International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 2762-2766

Received: 01-07-2019
Accepted: 03-08-2019

Pooja Upadhyay
Centre of Advanced Studies, Department of Plant Pathology, College of Agriculture, GBPUA&T Pantnagar, U.S. Nagar, Uttarakhand, India

AK Tewari
Centre of Advanced Studies, Department of Plant Pathology, College of Agriculture, GBPUA&T Pantnagar, U.S. Nagar, Uttarakhand, India

Corresponding Author:
Pooja Upadhyay
Centre of Advanced Studies, Department of Plant Pathology, College of Agriculture, GBPUA&T Pantnagar, U.S. Nagar, Uttarakhand, India

Evaluation of fungicides *in vitro* and under natural conditions against *Sclerotinia sclerotiorum* (Lib.) de Bary, the cause of Sclerotinia rot of rapeseed-mustard

Pooja Upadhyay and AK Tewari

Abstract
For the management study of Sclerotinia rot of rapeseed-mustard caused by *Sclerotinia sclerotiorum* (lib.) de Bary, different fungicides alone (6 no.) and in their combinations (15 no.) were tested *in vitro* at concentrations of 25, 50, 100, 250 µg a.i./ml and under field conditions against the pathogen. Among them, Iprodione was found most effective with (95.5% inhibition) of mycelial growth, followed by Propaconazole (94.4%) and Thiophenate methyl (88.8%) inhibition at 25 µg a.i./ml concentration. Among different fungicide combinations, at 25 µg a.i./.concentration, 95.3%, 94.4% and 86.6% inhibition found with Iprodione + Thiophenate methyl, Iprodione + Propaconazole and Iprodione + Carbendazim respectively, Under field conditions, Iprodione, Thiophenate methyl, Propaconazol, (Iprodione + Thiophenate methyl), (Iprodione + Propaconazol) and (Thiophenate methyl + Propaconazol) were found most effective with disease reduction, ranging between 61.7-66.3 per cent. Mencozeb was found least effective *in vitro* and under field conditions in managing the disease.

Keywords: Fungicides, *Sclerotinia sclerotiorum*, rapeseed-mustard, mycelial growth inhibition

Introduction
Rapeseed-mustard crop occupies a premier position in India accounting for 25 per cent of total oil seed production (Anonymous, 2006) [1]. It is grown mainly in North-Western and Central part of India in different ecosystem and cropping sequences. Rapeseed and Mustard seeds are the most important sources of edible oil with oil content ranging between 30 to 48 percent. Sclerotinia rot of Indian mustard (*Brassica juncea*) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has been reported from major rapeseed and mustard growing states of India eg. Assam, Uttar Pradesh, Haryana, Punjab, Rajasthan, Madhya Pradesh (Horning, 1983; Regnault and Pierre, 1984; Kang and Chahal, 2000) [6, 12, 7]. The disease was of minor importance till few years back, but recently it has assumed 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; Krishna *et al*., 2000; Shivpuri *et al*., 2000) [3, 10, 4, 7, 9, 18]. The pathogen is reported to have a wide host range, known to infect about 400 plant species (Kolte, 1985) [8]. Still disease has not been controlled consistently and economically. The explosive pathogenicity of the fungi under favourable conditions and the ability of the sclerotia to withstand adverse conditions allow them to be successful pathogen on many crops. Use of chemicals has been a practical and effective method of controlling the disease since years. 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; Mueller *et al.* 2002; Chattopadhyay *et al.* 2002) [13, 14-15, 17, 11, 2]. The present studies were conducted to screen out the effective fungicides and their combination which may successfully inhibit the pathogen growth and could be exploited for the effective management of the Sclerotinia rot under natural conditions.

Material and method
In vitro evaluation of fungicides
Six systemic and non systemic fungicides which were found effective against *Sclerotinia sclerotiorum* by earlier researchers in previous studies, were evaluated alone (6 no.) and in

their combination (15 no.) at four different concentration *viz.*, 25, 50, 100 and 250 µg a.i./ml against the pathogen under *in vitro* condition, to find out the relative efficacy of fungicides in inhibiting the radial growth of the pathogen. The effect of fungicides on radial growth of the pathogen was evaluated by 'Poisoned Food Technique' (Schmitz, 1930) [16]

Effect on mycelial 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 Petriplates. 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/check. 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 (Bliss, 1934).

$$\text{Per cent inhibition over control} = \frac{C - T}{T} \times 100$$

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

Evaluation of fungicides and their combination under field condition

Fungicides and their combinations were evaluated under field condition as seed treatment and foliar spray. For seed treatment, seeds were dipped in the fungicide solution for 1 hour before sowing. Plot with untreated seeds was served as control/check. For foliar spray, two sprays were done at 30 days and 45 days after sowing. After the 24 hrs of the first spray inoculation of the test pathogen, *Sclerotinia sclerotiorum* was done.

For preparation of fungicide spray solution, systemic fungicides viz. Bayleton, Thiophenate methyl, Carbendazim and non systemic fungicides viz. Iprodione and Propaconazole were applied @ 1gm/lit and @ 2 gm/ lit of water respectively. In combination of these fungicides half of the amount of both the fungicides was used for application.

Inoculation

Inoculation of the plants was done by mycelial disc method. Ten plants were inoculated in each replication. For inoculation, mycelial discs (2 no.) of 5.0 mm dia. were cut from the margin of a 3 days-old-culture of the pathogen grown on PDA and was placed at the middle portion of the main stem near to branching. Inoculated portion of the stem was covered with cotton, soaked in sterilized water and wrapped with plastic tape to conserve moisture.

Observation

Inoculated plants were examined periodically for the disease symptoms and final data were recorded 30 days after inoculation (DAI). Disease incidence was calculated by following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{No. of inoculated plants}} \times 100$$

Results and Discussion

Effect of fungicides and their combinations *in vitro*

Six fungicides (systemic and non-systemic) and their combinations (15 no.) were tested *in vitro* for their efficacy against the test pathogen. The data revealed that all the fungicide and their concentrations except Mancozeb considerably inhibited radial growth of the pathogen at 250 µg a.i./ml concentration. Among all the fungicides evaluated alone, Iprodione was found most effective in inhibiting radial growth of fungus (95.5%) followed by Propaconazole (94.4%) and Thiophenate methyl (88.8%) at 25 µg a.i./ml and were statistically at par but significantly different from other treatments. Similar trend of result was observed at other concentrations also. At maximum concentration of 250 µg a.i./ml Iprodione and Propiconazole showed 100% inhibition of radial growth followed by Thiophenate methyl (97.5).

Among different combinations of these fungicides with each other Iprodione + Thiophenate methyl showed 95.3 per cent mycelial inhibition over check followed by Iprodione + Propaconazole (94.4%) and Iprodione + Carbendazim (86.6%) at minimum concentration of 25 µg a.i./. These were at par with each other but significantly different from other treatments in inhibiting mycelial growth. Complete inhibition of radial growth was observed with Iprodione + Thiophenate methyl at 100 µg a.i./. Iprodione + Thiophenate methyl and Iprodione+ Propaconazole at 250 µg a.i./. Mencozeb was found least effective in inhibiting the growth of the pathogen alone as well as in combination with other fungicides. The results obtained in the present study are in close agreement with the findings of earlier workers in the effectiveness of the fungicides who also reported Carbendazim and Thiophenate methyl at 0.05 per cent (Shivpuri and Gupta, 2001) [17], Iprodione at 0.5% (Sharma and Kapoor, 1997) [15] Iprodione and Carbendazim at 0.2% (Singh, 1998) completely inhibited the mycelial growth of the *S. sclerotiorum.* However, in the present investigation complete inhibition of the pathogen was observed at lower concentrations (50/100/250 µg a.i./ml) as compared to earlier reports (0.05/0.2/0.5%).

Effect of fungicides and their combinations against Sclerotinia rot under field condition

Five fungicides along with their combinations (11 no.) which were found very effective *in vitro* were evaluated as seed treatment and as foliar spray (I^{st} at 24 hrs. before inoculation of the test pathogen at flowering stage and II^{nd} 15 days after Ist spray) against the pathogen under field conditions. Among all the fungicides and their combinations Iprodione, Thiophenate methyl, Propaconazol, (Iprodione + Thiophenate methyl), (Iprodione + Propaconazol) and (Thiophenate methyl + Propaconazol) were found more fungitoxic than others with significant reduction in disease incidence, ranging between 61.7 to 66.3 per cent followed by Carbendazim, (Iprodione + Carbendazim), (Iprodione + Bayleton) and (Propaconazol + Carbendazim) with a significant disease reduction ranging between 54.3 to 55.1 per cent. However, (Iprodione + Mancozeb) was found least effective against the pathogen with 18.2 per cent reduction in disease incidence.

The results obtained in the present investigation are more or less in accordance with the findings of the earlier workers (Vulsteke and Meeus, 1982; Singh, 1994; Zewain *et al*., 2005; and Herd *et al*., 2007) [21, 19, 22, 5] who reported the

effectiveness of Iprodione and Thiophenate methyl @ 0.1-0.2% (snap bean), carbendazim @ 0.1-0.2% (mustard), carbendazim @ of 0.1-0.15% (cauliflower), and Iprodione @ 0.2% (sun flower) respectively against Sclerotinia rot as seed treatment and/or foliar spay. Present study also supports earlier findings as fungicides treatment being a strong management tool against sclerotinia infestation. In earlier findings, fungicides viz. Carbendazim Ipridone and Thiophenate methyl were proved to be best against the pathogen in most of the crops but in the present study combinations of these fungicides were also found very effective against the disease that could be exploited for the crop health against a number of diseases/pathogens the crop.

Table 1: *In vitro* evaluation of fungicides against mycelial growth of *Sclerotinia sclerotiorum* (lib.) de Bary

Fungicide	Concentration (µg a.i./ml)							
	25		50		100		250	
	*RG (mm)	*I (%)	*RG (mm)	*I (%)	*RG (mm)	*I (%)	*RG (mm)	*I (%)
Iprodione	4.0	95.5	3.0	96.6	2.0	97.7	0.00	100
Thiophenate methyl	10.0	88.8	8.0	91.1	6.2	93.06	2.2	97.5
Carbendazim	10.5	88.2	9.5	89.4	7.0	92.2	4.0	95.5
Propaconazol	5.0	94.4	4.5	94.9	3.5	89.4	0.00	100
Bayleton	20.0	77.7	15.5	82.73	12.0	86.6	9.0	89.9
Mencozeb	90.0	0.00	85.0	5.50	35.5	60.53	18.0	79.96
Iprodione + Propaconazol	5.0	94.4	4.5	94.9	3.5	89.4	0.00	100
Iprodione + Thiophenate methyl	4.2	95.3	2.5	97.16	0.00	100	0.00	100
Iprodione + Bayleton	15.5	82.73	10.5	88.2	7.0	92.2	4.5	95.5
Iprodione + Carbendazim	12.0	86.6	7.9	91.19	6.2	93.06	4.0	86.6
Thiophenate methyl + Propaconazol	20.5	58.6	15.6	37.1	8.7	90.3	5.6	93.73
Thiophenate methyl + Carbendazim	40.5	55.06	36.0	59.9	15.5	82.7	12.0	86.6
Thiophenate methyl + Bayleton	65.5	27.2	40.5	54.9	30.0	66.6	20.6	77.06
Propaconazol + Bayleton	40.0	55.5	20.5	77.1	12.5	86.06	10.5	88.2
Propaconazol + Carbendazim	30.0	66.6	15.6	37.1	10.5	88.2	7.5	91.6
Carbendazim + Bayleton	70.0	22.2	42.0	53.3	35.0	61.1	20.0	77.7
Iprodione +Mencozeb	90.0	0.00	75.5	16.06	25.5	71.6	11.6	87.06
Thiophenate methyl + Mencozeb	85.0	5.5	60.5	32.7	25.0	72.2	13.5	84.9
Bayleton+ Mencozeb	85.5	4.96	70.0	22.2	50.0	44.4	42.6	52.59
Carbendazim+ Mencozeb	90.0	0.00	80.5	10.5	65.0	27.7	49.0	45.5
Propaconazol+ Mencozeb	87.0	3.30	84.5	6.06	70.0	22.2	55.0	38.8
Check	90.0	0.00	90.0	0.00	90.0	0.00	90.0	0.00
	Fungicide (A)			Concentration (B)			A X B	
Per cent inhibition								
CD (0.05)	0.87			2.01			4.02	
CV (%)	4.50							
Radial growth								
CD (0.05)	0.25			0.58			1.16	
CV (%)	2.36							

*Mean of three replications
RG- Radial growth,
I- inhibition
Transformed values were used for analysis

Table 2: Evaluation of fungicides against Sclerotinia rot of rapeseed-mustard under field/natural condition

Treatment	*Total plants (no)	*Inoculated plants (no)	* Disease Incidence (%)	*Disease Reduction (%)
Iprodione (50wp)	10.0	3.0	30	66.0 (54.6)
Thiophenate methyl (70wp)	10.0	3.0	30	66.0 (54.6)
Carbendazim (50wp)	10.0	4.0	40	55.1 (47.9)
Propaconazol (25EC)	10.0	3.0	30	65.9 (54.5)
Bayleton (25wp)	10.0	4.0	40	54.3 (47.5)
Iprodione (50wp)+ Thiophenate methyl (70wp)	10.0	3.0	30	66.3 (54.5)
Iprodione (50wp)+ Propaconazol (25EC)	10.0	3.0	30	66.0 (54.6)
Iprodione (50wp)+ Carbendazim (50wp)	10.0	4.0	40	54.8 (47.85)
Iprodione (50wp)+ Bayleton (25wp)	10.0	4.0	40	54.3 (47.56)
Thiophenate methyl (70wp)+ Propaconazol (25EC)	10.0	3.0	30	61.7 (52.12)
Thiophenate methyl (70wp)+ Carbendazim (50wp)	10.0	5.6	56	36.9 (37.4)
Thiophenate methyl (70wp)+ Bayleton (25wp)	10.0	5.0	50	43.1 (40.85)
Propaconazol (25EC)+ Bayleton (25wp)	10.0	5.6	56	35.1(31.05)
Propaconazol (25EC)+ Carbendazim (50wp)	10.0	4.0	40	55.1 (47.97)
Carbendazim (50wp)+ Bayleton (25wp)	10.0	4.3	43	51.4 (45.85)
Iprodione (50wp)+Mencozeb (75wp)	10.0	7.3	73	18.2 (25.12)
Check	10.0	9.0	90	0.00 (0.00)
CD (0.05)		1.52	15.28	12.5
CV (%)		20.8	20.8	17.2

*Mean of three replications; Values in parenthesis are angular transformed

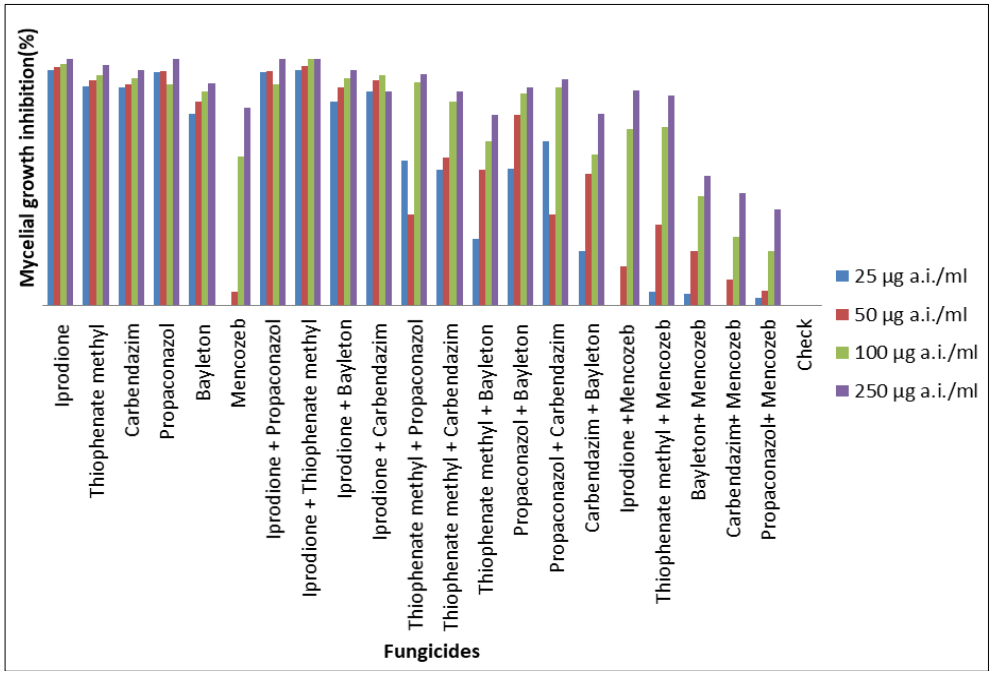

Fig 1: Evaluation of fungicides against *Sclerotinia sclerotiorum in vitro*

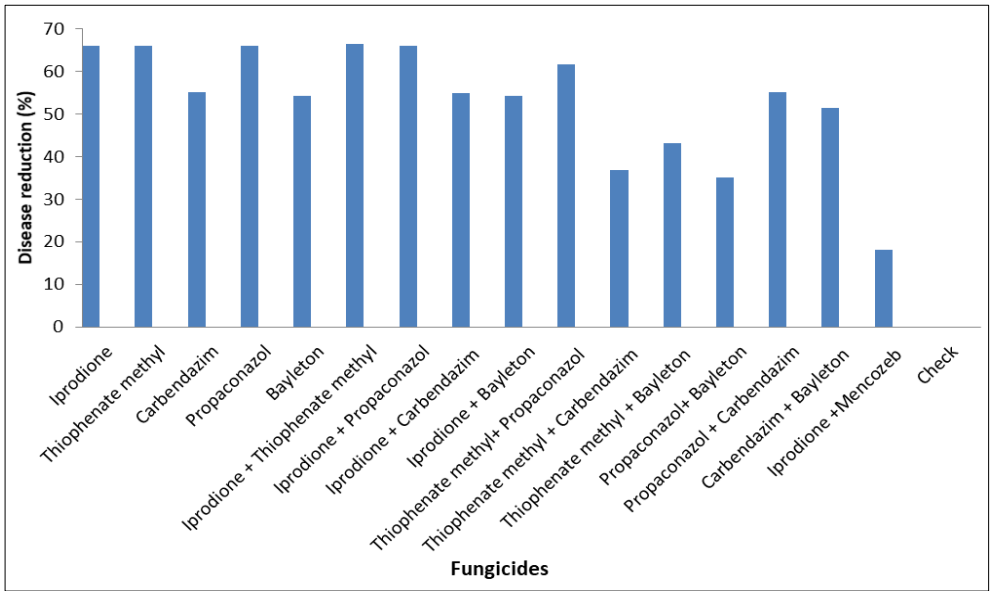

Fig 2: Screening of fungicides against *Sclerotinia sclerotiorum* under field

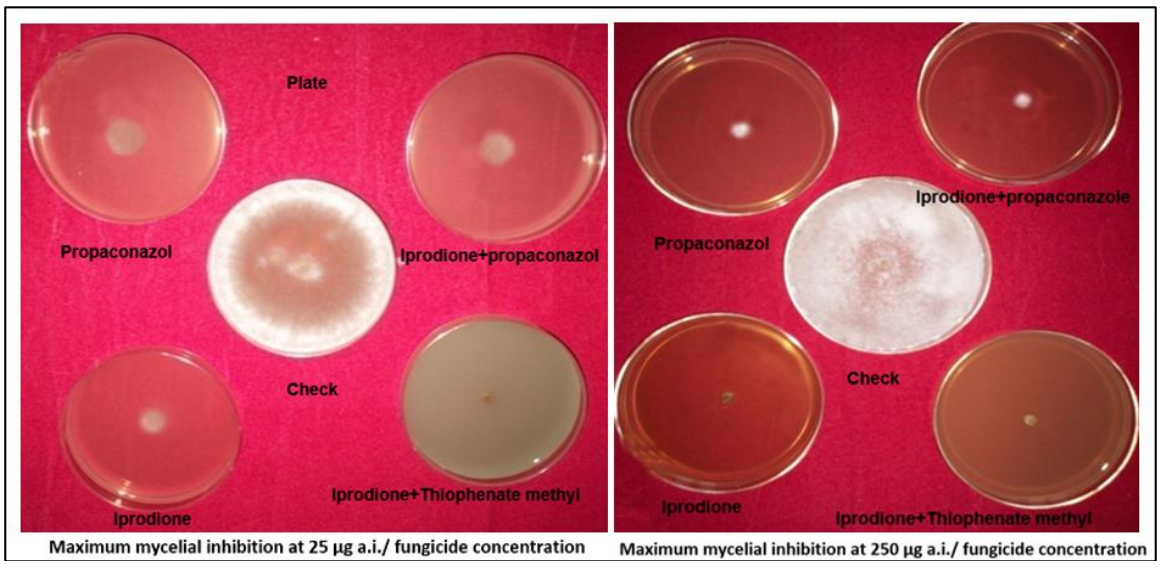

Fig 3: *In vitro* effect of fungicides and their combinations on *Sclerotinia sclerotiorum* (lib.) *de Bary*

References

1. Anonymous. Annual progress report 2006-07. All India co-ordinated research project (Rapeseed-Mustard). GBPUAT, Pantnager, 2006, pp. 127.
2. Chattopadhyay C, Meena PD, Sudeer K. Management of Sclerotinia stem rot of mustard using eco-freindly strategies. J. Mycol. and pl. pathol. 2002; 32:194-200.
3. Chauhan LS, Singh J, Chandra DR. Assesment of losses due to stem rot to yellow sarson. Proc. Natl Symp on Management of Microbes in Service of Mankind, 19-21 Nov 1992. Allahabad, India, 1992, pp. 65-66.
4. Ghasolia RP, Shivpuri A, Bhargava AK. Sclerotinia rot of Indian mustard (*Brassica juncea*) in Rajasthan. Indian Phytopath. 2004; 57:76-79.
5. Herd GW, Phillips AJL. Control of seed-borne *Sclerotinia sclerotiorum* by fungicidal treatment of Sunflower seed. Plant Pathology. 2007; 37(2):202-205.
6. Horning H. Zur epidemiology and Bekafungder Weibstengelikeit (*Sclerotinia sclerotiorum*). Raps. 1983; 1:32-34.
7. Kang IS, Chahal SS. Prevalence and incidence of white rot of rapeseed and mustard incited by *Sclerotinia sclerotiorum* in Punjab. Plant Dis. Res. 2000; 15:232-233.
8. Kolte SJ. Rapeseed-mustard and sesame diseases, In: Diseases of Annual Edible Oilseed Crops, CRC Press, Boca Raton, Florida, 1985, 135.
9. Krishna SK, Meena PD, Chattopadhyay C. Seed- yield and yield-attributes of Indian mustard affected by Sclerotinia rot. J. Mycol. Pl. Pathol. 2000; 30:265.
10. Lodha BC, Bhatanager MK, Mathur K, Doshi A, Mathur S, Bairwa LN *et al*. Plant Pathological thoughts and News. Department of Plant Pathology, Rajasthan Collage of Agric., Udaipur (India), 1992, 52.
11. Mueller DS, Dorrance AE, Derksen RC, Ozlan E, Kurle JE. Efficacy of fungicides on *Sclerotinia sclerotiorum* and their potential for control of sclerotinia stem rot on soybean. Plant Dis. 2002; 86:26-31.
12. Regnault Y, Pierre JG. Control of *Sclerotinia sclerotiorum* (Lib.) de Bary on oilseed rape in France. In: Aspect of Applied Biology 6. Agronomy, Physiology, Plant Breeding and Crop Protection of Oilseed Rape. Wellesbourne: AAB, 335-360, 1984.
13. Roy AK, Saikia UN. White blight of mustard and its control. Indian J. Agric. Sci. 1976; 46:274-277.
14. Sharma BK, Basandrai AK. Effect of biocontrol agents, fungicides and plant extracts on sclerotial viability of *Sclerotinia sclerotiorum*. Indian J Agric. Sci. 1997; 67:132-133.
15. Sharma CL, AS Kapoor. *In vitro* sensitivity of *Sclerotinia sclerotiorum* to fungicides. Himanchal J Agric. Res. 1997; 23:50-55.
16. Schmitz H. A suggested toximetric method for wood preservations. Inus and Engin. Chem. Anlyt. Ed. II, 1930, 361-663.
17. Shivpuri A, Gupta RBL. Evaluation of different fungicides and plant extracts against *Sclerotinia sclerotiorum* causing stem rot of mustard. Indian Phytopath. 2001; 54:272-274.
18. Shivpuri A, Sharma KB, Chhipa HP. Some studies on the stem rot (*Sclerotinia sclerotiorum*) disease of rapeseed/ mustard in Rajasthan. J Mycol. Pl. Pathol. 2000; 30:268.
19. Singh R, Tripathi NN, Kaushik CD. Management of Sclerotinia rot of Indian mustard (*Brassica juncea* (L.) Czern and Coss.) by fungicides. Crop Res. 1994; 7:276-281.
20. Singh Y. Management of Sclerotinia rot of rapeseed and mustard through chemicals. Plant Dis. Res. 1998; 13:149-150.
21. Vulsteke G, Meeus P. Chemical control of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on dwarf snap bean. Neth. J. Pl. Path. 1982; 88:79-85.
22. Zewain QA, Bahadur P, Sharma P. Integrated disease management of stalk rot of cauliflower. Indian Phytopath. 2005; 58(2):167-173.