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Efficacy of Fungicides against *Sclerotium rolfsii* Causing Collar Rot of Lentil (*Lens culinaris* Medik) in West Bengal

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Abstract

The experiment was conducted during *rabi* (November, 2018 to March, 2019) at the Plant Pathology Laboratory, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India to study the efficacy of fungicides against collar rot pathogen *Sclerotium rolfsii* isolate SRC1 under laboratory condition. The collar rot disease of lentil was found to be severe, with disease incidence and associated losses reaching up to about 60% in some parts of North India, where the crop was grown after rice, resulting into wilting of the seedling in the early stages of plant growth.. In this study we have used five fungicides to check their efficacy for inhibiting the growth of mycelia and to reduce the sclerotia formation by the isolate SRC 2 at different concentrations viz., 10, 25, 50, and 100 ppm. Fungicide efficacy was quantified by radial mycelial growth (mm) and percent inhibition at 2–4 DAI. Azoxystrobin 18.2% + Difenoconazole 11.4% SC showed the highest suppression of Sclerotium rolfsii, achieving 86.7–99.2% growth inhibition and reducing radial growth to ≤0.38 mm at 100 ppm. Mancozeb 75% WP recorded 79-83% inhibition, whereas other fungicides showed comparatively lower effects, confirming a clear dose-dependent inhibition of the pathogen.

Keywords: Lentil, collar rot, *Sclerotium rolfsii*, poisoned food method

1. Introduction

Lentil (*Lens culinaris* Medik.), is one of the India's oldest and most significant annual legume crops, which are provide protein, vitamins, and minerals to round out the daily human diet (Mandi et al., 2015; Dash et al., 2017; Patra et al., 2023; Meena et al., 2024). The lentil is growing in different districts of West Bengal as a paira and sole crop after harvesting of paddy in Rabi season. The Nadia district ranks first by claiming 42.15% of total lentil production of the state followed by Murshidabad (26.86%) and subsequently followed by Birbhum (6.58%) and Malda (6.17%) (Anonymous, 2016–2017). The *Sclerotium rolfsii* pathogen is obtained in soil for many years as dormant stages and to produce infection start into the host surface, when it becomes found the susceptible host in the fields (Leoni et al., 2014; Kushwaha et al., 2018; Ons et al., 2020; Nath and Patel, 2023; Sreechithra et al., 2024; Akber and Fang, 2024; Mondal et al., 2024). The pathogen causes a variety of plant diseases, including wilt, collar rot, stem rot, foot rot, damping off, and seedling blight (Gopalakrishnan et al., 2005; Xie et al., 2014;Islam et al., 2017; Sahu et al., 2019; Jiskani et al., 2021). . When the pathogen was artificially inoculated, it was discovered that the white fungal mycelium spread out into the soil surface as a thread-like structure and formed round to oval, small to medium-sized sclerotia (Suja et al., 2016; Rathi et al., 2018; Mondal et al., 2022). Presently, the cause for low yield and poor-quality pods is the result of soil-borne pathogen attack in the growing regions where it is widely grown (Schut et al., 2014; Chellemi et al., 2016; Panth et al., 2020; Kilani et al., 2021; Taylor et al., 2018; Khan et al., 2020). Soil-borne pathogen (*S. rolfsii*) is the most powerful pathogen because it has active in dormant stage under the soil surface up to 10–12 years, hence to difficult their controlling from soil (Amule et al., 2014; Kushwaha et al., 2018; Jiskani et al., 2021). The efficacy of fungicide can be used to suppress the sclerotia from the entire host plant root surface or soil surface (Amule et al., 2014; Suryawanshi et al., 2015; Rakesh et al., 2016; Bagul et al., 2024). Susceptible variety, weather, temperature, and humidity can increase the growth of the mycelium even after they form small light brown to dark-colored matured mustard seed-like sclerotia (Rakesh et al., 2016). The most effective fungicide used from ancient times is Penta Chloro Nitro Benzene (PCNB) fungicide which gives better results to remove the soil-borne pathogen (Fan et al., 2022; Corkley et al., 2022). But nowadays, the new formulation of fungicide can actively reduce the inoculums in the soil, which is a serious problem of lentil cultivation (Savary, 2014; Wavare et al., 2017; Corkley et al., 2022). *Sclerotium* is the most devastating pathogen to all the cultivated crops in India, which can reduce the economic value of lentil day to day (Pouralibaba et al., 2015; Chaudhary et al., 2017; Jiskani et al., 2021; Singh et al., 2023). The present study investigated that, the effect of systemic fungicide viz., Mancozeb 75% WP (Indofil M-45) was applied through poison food method from lower concentration to higher concentration at 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm, to estimate the growth of fungal mycelium from different isolates of the pathogen under in vitro condition.

2. Materials and Methods

2.1. Area of study

The experiment was conduct during *rabi* season in the month of November, 2018 to March, 2019 at the Plant Pathology Laboratory, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India. Our experiment was exhibitedto control the collar rot pathogen *Sclerotium rolfsii* isolate SRC2 through chemically using the poisoned food method,with isolate SRC2 selected due to its highest virulence. A pathogenicity test was conducted under controlled conditions, where SRC2 produced maximum disease incidence, severe collar lesions, and highest seedling mortality among all isolates (Mondal et al., 2022).

2.2. Isolation of fungal pathogen from eight different isolates

A total of eight isolates of *Sclerotium rolfsii* were collected from collar rot–infected lentil plants sampled from different lentil-growing areas of West Bengal (Table 1). All isolates were purified and maintained on potato dextrose agar (PDA) for further studies. To identify the most virulent isolate, a pathogenicity test was conducted under controlled conditions. Among the isolates tested, SRC-2 exhibited the highest virulence, producing maximum disease incidence, severe collar lesions, and the highest seedling mortality compared to the other isolates. Based on these pathogenicity parameters, SRC-2 was identified as the most pathogenic isolate and was therefore selected for subsequent in-vitro fungicide evaluation studies, in agreement with earlier findings (Mondal et al., 2022). The disease specimens were examined from the infected collar zone of lentil in the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur (Mondal et al., 2022). The uprooted plant parts were thoroughly washed with running tap water, and then the infected portion was cut into small pieces approximately 3–5 mm in size. The surface was sterilized by immersing it in a 1.0% sodium hypochlorite solution for 60 seconds, followed by two or three washes with sterile distilled water to get rid of any remaining chemicals. The sterilized pieces were aseptically transferred to PDA petri plates and incubated at 25±1˚C for 4 to 5 days for their full growth of mycelium. The pure culture tube was then stored at 4˚C for further use.

Table 1: Detailed description of diseased plant sample collection and GPS Location on different isolates

Isolation code	Place of collection	(GPS Location)
SRC1	From farmers field, Chakdaha, Nadia	23.08° (N) 88.52° (E)
SRC2	From Ramchandrapur, Murshidabad Krishi Vigyan Kendra, Murshidabad	24.18° (N) 88.27° (E)
SRC3	From farmers field at Saguni in patna, Masaurhi block, Bihar	25.02° (N) 87.14° (E)
SRC4	From farmers field Balagarh, Hoogly	23.12° (N) 88.46° (E)
SRC5	In-check farm, C block, Kalyani, Nadia	22.98° (N) 88.48° (E)
SRC6	From farmers field, Katwa, PurbaBurdwan	23.24° (N) 87.85° (E)
SRC7	From instructional farm, Palli Siksha Bhavana, (Institute of Agriculture) VisvaBharati, Sriniketan, Birbhum	23.67° (N) 87.72° (E)
SRC8	From Jalpaiguri KVK farm, Ramshai, Maynaguri, Jalpaiguri	26.52° (N) 88.73° (E)

2.3. In vitro efficacy of Fungicide against Sclerotium rolfsii

Using the poisoned food technique, it was clearly observed that fungicides inhibited the mycelial growth of soil-borne pathogens in a concentration-dependent manner, with higher concentrations resulting in greater growth suppression.Five fungicides, both contact and systemic, namely Carbendazim 50% WP, Carbendazim 12% WP+Mancozeb 63% WP, Azoxystrobin 18.2%+Difenoconazole 11.4% SC, Chlorothalonil 75% WP, and Mancozeb 75% WP, were tested for their bio-efficacy against *Sclerotium rolfsii* under in vitro conditions using the poisoned food technique (Nene and Thapliyal, 1973; Khan et al., 2020). For the experiment, a number of 105 sterilized Petri plates were used. Sterilization was carried out in a hot air oven at 180°C for 2 h. Potato Dextrose Agar

(PDA) medium was prepared, and fungicide stock solutions were prepared separately. For example, 1.0 g of Mancozeb 75% WP (contact fungicide) was dissolved in 99 ml of distilled water to obtain the stock solution. Four concentrations-10, 25, 50, and 100 ppm-were prepared and evaluated for bio-efficacy against *S. rolfsii* (Singh et al., 2023). Each treatment was replicated five times. Fungicide-amended PDA medium (20 ml) was poured into Petri plates, while fungicide-free PDA served as the control. A 5 mm diameter mycelial disc of *S. rolfsii* was aseptically inoculated at the center of each plate and incubated at 25±1°C. Mycelial growth was recorded at regular intervals. The radial growth data were converted into percent growth inhibition, calculated using Vincent's formula (Vincent, 1947).

Percent growth inhibition (I%)=C-T/C×100

Where,
C=Colony diameter in control plate (mm)/no. of sclerotia formed.
T=Colony diameter in the treated plate (mm)/no. of sclerotia formed.

2.4. Statistical analysis

The standard errors of means (SEm) of the replicate values obtained from the laboratory bioassays were computed using Microsoft Excel. Statistical analysis was carried out for all datasets to evaluate variability and significance of the results.

3. Results and Discussion

The phyto-efficacy study of different formulations of fungicides was conducted against the pathogen *Sclerotium rolfsii*. Among the five fungicides tested, Azoxystrobin 18.2%+Difenoconazole 11.4% SC showed the maximum inhibitory effect on mycelial growth. All tested concentrations of fungicides significantly inhibited the mycelial growth of the pathogen compared to the untreated control. At 4 days after inoculation (DAI), the inhibitory effect of Azoxystrobin 18.2%+Difenoconazole 11.4% SC ranged from 86% to 99%**,**followed by Mancozeb 75% WP (79–83%)**.** The least inhibition was recorded in Carbendazim 50% WP, followed by Carbendazim 12% WP+Mancozeb 63% WPand Chlorothalonil 75% WP**.**

The highest and lowest radial growth was observed at different days after inoculation which depending on the fungicide concentration. In general, higher fungicide concentrations resulted in lower radial growth compared to the control. Among the five fungicides, maximum radial growth (30.6 mm) was recorded at 2 DAI in Chlorothalonil 75% WP at 0.1% concentration, followed by Carbendazim 12% WP+Mancozeb 63% WP (30 mm). In contrast, the lowest growth (0.28 mm) was observed at 1.0% concentration of Azoxystrobin 18.2%+ Difenoconazole 11.4% SC, followed by Mancozeb 75% WP (6 mm).A similar trend in growth inhibition was observed at 3 and 4 DAI for all the fungicides, except for Azoxystrobin 18.2%+Difenoconazole 11.4% SC, which showed a minute increase in radial growth (0.38 mm) at 1.0% concentration (Table 2).

The present investigation revealed that Azoxystrobin 18.2%+Difenoconazole 11.4% SC (Godiwa Super) at 1.0% (100 ppm) concentration completely suppressed the mycelial

Table 2: *In vitro* efficacy of fungicides against isolate SRC-1 (*Sclerotium rolfsii*) in different days after inoculation

Treatments	Concentration (%)	Radial growth (mm) at DAI			Inhibition (%) at DAI		
		2 DAI	3 DAI	4 DAI	2 DAI	3 DAI	4 DAI
Carbendazim 50% WP (Dhanustin)	0.1	24.2	27.2	32	34.20 (35.77)	32.17 (34.51)	24.09 (29.36)
	0.25	23.2	24.8	31	36.93 (37.41)	38.16 (38.12)	26.46 (30.93)
	0.50	21.8	23.2	29	40.73 (39.65)	42.16 (40.47)	31.19 (33.92)
	1.00	20.0	22.2	26.8	45.65 (42.50)	44.67 (41.93)	36.35 (37.05)
	Control	36.8	40.2	42.2	0 (0.00)	0 (0.00)	0 (0.00)
Carbendazim 12% WP+Mancozeb 63% WP (Saaf)	0.1	22.2	24.8	30	39.63 (39.01)	38.16 (38.12)	28.87 (32.47)
	0.25	21.8	23.2	26.8	40.68 (39.61)	42.19 (40.49)	36.45 (37.13)
	0.50	23.6	23.6	24.4	35.79 (36.71)	41.16 (39.89)	42.04 (40.40)
	1.00	21	23.0	23	42.86 (40.88)	42.70 (40.80)	45.43 (42.37)
	Control	36.8	40.2	42.2	0 (0.00)	0 (0.00)	0 (0.00)
Azoxystrobin 18.2%+Difenoconazole 11.4% SC (Godiwa Super)	0.1	4.0	5.28	5.62	89.09 (70.78)	86.84 (68.74)	86.66 (68.58)
	0.25	2.86	2.86	3.46	92.22 (73.80)	92.86 (74.51)	91.79 (73.36)
	0.50	1.16	1.28	1.42	96.84 (79.79)	96.79 (79.71)	96.62 (79.43)
	1.00	0.28	0.38	0.38	99.23 (85.03)	99.06 (84.50)	99.10 (84.63)
	Control	36.8	40.2	42.2	0 (0.00)	0 (0.00)	0 (0.00)

Table 2: Continue...

Treatments	Concentration (%)	Radial growth (mm) at DAI			Inhibition (%) at DAI		
		2 DAI	3 DAI	4 DAI	2 DAI	3 DAI	4 DAI
Chlorothalonil 75% WP (Kavach)	0.1	30.6	33.4	35.2	16.86 (24.15)	16.88 (24.19)	16.53 (23.90)
	0.25	27.6	29.6	32.2	24.97 (29.94)	26.35 (30.87)	23.65 (29.09)
	0.50	22	23.2	25.4	40.14 (39.30)	42.15 (40.47)	39.67 (39.02)
	1.00	19.6	22.4	23.6	46.74 (43.12)	44.22 (41.67)	43.99 (41.53)
	Control	36.8	40.2	42.2	0 (0.00)	0 (0.00)	0 (0.00)
Mancozeb 75% WP (Indofil M-45)	0.1	8.2	15.4	18.6	48.75 (61.87)	30.00 (51.66)	79.33 (48.34)
	0.25	6.4	12.2	20.0	60 (65.42)	44.54 (56.53)	77.77 (46.51)
	0.50	6.2	12.6	15.2	61.25 (65.80)	42.72 (55.94)	83.11 (53.09)
	1.00	6.0	13.6	14.8	62.5 (66.20)	38.18 (54.53)	83.55 (53.73)
	Control	36.8	40.2	42.2	0 (0.00)	0 (0.00)	0 (0.00)
SEm±					0.90	0.78	1.56
CD (p<0.05)					2.58	4.47	

Average of five replications; with angular transformed values (p<0.05)

growth of *Sclerotium rolfsii* and prevented sclerotia formation (Plate 1). The efficacy of this fungicide was superior to the other formulations tested, followed by Mancozeb 75% WP. These findings are consistent with the reports of Sreechithra et al. (2024), who demonstrated that higher concentrations of fungicides markedly restricted the mycelial growth and sclerotia production of *S. rolfsii*, with Mancozeb 75% WP being particularly effective under in vitro conditions using the poisoned food technique.

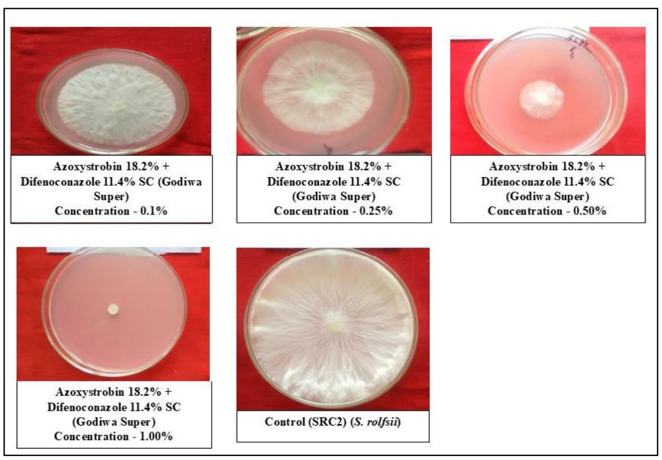

Plate 1: Activity of fungicide on radial growth of *Sclerotium rolfsii* (SRC2) in different concentration at 4 DAI

Similarly, Wavare et al. (2017) confirmed that fungicide concentrations of 100 ppm were highly effective in controlling *S. rolfsii*, the causal agent of collar rot, by restricting radial mycelial growth. Babariya and Nath (2023) further supported these results by showing that Mancozeb 75% WP and Azoxystrobin+Difenoconazole significantly reduced radial growth of *S. rolfsii* at different concentrations. There in vitro assays with Tebuconazole, Thiophanate Methyl, Ridomil Gold (Metalaxyl+Mancozeb), and Mancozeb also highlighted the superior inhibitory effect of higher concentrations.

The trend of higher fungicide concentrations exhibiting stronger inhibition was also reported by Corkley et al. (2022), who observed that lower doses were comparatively less effective in reducing mycelial growth. In line with this, Singh et al. (2023) and Jiskani et al. (2021) found that contact fungicides such as Mancozeb (75% WP), Thiram (75% WS), and Propineb (70% WP) effectively suppressed *S. rolfsii* only at higher concentrations (100 ppm). Likewise, Manjula et al. (2024) demonstrated that both fungicides and medicinal plant extracts exhibited dose-dependent inhibition of fungal growth under poisoned food technique assays.

In addition to fungicides, biological control agents and plant growth-promoting rhizobacteria (PGPR) have also been shown to be effective against *S. rolfsii*. Mondal et al. (2024) reported significant inhibition of mycelial growth by bio-control agents both in vitro and in pot culture, suggesting that integrated approaches mightenhance disease management.

Taken together, the present study confirmedthat synthetic fungicides, particularly Azoxystrobin+Difenoconazole and Mancozeb, werehighly effective in suppressing *S. rolfsii* growth at higher concentrations, in agreement with previous studies. These findings highlighted the potential of fungicides in managing collar rot under laboratory conditions and provided a basis for their further evaluation under field conditions.

4. Conclusion

Chemical control of *Sclerotium rolfsii* was effective in reducing both the mycelial growth *in vitro* and its proliferation in soil. Among the tested fungicides, Azoxystrobin 18.2%+ Difenoconazole 11.4% SC (Godiwa Super) and Mancozeb 75% WP (Indofil M-45) exhibited maximum efficacy in suppressing the pathogen. These results indicated that the tested fungicides hold strong potential for managing collar rot disease and might be recommended for subsequent field level evaluations to validate their practical effectiveness under natural conditions.

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