Original Research Article

Efficacy of Fungicides and Optimization of Application Timing for the Management of Sclerotinia Rot of Mustard Caused by *Sclerotiniasclerotiorum*

Abstract

Sclerotinia rot of rapeseed-mustard caused by *Sclerotiniasclerotiorum* (Lib.) de Baryhas gained significant importancein Rajasthan, Punjab, Haryana and other major rapeseed-mustard growing areas of India in recent years. Fungicides are extensively used for the control of this disease since no commercial cultivars have been found resistant to *S. sclerotiorum*and other management practices has not been found very effective. Therefore, thepresent investigations were carried out to evaluate the efficacy of fungicides and to optimize application timing for the effective management of Sclerotinia rot of mustard. Among 08 fungicides tested *in vitro*, Carbendazim 50% WP and Propiconazole 25% EC were found most efficient at completely inhibiting the mycelial growth of *S. sclerotiorum*at all the concentrations tested.In an*in vitro*sclerotial (carpogenic) germination test, Carbendazim 50% WP completely inhibited sclerotial germination at 100, 250 and 500 ppm. Under artificial inoculation conditions in thefield, Propiconazole 25% EC @0.1% was found most effective in reducing Sclerotinia rot incidence (87.04%) followed by Carbendazim 50% WP (83.33%) and Tebuconazole 25.9% EC (75.93%). Similarly, under natural epiphytotic conditions in field,Propiconazole 25% EC @0.1% was found best in diseaseincidence reduction (85.34%)with increased yield (40.00%)followed by Carbendazim 50% WP (81.25%& 38.09%) and Tebuconazole 25.9% EC (79.72%& 35.24%) in disease reduction and increased yield respectively. Differential time and frequency of application of Propiconazole @0.1% were tested and found that 02 applications i.e. 1st spray at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 2ndsprayat 4.3 flowering stage i.e. 50% bloom stage (85 DAS) was equally effectivein disease incidence reduction (82.70%) with 03 applications i.e. 1st spray at 4.1 flowering stage i.e. 10-20% bloom stage (55 DAS), 2nd at 4.2 flowering stage (70 DAS) and 3rd at 4.3 flowering stage (85 DAS).Therefore, the optimization of fungicide application timing and the number of sprays with adequate concentrations is advisable to increase the cost-effectiveness of crop production while controlling the disease.

Keywords: Sclerotinia rot, *Sclerotiniasclerotiorum*, rapeseed-mustard, fungicides and management

1 Introduction

Sclerotinia rot caused by *Sclerotiniasclerotiorum* (Lib.) de Baryis gaining increasedimportance over the years in major rapeseed-mustard growing regions of India **(Shekhawat *et al*., 2012; Trivedi*et al*., 2023)**. The disease incidence of Sclerotinia rot of rapeseed-mustard has been measured up to 72% in Rajasthan **(Ghasolia*et al*., 2004; Rathi*et al*., 2018)** and up to 80% in Punjab and Haryana **(Kang and Chahal, 2000; Sharma *et al*., 2023)**. Plants infected at or before flower initiation resulted in severe yield loss, whereas, infection after flowering stage caused more than 50% yield loss **(Shukla, 2005; Tiwari*et al*., 2021; Singh *et al*., 2022)**. *S.sclerotiorum* continues to be a very difficult pathogen to control due to its' high pathogenicity, wide host rangeand strong survival ability under adverse conditions. Fungicides are extensively being used for the control of Sclerotinia rot in rapeseed-mustard since no commercial cultivars have been found resistant to *S. sclerotiorum***(Bradley *et al*., 2006; Kamal *et al*., 2016; Khangura and van Burgel, 2021).**However, inconsistent and varying degrees of results have been reported with the use of different fungicidesin controlling this disease. Use of fungicides in inhibiting the carpogenic germination of sclerotia through soil application has also been reported **(Sumida *et al*., 2015).**Moreover, the efficacy of foliar application of fungicidesalsodepends onapplication timing i.e. during flowering stage/bloom stage **(Heffer Link and Johnson, 2007; Turkington *et al*., 2011; Khangura and van Burgel, 2021).**Therefore, the objective of this research wasto evaluate the efficacy of fungicides and to optimize the application number and appropriate timing for the effective management of Sclerotinia rot of mustard.

2 Materials and Methods

The *in vitro* studies were carried out in Oilseed Pathology laboratory and glasshouse, Department of Plant Pathology, College of Agriculture, GBPUAT and field experiments were conducted during Rabi seasons (2020-21, 2021-22 and 2022-23) at Norman E. Borlaug Crop Research Centre (NEBCRC), GBPUAT, Pantnagar, Udham Singh Nagar, Uttarakhand.

2.1 Evaluation of fungicides against *S. sclerotiorum*under *in vitro* conditions

2.1.1 Mycelial growth inhibition

Eight fungicides were evaluated at 04 different increasingconcentrations (25, 50, 100 and 250 ppm) to investigatemycelial growth inhibition of the test pathogen under laboratory conditions by following Poisoned Food Technique **(Schmitz, 1930)**. Stock solution of 10,000 ppm of each fungicide was prepared by adding therequired quantity of fungicide(as mentioned in List 1)in 10 mlsterilized distilled water. Then, the requisite concentrations of each fungicide were incorporated in sterilized PDA medium, thoroughly mixed by shaking prior to pouring in sterilized Petri plates and allowed to solidify. These Petri plates were inoculated with 5 mm mycelial disc of 4-days old culture of the test pathogen at the centre of the Petri plate and incubated at 20±1°C. Each treatment was replicated thrice with control (without any fungicide). The radial growth of the test pathogen was measured at 4 days after incubation and per cent mycelial growth inhibition was calculated by using the formula given:

$$\text{Mycelial Growth Inhibition (\%)} = {}^{C-T}/_{C} \times 100$$

where,

C = mycelial growth of test pathogen in control

T = mycelial growth of test pathogen in treatment

List 1. Details of Fungicides

Sl. No.	Name of the fungicide	Quantity reqd. to make 10,000 ppm
1	Carbendazim 50% WP	200 mg
2	Propiconazole 25% EC	400 µl
3	Tebuconazole 25.9% EC	386 µl
4	Thiophanate methyl 70% WP	143 mg
5	Carbendazim 12% + Mancozeb 63% WP	133 mg
6	Azoxystrobin 18.2% + Difenconazole 11.4% SC	338 µl
7	Mancozeb 75% WP	133 mg
8	Copper oxychloride 50% WP	200 mg

2.1.2Sclerotial germination (carpogenic) assay

The experiment was performed in Petri plates containing soil collected from the AICRP-Rapeseed-Mustard field of NEBCRC, GBPUAT, Pantnagar, by following the method described by **Sumida *et al*. (2015)** with some modifications. The soil was prepared by sieving, moistening

and autoclaving for 1 h at 121°C on two successive days. Seventy (70)g of the sterilized soil was filled in Petriplates. Then, 15 sclerotia/Petriplatewere buried and covered with soil of 2.0 mm thickness over sclerotia. Stock solution of 10,000 ppm was prepared by adding therequired quantity of fungicide in sterilized distilled water which wasused for the preparation of different concentrations (50, 100, 250 and 500 ppm). 15 ml of each fungicide concentration was poured separately in Petriplates and incubated at 20±1°C for 45 days. Each treatment was replicated thrice with control (without any fungicide). The formation of apothecia from each sclerotium was recorded and the levels ofinhibition of sclerotial germination (carpogenic) werecalculated by using the formula:

$$\text{Inhibition (\%)} = {}^{C-T}/_{C} \times 100$$

where,

C = Number of sclerotia which produced stipes/apothecia in control

T = Number of sclerotia which produced stipes/apothecia in treatment.

2.2 Evaluation of promising fungicides against the Sclerotiniastemrot in field conditions

The soil of experimental field was sandy loam of average fertility with good drainage facilities. One deep ploughing was done with disc plough and subsequent light ploughings were done with rotavator followed by planking. Plot size (3×1.5 m^2 area) with 5 rows was prepared for each treatment according to the layout plan. The variety i.e. Varuna (*Brassica juncea*) was used for the field experiments. All the plants received uniform cultural operations throughout the experimental period and whole of the experimental field was kept clean and well maintained. In all the plots, the recommended dose of fertilizer (80kg- N, 40kg- P and 20kg-K/ha) was applied at the time of sowing. All the field experiments were laid out in Randomized Block Design (RBD) with 3 replications in each treatment.

2.2.1 Under artificial inoculation conditions

Ten plants per plot were randomly selected and inoculated with 10 mm mycelial bits taken from 10 days old fresh culture of *S. Sclerotiorum*. Aerial spray of each fungicide @ 0.1% concentration was given 03 times i.e. 1st spray at 2 days before inoculation of the test pathogen i.e. 60 days after sowing (DAS) and the 2nd and 3rd spray at 4 and 14 days after pathogen

inoculation (66 DAS and 76 DAS) respectively. The plots without fungicide application were served as control. Three replications were maintained for each treatment. At 110 DAS, infected lesion length (cm) and girth (cm) were measured with a measuring scale. The disease incidence was calculated by using the formula given below:

$$\text{Disease Incidence (\%)} = \left(\text{number of infected plants} / \text{total number of inoculated plants}\right) \times 100$$

2.2.2Undernatural epiphytotic conditions

The aerial spray of effective fungicides was given @ 0.1% for three times i.e. 1st spray (55 DAS), 2nd spray (70 DAS) and 3rd spray (85 DAS). The plots without fungicide application were served as control. The observations on number of infected and non-infected plants in each plot were recorded at 110 DASand percent disease incidence was calculated. The yield (kg/ha) was also recorded.

$$\text{Disease Incidence (\%)} = \left(\text{number of infected plants} / \text{total number of plants}\right) \times 100$$

2.3 Optimization of stage of flowering for foliar application

The fungicide which was found most effective under field conditions was further applied as aerial spray at different flowering stages viz. 4.1, 4.2, 4.3 and 4.4 stage of mustard plant as reported by **Harper and Berkenkamp (1975)**or at 10-20%, 30%, 50% and >50% bloom stage of canola plant as given by **Anonymous (2010)**, to select appropriate stage of flowering for the aerial spray for cost effective management of Sclerotinia stem rot of rapeseed-mustard. The disease incidence was calculated by using the formula given below:

$$\text{Disease Incidence (\%)} = \left(\text{number of infected plants} / \text{total number of plants}\right) \times 100$$

List 2.Details of experiment and spray schedule

Treatment		Timing and number of application in relation to stage of crop
T1	Propiconazole @0.1%	1st Spray at 4.1 flowering stage i.e. 10-20% bloom stage (55 DAS) and 2nd at 4.2 flowering stage i.e. 30%bloom stage (70 DAS)
T2	Propiconazole @0.1%	1st Spray at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 2nd at 4.3 flowering stage i.e. 50%bloom stage (85 DAS)
T3	Propiconazole @0.1%	1st Spray at 4.3 flowering stage i.e. 50% bloom stage (85 DAS) and

		2nd at 4.4 flowering stage i.e. >50%bloom stage (100 DAS)
T4	Propiconazole @0.1%	1st Spray at 4.1flowering stage i.e. 10-20% bloom stage (55 DAS), 2nd at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 3rd at 4.3 flowering stage i.e. 50% bloom stage (85 DAS)
T5	Control	No fungicide spray

2.4 Statistical analysis

The statistical analysis of the experimental data was carried out using OPSTAT and WASP 2.0 software package. The data obtained from the laboratory experiments and the field experiments were analyzed statistically with Completely Randomized Design (CRD) and Randomized Block Design (RBD) respectively. Different treatments were compared using critical difference (CD) value at 0.05 (5%) level of significance.

3Results and Discussion

3.1 Evaluation of fungicides against the test pathogen under *in vitro*conditions

3.1.1 Mycelial growth inhibition

Eight fungicides were evaluated for their ability to inhibit the mycelial growth and sclerotia formation of *S. sclerotiorum* under *in vitro* conditions by following thePoisoned Food Technique. The outcome of the study exhibited in Table 1 and Plate 1 revealed that among the fungicides, Carbendazim 50% WP and Propiconazole 25% EC were found best effective with no mycelial growth resulting in complete inhibition of mycelial growth (100.00%) and sclerotia formation (0.00 no.) at all the concentrations (25, 50, 100 and 250 ppm) which were significantly different in terms of mycelial growth inhibition but at par in sclerotia formation from Carbendazim 12% + Mancozeb 63% WP (84.44%) and Thiophanate methyl 70% WP (84.44%) at 25 ppm.However,thesewere at par with each other at 50, 100 and 250 ppm in mycelial growth inhibition (100.00%) and sclerotia formation (ranged: 0.00-1.67 no.). The least effective fungicide was noted as Copper oxychloride 50% WP causing no mycelial growth inhibition (0.00%) and sclerotia formation (11.33, 17.00, 13.67 & 14.00 no.) at 25, 50, 100 & 250 ppm, respectivelyand was at par with control in mycelial growth inhibition (0.00%) but significantly different in sclerotia formation (31.00 no.).

Among the various concentrations of fungicide tested, no significant differencein mycelial growth inhibition at all concentrations was observed in Carbendazim 50% WP,

Propiconazole 25% EC and Copper oxychloride 50% WP.In sclerotia formation, no significant differences were observed at all concentrations in Carbendazim 50% WP, Propiconazole 25% EC, Thiophanate methyl 70% WP and Azoxystrobin 18.2% + Difenconazole 11.4% SC.

The present study revealed that Carbendazim 50% WP and Propiconazole 25% EC as the most efficient fungicide which completely inhibited the mycelial growth of *S. sclerotiorum*at all the concentrations tested. Similar results were also obtained by **Shivpuri and Gupta (2001),Chand *et al*. (2009), Bharti *et al*. (2015),Rakesh *et al*. (2016) and Sharma *et al*. (2022).** In addition, Carbendazim 12% + Mancozeb 63% WP and Thiophanate methyl 70% WP also resulted complete inhibition of mycelial growthat 50, 100 and 250 ppmwhich was in agreement with **Goswami *et al*. (2020)** who reported that Thiophanate methyl and Carbendazim + Mancozeb (SAAF) completely inhibited the mycelial growth of *S. sclerotiorum* at 250 µg a.i./ml.

Table 1: *In vitro* evaluation fungicides against mycelial growth of *S. sclerotiorum*

Fungicide	Mycelial growth (mm)				Mycelial growth inhibition (%)				Sclerotia formation (no.)			
	25ppm	50ppm	100ppm	250ppm	25ppm	50ppm	100ppm	250ppm	25ppm	50ppm	100ppm	250ppm
Carbendazim 50% WP	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00
Propiconazole 25% EC	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00
Tebuconazole 25.9% EC	15.00	11.00	7.33	0.00	83.33	87.78	91.85	100.00	3.67	2.33	0.67	0.33
Thiophanate methyl 70% WP	14.00	0.00	0.00	0.00	84.44	100.00	100.00	100.00	0.67	0.33	0.00	0.00
Carbendazim 12% + Mancozeb 63% WP	9.00	0.00	0.00	0.00	90.00	100.00	100.00	100.00	1.67	0.00	0.00	0.00
Azoxystrobin 18.2% + Difenconazole 11.4% SC	20.00	17.33	13.00	09.00	77.78	80.74	85.56	90.00	1.67	2.00	1.67	0.67
Mancozeb 75% WP	33.00	25.00	14.67	09.33	63.33	72.22	83.70	89.63	8.00	6.00	0.67	0.67
Copper oxychloride 50% WP	90.00	90.00	90.00	90.00	0.00	0.00	0.00	0.00	11.33	17.00	13.67	14.00

Control	90.00	90.00	90.00	90.00	0.00	0.00	0.00	0.00	31.00	31.00	31.00	31.00
Factors	**C.D. (5%)**		**S.E. (m)**		**C.D. (5%)**		**S.E. (m)**		**C.D. (5%)**		**S.E. (m)**	
Factor-A (Fungicide)	1.15		0.41		1.28		0.45		2.23		0.79	
Factor-B (Concentration)	0.77		0.27		0.85		0.30		1.48		0.53	

3.1.2Sclerotial germination (carpogenic) assay

Six fungicides viz. Carbendazim 50% WP, Propiconazole 25% EC, Tebuconazole 25.9% EC, Thiophanate methyl 70% WP, Carbendazim 12% + Mancozeb 63% WP and Azoxystrobin 18.2% + Difenconazole 11.4% SC which showedhigh effectivityin inhibiting mycelial growth and sclerotial formation were selected and the results are shown in Table 2 and Plate 2. All the fungicides were found to besignificantly effective at all concentrations over control regarding inhibition of sclerotial germination of the test pathogen.

At 50 ppm, Carbendazim 50% WP was recorded most effective as only 2.67 no. sclerotia germinated (out of 15 no.) leading to highest sclerotial germination inhibition of 82.22% which was found significantly different from Thiophanate methyl 70% WP (60.00%), Propiconazole 25% EC (51.11%) and other treatments. At 100 ppm, Carbendazim 50% WP showed complete sclerotial germination inhibition (100.00%) which was found significantly different from Thiophanate methyl 70% WP, Propiconazole 25% EC (68.89% in each), Tebuconazole 25.9% EC (60.00%) and other treatments. Similar results were obtained at 250 ppm, where Carbendazim 50% WP again showed complete sclerotial germination inhibition (100.00%) and was significantly different from Tebuconazole 25.9% EC (88.89%), Thiophanate methyl 70% WP and Propiconazole 25% EC (84.45% each) and other treatments. At 500 ppm, complete sclerotial germination inhibition (100.00%) was recorded in Carbendazim 50% WP and Propiconazole 25% EC and were at par with each other but significantly different from Tebuconazole 25.9% EC, Thiophanate methyl 70% WP and Carbendazim 12% + Mancozeb 63% WP (95.55% in each) and other treatments. Among the six fungicides, the least sclerotial germination inhibition of 6.67% and 20.00% at 50 ppm and 100 ppm respectively were recorded in Carbendazim 12% + Mancozeb 63% WP whileAzoxystrobin 18.2% + Difenconazole 11.4%

showed least sclerotial germination inhibition of 51.11% and 64.44% at 250 ppm and 500 ppm respectively and no sclerotial germination inhibition (0.00%) was observed control.

Of the various fungicide concentrations evaluated, only Carbendazim 50% WP displayed non-significant differences at 100, 250 and 500 ppm while all other fungicides showed significant differences at all the concentrations in terms of sclerotial germination inhibition. It is evident from the results that inhibition of sclerotial germination gradually increases with increasing fungicide concentration.

In the present investigation,Carbendazim 50% WP was proved as the best fungicide in inhibiting the sclerotial germination of *S. sclerotiorum* when tested 50, 100, 250 and 500 ppm concentrations. Similarly, **Sumida *et al.* (2015)** reported inhibition of sclerotia germination (44.4%) of *S. sclerotiorum* by Carbendazim @0.1%. In addition, Propiconazole 25% EC, Tebuconazole 25.9% EC, Thiophanate methyl 70% WP and Carbendazim 12% + Mancozeb 63% WP also gave good results at higher concentrations (250 and 500 ppm) with more than 80% inhibition of sclerotial germination.

Table 2: *In vitro* evaluation of fungicides against sclerotial germination (carpogenic) of *S. sclerotiorum*

Fungicide	Sclerotia germination(no.)				Sclerotia germination(%)				Germination inhibition(%)			
	50 ppm	100 ppm	250 ppm	500 ppm	50 ppm	100 ppm	250 ppm	500 ppm	50 ppm	100 ppm	250 ppm	500 ppm
Carbendazim 50% WP	2.67	0.00	0.00	0.00	17.78	0.00	0.00	0.00	82.22	100.00	100.00	100.00
Propiconazole 25% EC	7.33	4.67	2.33	0.00	48.89	31.11	15.55	0.00	51.11	68.89	84.45	100.00
Tebuconazole 25.9% EC	12.33	6.00	1.67	0.67	82.22	40.00	11.11	4.45	17.78	60.00	88.89	95.55
Thiophanate methyl 70% WP	6.00	4.67	2.33	0.67	40.00	31.11	15.55	4.45	60.00	68.89	84.45	95.55
Carbendazim 12% + Mancozeb 63% WP	14.00	12.00	2.33	0.67	93.33	80.00	15.56	4.45	6.67	20.00	84.44	95.55
Azoxystrobin 18.2% + Difenconazole 11.4% SC	13.33	8.67	7.33	5.33	88.89	57.78	48.89	35.56	11.11	42.22	51.11	64.44

Control	15.00	15.00	15.00	15.00	100.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00
Factors	**C.D. (5%)**		**S.E. (m)**		**C.D. (5%)**		**S.E. (m)**		**C.D. (5%)**		**S.E. (m)**	
Factor A (Fungicide)	0.98		0.35		6.56		2.31		6.56		2.31	
Factor B (Concentration)	0.74		0.26		4.96		1.75		4.96		1.75	

3.2 Evaluation of promising fungicides against Sclerotinia stem rot in field

3.2.1 Under artificial inoculation conditions

Six effective fungicides (as mentioned in3.1.2) at a0.1% concentration were applied 03times as aerial spray on whole of the plants including inoculated portion.The results presented in Table 3. and Fig. 1 showedthat all the fungicides were found to be significantly and equally effective over control in accordance to disease incidence and disease reduction in both two Rabi seasons (2020-21&2021-22). In 2020-21,Propiconazole 25% EC was found best which showed minimum disease incidence (13.33%) with disease reduction over control (84.62%). The other fungicides also exhibited promising results viz. Carbendazim 50% WP, Tebuconazole 25.9% EC with disease incidence (16.67% and 20.00%) and disease reduction (80.77% and 76.92%) respectively while the maximum disease incidence (86.67%) was recorded in Control.

Similar trendswere also observed in 2021-22, where minimum disease incidence (10.00%) and disease reduction over control (89.29%) was observed in Propiconazole 25% EC followed by Carbendazim 50% WP (13.33% and 85.71%), Thiophanate methyl 70% WP and Carbendazim 12% + Mancozeb 63% WP (20.00% in each and 78.57% in each) in terms of disease incidence and disease reduction over control, respectively. The highest disease incidence (93.33%) was observed in Control.

Additionally, pooled data also revealed that all the fungicides were at par with each other and found significantly effective in their ability to decrease disease incidence and severity. The least disease incidence (11.67%) and highest disease reduction over control (87.04%) was recorded in Propiconazole 25% EC followed by Carbendazim 50% WP (15.00% and 83.33%) and Tebuconazole 25.9% EC (21.66% and 75.93%) while the highest disease incidence (90.00%) was observed in Control.

In the present investigation, Propiconazole 25% EC and Carbendazim 50% WP gave best results when tested against Sclerotinia rot under artificial inoculation condition. **Rakesh *et al.* (2016)**reported that foliar spray of Carbendazim @ 0.1% and Propiconazole @ 0.05% twice at 45 and 60 DAS were found effective in controlling disease incidence of Sclerotinia stem rot of Indian mustard under sick plot condition. **Zamani-Noor (2021)** also stated that foliar application of Tebuconazole @ 1.5 l/ha and Azoxystrobin @ 0.5 kg/ha one day after artificial inoculation of *S. sclerotiorum* resulted disease reduction of 58.3 and 57.2% respectively over untreated control.

Table 3: Evaluation of Fungicides against Sclerotinia stem rot pathogen under artificial inoculation conditions in field

Fungicide	Rabi season (2020-21)		Rabi season (2021-22)		Pooled data (2020-21 & 2021-22)	
	Disease Incidence (%)	Disease reduction over control (%)	Disease Incidence (%)	Disease reduction over control (%)	Disease Incidence (%)	Disease reduction over control (%)
Carbendazim 50% WP	16.67 (23.85)	80.77	13.33 (17.70)	85.71	15.00 (22.75)	83.33
Propiconazole 25% EC	13.33 (21.14)	84.62	10.00 (14.99)	89.29	11.67 (19.95)	87.04
Tebuconazole 25.9% EC	20.00 (26.55)	76.92	23.33 (28.27)	75.00	21.66 (27.72)	75.93
Thiophanate methyl 70% WP	26.67 (30.78)	69.23	20.00 (26.06)	78.57	23.33 (28.87)	74.07
Carbendazim 12% + Mancozeb 63% WP	30.00 (32.99)	65.39	20.00 (26.55)	78.57	25.00 (29.99)	72.22
Azoxystrobin 18.2% + Difenconazole 11.4% SC	26.67 (30.28)	69.23	23.33 (28.77)	75.00	25.00 (29.98)	72.22
Control	86.67 (68.83)		93.33 (81.14)		90.00 (72.32)	
C.D. (5%)	17.26 (12.01)		17.55 (20.31)		16.71 (17.19)	
S.E. (m)	5.54 (3.86)		5.63 (6.52)		5.37 (5.53)	
C.V.	30.53 (19.94)		33.60 (35.36)		30.75 (28.88)	

3.2.2 Under natural epiphytotic conditions

Six fungicides (as mentioned in 3.1.2) were further investigated against Sclerotinia rot under natural epiphytotic conditions in field where each fungicide was applied at 55, 70 and 85 DAS as aerial spray. The results presented in Table 4and Fig. 2 revealed that all the fungicides were significantly effective over control in reducing the disease incidence. In Rabi season2020-21 as well as 2021-22, less disease incidence was observed in fungicide treatment (4.00-7.20%) as compared to control (75.83-76.00%).

In 2020-21, among the fungicides, Propiconazole 25% EC was found most effective resulting in least disease incidence and maximum disease reduction over control (4.00% & 86.05%, respectively) followed by Carbendazim 50% WP (5.20% & 81.86%) and Tebuconazole 25.9% EC (5.60% &80.47%) and were at par with each other but significantly different withcontrol (28.67% DI).

Similarly, in 2021-22, minimum disease incidence and maximum disease reduction over control were recorded in Propiconazole 25% EC (4.60% & 84.67%, respectively) followed by Carbendazim 50% WP (5.80% & 80.67%) and Thiophanate methyl 70% WP (6.10% & 79.67%) and were par with each other but significantly different with control (30.00% DI).

The pooled analysis data also showed Propiconazole 25% EC as the best fungicide with least disease incidence (4.30%) and maximum disease reduction over control (85.34%) followed by Carbendazim 50% WP (5.50% & 81.25%) and Tebuconazole 25.9% EC (5.95% & 79.72%) and were at par with each otherin disease incidence and disease reduction but significantly different with control (29.34% DI).

The yield (kg/ha) of different treatments presented in Table 5 and Fig. 3 showed non-significant differences among the fungicide treatmentsbutshowed significant increased yield over control. During both the years 2020-21 and 2021-22, the maximum yield (1644.44 &1622.22 kg/ha, respectively) was recorded in Propiconazole 25% EC followed by Carbendazim 50% WP and Tebuconazole 25.9% EC (1622.22 & 1600.00 kg/harespectively in 2020-21) and also by Carbendazim 50% WP and Thiophanate methyl 70% WP (1600.00 and 1577.78 kg/ha respectively in 2021-22). However, the minimum yield was displayed in Control (1177.78 and 1155.56 kg/ha) in 2020-21 and 2021-22 respectively.

Moreover, the pooled data showed maximum yield (1644.44 kg/ha) in Propiconazole 25% EC followed by Carbendazim 50% WP (1622.22 kg/ha), Tebuconazole 25.9% EC and Thiophanate methyl 70% WP (1577.78 kg/ha each) which were at par with each other but significantly different with Control (1177.78 kg/ha).

The present investigation revealed that application of Propiconazole 25% EC, Carbendazim 50% WP, Tebuconazole 25.9% EC and Thiophanate methyl 70% WP at 55, 70 and 85 DAS as aerial spray @ 0.1% effectively controlled the disease incidence of Sclerotinia rot of mustard.Several workers viz., **Sharma *et al.* (2006)**, **Kumar and Prasad (2007)**, **Ghasolia and Shivpuri (2008)**, **Rathi *et al*. (2012)**, **Sharma *et al*. (2017) and Roy *et al.* (2021)** also reported Carbendazim as effective fungicide against Sclerotinia rot of Indian mustard. **Rakesh *et al.* (2016)**unveiled that foliar spray of Carbendazim @ 0.1% and Propiconazole @ 0.05% twice at 45 and 60 DAS were found effective in controlling disease incidence of Sclerotinia stem rot of Indian mustard by 76.3% and 69.0% with increasing seed yield by 31.1% and 23.8% respectively as compared to untreated control.

Table 4: Evaluation of Fungicides against Sclerotinia rot disease under natural epiphytotic conditions in field

Fungicide	Rabi season (2020-21)		Rabi season (2021-22)		Pooled data (2020-21 & 2021-22)	
	Disease Incidence (%)	Disease reduction over control (%)	Disease Incidence (%)	Disease reduction over control (%)	Disease Incidence (%)	Disease reduction over control (%)
Carbendazim 50% WP	5.20 (13.16)	81.86	5.80 (13.91)	80.67	5.50 (13.54)	81.25
Propiconazole 25% EC	4.00 (11.53)	86.05	4.60 (12.37)	84.67	4.30 (11.96)	85.34
Tebuconazole 25.9% EC	5.60 (13.68)	80.47	6.30 (14.53)	79.00	5.95 (14.11)	79.72
Thiophanate methyl 70% WP	6.00 (14.17)	79.07	6.10 (14.29)	79.67	6.05 (14.23)	79.38
Carbendazim 12% + Mancozeb 63% WP	6.60 (14.88)	76.98	6.80 (15.11)	77.33	6.70 (15.00)	77.16
Azoxystrobin 18.2% + Difenconazole1	6.93 (15.26)	75.83	7.20 (15.55)	76.00	7.07 (15.41)	75.90

1.4% SC						
Control	28.67 (32.35)		30.00 (33.18)		29.34 (32.77)	
C.D. (5%)	1.74 (1.29)		1.88 (1.34)		1.79 (1.31)	
S.E. (m)	0.56 (0.41)		0.59 (0.43)		0.57 (0.42)	
C.V.	10.75 (4.35)		10.64 (4.38)		10.72 (4.37)	

Note: Numbers in parentheses are angular transformed values

Table 5: Yield in different Fungicides tested against Sclerotinia rot disease under natural epiphytotic conditions in field

Fungicide	Rabi season (2020-21)			Rabi season (2021-22)			Pooled data (2020-21 & 2021-22)		
	Kg/ plot	**Kg/ha**	**Increased yield (%)**	**Kg/ plot**	**Kg/ha**	**Increased yield (%)**	**Kg/ plot**	**Kg/ha**	**Increased yield (%)**
Carbendazim 50% WP	0.73	1622.22	37.74	0.72	1600.00	38.46	0.73	1611.11	38.09
Propiconazole 25% EC	0.74	1644.44	39.62	0.73	1622.22	40.38	0.74	1633.33	40.00
Tebuconazole 25.9% EC	0.72	1600.00	35.85	0.70	1555.56	34.62	0.71	1577.78	35.24
Thiophanate methyl 70% WP	0.71	1577.78	33.96	0.71	1577.78	36.54	0.71	1577.78	35.24
Carbendazim 12% + Mancozeb 63% WP	0.70	1555.56	32.08	0.69	1533.33	32.69	0.70	1544.45	32.38
Azoxystrobin 18.2% + Difenconazole 11.4% SC	0.69	1533.33	30.19	0.68	1511.11	30.77	0.69	1522.22	30.48
Control	0.53	1177.78		0.52	1155.56		0.53	1166.67	
C.D. (5%)	0.09	204.57		0.09	201.48		0.09	204.27	
S.E. (m)	0.03	65.67		0.03	64.67		0.03	65.57	
C.V.	7.33	7.43		7.31	7.43		7.34	7.44	

3.3 Optimization of stage of flowering for foliar application

To optimize appropriate time of applicationfor the aerial sprayingof fungicides at specific stages of flowering of mustard, 4 different spray schedules were investigated using Propiconazole 25% EC @ 0.1% which was found most effective under field conditions. The results presented in Table 6 and Fig. 4 showed significant difference among the treatments.

The best effective spray schedule was observed in T4 [i.e. 1st Spray at 4.1 flowering stage i.e. 10-20% bloom stage (55 DAS), 2nd at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 3rd at 4.3 flowering stage i.e. 50% bloom stage (85 DAS)] with least disease incidence (4.60% & 3.90%) and disease reduction over control by 84.82% and 83.54% during Rabi seasons (2021-22 and 2022-23) respectively followed by T2 [i.e. 1st Spray at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 2nd at 4.3 flowering stage i.e. 50% bloom stage (85 DAS)] with disease incidence (5.00% in 2021-22 and 4.33% in 2022-23) and was at par with each other but significantly different from other treatments and control (30.30% &23.70% DIin 2021-22 & 2022-23 respectively).

The pooled data of both the years also showed treatment T4as the most effective treatment by showing least disease incidence (4.25%) and was found at par with treatment T2with disease incidence (4.67%) but significantly different other treatments andcontrol (27.00%).

The present study revealed that two aerial spray of Propiconazole @ 0.1% with 1st Spray at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 2nd at 4.3 flowering stage i.e. 50% bloom stage (85 DAS) was at par with three aerial spray of Propiconazole @0.1% with 1st Spray at 4.1 flowering stage i.e. 10-20% bloom stage (55 DAS), 2nd at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 3rd at 4.3 flowering stage i.e. 50% bloom stage (85 DAS) and therefore, two aerial spray is recommended for the management of Sclerotinia rot of mustard.**Khangura and van Burgel (2021)**also reported that the fungicides sprayed in between 10% and 50% bloom stage were more effective in reducing disease incidence of Sclerotinia stem rot of canola as compared to earlier and late stage application.

Table 6: Effect of Fungicide against Sclerotinia rot disease at different flowering/bloom stage of mustard

Treatment	Rabi season (2021-22)		Rabi season (2022-23)		Pooled data (2021-22 & 2022-23)	
	Disease Incidence (%)	Disease reduction over control (%)	Disease Incidence (%)	Disease reduction over control (%)	Disease Incidence (%)	Disease reduction over control (%)
T1	6.60 (14.86)	78.22	5.70 (13.79)	75.95	6.15 (14.33)	77.22

T2	5.00 (12.91)	83.50	4.33 (12.00)	81.73	4.67 (12.47)	82.70
T3	7.67 (16.06)	74.69	7.10 (15.44)	70.04	7.39 (15.76)	72.63
T4	4.60 (12.38)	84.82	3.90 (11.38)	83.54	4.25 (11.89)	84.26
Control	30.30 (33.38)		23.70 (29.12)		27.00 (31.29)	
C.D. (5%)	1.72 (1.41)		1.40 (1.29)		1.11 (1.10)	
S.E. (m)	0.52 (0.43)		0.42 (0.39)		0.34 (0.33)	
C.V.	8.31 (4.13)		8.16 (4.12)		5.89 (3.36)	

Note: Numbers in parentheses are angular transformed values

4Conclusion

From the present investigation, it is concluded that Propiconazole 25% EC was most efficient fungicide under *in vitro*in mycelial growth inhibition and sclerotial germination and in field in managing *S. sclerotiorum*pathogen causing Sclerotinia rot of rapeseed-mustard. Among various flowering stages two applications of Propiconazole @0.1% i.e. 1st spray at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 2nd at 4.3 flowering stage i.e. 50% bloom stage (85 DAS) was at par with three aerial applicationsat three different stages in controlling the disease.

The fungicides viz.Carbendazim 50% WP, Propiconazole 25% EC, Tebuconazole 25.9% EC, Thiophanate methyl 70% WP, Carbendazim 12% + Mancozeb 63% WP and Azoxystrobin 18.2% + Difenconazole 11.4% SC which were found effective in inhibiting the sclerotial germination under *in vitro* condition could be used as soil application to reduce the primary inoculumin the field.

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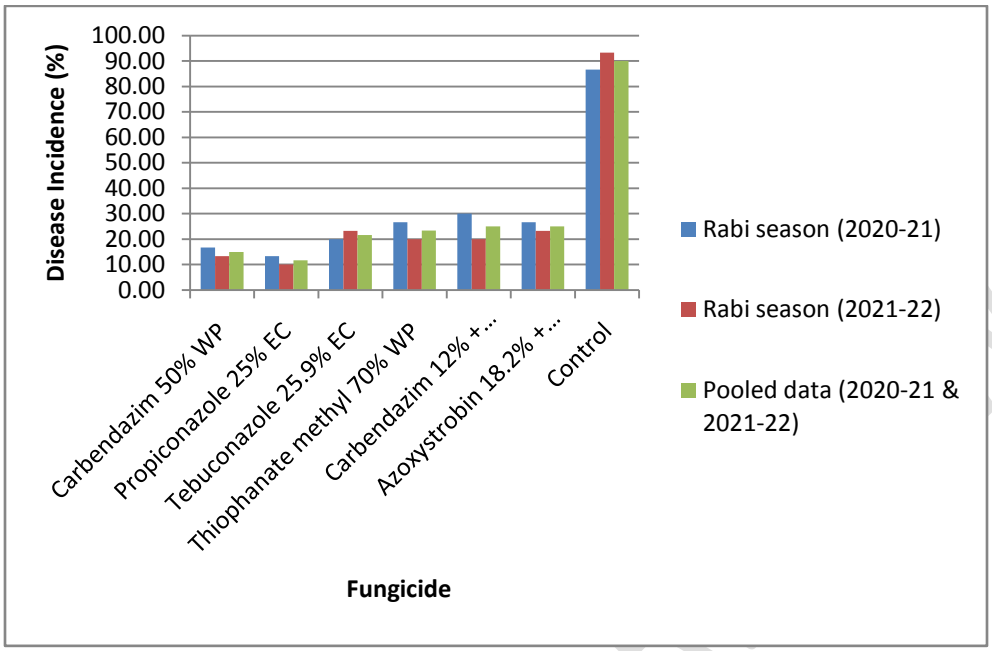


Fig. 1: Effect of Fungicides against Sclerotinia stem rot pathogen under artificial inoculation conditions in field

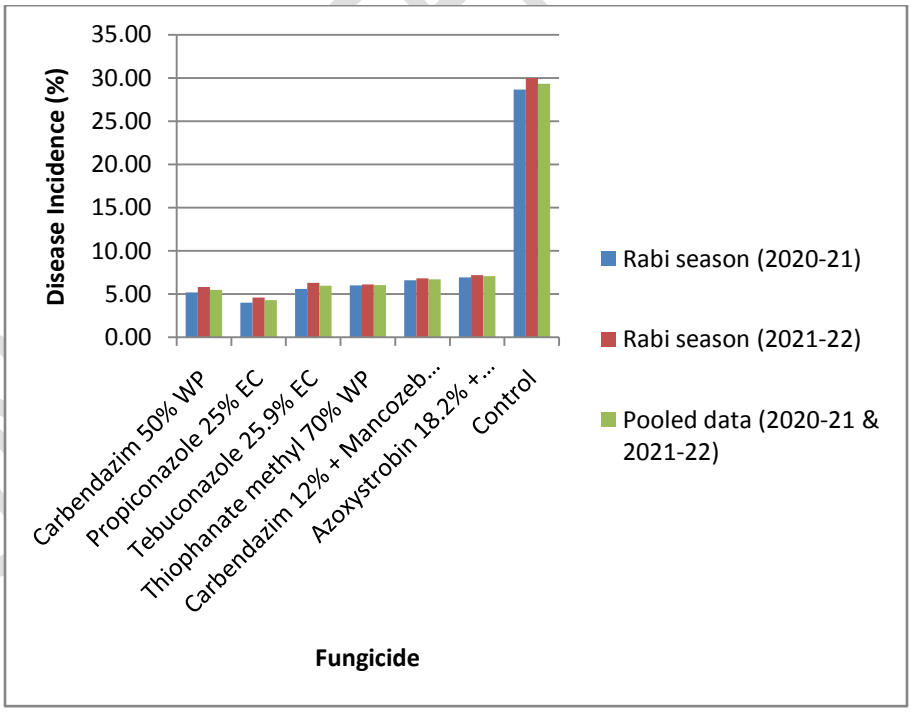


Fig. 2: Effect of fungicides against Sclerotinia rot disease under natural epiphytotic conditions in field

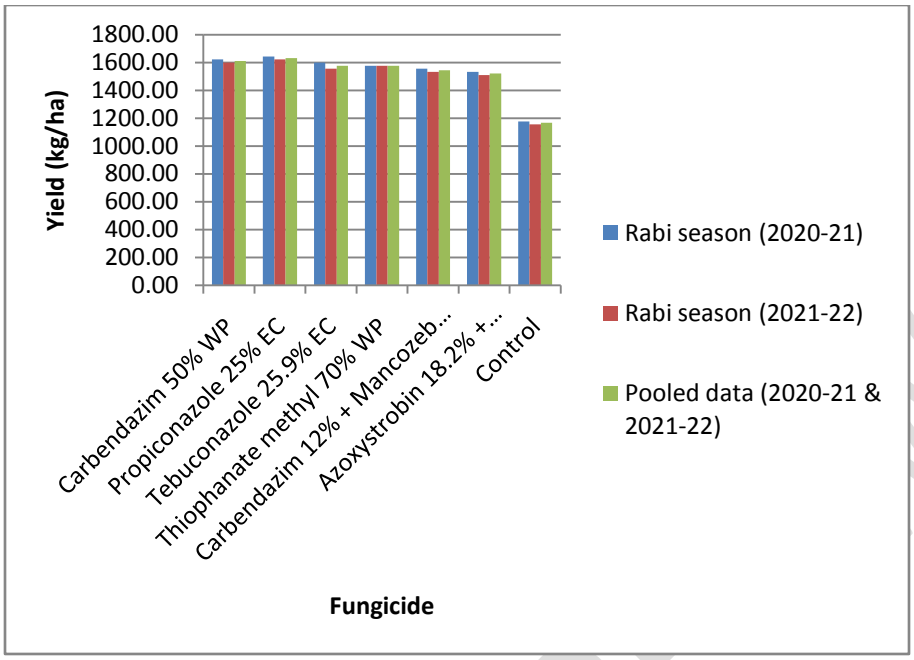


Fig. 3: Yield in different Fungicides tested against Sclerotinia rot disease under natural epiphytotic conditions in field

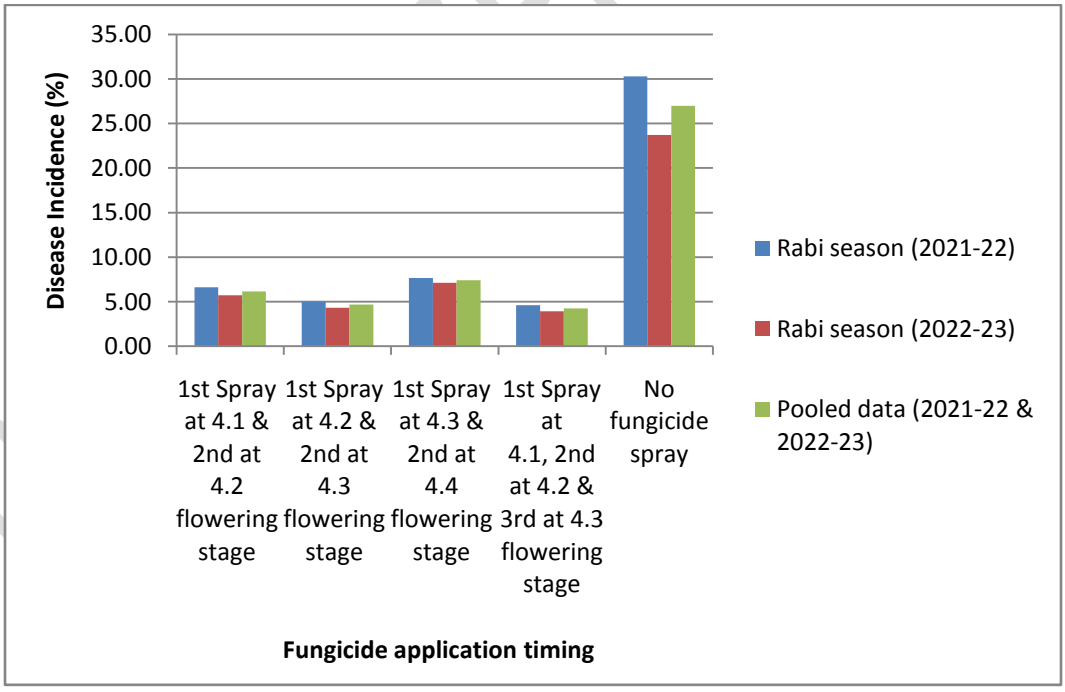
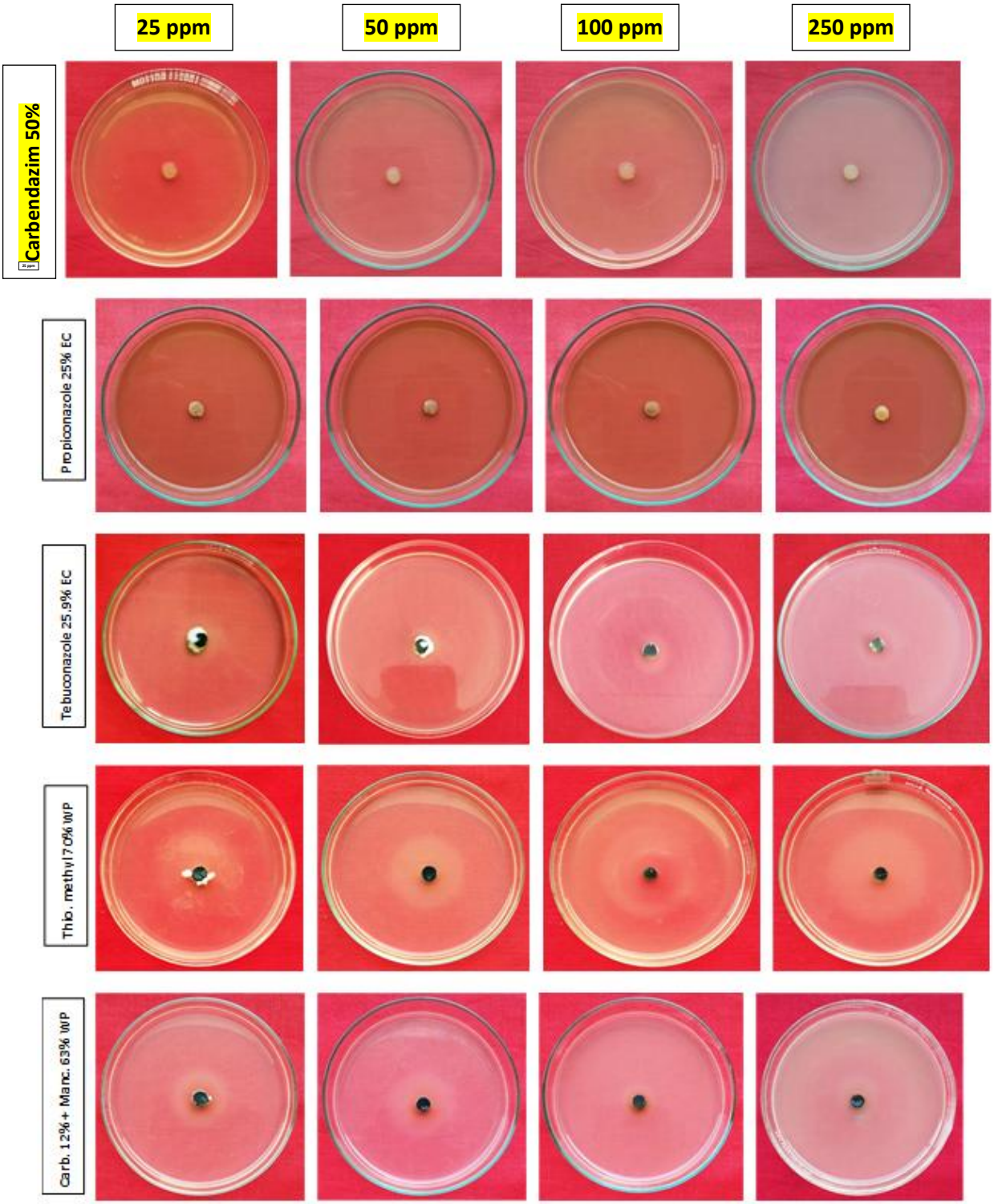


Fig. 4: Effect of Fungicide against Sclerotinia rot disease at different flowering stage of mustard


25 ppm
50 ppm
100 ppm
250 ppm
Carbendazim 50%
Propiconazole 25% EC
Tebuconazole 25.9% EC
Thio. methyl 70% WP
Carb. 12% + Manc. 63% WP

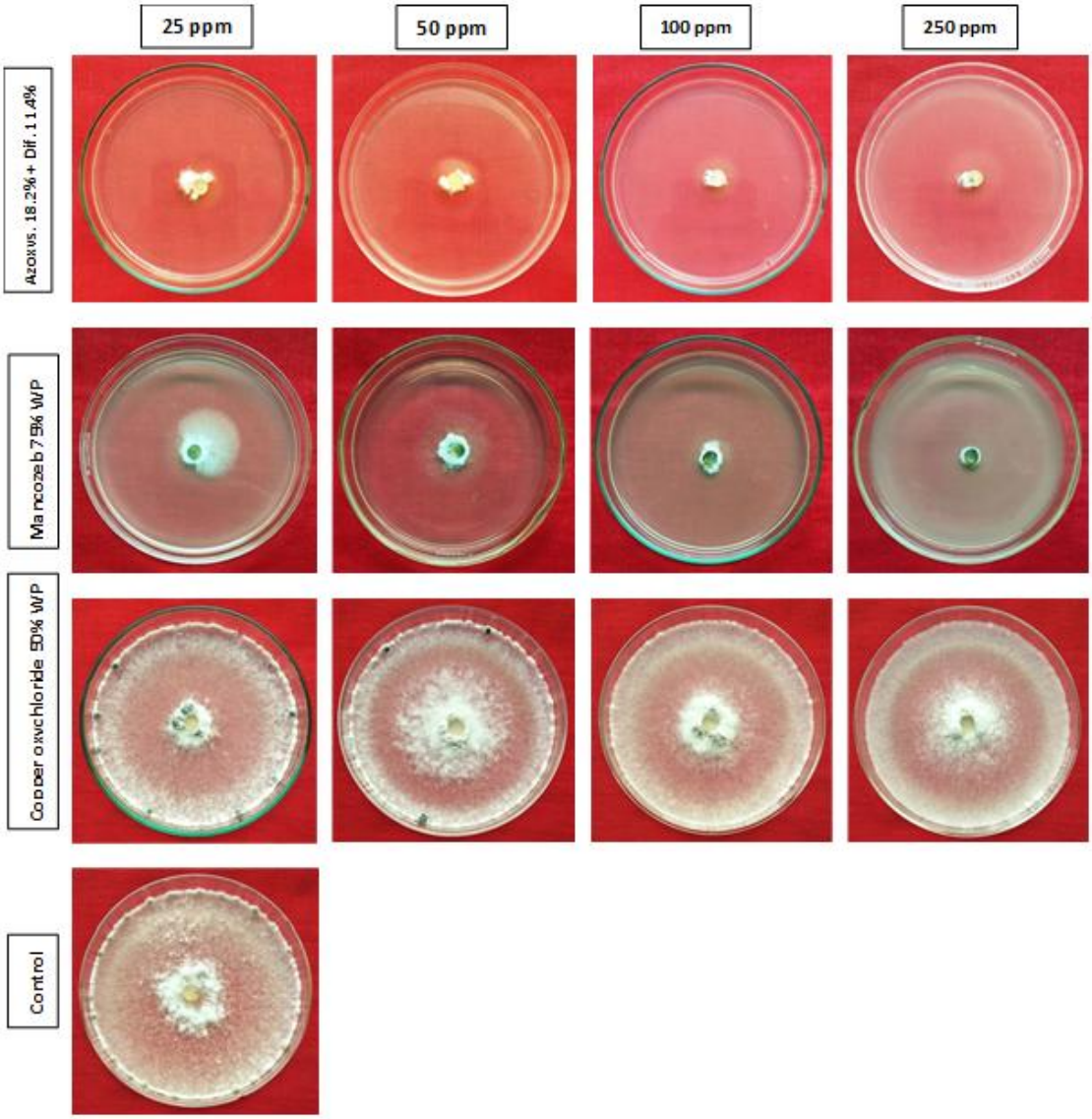


Plate 1: Effect of fungicides against mycelial growth of *S. sclerotiorum* at different concentrations

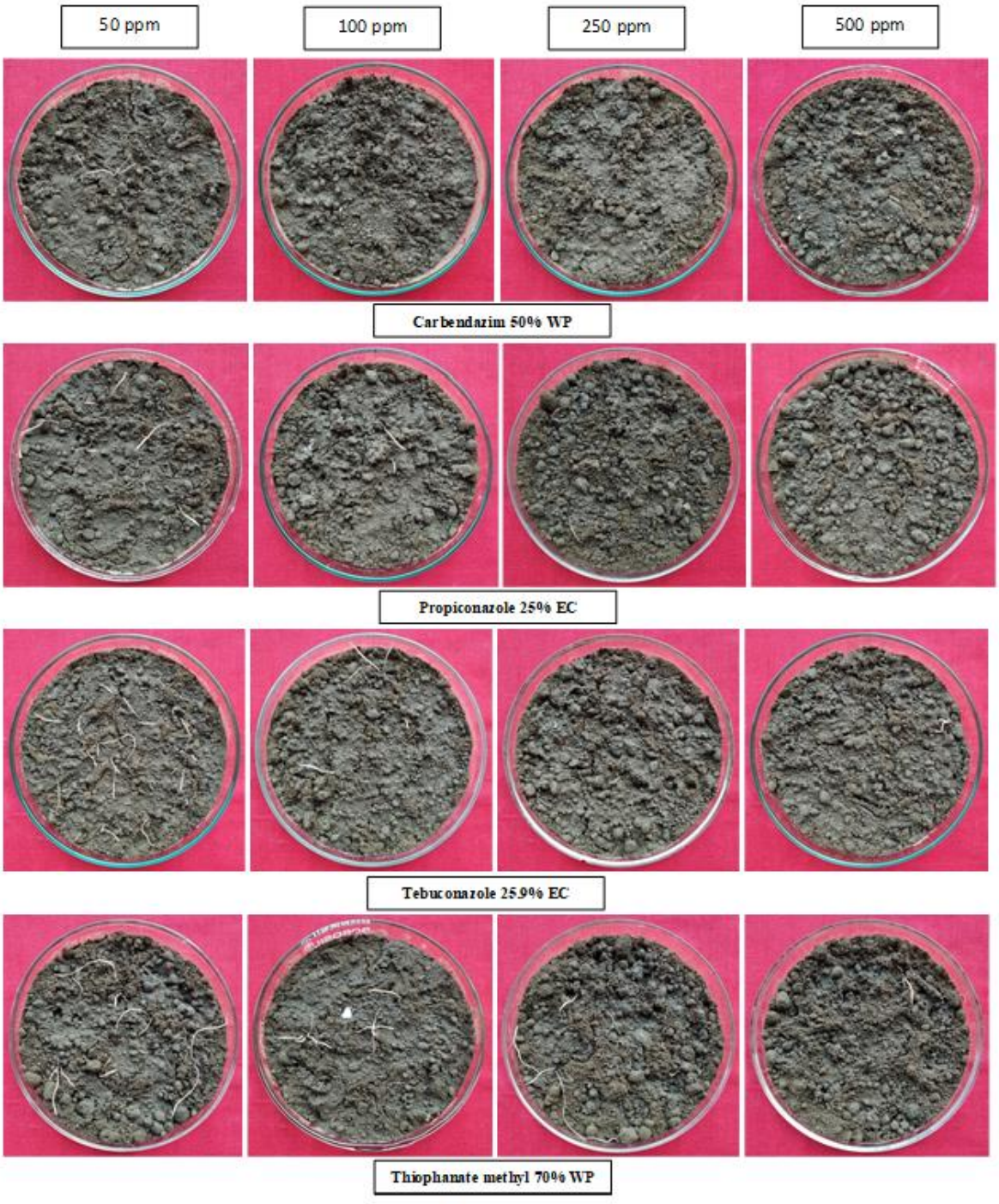

50 ppm
100 ppm
250 ppm
500 ppm
Carbendazim 50% WP
Propiconazole 25% EC
Tebuconazole 25.9% EC
Thiophanate methyl 70% WP

Plate 2: Effect of fungicides against sclerotial germination (carpogenic) of *S. sclerotiorum* at different concentrations