Journal of Oilseed Brassica, 16 (2) : 174-178, July 2025

Management of Sclerotinia rot of Indian mustard through novel combi-formulations of fungicides

A Kumawat*, RP Ghasolia, K Kansotia, G Kumawat[1], Anupriya, S Godika and SK Goyal
Department of Plant Pathology, Sri Karan Narendra College of Agriculture (SKNAU), Jobner, Jaipur, Raj., India
[1]Live Stock Feed Resource Management and Technology Centre, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Raj., India
**Corresponding author: archukumawat8@gmail.com*
https://doi.org/10.56093/job.v16i2.4

Abstract

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an important and vital oilseed crop which suffers from various pests and diseases. Sclerotinia rot caused by *Sclerotinia sclerotiorum* has emerged as a significant threat, leading to economic and quality losses. The present research aimed to reduce the losses due to this dreaded and notorious pathogen through old and novel combi-formulations of fungicides by assessing under *in vitro* and field conditions for two consecutive years (2023-24 and 2024-25). All tested fungicides inhibited mycelial growth cent per cent except metiram + pyraclostrobin under *in vitro* conditions at all three concentrations (50, 100, and 200 ppm).Under field conditions, two foliar applications (first spray at disease appearance and second spray at 15 days after of first spray) on the Indian mustard susceptible var. NRCHB-101. Among these fungicides, two foliar sprays of tebuconazole + trifloxystrobin (@ 0.08%) at the time of disease appearance and 15 days after it, was proved to be the most effective in reducing disease incidence (78.69%) and in increasing seed yield (42.38%) over check followed by carbendazim and captan + hexaconazole. Conclusively, two sprays of tebuconazole + trifloxystrobin (0.08%) may be sprayed to minimize losses to get more benefits.

Keywords: *Fungicides, Indian mustard, sclerotinia rot, sclerotinia sclerotiorum*

Introduction

India with its growing demand became the third-largest consumer market for edible oils worldwide. However, the country currently imports over 60 per cent of its edible oil requirements. This significant gap in the availability of edible oils can be attributed to several factors, including the steadily rising population, abrupt climate changes, low productivity of oilseed crops, and the prevalence of complex disease-pest syndromes (Renjini and Jha, 2019; Singh *et al*., 2021).India is the fourth largest oilseeds producer in the world with 20.8 per cent of the total area under cultivation globally which accounting for 10 per cent of global production (Anonymous, 2023-24). *Brassica juncea* (L.) commonly known as Indian mustard or*Rai* or *Laha* in Hindi and belongs to the family *Brassicaceae* (*Crucifereae*). It is herbaceous and self-pollinated crop but certain amount of pollination (2-15%) occurs due to insects and other factors. However, the low productivity of oilseed crops is the primary reason for the significant supply-demand imbalance in India. However, the crop faces significant yield and production instability due to its sensitivity to various abiotic and biotic stresses with changing climatic conditions. These challenges are expected to worsen, posing a risk to the stability of Indian mustard cultivation and edible oil production in India (Sharma *et al*., 2018).

Among the multiple stresses, fungal diseases such as black leaf spot and blight (*Alternaria brassicae* and *Alternaria brassicicola*), white rust (*Albugo candida*), Sclerotinia rot (*Sclerotinia sclerotiorum*) and powdery mildew (*Erysiphe cruciferarum*) have emerged as major factors impacting crop productivity (Kumar *et al.*, 2015). The Sclerotinia rot, in particular, has transitioned from minor significance to a highly devastating disease in the past decade. It is currently one of the most destructive diseases affecting mustard globally, leading to yield losses ranging from 5 per cent to 100 per cent (Uloth *et al*., 2016; Sharma *et al*., 2018; Singh *et al*., 2021). This disease also impacts the oil content (up to 35%) and quality (Inturrisi *et al.*, 2021). *S. sclerotiorum* belonged to the *Sclerotiniaceae*, a family of the phylum *Ascomycota*. The pathogen is characterized by the formation of hard blackish sclerotia, which on germination produce cup-shaped brown coloured apothecia. Sclerotia can germinate either myceliogenically,causing soil-borne infection at basal region or carpogenically, causing air-borne infection on aerial parts which serve as major source of inoculum for primary infection in the standing crop infecting the leaves and siliquae.The spread of Sclerotinia rot of Indian mustard depends on the prevailing weather conditions (Sharma *et al*., 2015)

This disease is gaining importance in the mustard growing

areas and leading to disastrous crop failure as the disease incidence was recorded up to 73.8 per cent in some districts of Punjab and Haryana and at a few locations it went up to 80 per cent (Kang and Chahal, 2000; Sharma *et al*., 2001; Ghasolia *et al*., 2004). Non-chemical agents provide tenable and eco-friendly alternatives for the management of Sclerotinia rot throughseed treatments as well as foliar spray (Meena *et al*., 2013). However, controlling this pathogen using cultural, biological and existing chemical methods is challenging due to its complex infection process and the ability of its resting structures to survive in the soil for up to 10 years (Brustolin *et al*., 2016).The present investigation was carried out with the objective to test the efficacy of old and newer combi-formulations of fungicides against *S.Sclerotiorum* under *in vivo* and *in vitro* conditions so that these can be used as a component of integrated Sclerotinia rotdisease managementin mustard.

Materials and Methods

Collection and isolation of *S. Sclerotiorum*

Plants of Indian mustard affected by Sclerotinia rot, exhibiting partial or complete wilting of stems and branches, were collect from Agronomy Farm of SKNAU, Jobner. Isolations of the pathogen was done on potato dextrose agar (PDA) medium using black sclerotia found inside the infected stem, as well as from individual stem rot lesions. The culture was then purified through the hyphal tip method (Riker and Riker, 1936) and identified as *S. sclerotiorum*.

In vitro efficacy of fungicides against *S. sclerotiorum*

A laboratory experiment was conducted to evaluate the efficacy of six old and newer molecules of fungicides such as carbendazim 50% WP,metiram 55% + pyraclostrobin 5% WG, azoxystrobin 23% SC, captan 70% + hexaconazole 5% WP, tebuconazole 25.9% EC and tebuconazole 50% + trifloxystrobin 25% WG through poisoned food technique (Schmitz, 1930) with three concentrations (50, 100, and 200 ppm). Each fungicide was incorporated into 100 ml of sterilized PDA medium, thoroughly mixed, and poured into sterilized Petri plates, allowing the medium to solidify. A 5 mm diameter disc from a seven-day old *S. sclerotiorum* culture was placed at the centre of each plate, which was then incubated at 25 ± 1°C. The experiment was set up in a completely randomized design with three replications. Mycelial growth inhibition was assessed by measuring fungal growth in each treatment and calculating the average across replications. The percentage inhibition of mycelial growth was determined using Bliss's (1934) formula.

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent mycelial growth inhibition, C = Growth of fungus in control (average of both diagonals), T = Growth of fungus in treatment (average of both diagonals).

In vivo efficacy of fungicides against Sclerotinia rot

Aforementionedfungicides were also evaluated under field conditions to know their efficacy in disease management by applying through foliar spray on susceptible var. NRCHB-101. First spray was provided at the time of disease appearance and second at 15 days after of first spray under RBD with three replications. Inoculum (multiplied on sorghum grains) was added (20g/ m row length) at the time of sowing. Observations on disease incidence and intensity were recorded at 90 DAS and yield at harvest. For disease incidence, the plants showing even a minute lesions on stem due to disease was considered as a diseased plant.

Disease rating (0-4) scale of Lesovoi *et al*. (1987) and Sansford (1995) with a slight modification was followed to assess intensity as: 0 = healthy (no visible lesion); 1 = 0.1-2 cm lesion length on stem; 2 = 2.1-4.0 cm; 3 = 4.1-6.0 cm; 4 = > 6.1 cm lesion length on stem or complete dried plant. The length of lesion on infected stem was considered for recording the disease intensity. The per cent disease intensity was calculated using the formula of Wheeler (1969).

$$\text{Per cent Disease Intensity (PDI)} = \frac{\text{Sum of all the individual ratings}}{\text{No. of plants observed x Maximum disease rating}} \times 100$$

The per cent disease reduction over control was calculated by using the following formula:

$$\text{Per cent disease reduction over control} = \frac{\text{Disease in control} - \text{Disease in treatment}}{\text{Disease in control}} \times 100$$

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

$$\text{Increase in yield over control (\%)} = \frac{\text{Yield of plants in treatment} - \text{Yields of plants in inoculated control}}{\text{Yield of plants in inoculated control}} \times 100$$

Results and Discussion

In vitro efficacy of fungicides against *S. sclerotiorum*

The fungicides evaluated through poisoned food

Table 1: Efficacy of fungicides against *S .sclerotiorum* causing Sclerotinia rot of Indian mustard (*in vitro*)

Fungicides	Per cent inhibition of mycelial growth (4th DAI) at different concentration			* Mean
	50 ppm	100 ppm	200 ppm	
Carbendazim	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Metiram + Pyraclostrobin	78.15(58.29)	80.37(65.62)	85.56(90.00)	85.11(71.30)
Azoxystrobin	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Captan + Hexaconazole	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Tebuconazole	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Tebuconazole + Trifloxystrobin	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Control	0.00	0.00	0.00	0.00
Mean	96.35(72.61)	96.72(73.66)	97.59(77.14)	-
	SEm±	LSD (p=0.05)		
F (Fungicides)	0.04	0.15		
C (Concentration)	0.03	0.13		
F×C	0.08	0.22		

*Average of three replications, DAI- Days after inoculation, Figures given in parentheses are angular transformed values

techniquewere found significantly superior in inhibiting vegetative growth of fungus over check. Data (Table 1) showed that carbendazim, azoxystrobin, captan+ hexaconazole, tebuconazoleand tebuconazole + trifloxystrobin were inhibited mycelial growth cent per cent at all three concentrations (50, 100, and 200 ppm) (Fig.1) while metiram + pyraclostrobin depicted 78.15, 80.37 and 85.56 per cent inhibition at 50, 100 and 200 ppm, respectively. The present results are parallel to the findings of Roy *et al*. (2021) and Manhas *et al*.(2022) whohave reported carbendazimand captan + hexaconazoleas significantly superior in inhibiting mycelial growth of *S. sclerotiorum.*

In vivo efficacy of fungicides against Sclerotinia rot

Results of efficacy of fungicides under field conditions (Table 2) were also found significantly superior over control in reducing disease incidence and intensity and in increasing seed yield during both the years (2023-24 and 2024-25) of testing. Two foliar sprays of the tebuconazole + trifloxystrobin(@ 0.08%) at the time of disease appearance and 15 days after of first spray was proved to be the most effective in reducing disease

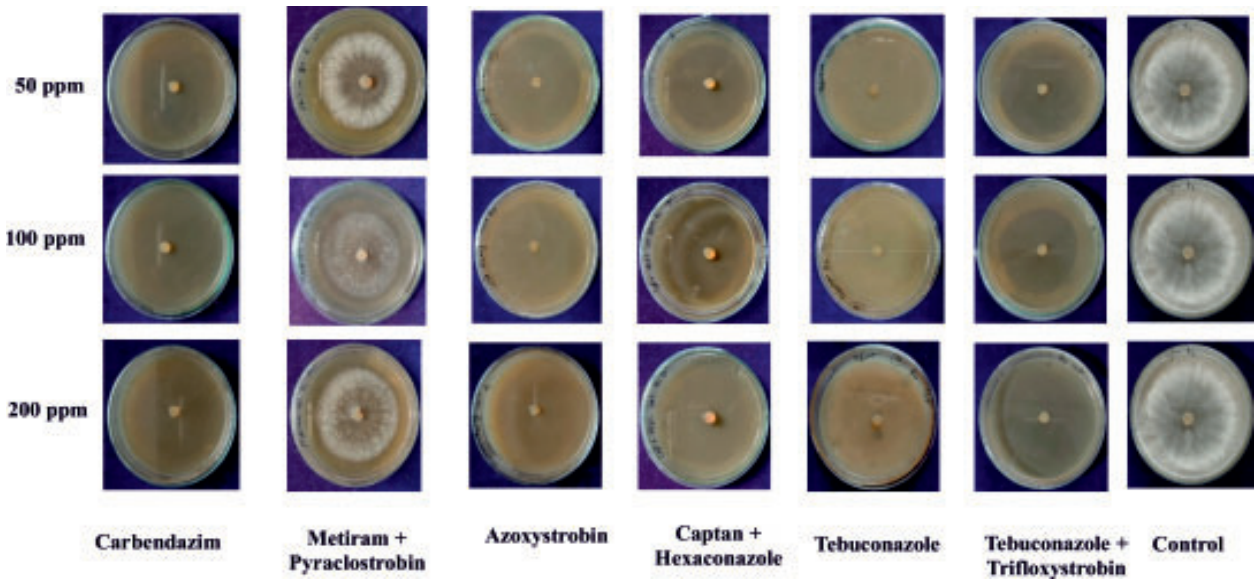

Fig. 1: Efficacy of fungicides against *S. sclerotiorum* causing Sclerotinia rot of Indian mustard (*in vitro*)

Table 2:Efficacy of fungicides in managing Sclerotinia rot of Indian mustard under field conditions

Fungicide	Con. (%)	Disease incidence (%)*			Disease reduction (%) over	Disease intensity (%)*			Yield (q/ha)*		Increase in yield (%)
		2023-24	2024-25	Pooled	control	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	over check
Carbendazim	0.1	12.09(20.33)	10.06(18.47)	11.07(19.42)	73.45	9.00(17.33)	7.67(16.02)	8.33(16.74)	13.40	13.95	13.68	41.54
Metiram + Pyraclostrobin	0.2	15.84(23.43)	14.16(22.04)	15.00(22.77)	64.04	14.33(22.20)	11.67(19.95)	13.00(21.10)	12.36	13.06	12.71	31.56
Azoxystrobin	0.1	13.92(21.86)	12.02(20.17)	12.97(21.09)	68.90	11.33(19.60)	10.00(18.42)	10.67(19.06)	13.24	13.54	13.39	38.59
Captan + Hexaconazole	0.2	13.06(21.16)	10.35(18.70)	11.70(20.00)	71.94	9.67(18.08)	8.00(16.35)	8.83(17.24)	13.30	13.90	13.60	40.77
Tebuconazole	0.1	13.27(21.30)	11.00(19.33)	12.13(20.38)	70.90	11.00(19.29)	8.67(17.08)	9.83(18.23)	13.28	13.76	13.52	39.92
Tebuconazole + Trifloxystrobin	0.08	9.76(18.16)	8.02(16.38)	8.89(17.33)	78.69	7.67(16.02)	4.33(11.94)	6.00(14.16)	14.28	14.59	14.43	49.38
Control	-	41.22(39.90)	42.18(40.45)	41.70(40.18)	0.00	31.33(34.03)	32.33(34.65)	31.83(34.35)	9.89	9.43	9.66	0.00
SEm(±)	-	1.34	1.42	1.07	-	1.10	0.97	0.74	0.56	0.60	0.43	-
LSD (p=0.05)	-	4.15	4.38	3.30	-	3.39	2.97	2.30	1.72	1.86	1.33	-
CV (%)	-	9.83	11.09	8.05	-	9.12	8.70	6.44	7.57	7.95	5.76	-

*Average of three replications, Figures given in parentheses are angular transformed values

incidence (78.69%) and in increasing yield (49.38%) over controlfollowed by carbendazim and captan + hexaconazole. Our findings are in accordance with the results of Bharti (2021), Roy *et al*. (2021) and Humauan *et al*. (2022). Humauan *et al*. (2022) reported tebuconazole 250 EC (2 ml/l) as most successful in reducing incidence of white mold disease of mustard compared to unsprayed control. Similar trends of reduction inincidence and intensityand in increased seed yield was also reported by Bharti (2021) and Roy *et al*. (2021) under field conditions.

Conclusion

Conclusively, two sprays of tebuconazole 50% + trifloxystrobin 25% WG (@ 0.08%) at 15 days interval after appearance of disease was rated most effective in reducing disease incidence and in increasing seed yield and it can be included with integrated disease management practices to get more benefits.

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