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# Efficacy of Fungicides and Optimization of Application Timing for the Management of Sclerotinia Rot of Mustard Caused by Sclerotinia sclerotiorum

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Sclerotinia rot of rapeseed-mustard caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has gained significant importance in 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 have not been found very effective. Therefore, the present investigations were carried out

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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 the field, 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 disease incidence 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 vield 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 2<sup>nd</sup> spray at 4.3 flowering stage i.e. 50% bloom stage (85 DAS) was equally effective in disease incidence reduction (82.70%) with 03 applications i.e. 1<sup>st</sup> spray at 4.1 flowering stage i.e. 10-20% bloom stage (55 DAS), 2<sup>nd</sup> at 4.2 flowering stage (70 DAS) and 3<sup>rd</sup> 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; Sclerotinia sclerotiorum; rapeseed-mustard; fungicides; management.

## 1. INTRODUCTION

Sclerotinia rot caused by Sclerotinia sclerotiorum (Lib.) de Bary is gaining increased importance over the years in major rapeseed-mustard growing regions of India [1,2]. The disease incidence of Sclerotinia rot of rapeseed-mustard has been measured up to 72% in Rajasthan [3,4] and up to 80% in Punjab and Haryana [5,6]. Plants infected at or before flower initiation resulted in severe vield loss, whereas, infection after flowering stage caused more than 50% vield loss [7,8,9]. S. sclerotiorum continues to be a very difficult pathogen to control due to its' high pathogenicity, wide host range and strong adverse survival ability under 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 [10,11,12]. However, inconsistent and varying degrees of results have been reported with the use of different fungicides in controlling this disease. Use of fungicides in inhibiting the carpogenic germination of sclerotia through soil application has also been reported [13]. Moreover, the efficacy of foliar application of fungicides also depends on application timing i.e. during flowering stage/bloom stage [14,15,12]. Therefore, the objective of this research was to 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 increasing concentrations (25, 50, 100 and 250 ppm) to investigate mycelial growth inhibition of the test pathogen under laboratory conditions by following Poisoned Food Technique [16]. Stock solution of 10,000 ppm of each fungicide was prepared by adding the required quantity of fungicide (as mentioned in List 1) in 10 ml sterilized distilled water. Then, the requisite fungicide concentrations of each 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:

Mycelial Growth Inhibition (%) = 
$$\frac{C - T}{C} \times 100$$

where,

C = mycelial growth of test pathogen in control T = mycelial growth of test pathogen in treatment

## 2.1.2 Sclerotial 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. [13] 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 Petri plates. Then, 15 sclerotia/Petri plate were buried and covered with soil of 2.0 mm thickness over sclerotia. Stock solution of 10,000 ppm was prepared by adding the required quantity of fungicide in sterilized distilled water which was used for the preparation of different concentrations (50, 100, 250 and 500 ppm), 15 ml of each fungicide concentration was poured separately in Petri plates 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 of inhibition of sclerotial germination (carpogenic) were calculated by using the formula:

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

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 Sclerotinia Stem rot 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 (3x1.5 m<sup>2</sup> 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 2<sup>nd</sup> and 3<sup>rd</sup> 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 aiven below:

 $\label{eq:Disease Incidence (%)} \text{Disease Incidence (%)} = \frac{\text{number of infected plants}}{\text{total number of inoculated plants}} \times 100$ 

#### 2.2.2 Under natural epiphytotic conditions

The aerial spray of effective fungicides was given @ 0.1% for three times i.e. 1<sup>st</sup> spray (55 DAS), 2<sup>nd</sup> spray (70 DAS) and 3<sup>rd</sup> 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 DAS and percent disease incidence was calculated. The yield (kg/ha) was also recorded.

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

SI. 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

List 1. Details of fungicides

#### List 2. Details of experiment and spray schedule

Trea	atment	Timing and number of application in relation to stage of crop
T1	Propiconazole @ 0.1%	1 <sup>st</sup> Spray at 4.1 flowering stage i.e. 10-20% bloom stage (55 DAS)
		and 2 <sup>nd</sup> at 4.2 flowering stage i.e. 30% bloom stage (70 DAS)
T2	Propiconazole @ 0.1%	1 <sup>st</sup> Spray at 4.2 flowering stage i.e. 30% bloom stage (70 DAS)
		and 2 <sup>nd</sup> at 4.3 flowering stage i.e. 50% bloom stage (85 DAS)
Т3	Propiconazole @ 0.1%	1 <sup>st</sup> Spray at 4.3 flowering stage i.e. 50% bloom stage (85 DAS)
		and 2 <sup>nd</sup> at 4.4 flowering stage i.e. >50% bloom stage (100 DAS)
Τ4	Propiconazole @ 0.1%	1 <sup>st</sup> Spray at 4.1 flowering stage i.e. 10-20% bloom stage (55 DAS),
	-	2 <sup>nd</sup> at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 3 <sup>rd</sup> at
		4.3 flowering stage i.e. 50% bloom stage (85 DAS)
T5	Control	No fungicide spray

## 2.3 Optimization of Stage of Flowering 3 RESULTS AND DISCUSSION 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 [17] or at 10-20%, 30%, 50% and >50% bloom stage of canola plant as given by Anonymous [18], 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:

Disease Incidence (%) =  $\frac{\text{number of infected plants}}{\text{number of infected plants}} \times 100$ 

## 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

## 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 the Poisoned 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, these were 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, respectively and 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 difference in mycelial growth inhibition at all concentrations was Carbendazim WP, observed in 50% 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 [19], Chand et al. [20], Bharti et al. [21], Rakesh et al. [22] and Sharma et al. [23]. In addition, Carbendazim 12% + Mancozeb 63% WP and Thiophanate methyl 70% WP also resulted complete inhibition of mycelial growth at 50, 100 and 250 ppm which was in agreement with Goswami et al. [24] who that Thiophanate methyl reported and Carbendazim + Mancozeb (SAAF) completely inhibited the mycelial growth of S. sclerotiorum at 250 µg a.i./ml.

## 3.1.2 Sclerotial 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 showed high effectivity in 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 be significantly 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 while Azoxystrobin 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. [13] reported inhibition of sclerotia germination (44.4%) of S. sclerotiorum Carbendazim @0.1%. In by 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.

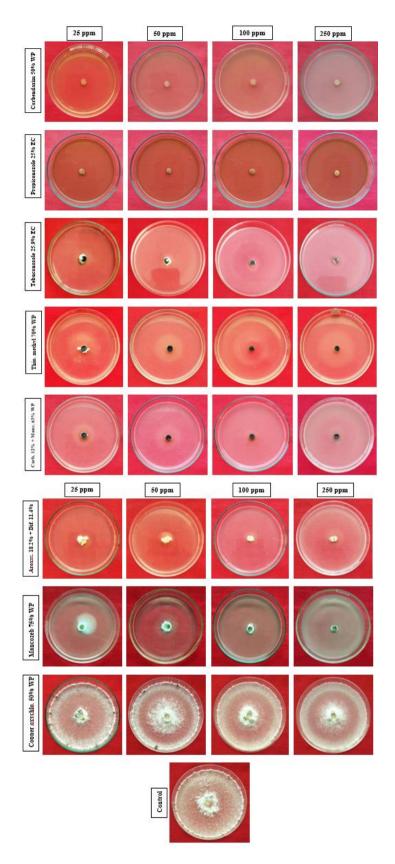


Plate 1. Effect of fungicides against mycelial growth of S. sclerotiorum at different concentrations

Fungicide	Mycelial growth (mm)				Mycelial growth inhibition (%)				Sclerotia formation (no.)			
	25 ppm	50 ppm	100 ppm	250 ppm	25 ppm	50 ppm	100 ppm	250 ppm	25 ppm	50 ppm	100 ppm	250 ppm
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% +	9.00	0.00	0.00	0.00	90.00	100.00	100.00	100.00	1.67	0.00	0.00	0.00
Mancozeb 63% WP												
Azoxystrobin 18.2% +	20.00	17.33	13.00	09.00	77.78	80.74	85.56	90.00	1.67	2.00	1.67	0.67
Difenconazole 11.4% SC												
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	

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

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

Fungicide		Sclerotia g	ermination	(no.)	Sclerotia germination (%)				Germination inhibition (%)			
-	50	100	250	500	50	100	250	500	50	100	250	500
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	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%	6)	S.E. (m)		C.D. (5%)		S.E. (m)		C.D. (5%	6)	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	

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

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



Plate 2. Effect of fungicid es against sclerotial germination (carp ogenic) of S. sclerotiorum at different concentrations

#### 3.2 Evaluation of Promising Fungicides against Sclerotinia Stem rot in Field

#### 3.2.1 Under artificial inoculation conditions

Six effective fungicides (as mentioned in 3.1.2) at a 0.1% concentration were applied 03 times as aerial spray on whole of the plants including inoculated portion. The results presented in Table 3. and Fig. 1 showed that 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 Carbendazim 50% WP. viz. 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 trends were 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.

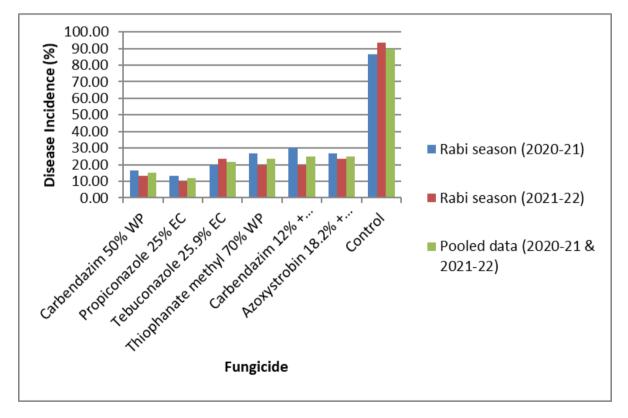


Fig. 1. Effect of Fungicides against Sclerotinia stem rot pathogen under artificial inoculation conditions in field

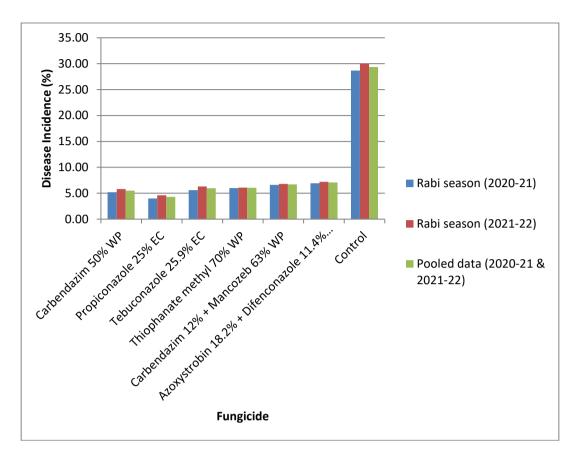


Fig. 2. Effect of fungicides against Sclerotinia rot disease under natural epiphytotic conditions in field

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. [22] 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 [25] also stated that foliar application of Tebuconazole @ 1.5 I/ha and Azoxystrobin @ 0.5 kg/ha one day after artificial inoculation of 58.3 and 57.2% respectively over untreated control.

#### 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 4 and Fig. 2 revealed that all the fungicides were significantly effective over control in reducing the disease incidence. In Rabi season 2020-21 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 with control (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 other in disease incidence and disease reduction but significantly different with control (29.34% DI).

yield (kg/ha) of different treatments The presented in Table 5 and Fig. 3 showed nonsignificant differences among the fungicide treatments but showed 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/ha respectively 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 EC, application of Propiconazole 25% 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. [26], Kumar and Prasad [27], Ghasolia and Shivpuri [28], Rathi et al. [29] Sharma et al. [30] and Roy et al. [31] also reported Carbendazim as effective fungicide against Sclerotinia rot of Indian mustard. Rakesh et al. [22] 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.

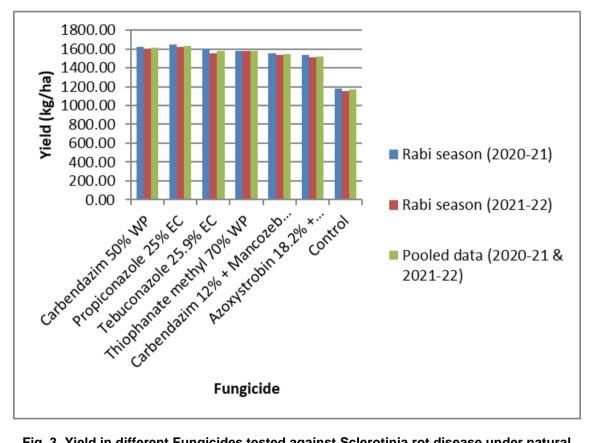


Fig. 3. Yield in different Fungicides tested against Sclerotinia rot disease under natural epiphytotic conditions in field

Fungicide	Rabi season (20	)20-21)	Rabi season (20)	21-22)	Pooled data (202	0-21 & 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% + Difenconazole 11.4% SC Control	6.93 (15.26) 28.67	75.83	7.20 (15.55) 30.00	76.00	7.07 (15.41) 29.34	75.90
	(32.35)		(33.18)		(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)	

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

Note: Numbers in parentheses are angular transformed values

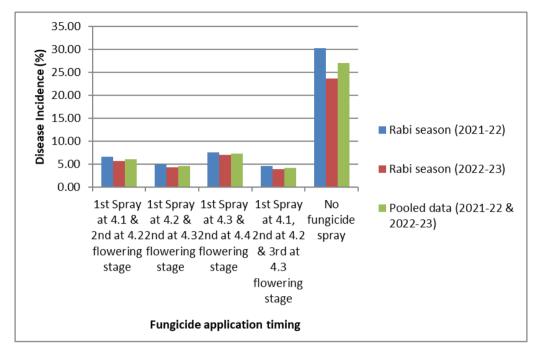
Fungicide	Rabi season (2020-21)			Rabi season (2021-22)			Pooled data (2020-21 & 2021-22)		
-	Kg/ plot	Kg/ha	Increased yield (%)	Kg/plot	Kg/ha	Increased yield (%)	Kg/plot	Kg/ha	Increased yield (%)
Carbendazim 50% WP	0.73	1622.22	37.74	0.72	1600.00	38.46	0.73	1611.11	38.09
Propiconazole 25% EC	0.74	1644.44	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% +	0.70	1555.56	32.08	0.69	1533.33	32.69	0.70	1544.45	32.38
Mancozeb 63% WP									
Azoxystrobin 18.2% +	0.69	1533.33	30.19	0.68	1511.11	30.77	0.69	1522.22	30.48
Difenconazole 11.4% SC									
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	

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

## 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 &	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		
Т3	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



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

## 3.3 Optimization of Stage of Flowering for Foliar Application

To optimize appropriate time of application for the aerial spraying of 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), 2<sup>nd</sup> 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. 1<sup>st</sup> Spray at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 2<sup>nd</sup> 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% DI in 2021-22 & 2022-23 respectively).

The pooled data of both the years also showed treatment T4 as the most effective treatment by

showing least disease incidence (4.25%) and was found at par with treatment T2 with disease incidence (4.67%) but significantly different other treatments and control (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 2<sup>nd</sup> 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), 2<sup>nd</sup> at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 3<sup>rd</sup> at 4.3 flowering stage i.e. 50% bloom stage (85 DAS) and therefore, two aerial is recommended for spray the management of Sclerotinia rot of mustard. Khangura and van Burgel [12] 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.

## 4. CONCLUSION

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 the

different spray schedules two applications of Propiconazole @0.1% i.e. 1<sup>st</sup> spray at 4.2 flowering stage i.e. 30% bloom stage (70 DAS) and 2<sup>nd</sup> at 4.3 flowering stage i.e. 50% bloom stage (85 DAS) was at par with three aerial applications at 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 inoculum in the field.

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## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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