IN VITRO EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS AGAINST *Sclerotium rolfsii* AND *Sclerotinia sclerotiorum*

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Abstract

An experiment was conducted in the laboratory of Plant Pathology Division of Bangladesh Institute of Nuclear Agriculture (BINA) during September 2021 to February 2022 to evaluate the efficacy of five fungicides and three biocontrol agents against two soil borne fungi, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. Five chemical fungicides viz. Dithane M-45, Bavistin 50 DF, Ridomil Gold MZ 68 WP, Antracol 70 WP and Secure 600 WG and three biocontrol agents viz. BC1 (*Trichoderma harzianum*), BC2 (*T. harzianum)* and BC3 (*T. viride*) were evaluated. The sensitivity of the fungicides against *S. rolfsii* and *S. sclerotiorum* was tested by poison food technique. The antagonistic activity of the biocontrol agents was evaluated against the fungi through dual culture technique. Among the tested fungicides, the highest inhibition of mycelial growth (83.2% for *S. rolfsii*; 71.9% for *S. sclerotiorum*) and reduction of sclerotia production (44.8% for *S. rolfsii*; 34.4% for *S. sclerotiorum*) was recorded for Bavistin 50 DF. The biocontrol agent BC1 showed the highest inhibition of mycelial growth (78.2% for *S. rolfsii*; 39.4% for *S. sclerotiorum*) and reduction of sclerotia production (40.6% for *S. rolfsii*; 37.5% for *S. sclerotiorum*) for both fungi. Therefore, the biocontrol agent BC1 can be useful to suppress *S. rolfsii* and *S. sclerotiorum*.

Key words: Fungicide, Biocontrol agent, *Thichoderma*, *Sclerotium*, *Sclerotinia*

Introduction

Sclerotium rolfsii and *Sclerotinia sclerotiorum* are two most devastating soil borne fungi causing major diseases of different important crops in the tropic, sub tropic and temperate regions. Both the pathogens have an extensive host range including legume, cucurbit and crucifer crops. Crops in 500 species of 100 families are susceptible to *S. rolfsill* (Hemanth *et al.*, 2016). This fungus is responsible for collar rot disease in chili, tomato, lentil and causes 5-20% crop loss in peppermint under field condition (Anand and Singh, 2004). *S. sclerotiorum* is another economically important fungus having a wide host range and is capable of infecting 400 plant species (Kolte, 1985). The fungus causes white mold disease in soybean and mustard, head rot, stalk rot in sunflower (Mathew *et al*., 2020). In the field of sunflower, yield loss of 10 to 20% has been observed for head rot (Gulya *et al.*, 2019) and 5 to 70% for stalk rot (Kolte, 1985). These two fungi are capable of producing sclerotia through which they overwinter in soil or crop debris and persist in soil for three to five years. Under favourable condition the sclerotia germinate to produce mycelia

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that make infection to the plant. Therefore, management of these two fungi is a great challenge for crop production. Use of chemicals in controlling plant diseases has been practicing since years as an effective tool. Fungicides belong to hexaconazole, propiconazole, mancozeb, carbendazim can inhibit the growth of *S. rolfsii* and *S. sclerotiorum* (Johnson *et al.*, 2008). However, many results from the efficacy of fungicides in controlling plant pathogens are not consistent (Keinath and Batson, 2020). Moreover, the same fungicide may not be equally effective to all strains of fungus due to genetic variability. Therefore, a continuous study to evaluate the efficacy of fungicides that are popularly used in the country in controlling plant diseases is needed. Use of biocontrol agent like *Trichoderma* sp. is considered as an environment friendly strategy for the management of soil borne plant pathogenic fungi (Amin *et al.*, 2010). *Trichoderma* sp. colonizes the hyphae of *S. rolfsii*, disrupts mycelia and finally kills the pathogen (Chet *et al*., 2009). The antagonist fungus has the ability to suppress *S. rolfsii* and *S. sclerotiorum* through the mechanism of lysis of sclerotia and production of volatile metabolites which paralyze the hyphal trend of the pathogens (Shaigan *et al.*, 2008). As these two pathogens are soil borne in nature and they have a wide host range, their management through chemicals as well as bio control agents is important. Keeping this in mind, the present study was undertaken to investigate the efficacy of five different chemical fungicides and three bioagents on mycelia growth and production of sclerotia of *S. rolfsii* and *S. sclerotiorum*.

Materials and Methods

The experiment was conducted in the laboratory of Plant Pathology Division of Bangladesh Institute of Nuclear Agriculture (BINA) during September 2021 to February 2022. Five chemical fungicides viz. Dithane M-45, Bavistin 50 DF, Ridomil Gold MZ 68 WP, Antracol 70 WP, and Secure 600 WG were evaluated. The fungicides were purchased from the local market in Mymensingh city. Details of the fungicides have been presented in Table 1.

Table 1. List of fungicides used in the study

Commercial name	Active ingredient	Rate of application
Dithane M-45	Mancozeb (80%)	$2gL^{-1}$
Bavistin 50 DF	Carbendazim (50%)	$1gL^{-1}$
Ridomil Gold MZ 68 WP	Mancozeb (64%) + Metalaxyl (18%)	$2gL^{-1}$
Antracol 70 WP	Propineb (70%)	$2gL^{-1}$
Secure 600 WG	Mancozeb (50%) and Fluazinam (40%)	$1gL^{-1}$

Three commercial biocontrol agents coded as BC_1 (*Trichoderma harzianum* based), BC_2 (*T. harzianum* based) and BC_3 (*T. viride* based) were provided by the Plant Pathology Division of BINA. Pure culture of the fungi was grown on Potato Dextrose Agar (PDA) medium in petridishes (9 cm diameter) and was kept in a refrigerator (5°C) for further use.

The isolates of *S. rolfsii* and *S. sclerotiorum* were obtained from infected soybean and mustard plant, respectively. Plant samples showing typical symptom of collar rot in soybean and white rot in mustard were collected from the experimental field of BINA at

Mymensingh. Infected plant parts were cut into 3 mm segments including the advancing margins of infection. The segments were surface sterilized with 0.5% sodium hypochlorite solution for 2 minutes were washed thoroughly with sterilized water. After drying the sterilized segments between folds of filter paper, these were transferred in PDA plates and incubated for 7 days at 28ºC. Pure culture of the fungi was made by sub-culturing three times. The pure culture of the fungi was maintained on PDA medium in petridishes (9 cm diameter) and kept in the refrigerator (5ºC) until required.

The sensitivity of the fungicides against the fungi was tested by poison food technique (Grover and Moore, 1962). Mother culture of the fungi was prepared from pure culture that was used as the source of mycelium of the fungi. The concentration of the fungicides was used according to the recommended dose mentioned in the fungicide packets. Stock solution of each fungicide was prepared by adding the appropriate weight of the fungicide to 1L of sterile distilled water. Then 60 ml of stock solution for each fungicide was mixed with 60 ml of double-strength PDA in separate Erlenmeyer flask of 250 ml before sterilization. Twenty ml of autoclaved PDA and fungicide mixture (autoclaved at 121°C, 15 lb/inch^2 pressure for 15 minutes) was poured into a petridish (9 cm diameter) and allowed to solidify. Five plates were maintained for each fungicide. After solidification, a mycelium block of 5 mm was taken from the edge of five days old culture of the fungi and kept at the center of petridishes containing fungicide mixed PDA. For having direct contact of mycelia with poisoned media the fungal block was placed in an inverted position. The control plates were made without the addition of fungicides to PDA. The inoculated plates were incubated at 26±1°C for seven days. Data on radial mycelial growth was taken at 24 hours interval for seven days. The percent inhibition of the fungi was calculated by using the following formula (Bashar, 1990).

$$\text{Percent growth inhibition (I)} = \{(A-B)/A\} \times 100$$

Where,

A = Radial growth of the fungus in control plate, B = Radial growth of the fungus in fungicide treated plate.

The antagonistic activity of three biocontrol agents against *S. rolfsii* and *S. sclerotiorum* was evaluated through dual culture assay modified from Mello *et al.*, 2007. The fungi *Trichoderma* sp., *S. rolfsii* and *S. sclerotiorum* were cultured individually on PDA media in petridishes. A disc (5mm diameter) of *Trichoderma* sp. of five days old culture was inoculated in one side of PDA plate (9 cm diameter) and another disc of *S. rolfsii* or *S. sclerotiorum* of the same size from seven days old culture was inoculated at the opposite side of plate. The distance between two discs was approximately 5 cm. A sterile agar disc of 5mm diameter was placed instead of *Trichoderma* sp. in the control treatment. The plates were incubated at 26±1ºC for 7 days. The experimental design used in *in vitro* experiment was Completely Randomized Design (CRD) with five replications. The percentage of growth inhibition of the pathogens by *Trichoderma* sp. was calculated according to the following formula (Rini and Sulochana, 2007).

$$\text{Percentage growth inhibition} = (C-T)/C \times 100$$

Here,

C = The radial mycelia growth of *S. rolfsii* or *S. sclerotiorum* in control plate (mm)

T = The radial mycelia growth of *S. rolfsii* or *S. sclerotiorum* in presence of *Trichoderma* sp. (mm)

Data were analyzed statistically and the means were separated by LSD following MSTAT-C program.

Results and Discussion

The efficacy of five fungicides on mycelial growth of *S. rolfsii* and *S. sclerotiorum* is presented in Table 2. At 5 days after inoculation (DAI), the highest inhibition of mycelial growth of *S. rolfsii* (83.2%) and *S. sclerotiorum* (71.9%) was recorded for Bavistin 50 DF followed by Dithane M-45 (77.4% for *S. rolfsii* and 34.7% % for *S. sclerotiorum*). Among the tested fungicides, Antracol was found to be the least effective in inhibiting *S. rolfsii* since it showed only 31.4% growth inhibition at 5 DAI. For *S. sclerotiorum*, Secure gave the least inhibition of mycelial growth (26.8%). Bavistin was observed as the most effective one among five tested fungicides in an *in vitro* evaluation of mycelial growth inhibition of *S. rolfsii* (Siddique *et al.*, 2016). In another *in vitro* evaluation Goswami *et al.* (2020) observed more than 90% inhibition of *S. sclerotiorum* by carbendazim fungicide.

Table 2. Effect of five different fungicides on the inhibition of radial mycelial growth of *S. rolfsii* and *S. sclerotiorum*

Fungi	Fungicides	Inhibition growth of mycelia (%)				
		1 DAI	2 DAI	3 DAI	4 DAI	5 DAI
S. rolfsii	Dithane M-45	23.4b	39.5a	50.8b	67.6b	77.4b
	Bavistin 50 DF	30.1a	40.6a	57.5a	73.2a	83.2a
	Ridomil Gold MZ 68 WP	19.4c	21.3b	23.6d	32.3c	35.2c
	Antracol 70 WP	17.8c	19.7c	24.4c	28.4d	31.4d
	Secure 600 WG	19.5c	22.6b	25.0c	31.5c	32.3d
S. sclerotiorum	Dithane M-45	17.2b	22.2b	23.3b	30.1b	34.7b
	Bavistin 50 DF	21.8a	36.6a	54.3a	66.5a	71.9a
	Ridomil Gold MZ 68 WP	12.3c	16.5c	22.2b	29.5b	33.3b
	Antracol 70 WP	16.7b	17.9c	24.1b	25.3c	28.5c
	Secure 600 WG	13.6c	16.5c	19.7c	24.8c	26.8c

DAI = Days after inoculation, Values followed by the same letter in a column do not differ significantly at 5% level of significance

Mycelial growth of *S. rolfsii* and *S. sclerotiorum* was inhibited by the three biocontrol agents BC1, BC2 and BC3 (Fig. 1 and 2). The efficacy of three biocontrol agents on mycelial growth inhibition of *S. rolfsii* and *S. sclerotiorum* is presented in Table 3. In *S. rolfsii*, the highest inhibition of mycelial growth (78.2%) was recorded by BC1 which was significantly higher than the inhibition growth by BC3 (73.4%) and BC2 (34.2%) at 5DAI. In *S. sclerotiorum*, the highest inhibition of mycelial growth was recorded by BC1 followed by BC2. At 5DAI the inhibition of mycelial growth by BC1 was 39.4% and the least inhibition was found by BC3 (31.4%). Results of the present study is in agreement with the

findings of other workers. Mycelial growth inhibition of *S. rolfsii* by *T. harzianum* was observed 56.25% by Darvin *et al.* (2013) and 70% by Sab *et al.* (2014). In an *in vitro* study, Barai and Dalili (2016) investigated the effect of 14 isolates of *T. harzianum* on the control of *S. rolfsii* where two isolates had the most effect on mycelium inhibition of the pathogen. An isolate of *T. asperellum* was found effective to inhibit the mycelial growth of *S. sclerotiorum* (Vinodkumar *et al.*, 2017).

Table 3. Effect of three biocontrol agents on the inhibition of radial mycelial growth of *S. rolfsii* and *S. sclerotiorum*

Fungi	Biocontrol agents	Inhibition growth of mycelia (%)				
		1 DAI	2 DAI	3 DAI	4 DAI	5 DAI
S. rolfsii	BC1	26.2a	35.0a	47.3a	67.2a	78.2a
	BC2	19.4c	21.3c	22.6c	33.3c	34.2c
	BC3	23.4b	39.5b	50.8b	64.6b	73.4b
S. sclerotiorum	BC1	19.5a	22.6a	25.0a	31.5a	39.4a
	BC2	16.8b	19.6b	23.4b	30.4a	36.3b
	BC3	15.6b	18.5b	21.8c	26.3b	31.4c

DAI = Days after inoculation, Values followed by the same letter in a column do not differ significantly at 5% level of significance

The number of sclerotia production on PDA plates of *S. rolfsii* and *S. sclerotiorum* was counted at 20 DAI (Figure 3). In *S. rolfsii* the number of sclerotia ranged from 53-70 for five different fungicides (Table 4). The highest sclerotia reduction was obtained by Bavistin 50 DF (44.8%) followed by Dithane M-45 (43.8%). For the biocontrol agents the highest number of sclerotia (68) was obtained with BC2 while the least number (57) was with BC1 compared to the control. In *S. sclerotiorum* the highest reduction of sclerotia production was recorded by Bavistin 50 DF (34.4%) followed by Dithane M-45 (28.1%) (Table 5).

Table 4. Effect of five different fungicides and three biocontrol agents on sclerotia production of *S. rolfsii*

Fungicides/ biocontrol agents	Number of sclerotia production at 20 DAI	Sclerotia reduction (%) over control at 20 DAI
Dithane M-45	54d	43.8
Bavistin 50 DF	53d	44.8
Ridomil Gold MZ 68 WP	63c	34.4
Antracol 70 WP	68b	29.2
Secure 600 WG	70b	27.1
Control (without fungicide)	96a	--
BC1	57d	40.6
BC2	68b	29.2
BC3	60c	37.5
Control (without biocontrol agent)	96a	--

DAI = Days after inoculation, Values followed by the same letter in a column do not differ significantly at 5% level of significance

Table 5. Effect of five different fungicides and three biocontrol agents on sclerotia production of *S. sclerotiorum*

Fungicides/ biocontrol agents	Number of sclerotia production at 20 DAI	Sclerotia reduction (%) over control at 20 DAI
Dithane M-45	69d	28.1
Bavistin 50 DF	63e	34.4
Ridomil Gold MZ 68 WP	72c	25.0
Antracol 70 WP	76b	20.8
Secure 600 WG	71cd	26.0
Control (without fungicide)	96a	--
BC1	60d	37.5
BC2	63c	34.3
BC3	67b	30.2
Control (without biocontrol agent)	96a	--

DAI = Days after inoculation, Values followed by the same letter in a column do not differ significantly at 5% level of significance

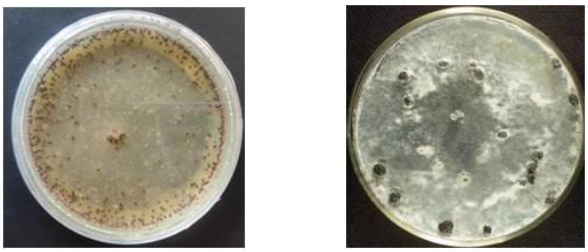
The biocontrol agent BC1 performed the best in reduction of sclerotia production (37.5%) compared to other two biocontrol agents. Inhibition of sclerotia production of *S. rolfsii* and *S. sclerotiorum* was observed by other worker (Haddad *et al.*, 2017). In a study Jegathambigai *et al.* (2010) found that at 14 DAI, sclerotia of *S. rolfsii* lost the rigidity due to the effect of *T. viride* and finally the sclerotia degraded after four weeks of inoculation. In another study Troian *et al.* (2014) observed that an isolatre of *Trichoderma* was able to multiply abundantly at the sclerotia of *S. sclerotiorum* and penetrate the inner surface of this structure and thus the iability of the sclerotia was lost.

A. BC1 B. BC3 C. BC2

Fig. 1. Inhibition of mycelial growth of *S. rolfsii* by three biocontrol agents

A. BC1 B. BC2 C. BC3

Fig. 2. Inhibition of mycelial growth of *S. sclerotiorum* by three biocontrol agents



A B

Fig. 3. Production of sclerotia of (A) *S. rolfsii* and (B) *S. sclerotiorum* on Potato Dextrose Agar (PDA) medium

Conclusion

Among the tested fungicides, the highest inhibition of mycelial growth and reduction of sclerotia production of *S. rolfsii* and *S. sclerotiorum* was recorded for Bavistin 50 DF followed by Dithane M-45. The biocontrol agent BC1 (*T. harzianum*) showed the highest inhibition of mycelial growth and reduction of sclerotia production for both *S. rolfsii* and *S. sclerotiorum*. However, further trial is needed to see the efficacy of the fungicides and biocontrol agents against *S. rolfsii* and *S. sclerotiorum* under field condition.

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