungicides (Table 1) at four different concentrations *viz.*,

25, 50, 100 and 250 µg a.i./ml were evaluated against *S. sclerotiorum* pathogen *in vitro* to evaluate the relative efficacy of fungicides in inhibiting the radial growth and sclerotial viability. The effect of fungicides on radial growth of the pathogen was evaluated by 'Food Poison Technique' (Schmitz, 1930) [14] and sclerotial viability by dipping the surface sterilized sclerotia in the fungicide solution for 5 and 15 minutes.

2.1 Effect on radial growth

In poisoned food technique, requisite concentration of each fungicide (prepared by stock solution) was incorporated into PDA flasks, mixed thoroughly by shaking and poured 20 ml in each Petri plates. The medium was allowed to solidify and then inoculated with 5 mm discs of 5 day-old culture of the pathogen. The mycelial disc was placed at the centre of each Petri plate and incubated at 20 ± 1^0C. The Petri plates inoculated with the pathogen but without any fungicide were served as control. Three replications were kept for each treatment. The observations on radial growth (mm) of fungal colony were measured 5 days after incubation when control Petriplates was filled with the growth of the pathogen. The mycelial inhibition of the pathogen over control was calculated by using formula.

Per cent inhibition over control

Where,
C = growth of fungus in control
T = growth of fungus in treatment

2.2 Effect on sclerotial viability (germination)

The effect of fungicides and their concentrations on sclerotial viability of the pathogen were evaluated by dip treatment of surface sterilized sclerotia for 5 and 15 minutes. Stock solution of 10,000 µg a.i./ml was prepared by adding required quantity of fungicide in sterilized distilled water and used for the preparation of different concentrations. The treated sclerotia (15 no.) dried under aseptic conditions were placed in Petri plates. Each treatment was replicated thrice. Petri plates inoculated with untreated sclerotia (dipped in sterilized distilled water) were served as control. These Petri plates were incubated at 20 ± 1^0C for 5 days. Observations on number of germinated sclerotia (myceliogenic) were recorded and per cent inhibition of sclerotial germination over control was calculated.

3. Results and Discussion

3.1 Effect on radial growth

Pathogen growth in various fungicides was measured and average colony dia. (mm) of the colony in each fungicide was recorded. The data (Table 1 and Fig.1) revealed that all fungicides invariably inhibited radial growth of the pathogen at different concentration (Plate 1). Among all the fungicides evaluated, thiophenate methyl was found most effective in inhibiting radial growth of fungus (95.5%) followed by iprodione (92.6%) and hexaconazole (90.7%) at 25 µg a.i./ml and were statistically at par but significantly different from other treatments. Same results were obtained at 50 µg a.i./ml. Thiophenate methyl showed 97.7 per cent mycelial inhibition over check at 100 µg a.i./ml followed by iprodione, triademefon (95.1%) and SAAF (94.0%) and were at par with each other but significantly different from other treatments in inhibiting mycelial growth. Complete inhibition of radial growth was observed with thiophenate methyl and SAAF at 250 µg a.i./ml and were at par with each other but significantly different from other treatments whereas, ridomil 72 MZ was found least effective in inhibiting the growth of the pathogen. Among the various concentration of fungicide, no significant differences were observed at 50, 100 and 250 µg a.i./ml in propiconazole, thiophenate methyl, benomyl, iprodione, tebuconazole, hexaconazole and at 100 & 250 µg a.i./ml in triademefon and carbendazim. However, significant differences were observed at 100 and 250 µg a.i./ml with mancozeb and SAAF in inhibiting mycelial growth of the pathogen. Studies made *in vitro* resulted that thiophenate methyl and SAAF (carbendazim + mancozeb) proved to be the best among eleven fungicides evaluated. These two fungicides completely inhibited the mycelial growth of the pathogen at 250 µg a.i./ml. The fungicides *viz.*, iprodione, hexaconazole, triademefon, tebuconazole, carbendazim, benomyl, propiconazole and mancozeb were also found effective at 100 µg a.i./ml as they inhibited more than 90 per cent fungal growth.

The results obtained in the present study are in close agreement with the findings of earlier workers who also reported that benomyl (Roy and Saikia, 1979) [11] and carbendazim and thiophenate methyl (Shivpuri and Gupta, 2001) [15] completely inhibited the mycelial growth of the fungus at 0.05 per cent. However, Sharma and Kapoor (1997) [13] observed, complete inhibition of mycelial growth of the pathogen at higher dose of iprodione (0.5%). Chattopadhyay *et al. (*2002) [2] also reported complete inhibition of fungal growth at higher dose of carbendazim (0.1% a.i.) and mancozeb (0.2% a.i.) Singh (1998) [27] also observed that, iprodione vinclozolin, benomyl, metalaxyl and carbendazim completely inhibited the growth of the fungus only at higher dose (0.2%). Benomyl completely inhibited the fungal growth even at 10 ppm (Singh *et al*., 1994) [17] while vinclozolin inhibited the growth even at 1.0 µg a.i./ml (Mueller *et al*., 2002). Goswami *et al*. (2007) [10, 5] observed thiophenate methyl and SAAF (carbendazim + mancozeb) proved to be the best as they completely inhibited the mycelial growth of the pathogen at 250 µg a.i./ml. The hexaconazole and SAAF which not reported earlier in the literature were found effective in the present investigation against the pathogen.

Table 1: Effect of fungicides on radial growth of *S. sclerotiorum* (5 DAI)

	Concentration (µg a.i./ml)							
	25		50		100		250	
Fungicide	*RG	I (%)	*RG	I (%)	*RG	I (%)	*RG	I (%)
Propiconazole (25% EC)	22.0	75.5	10.3	88.5	6.3	92.6	6.3	92.6
Mancozeb (75% WP)	90.0	0.0	86.6	3.7	7.3	91.8	2.0	97.7
Metalaxyl 8% WP + MZ 64% WP	90.0	0.0	90.0	0.0	50.6	43.7	10.3	88.5
Thiophenate methyl (70% WP)	4.0	95.5	4.0	95.5	2.0	97.7	0.0	100.0
Tebuconazole (25.9% EC)	26.0	71.1	17.3	80.7	4.0	93.3	6.0	95.5
Benomyl (50% WP)	9.0	90.0	7.6	91.4	7.3	91.8	4.6	94.8
Triademefon (25% WP)	17.3	80.7	7.0	92.2	4.3	95.1	2.0	97.7

Iprodione (50% WP)	6.6	92.6	6.3	92.9	6.3	95.1	4.0	95.6
Carbendazim 12% WP + MZ 63% WP	10.6	88.1	9.0	90.0	4.6	93.7	0.0	100.0
Carbendazim (50% WP)	17.6	80.3	8.3	90.7	5.3	94.0	3.3	96.3
Hexaconazole (5% SC)	8.3	90.7	7.6	91.4	6.6	92.5	3.6	95.9
Control	90.0							
CD (P=0.05)	Fungicides 7.1		Concentrations 4.3			Fungicide x Concentration 14.1		
CV (%)	12.7							

* Mean of three replications
Transformed values are used for analysis
RG – Radial growth (mm) I- Inhibition
MZ- Mencozeb

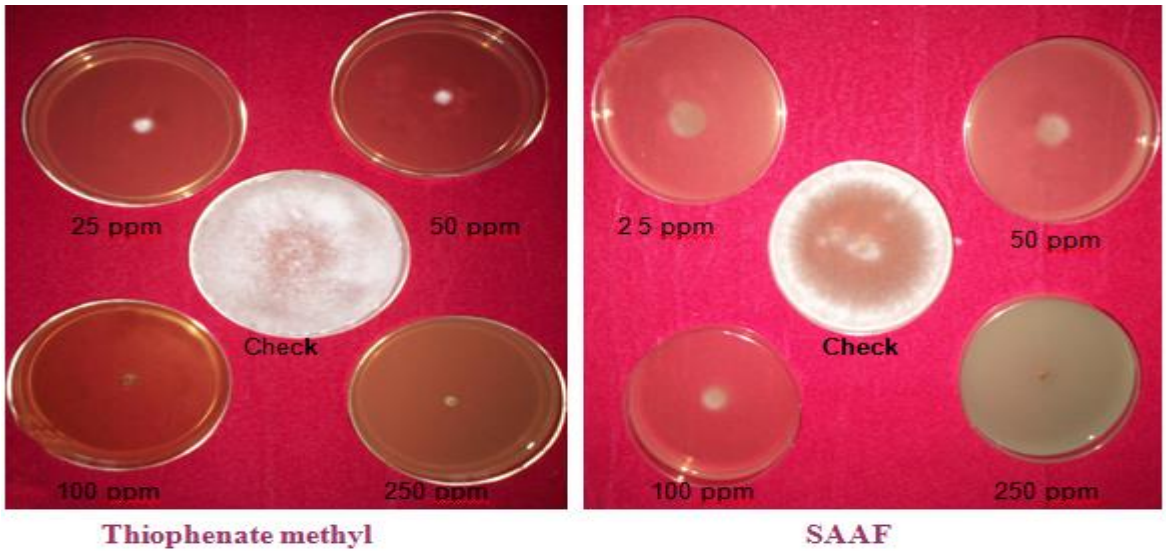

Plate 1: Effect of fungicides on radial growth of *S. sclerotiorum*

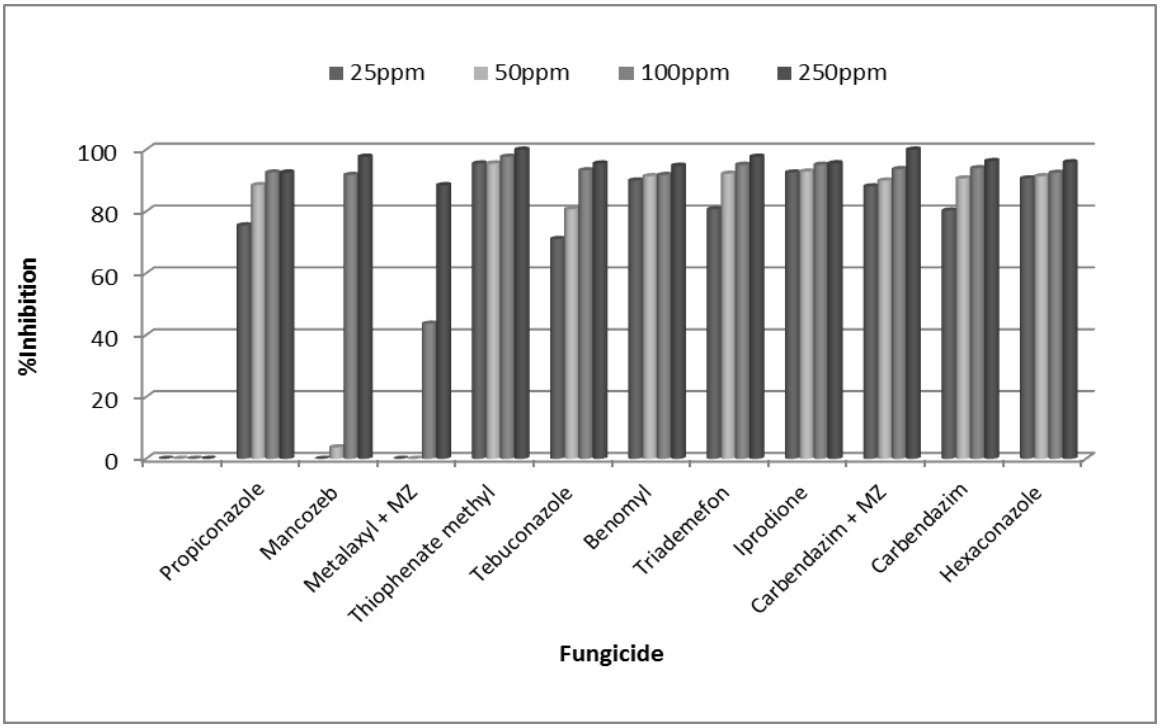

Fig 1: Effect of fungicides on radial growth of *S. sclerotiorum*

3.2 Effect on sclerotial viability

The observation on myceliogenic germination of sclerotia was recorded after 5 days of incubation in sclerotia treated separately with each fungicide by dipping at different concentration for 5 (Table 2a and Fig.2) and 15 minutes (Table 2b and Fig.3).

3.2.1 Effect of 5 minutes dip treatment:

During 5 minute dip treatment among various fungicides, only benomyl at 100 µg a.i./ml completely inhibited the sclerotial germination treated for 5 minutes followed by carbendazim (73.3 & 93.3%), thiophenate methyl (50.0 & 66.6%) and SAAF (46.6 & 63.3%) at 100 & 250 µg a.i./ml respectively. However, other fungicides were found ineffective (Plate 2, Table 2a and Fig.2).

Table 2a: Effect of fungicide on sclerotial viability (5 minutes dip treatment)

	Concentration (µg a.i./ml)							
	25		50		100		250	
Fungicide	***SG**	**I (%)**	***SG**	**I (%)**	***SG**	**I (%)**	***SG**	**I (%)**
Propiconazole (25% EC)	15.0	0.0)	15.0	0.0	15.0	0.0	15.0	0.0
Mancozeb (75% WP)	15.0	0.0	15.0	0.0	15.0	0.0	15.0	0.0
Metalaxyl 8% WP + MZ 64% WP	15.0	0.0	15.0	0.0	15.0	0.0	15.0	0.0
Thiophenate methyl (70% WP)	14.0	6.6	8.0	46.6	7.5	50.0	5.0	66.6
Tebuconazole (25.9% EC)	15.0	0.0	15.0	0.0	14.0	6.6	13.5	10.0
Benomyl (50% WP)	10.5	30.0	7.0	53.3	0.0	100.0	0.0	100.0
Triademefon (25% WP)	15.0	0.0	15.0	0.0	15.0	0.0	15.0	0.0
Iprodione (50% WP)	15.0	0.0	15.0	0.0	15.0	0.0	15.0	0.0
Carbendazim 12% WP + MZ 63% WP	15.0	0.0	9.5	36.6	8.0	46.6	5.5	63.3
Carbendazim (50% WP)	6.6	56.6	5.0	66.6	4.0	73.3	1.0	93.3
Hexaconazole (5% SC)	15.0	0.0	14.0	6.6	14.0	6.6	11.5	23.3
Control	15.0							
CD (P=0.05)	Fungicides-6.0		Concentrations – 3.6;			Fungicide x Concentration - 12.0		
CV (%)	30.0							

* Mean of three replications (15 sclerotia / plate)
Transformed values are used for analysis
SG – Sclerotia germinated I- Inhibition
MZ- Mencozeb

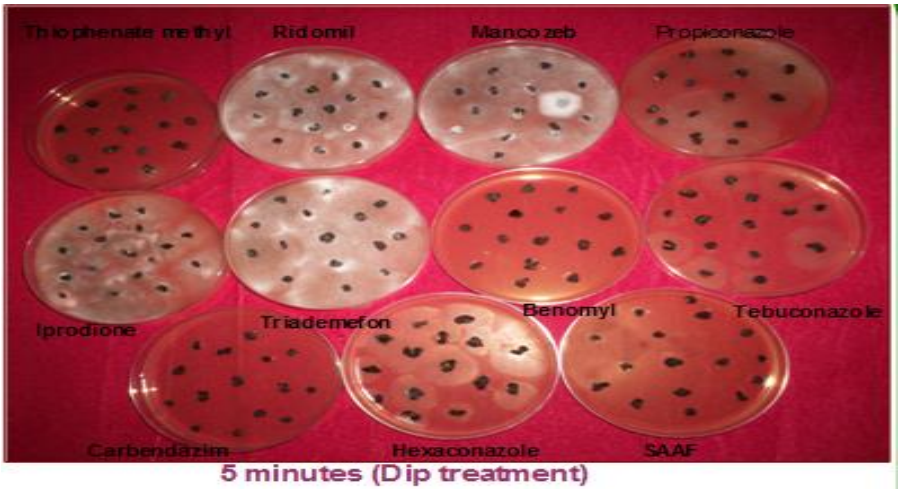

Plate 2: Effect of fungicides on sclerotial germination (5 minutes dip treatment)

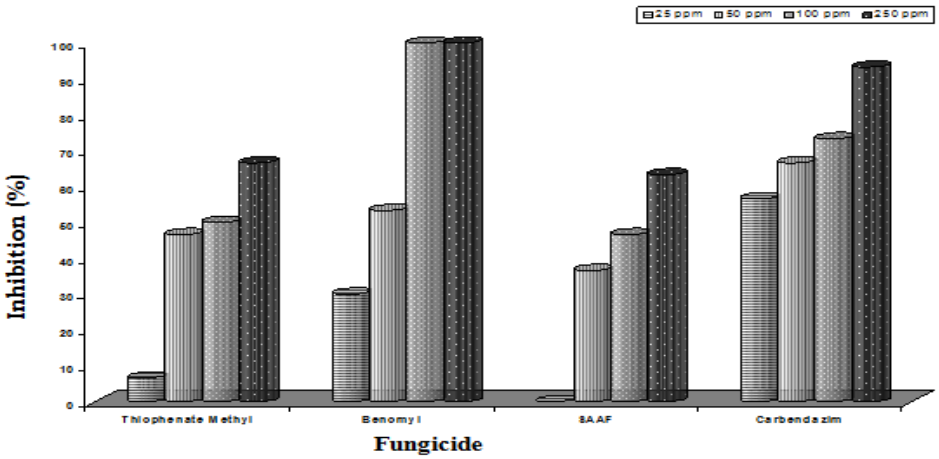

Fig.2: Effect of fungicides on sclerotial germination (5 minutes dip treatment)

3.2.2 Effect of 15 minutes dip treatment:

When sclerotia were treated for 15 minutes, benomyl and carbendazim were found significantly most effective in inhibiting sclerotial germination (66.6 and 66.6%) at 25 µg a.i./ml and 91.6 and 75.0 per cent at 50 µg a.i./ml respectively, as compared to remaining fungicides. Significantly higher inhibition of sclerotial germination was observed at 100 µg a.i./ml with SAAF (66.6%) followed by thiophenate methyl (41.6%) and propiconazole (41.6%). Complete inhibition of sclerotial germination was observed with benomyl and carbendazim followed by propiconazole (91.6%), thiophenate methyl (75.0%), tebuconazole (75.0%), SAAF (66.6%) and hexaconazole (58.7%) at 250 µg a.i./ml (Plate 3, Table 2b and Fig.3). However, mancozeb,

triademefon and iprodione were found significantly less effective. Ridomil 72 MZ was found ineffective even at higher concentration. Among various concentration of effective fungicides no significant differences were observed at 100 & 250 µg a.i./ml with benomyl and SAAF in inhibiting sclerotial germination. However, significant differences were observed with carbendazim at 100 & 250 µg a.i./ml.

Table 2b: Effect of fungicide on sclerotial viability (15 minutes dip treatment)

	Concentration (µg a.i./ml)							
	25		50		100		250	
Fungicide	***SG**	**I (%)**	***SG**	**I (%)**	***SG**	**I (%)**	***SG**	**I (%)**
Propiconazole (25% EC)	5.5	8.3	5.0	16.3	3.5	41.6	0.5	91.6
Mancozeb (75% WP)	6.0	0.0	6.0	0.0	5.5	8.3	5.0	16.6
Metalaxyl 8% WP + MZ 64% WP	6.0	0.0	6.0	0.0	6.0	0.0	6.6	0.0
Thiophenate methyl (70% WP)	6.0	0.0	5.0	16.6	3.5	41.6	1.5	75.0
Tebuconazole (25.9% EC)	5.5	8.3	5.0	16.6	4.5	25.0	1.5	75.0
Benomyl (50% WP)	2.0	66.6	0.5	91.6	0.0	100.0	0.0	100.0
Triademefon (25% WP)	5.5	8.3	5.5	16.6	4.5	25.0	2.5	33.3
Iprodione (50% WP)	5.5	8.3	5.0	8.3	5.0	25.0	4.0	25.0
Carbendazim 12% WP + MZ 63% WP	6.0	0.0	2.5	58.3	2.0	66.6	2.0	66.6
Carbendazim (50% WP)	2.0	66.6	1.5	75.0	1.5	75.0	0.0	100.0
Hexaconazole (5% SC)	6.0	0.0	4.5	25.0	4.5	25.0	2.5	58.7
Control	6.0	0.0	6.0	0.0	6.0	0.0	6.0	0.0
CD (P=0.05)	Fungicides 15.2		Concentrations 9.2			Fungicide x Concentration 30.5		
CV (%)	30.0							

* Mean of three replications (15 sclerotia / plate)
Transformed values are used for analysis
SG – Sclerotia germinated I- Inhibition
MZ- Mencozeb

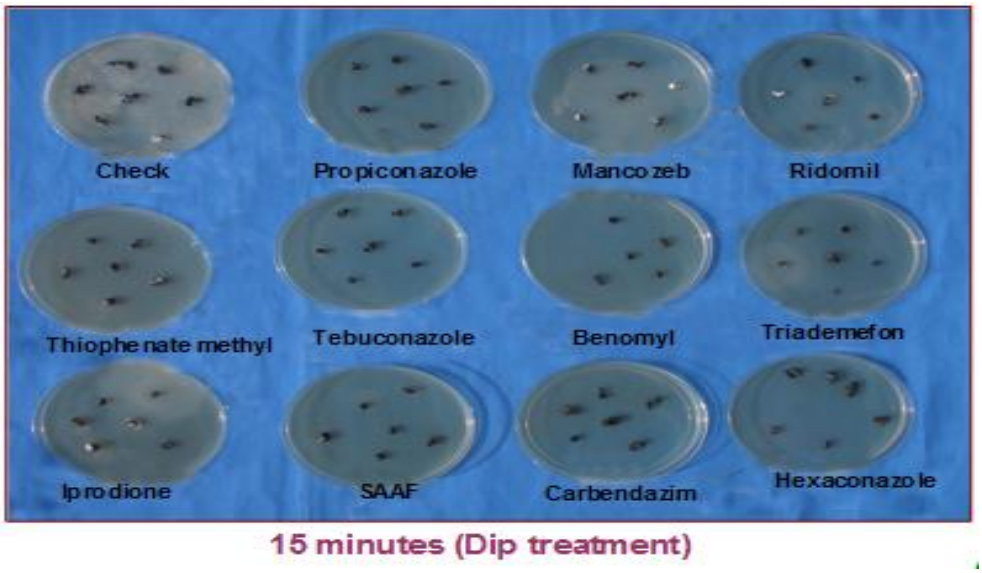

Plate 3: Effect of fungicides on sclerotial germination (15 minutes dip treatment)

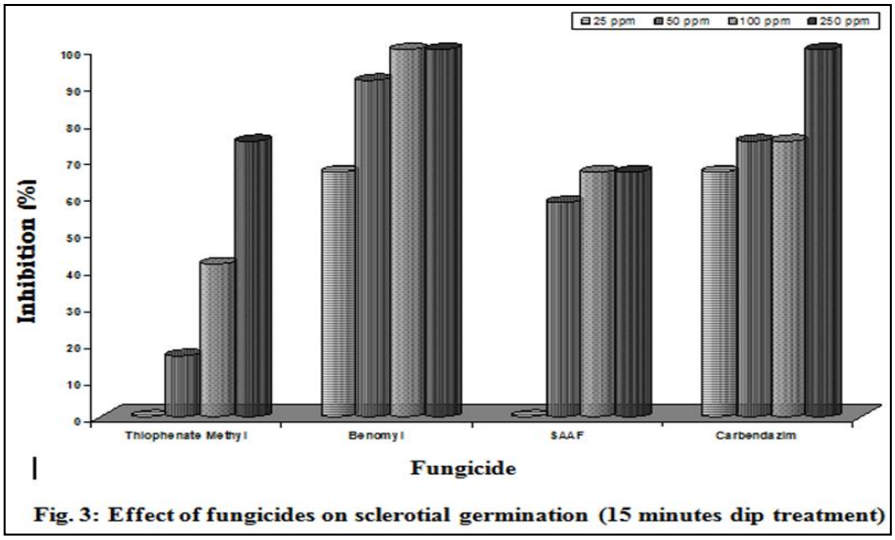

Fig. 3: Effect of fungicides on sclerotial germination (15 minutes dip treatment)

In the present study, complete inhibition of sclerotial germination was found with benomyl at 100 µg a. i./ml in both the treatments (5 and 15 minutes) sclerotial dip treatment. However, carbendazim at 250 µg a. i./ml completely inhibited the sclerotial germination only in 15 minutes dip treatment. The other fungicides were found ineffective in inhibiting sclerotial germination. Sharma and Basandrai (1997) [12] also reported carbendazim as most effective in inhibiting sclerotial germination (90%) at 100 ppm. However, Goswami *et al*. (2008) [6] observed 100 per cent inhibition of sclerotial germination with benomyl and carbendazim. In the present study, the increased efficacy of fungicides was observed when sclerotia were treated for 15 minutes. Thus it is possible to reduce sclerotia viability by judicious use of fungicides. Not much of the works has been done on sclerotial dip treatment against the pathogen.

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