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Full Research Article

In Vitro Efficacy of Fungicides on *Sclerotinia sclerotiorum* and their Potential for Control of Stem Rot in Indian Mustard (*Brassica juncea* L.)

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Abstract

Sclerotinia stem rot or stem blight or white rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a serious problem in mustard crop in northern India. Efficacy of six fungicides, carbendazim 50 WP, propiconazole 25 EC, hexaconazole 5 EC, difenaconazole 25 EC, carbendazim 12%+mancozeb 63% 75WP and penconazole 10 EC were tested against *S. sclerotiorum*. Under *in vitro* studies, fungicides carbendazim 50 WP and carbendazim 12%+mancozeb 63% mixture 75 WP at 0.1% concentration completely inhibited the mycelial growth, sclerotial formation and germination of sclerotia. Propiconazole 25 EC, hexaconazole 5 EC, difenaconazole 25 EC, penconazole 10 EC were also found effective at higher concentration (0.05% and 0.1%) but at lower concentrations (less than 0.05%), these fungicides were observed less effective to inhibit the mycelial growth, sclerotial formation and germination of sclerotia. In field experiments during *rabi* 2007-08 and 2008-09, seed treatment with carbendazim 50 WP @ 2 g kg^{-1} and two foliar sprays of same at 0.1% was most effective for disease control and increased seed yield in both the years. In this treatment lowest disease incidence (9.32%), minimum disease intensity (7.50%), highest disease control (76.92%), maximum average production (14.89 q ha^{-1}) and maximum net profit as well as benefit-cost ratio (2.01:1) was recorded. Seed treatment with carbendazim 50 WP @ 2 g kg^{-1} and two foliar sprays of carbendazim 12%+mancozeb 63% 75 WP at 0.2% was also found effective in disease control (59.18%) and increased seed yield (12.96 q ha^{-1}) over untreated control.

1. Introduction

Indian mustard [*Brassica juncea* (L.) Czern and Coss] is a major oilseed crop grown in *rabi* season in various regions of India. Rapeseed and mustard are the major oilseed crops of India with oil contents ranging between 30 to 46%. Among the annual edible oilseeds, rapeseed and mustard contributes about 23% in acreage and over 25% in production for the last five years in India. India holds a leading position in area and production rapeseed and mustard i.e., 6.70 m ha and 7.96 mt, respectively, with an average productivity of 1188 kg ha^{-1}. The main mustard producing states in India are Rajasthan, Madhya Pradesh, Haryana, Uttar Pradesh, Punjab, etc., Among these states, Rajasthan stands first both in area and production i.e., 3.08 m ha and 3.83 mt, respectively, with an average productivity of 1243 kg ha^{-1}. (Directorate of Economics and Statistics, 2014). Some cultural and biotic factors directly influence the production of mustard. Ray et al. (2014) reported that number of irrigations with time period along with sulphur application influenced growth attributes and seed yield in mustard. Under biotic factors diseases directly influence the yield of mustard. Stem rot of Indian mustard is an economically important yield reducing disease that has been widely reported in the last few years in India and elsewhere with the high disease incidence and severe yield losses leading to discouragement of growers of the crop (Krishnia et al., 2000). The disease symptoms usually appear four to six weeks after sowing or at flowering stage, when significant damage has already been done. Sudden drooping of leaves followed by drying of plants are characteristic features of the disease. This disease is gaining importance in the mustard growing areas, which may cause crop failure as the disease incidence was recorded up to 73.8% in some districts of Punjab and Haryana (Kang and Chahal, 2000; Sharma et al., 2001). In Rajasthan, the disease incidence was recorded up to 60% (Ghasolia et al., 2004; Shukla, 2005). Sclerotia are the primary survival structures of *S. sclerotiorum* and act as a source of infection for many years in the field.

The explosive pathogenicity of this fungus under favorable conditions and the ability of its sclerotia to withstand adverse conditions allow it to be a successful pathogen. Due to strict soil borne nature and wide host range of the pathogen, it is difficult to manage the disease only through host resistance or chemical control. The effectiveness of chemicals is erratic because of soil borne nature of the pathogen which makes accessibility of fungicides to sclerotia rather difficult only a small proportion of the fungicide applied that too in a diluted concentration may be ineffective in rendering the sclerotia non-viable. The aim of the present study was to find out the better disease management practice through fungicides to minimize the yield losses. Laboratory and field studies were therefore conducted to evaluate the best fungicide for controlling this disease.

2. Materials and Methods

2.1. In vitro testing of fungicides

2.1.1. Inoculum

An isolate of *S. sclerotiorum* was obtained from diseased mustard plant collected from farmer's field. Small pieces of diseased tissues along with adjoining healthy area and sclerotia found in diseased stem were surface sterilized by dipping in mercuric chloride solution (1:1000) for two minutes followed by three washings with sterile water and blot dried then plated aseptically on Potato Dextrose Agar (PDA)in Petri dishes. These were incubated in BOD incubator for growth of the fungus at 27±2 °C. Sub cultures from pure peripheral growth were made on PDA slants. The pathogenicity of the isolated fungus was proved by mixing 15 day old inoculum (grown on sterilized sorghum grains) at the rate of 50 g pot^{-1} in the upper 3-5 cm layer of the sterilized soil of each pot. The soil of the pots were moistened and covered with polythene bags and left for 24 hours in green house. On next day, apparently healthy surface sterilized mustard seeds were sown in these pots. Re-isolations from the diseased seedlings yielded the same fungus.

2.1.2. Inhibition of mycelial growth

Six fungicides viz., carbendazim 50 WP, propiconazole 25 EC, hexaconazole 5 EC, difenaconazole 25 EC, carbendazim 12%+mancozeb 63% mixture 75 WP and penconazole 10 EC were tested by poison food technique. The test fungus was grown on PDA amended with desired quantity of fungicides to obtain five different concentrations viz., 0.001, 0.005, 0.01, 0.05 and 0.1%. The mycelial disc (5 mm) of *S. sclerotiorum* culture (5 day old) were transferred at the centre of agar surface in Petri dishes. PDA without fungicides served as control. The inoculated Petri dishes were kept in BOD incubator at 27±2 °C. Three replications were kept for each treatment. The mycelial growth of the test fungus was recorded at 24 hour intervals upto 7 days of inoculation i.e., when the full growth of the pathogen was observed in control. Per cent inhibition of radial growth was calculated.

2.1.3. Inhibition of sclerotia germination

Fungal sclerotia were treated with each fungicide separately and the test fungicides of 0.001, 0.005, 0.01, 0.05 and 0.1% concentrations were also mixed with the potato dextrose agar (PDA) medium and poured in sterilized Petri dishes. Ten treated sclerotia were placed on each PDA plate aseptically and replicated three times. Inoculated plates were incubated at 27±2 °C for 15 days. Observation was recorded on myceliogenic germination of the sclerotia. Inhibition of sclerotia germination were calculated.

2.2. In vivo testing of fungicides

A field experiment was executed during *rabi* season of 2007-08 and 2008-09 at the research farm of the College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. A randomized block design was used with three replications in 4×3 m^2 plots. The susceptible mustard cultivar 'varuna' was used for all experiments. Experiment was artificially inoculated with 20 g inoculums per meter row. The fungus inoculum was multiplied on sterilized sorghum grains. The sorghum grains were soaked in sterilized water overnight. The excess water drained out and 40 grams of grains was taken in each 250 ml conical flask and sterilized in autoclave at 1.045 kg cm^{-2} pressure for 20 minutes. The sorghum grains in flasks were inoculated aseptically with 5 days old mycelial discs (5 mm) of the pathogen and inoculated for 15 days at 20±2 °C. The inoculum was mixed in rows at the time of sowing. All the recommended agronomic practices were followed to raise the crop. The fungicide carbendazim 50 WP at 2 g kg^{-1} was used as seed treatment in all treatments except control. One and two foliar spray was given by test fungicides viz., carbendazim 50 WP, propiconazole 25 EC, hexaconazole 5 EC, difenaconazole 25 EC, penconazole 10 EC at 0.1% and carbendazim 12%+mancozeb 63% mixture 75 WP at 0.2%. The first foliar spray of all six fungicides was done at first appearance of disease or 60 days after sowing and the second foliar spray done at 15 days after the first spray. Plots sprayed with plain water served as check treatment.

The observations of disease intensity, incidence and seed yield were recorded on plot basis. The results of experiments were statistically analyzed by analysis of variances (ANOVA). To assess the Sclerotinia rot intensity, the rating (0-4) scale (Lesovoi et al., 1987; Sansford, 1995) was followed. Scale 0 for healthy (No visible lesion), 1 for 0.1-2.0 cm lesion length on the stem, 2 for 2.1-4.0 cm lesion length on the stem, 3 for 4.1-6.0 cm lesion length on the stem and 4 for more than 6

cm lesion length on the stem or complete griddle plant. The length of lesion on infected stem was considered for recording the disease intensity (Sharma, 1987). The infected area was calculated from five randomly selected plants in each plot and then the average for each treatment was worked out. Per cent disease intensity was calculated by using the formula [(sum of individual ratings/No. of plants observed×maximum disease rating)×100]. The per cent disease control was calculated by using the formula [(Disease in control-Disease in treatment/ Disease in Control)×100] (McKinney, 1923).

3. Results and Discussion

3.1. Effect of fungicides on mycelia growth of the pathogen

In vitro studies of six fungicides tested by poisoned food technique revealed that carbendazim 50 WP and carbendazim12%+mancozeb 63% mixture 75 WP were found highly effective, which inhibited cent per cent growth of *S. sclerotiorum* at 1000 ppm (Table 1). Fungicides, such as propiconazole 25 EC, hexaconazole 5 EC, difenaconazole 25 EC, penconazole 10 EC were also found effective at higher concentration but at lower concentrations, all fungicides were observed less effective. No sclerotial production was recorded on PDA plates amended with carbendazim 50 WP and carbendazim12%+mancozeb 63% mixture at 1000 ppm (Table 1). These finding are in agreement with reports made by Shivpuri and Gupta (2001); Mullar et al. (2002); Phool Chand et al. (2009); Tripathi and Tripathi (2010) while working on *S. sclerotiorum in vitro*, who reported that carbendazim

Table 1: Effect of fungicides on inhibition of mycelial growth, Sclerotia formation and myceliogenic germination of *S. sclerotiorum* on potato dextrose agar

Fungicides	% mycelial growth inhibition					Sclerorita formation (no.)					% myceliogenic germination inhibition				
	Concentration (%)					Concentration (%)					Concentration (%)				
	0.001	0.005	0.01	0.05	0.1	0.001	0.005	0.01	0.05	0.1	0.001	0.005	0.01	0.05	0.1
Carbendazim 50 WP	5.93 (13.54)*	31.11 (33.75)	68.15 (55.62)	90.37 (71.90)	100.00 (89.96)	19.67	9.67	1.33	0.00	0.00	24.44 (29.54)	60.00 (50.83)	84.44 (66.94)	94.44 (78.84)	100.00 (89.96)
Propiconazole 25 EC	2.96 (9.58)	15.19 (22.81)	30.00 (33.18)	52.96 (46.68)	71.85 (57.95)	25.33	17.67	13.33	7.00	4.00	20.00 (26.06)	34.44 (35.89)	46.67 (43.06)	65.56 (54.08)	88.89 (70.54)
Hexaconazole 5 EC	3.33 (10.42)	8.15 (16.46)	14.07 (22.00)	32.96 (35.02)	50.37 (45.19)	25.67	20.33	14.33	10.33	5.00	14.44 (22.13)	24.44 (29.54)	55.56 (48.18)	64.44 (53.41)	70.00 (56.77)
Difenconazole 25 EC	2.59 (9.22)	7.78 (15.86)	12.96 (20.96)	22.59 (28.36)	45.93 (42.64)	28.00	18.33	17.00	12.00	7.67	24.44 (29.54)	45.56 (42.42)	64.44 (53.41)	70.00 (56.97)	75.56 (60.42)
Carb.12%+manco.63% mixture 75 WP	8.89 (17.20)	29.26 (32.61)	53.70 (47.11)	86.30 (68.25)	100.00 (89.96)	20.33	15.00	6.67	2.67	0.00	25.56 (30.28)	54.44 (47.54)	65.56 (54.08)	85.56 (67.83)	100.00 (89.96)
Penconazole 10 EC	5.93 (13.54)	9.26 (17.58)	26.30 (30.71)	37.41 (37.65)	52.22 (46.26)	24.67	20.67	15.00	10.33	6.33	28.89 (32.49)	34.44 (35.89)	64.44 (53.41)	65.56 (54.08)	71.11 (57.47)
	SEm±		CD (p=0.05)			SEm±		CD (p= 0.05)			SEm±		CD (p=0.05)		
Fungicide	0.69		1.94			0.34		0.95			0.91		2.56		
Concentration	0.69		1.94			0.34		0.95			0.91		2.56		
Fungicide× concentration	1.54		4.34			0.75		2.12			2.03		5.72		

*Figures in parentheses are angular transformed values

completely inhibited the mycelial growth and sclerotial production whereas, carbendazim 12%+mancozeb 63% mixture was effective at higher concentration but literature is silent on use of propiconazole, hexaconazole, difenaconazole and penconazole against *S. sclerotiorum*.

3.2. Effect of fungicides on myceliogenic germination of sclerotia

Studies on myceleogenic germination indicate that both carbendazim 50 WP and carbendazim 12%+mancozeb 63% 75 WP were highly effective in inhibiting the germination of sclerotia. The results presented in Table 1 showed no sclerotia germinate at 1% concentration and gave 100% inhibition in both fungicides. This observation is in accordance with earlier findings of Hawthorne and Jarvis (1973), who reported that carbendazim at 100 µg ml^{-1} reduced the sclerotial viability to 5%. Singh and Kapoor (1996) found that carbendazim (100 µg ml^{-1}) treatment of sclerotia of *S. sclerotiorum* for 20 min. inhibited 93.88% myceliogenic germination.

3.3. In vivo studies

The efficacy of non systemic and systemic fungicides carbendazim 50 WP, propiconazole 25 EC, hexaconazole 5 EC, difenaconazole 25 EC, carbendazim 12%+mancozeb 63% mixture 75 WP and penconazole 10 EC were tested against Sclerotinia stem rot of Indian mustard under field conditions. All the fungicides tested were found to be significantly superior over untreated control in reducing disease incidence, disease intensity and in increasing seed yield of Indian mustard during both crop seasons *Rabi* 2007-08 and 2008-09. The data presented in Table 2 revealed that during 2007-08 minimum disease incidence (9.20%), disease intensity (8.33%) and maximum seed yield (14.61 q ha^{-1}) was recorded with seed treatment followed by two foiliar spray of carbendazim 50 WP. The similar trend was also recorded during 2008-09. Two foliar sprays with carbendazim 50 WP found significantly superior over control. Average of two years (Table 2) revealed that seed treatment with carbendazim (2 g kg^{-1}) and two foliar sprays at 0.1% was proved most effective in reducing disease intensity (7.50%) and increasing seed yield (101.73%) over untreated control. Maximum benefit cost ratio (2.01:1) was also recorded in this treatment. Seed treatment with carbendazim 50WP (2 g kg^{-1}) and one foliar spray (0.1%) of the same fungicide and seed treatment with carbendazim 50 WP and two foliar spray with carbendazim 12%+mancozeb 63% mixture 75 WP (0.2%) were also found effective in reducing disease intensity and increasing seed yield over control. In corroborate to present findings, Sharma et al. (2011) reported that seed treatment with carbendazim and foliar spray of the same at 65 DAS was proved to be significantly effective which reduced the disease incidence and increased seed yield against Sclerotinia stem rot of Indian mustard. Chattopadhyay et al. (2002) reported that seed treatment and foliar spray with carbendazim

Table 2: Efficacy of fungicides against Sclerotinia stem rot of Indian mustard under field conditions

Treatments	No. of spray	Disease incidence (%)			Disease intensity (%)			Disease control (%)	Seed yield (q ha^{-1})			Increase in seed yield over control (%)	B:C ratio
		2007-08	2008-09	Average	2007-08	2008-09	Average		2007-08	2008-09	Average		
Carbendazim 50 WP	1	13.63 (21.64)*	11.62 (19.92)	13.62 (21.58)	13.33 (21.33)	10.00 (18.43)	11.67 (19.88)	68.18	12.59	13.28	12.94	75.21	1.89:1
Carbendazim 50 WP	2	9.20 (17.64)	9.44 (17.88)	9.32 (17.76)	8.33 (16.59)	6.67 (14.75)	7.50 (15.67)	76.92	14.61	15.18	14.89	101.73	2.01:1
Propiconazole 5 EC	1	34.67 (36.05)	35.79 (36.71)	35.23 (36.37)	31.67 (34.22)	31.67 (34.22)	31.67 (34.22)	22.45	7.45	8.03	8.05	8.97	1.22:1
Propiconazole 25 EC	2	29.76 (33.05)	25.99 (30.60)	27.88 (31.83)	28.33 (32.13)	23.33 (28.84)	25.83 (30.49)	36.73	8.08	9.22	8.65	17.12	1.25:1
Hexaconazole 5 EC	1	39.55 (38.95)	39.99 (39.21)	39.77 (39.08)	30.00 (33.20)	31.67 (34.22)	30.83 (33.71)	24.49	7.44	7.87	7.66	3.69	1.14:1
Hexaconazole 5 EC	2	31.58 (34.17)	25.38 (30.22)	29.14 (32.60)	26.67 (31.06)	25.00 (29.99)	25.83 (30.52)	36.73	7.80	8.64	8.22	11.36	1.16:1
Difenconazole 25 EC	1	36.64 37.23)	35.30 (36.43)	35.97 (36.83)	33.33 (35.24)	33.33 (35.20)	33.33 (35.22)	18.37	8.49	10.48	9.48	28.46	1.33:1

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Treatments	No. of spray	Disease incidence (%)			Disease intensity (%)			Disease control (%)	Seed yield (q ha^{-1})			Increase in seed yield over control (%)	B:C ratio
		2007-08	2008-09	Average	2007-08	2008-09	Average		2007-08	2008-09	Average		
Difenconazole 25 EC	2	28.53 (32.27)	31.16 (33.91)	29.84 (33.09)	30.00 (33.15)	28.33 (32.08)	29.17 (32.61)	28.57	9.23	12.31	10.77	45.92	1.36:1
Carb.12%+ Mencozeb 63% mixture 75 WP	1	20.19 (26.88)	24.26 (29.48)	22.23 (28.08)	18.33 (25.30)	21.67 (27.70)	20.00 (26.50)	51.02	10.52	13.07	11.79	59.73	1.77:1
Carb.12%+ Mencozeb 63% mixture 75 WP	2	17.24 (24.49)	19.08 (25.83)	18.16 (25.16)	16.67 (24.04)	16.67 (24.04)	16.67 (24.04)	59.18	11.77	14.15	12.96	75.55	1.85:1
Penconazole 10 EC	1	48.43 (44.08)	44.49 (41.82)	46.46 (42.95)	36.67 (37.24)	36.67 (37.24)	36.67 (37.24)	10.20	7.35	7.74	7.55	2.19	1.10:1
Penconazole 10 EC	2	42.90 (40.90)	40.21 (39.33)	41.55 (40.11)	31.67 (34.22)	33.33 (35.24)	32.50 (34.73)	20.41	7.68	7.78	7.73	4.72	1.04:1
Control (without fungicides)		72.58 (58.40)	70.05 (58.84)	71.31 (57.62)	38.33 (38.23)	43.33 (41.15)	40.83 (39.69)	-	7.23	7.53	7.38	-	1.17:1
SEm±		0.96	1.00	0.70	1.73	1.81	0.90		0.27	0.29	0.20	-	-
CD (p=0.05)		2.80	2.92	1.98	3.60	3.78	2.55	-	0.78	0.86	0.57	-	-

*Figures in parentheses are angular transformed values

(0.1%) found significant reduction in Sclerotinia rot disease of Indian mustard in Bharatpur district of Rajasthan. Similar findings were also reported by Tripathi and Tripathi (2010) and Chaudhary et al. (2010), who reported carbendazim was most effective as seed treatment and foliar spray to control sclerotinia rot of Indian mustard.

4. Conclusion

Seed treatment with carbendazim 50 WP at 2g kg^{-1} and two foliar sprays at 60 and 75 days after sowing with carbendazim 50 WP at 0.1% or carbendazim12%+mancozeb 63% mixture 75 WP at 0.2% was highly effective to manage the Sclerotinia stem rot in mustard crop and by the use of these chemicals farmers can minimize the yield loss caused by *S. sclerotiorum*.

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