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Integrated approaches for managing collar rot disease and increasing soybean yield

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ABSTRACT

The goal of this study was to assess the effectiveness of utilizing a bio-agent in combination with a fungicide and an organic amendment to reduce collar rot disease of soybean caused by *Sclerotium rolfsii*. Before conducting field studies, *in vitro* tests were used to identify the most virulent *S. rolfsii* isolate, effective antagonistic isolate of *Trichoderma harzianum*, fungicide, and organic amendment. The SSC-1 isolate was identified as the test pathogen after seven *S. rolfsii* isolates were exposed to the pathogenicity test. The ISR-26 strain of *T. harzianum* was the most efficient of the 87 *T. harzianum* isolates which could inhibit the radial development of pathogen by 81.85%. The most effective seed-treating fungicide in the fungicidal assessment trial, Provax 200 was found at a moderate dosage (150 ppm), while Conza 5%EC required at 300 ppm to inhibit the radial growth of the *S. rolfsii* isolate SSC-1. The test pathogen's growth and development were stunted about 65.92% by mustard oil cake at a concentration of 3% which was the most effective organic amendment as compared to others. In the field trial, treatment T_9 (pathogen inoculated soil+wheat grains colonized *T. harzianum* isolate ISR–26+organic amendment+fungicide treated seeds) was the most efficient in dropping seedling mortality (80.24%), disease incidence (86.06%), and disease severity (84.8%). This T_9 treatment was the most effective procedure of collar rot disease control in soybean and it also resulted in a substantial increase in yield (355.32%). Growers of soybean around the world might be benefitted more from this holistic strategy.

Keywords: *S. rolfsii*, *T. harzianum*, Fungicides, Organic amendments, and Soybean.

INTRODUCTION

Soybean (*Glycine max* L.) is a member of the Leguminosae family. It contributes roughly 25 percent to the production of edible oil on a global scale and provides about two-thirds of the protein concentrate used in the world's livestock feed (Krishnamurthy and Shivashankar, 1975). Soybean meal is also a valuable ingredient in feeds formulated for fish and poultry. However, soybeans are one of the best plant sources of protein, containing around 36 grams of protein per 100 grams of raw soybean. This makes soybean an important food source for vegetarians and vegans. It also a good source of dietary fiber, with around 17% grams of fiber in 35% carbohydrate (Liu, 1997). That is important for digestive health and can help lower cholesterol levels. Not only that soybeans are rich in a variety of vitamins and minerals, including iron, calcium, magnesium, phosphorus, and potassium. They also contain vitamin C, vitamin K, and B vitamins such as folate and thiamine. It's a source of isoflavones, which are a type of plant compound that can have beneficial health effects. Isoflavones have been shown to have anti-inflammatory properties, and may also help lower cholesterol levels and reduce the risk of certain types of cancer. Moreover, soybeans are a good source of healthy unsaturated fats, including both polyunsaturated and monounsaturated fats (Wolke, 2007).

Growing interest in soybean among Bangladeshi farmers has led to the crop's rise in popularity as a winter staple there. In the 2020-21 season, the total cultivable land for soybean in Bangladesh was 57.47 thousand hectares, with a yield production of 91 thousand tons and an average yield of 1.58 t ha^{-1} (Anonymous, 2022). However, there are many obstacles on the way to a successful soybean harvest, such as poor seed quality,

extreme weather, erratic rainfall, and the presence of pests and diseases. More than a hundred different plant pathogens, such as sixty-six fungi, six bacteria, eight viruses, and seven nematodes, have been identified as posing a threat to soybean cultivation (Sinclair, 1988). The global soybean industry faces a serious threat from soil-borne diseases. Soybean yields and economic value can be severely impacted by soil-borne pathogens like fungi, bacteria, and nematodes. Collar rot is one of the most damaging soil-borne diseases to soybean production in the field. The fungus *S. rolfsii* is responsible for this terrible plant disease. *S. rolfsii* is one of the most destructive soil-dwelling fungi. It can infect many different types of plants, including peanuts, tomatoes, peppers, eggplants, and even other vegetables. This fungus lives in the soil and overwinters as dark, hard structures called sclerotia. Wilting, stem rot, leaf blight, and fruit rot are the symptoms of this fungus. In warm and humid conditions, the infection can spread rapidly, resulting in severe crop losses. Because of its versatility, S*. rolfsii* is difficult to eradicate from the arable land. This organism can degrade plant cell walls and other plant tissues by producing wide variety of enzymes. Secondary metabolites, such as oxalic acid, are produced by this fungus and are toxic to plant tissues and aid in the fungus' ability to colonize and spread (Paramasivan *et al.,* 2013).

However, due to its long persistence in a wide host range, this pathogen is challenging to manage. Fungicides that use chemicals are effective against the fungus, but it's costly, dangerous to humans and animals, and even pollutes the environment. Additionally, several problems have surfaced due to the reckless use of chemical pesticides and fertilizers in modern agriculture. Chemical residue has plethora of detrimental effects such as enter into the food chain, resurgence of both intended and unintended pests, and elimination of helpful creatures like honeybees. However, it is possible that the pathogen can be managed most effectively through the use of an Integrated Disease Management (IDM) strategy that combines chemical, cultural, and biological methods of control. Soybean collar rot caused by *S. rolfsii* might be managed through the strategic and skillful application of chemicals, bio-control agents, and organic amendments. Soil-borne plant pathogens can be effectively managed during crop cultivation by applying a fungicide, an organic amendment (mustard oilcake), and a biocontrol agent (*Trichoderma* sp.) (Das *et al.,* 2019). Reduced plant disease, better soil, and increased yield production; those are the results of this comprehensive package. There has not been published information regarding the integrated management of collar rot disease of soybean in Bangladesh. The goal of this study is to find out how well a bio-agent, fungicide, and an organic amendment combinedly work against the *S. rolfsii* caused collar rot disease of soybean.

MATERIAL AND METHODS

Cultural characterization of *Sclerotium rolfsii*:
Leguminous crops like soybean, bush bean, and pea were selected as sources of ten different *S. rolfsii* isolates: SSC-1, SSC-2, SBR-1, SBR-2, SBR-3, SPR-1, SPR-2, SCB-1, SCB-2, and SCB-3. Root rot symptoms were carefully chosen from infected fields. Isolates of the fungi were obtained using Mian's (1995) technique. The fungus colonies were then cultured on Potato Dextrose Agar (PDA) medium and identified using the methods described by Barnet and Hunter (1972). Finally, the chosen *S. rolfsii* isolates were incubated in triplicate on PDA plates (Rahman *et al.,* 2020a). Pathogen colony structure, zonation, colony colour, population/number of sclerotia per plate, sclerotia colour, sclerotia shape/size, and sclerotial distribution pattern were used to characterize the cultures.

Pathogenicity test:
Pathogenicity tests were conducted on soybean seedlings grown in pot culture using inocula of the test pathogen isolates (Rubayet and Bhuiyan, 2016; Rubayet *et al.,* 2017; Liton *et al.,* 2019).

Screening of *T. harzianum* isolates:
The purpose of this research was to isolate *T. harzianum* from various regions of Bangladesh and evaluated its efficacy as a biocontrol agent against a test pathogen. Thirty-seven *T. harzianum* isolates were obtained from agricultural fields in the districts of Gazipur, Chuadanga, and Meherpur of Bangladesh using the soil dilution plate technique (Dhingra and Sinclair, 1985). Fifty additional isolates were taken from the plant pathology lab at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh and subsequently confirmed to be *T. harzianum* based on morphological features. Finally, after seven days of incubation, the radial growth of the test pathogen was measured to see how well each of the 87 isolates inhibited the growth of the pathogen. The percentage of inhibition was calculated by Sundar *et al.* (1995) formula.

Effect of fungicides and organic amendments on test pathogen:

Radial colony growth:

Two experiments were conducted to evaluate the effectiveness of different treatments in inhibiting the radial colony growth of a fungal pathogen. The first experiment assessed the impact of five fungicides such as Conza 5%EC, Cabrio*^{Top} 60WP, Provax 200, Bavistin and Dithane M-45 at different concentrations (viz., 75, 150, and 300 ppm) using the poison food technique (Dhingra and Sinclair, 1985) and the second experiment assessed the effect of five organic amendments for instance oil cake of mustard (*Brassica nigra*), sesame (*Sesamum indicum*), soybean (*Glycine max*), coconut (*Cocos nucifera*) and tea (*Camellia sinensis*) waste at different concentrations (concentrations viz. 1, 2, and 3%) using standard techniques (Dhingra and Sinclair, 1985; Rubayet *et al.,* 2011; Rubayet *et al.,* 2018). After 3 days of incubation, both sets of experiments looked at how well the treatments inhibited radial colony growth of the fungal pathogen (Sundar *et al.,* 1995).

Mycelial dry weight:

The impact of the aforementioned five fungicides on the mycelial dry weight of *S. rolfsii* isolate SSC-1 was evaluated using a method similar to that developed by Rahman *et al.* (2020b). Using the same method described by Dhingra and Sinclair (1995), the same fungal isolates were grown in Potato Dextrose Broth (PDB) amended with 1, 2, and 3% (v/v) of individual organic amendments to determine their effect on mycelial dry weight. With the same formula was applied to the dry weight of the control treatment. After 20 days of incubation, the effect of sclerotia formation by *S. rolfsii* was also recorded.

Compatibility test of *T. harzianum* isolate ISR-26:

The rationale for this compatibility test of *T. harzianum* isolate ISR-26 with fungicides and organic amendments was provided by Rubayet and Bhuiyan (2012) and Rahman *et al.* (2020a).

Preparation of inoculum:

Two different isolates, *S. rolfsii* SSC-1 and *T. harzianum* ISR-26 were used in standard procedures to colonize wheat grains (Rubayet and Bhuiyan, 2016).

Integrated management of test pathogen:

Field tests were conducted to determine the efficacy of a combination of *T. harzianum* isolate ISR-26, Provax-200 seed treatment fungicide, and mustard oil cake in combating collar rot disease in soybean, caused by *S. rolfsii* Isolate SSC-1. Soybean production was also observed as a result of the experiment. The intended pathogen was deliberately introduced into the experimental field prior to seed planting.

Soybean production:

The cultivable land was readied and divided into plots following Rahman *et al.* (2018) method. Nine different treatments were assigned at random to each of the nine-unit plots (2.0 m × 1.5 m) per block. Prior to planting, the seeds were soaked for 24 hours to enhance germination and then dried to prevent excess moisture. Each treatment was administered to the seeds by treating them with Provax 200 at a rate of 0.2 g per 100 g of seeds. The seeds were then sown uniformly in rows by hand, with a row-to-row distance of 25 centimeters and a seeding rate of 45 kilograms per hectare. Weeding, mulching, and irrigation were conducted as needed throughout the experiment in the field.

Treatments application methods:

Nine different treatments were evaluated in an outdoor field experiment that was deliberately inoculated with a targeted strain of *S. rolfsii*. The first treatment, Control-1, involved the use of 5% formaldehyde to sterilize the soil by saturating it and then covering it with clear polyethylene sheets. The sheets were removed 48 hours later and allowed to air out for seven days before sowing. The targeted fungal colonized wheat grain was mixed thoroughly with the soil at a rate of 90 grams per square meter according to Yuen *et al.* (1994) design and layout. In the control plots, sterilized and air-dried wheat grains that were not colonized by the fungal isolate were inoculated at the same rate. Mustard oil cake was added to the soil of relevant treatment plots (2.0 m × 1.5 m) at a rate of 5 tons per hectare. After 21 days, *T. harzianum* isolate ISR-26 colonized wheat grains were added to the soil of the selected treatments at a rate of 50 grams per square meter (Abd-El-Khair *et al.,* 2010). Soybean seeds were then sowed in the plots of all treatments three days later. While treating seeds with fungicide, 100 g of seeds were placed in a conical flask along with 0.2 g of Provax 200, which was then thoroughly mixed before seeding.

Data recording and analysis:

The number of emerging seedlings were counted 15 days after sowing and determined the percentage of total seedling mortality that occurred both before and after the seedlings emerged. Just 30 days after planting, every other day counts were taken of infected seedlings (Rahman *et al.,* 2018). Percentages of germination and seedling mortality were calculated based on the total number of seeds planted. Following standard equations, the disease incidence (DI), percent disease index (PDI), and total yield were calculated (Rahman *et al.,* 2013; Razaq *et al.,* 2015). MSTAT-C was used to perform statistical analysis on the collected data. In cases where this was necessary, data transformations were performed prior to analysis. Duncan's Multiple Range Test was used to compare the means of the treatments (Gomez and Gomez, 1984).

RESULTS

Cultural characterization of *S. rolfsii*:

The isolates were identified based on their morphological characteristics, specifically the formation of mycelia, size and shape of sclerotia, and color. Sclerotia are compact masses of hardened mycelium that are produced by some fungi and it can be used as a distinguishing characteristic for different species. The immature sclerotia were described as round and white, while the mature sclerotia resembled mustard seeds and were dark brown in color. It is noted that all of the isolates produced a large number of small sclerotia, ranging from 426 to 578. Additionally, the color of the sclerotia was mostly dark brown (Table 1 and Fig. 1).

Table 1. Cultural characterization of *S. rolfsii* isolates on PDA medium

Isolates	Source Crops	No. of sclerotia / plate	Pattern of sclerotia formation
SSC-1^{Ø}	Soybean	578	Mostly in the periphery
SSC-2^{Ø}	Bushbean	512	Spread all over the plate
SBR-1^{Ø}	Soybean	456	Cover the plate, preferably in the margins
SBR-2^{Ö}	Soybean	562	Mostly in the periphery
SBR-3^{Ø}	Carrot	471	Cover the plate, preferably in the margins
SPR-1^{Ø}	Potato	543	Cover the plate, preferably in the margins
SPR-2^{ß}	Soybean	426	More in the periphery
SCB-1^{Ø}	Pea	459	More in the periphery
SCB-2^{Ö}	Lentil	534	More in the periphery
SCB-3^{Ø}	Brinjal	451	More in the periphery

Location: ^{Ø} = BSMRAU, ^{Ö} = Chuadanga, ^{ß} = Meherpur. Colony type: Whitish, Radiate and Fluffy and Sclerotia colour: Dark brown

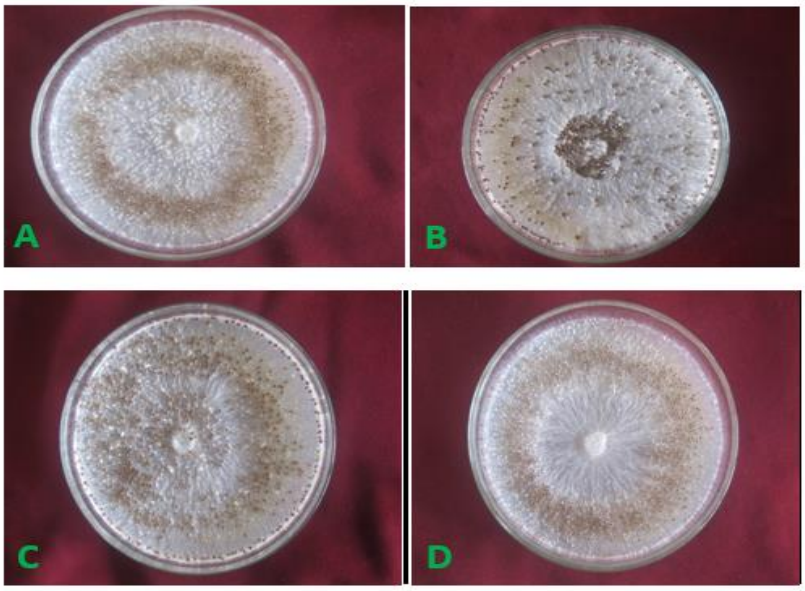

Fig. 1. Cultural variation of *S. rolfsii* isolates on PDA medium (A-D; 7 days old cultures).

Pathogenicity test:

Isolate SSC-1 was responsible for the highest rate of pre-emergence mortality in soybean (96.29 %), followed by strain SPR-2. On sterile soil without any *S. rolfsii* inoculum seedling mortality was null. Soybean seedling post-emergence mortality was highest (22.22%) in SCB-3, followed by SPR-1 (18.52%) and SCB-2 (18.52%). As for total seedling death, SSC-1 infection accounted for 96.29% of cases, SPR-2 infection for 85.18%, and sterile soil without an inoculum of *S. rolfsii* in control pots resulted in zero mortalities. In all other isolates, mortality was over 59%. (Table 2 and Fig. 2). It was found in this investigation that all of the isolates were harmful to soybean seed and seedlings, however the virulence of the isolates varied.

Table 2. Pathogenicity test of *S. rolfsii* isolates on soybean variety shohag in pot culture

Isolates	% seedling mortality		
	Pre- emergence	Post-emergence	Total
SSC-1	96.29	0.00	96.29 ^{a} $_{(82.40)*}$
SSC-2	59.26	11.11	70.37 ^{bc**} $_{(57.12)}$
SBR-1	70.36	11.11	81.47 ^{bc} $_{(64.75)}$
SBR-2	66.66	7.40	74.06 ^{bc} $_{(60.20)}$
SBR-3	62.97	14.81	77.78 ^{bc} $_{(62.38)}$
SPR-1	51.84	18.52	70.36 ^{bc} $_{(57.31)}$
SPR-2	77.78	7.40	85.18 ^{b} $_{(67.65)}$
SCB-1	59.26	3.70	62.96 ^{c} $_{(52.56)}$
SCB-2	40.73	18.52	59.25 ^{c} $_{(50.37)}$
SCB-3	44.44	22.22	66.66 ^{bc} $_{(55.59)}$
Control	0.00	0.00	0.00 ^{d} $_{(13.53)}$

* The converted arcsine values are indicated by the figures in parenthesis. **It has been determined by using the Duncan Multiple Range Test that there is no statistically significant difference (*P>0.05*) between the means of the same column followed by common letter(s).

Fig. 2. Pathogenicity test of *S. rolfsii* isolates in pot culture.

Screening of *Trichoderma* isolates against test pathogen:

The antagonistic effectiveness of 87 *T. harzianum* isolates was tested *in vitro* using the dual plate culture method against the SSC-1 isolate of *S. rolfsii*. More than half of *S. rolfsii's* radial growth was inhibited by all *Trichoderma* isolates studied *in vitro*, and their overgrowth pattern was almost identical. Eight of the nine isolates (ISR-26, ISR-25, GBRT-4, GT-71, THC-5, MYT-75, THC-3, DT-5, and GT-76) were able to limit growth of test pathogen by greater than 75%. The *T. harzianum* isolate ISR-26 was the most effective at inhibiting the growth of *S. rolfsii* isolate SSC-1 (83.51%), followed by the isolate ISR-25 (82.04%). It was found that THC-6 (51.85%) and BTG-1 (52.78%) inhibited *S. rolfsii* radial growth the least. However, only nine isolates (10.35%) showed antagonism class 1, 23 isolates (26.44%) showed antagonism class 2, and 55 isolates (63.21%) showed antagonism class 3, No isolates showed antagonism class number 4 or 5 (Table 3 and Fig. 3).

Table 3. Antagonism of *T. harzianum* isolates against *S. rolfsii* isolate SSC-1 on PDA

Class	*T. harzianum* isolates	Isolates No.	% isolates
1	ISR- 25; 26, GT-76, GBRT-4, GT-71, MYT-75, THC-3; 5, DT-5	9	10.35
2	THC-2; 4, NT-65; 66, GT- 34; 36, RTI-1; 4; 5; 7; 9; 10, ISR-24, MT -55; 59; 105, GT-35; 44, GSET-11, ISDT-13, BTG-2, TT-112, CT-102	23	26.44
3	BTG-1; 3; 4; 5; 6; 7, CT-99; 100; 101, DT-6; 7, GBRT-3; 31, GBUT-1; 2, GHT-97; 98, GSET-10, GT-20; 23, GT-74; 77; 80; 93, GUT-19, ISDT-15;16, ISR-21; 22; 23; 27; 28, ISRC-11; 12, MT-51; 52; 53; 57; 58; 104; 106; 107, NT-63; 64, RT-90, RTI-2; 3; 6; 8, THC-1; 6; 7; 8; 9; 10	55	63.21
4	_	_	_
5	_	_	_
Total	_	87	100

A B

Fig. 3. Antagonism of *Trichoderma* isolates (A= ISR-26, B = ISR-25) against *S. rolfsii* isolate SSC-1 on PDA (7 days old).

Efficacy of fungicides against test pathogen:

Complete inhibition of radial colony growth was obtained at all the concentrations of Provax-200 and Conza 5%EC at 150 ppm and 300 ppm concentration which were significantly identical and superior in comparison to the other fungicides. Bavistin 50WP was found less effective against *S. rolfsii* even at highest concentration it inhibited only 52.59% radial growth of the test pathogen. At 300 ppm Cabrio^{top} inhibited 86.29% radial growth followed by 300 ppm of Dithane M45 (66.29%). In case of mycelial dry weight, 100% inhibition was achieved with all concentration of Provax 200 and Conza 5% EC at 150 ppm and 300 ppm. Cabrio^{top} inhibited 97.22% mycelial dry weight of *S. rolfsii* followed by 300 ppm of Dithane M45 (77.34%). Bavistin 50WP and Dithane M45 showed lower inhibition of mycelial dry weight of *S. rolfsii* at 75 ppm concentration. While investigating with inhibition of sclerotia formation Provax 200 and Conza 5%EC even at lowest concentration and Cabrio^{top} at 300 ppm concentration completely (100%) inhibited sclerotia formation of the test pathogen (Table 4 and Fig. 4).

Table 4. Laboratory evaluation of fungicides against the growth and sclerotia formation of test pathogen

Fungicides (Group)	Conc. (ppm)	% inhibition in *S. rolfsii*		
		radial growth	mycelial dry weight	sclerotia
Conza 5%EC (Hexaconazole 5% EC)	75	67.03 ^{c} (54.97)	75.44 ^{b} (60.33)*	100 ^{a} (88.89)
	150	100 ^{a} (88.35)	100 ^{a} (88.35)	100 ^{a} (88.89)
	300	100 ^{a} (88.35)	100 ^{a} (88.35)	100 ^{a} (88.89)
Cabrio^{*Top} (Pyroclostribin 5%+ Metirum 55% WP)	75	39.25 ^{ef} (38.80)	52.13 ^{de} (46.23)	60.90 ^{cd} (50.72)
	150	54.07 ^{d} (47.34)	64.57 ^{c} (53.49)	80.39 ^{bc} 62.84
	300	86.29 ^{b} (68.34)	97.22 ^{a} (83.86)	100 ^{a} (88.89)
Bavistin 50WP (Carbendazim 50% WP)	75	14.81 ^{h} (22.60)	17.40 ^{g} (24.64)	26.02 ^{e} (30.74)
	150	41.11 ^{e} (39.88)	48.57 ^{e} (44.18)	54.89 $^{c-e}$ (47.03)
	300	52.59 ^{d} (46.49)	59.94 ^{cd} (50.74)	78.75 ^{bc} (61.55)
Provax 200 (Carboxin 37.5% + Thiram 37.5% WP)	75	100 ^{a} (88.35)	100 ^{a} (88.35)	100 ^{a} (88.89)
	150	100 ^{a} (88.35)	100 ^{a} (88.35)	100 ^{a} (88.89)
	300	100 ^{a} (88.35)	100 ^{a} (88.35)	100 ^{a} (88.89)
Dithane M-45 (Mancozeb 80% WP)	75	17.77 ^{g} (24.93)	26.49 ^{f} (30.98)	33.59 ^{df} (35.29)
	150	37.40 ^{f} (37.69)	51.08 ^{e} (45.62)	61.55 ^{cd} (51.37)
	300	66.29 ^{c} (54.51)	77.34 ^{b} (61.64)	89.19 ^{ab} (71.23)
Control		90.00 mm	1.013 g	575

* The converted arcsine values are indicated by the figures in parenthesis. **It has been determined by using the Duncan Multiple Range Test that there is no statistically significant difference (*P>0.05*) between the means of the same column followed by common letter(s).

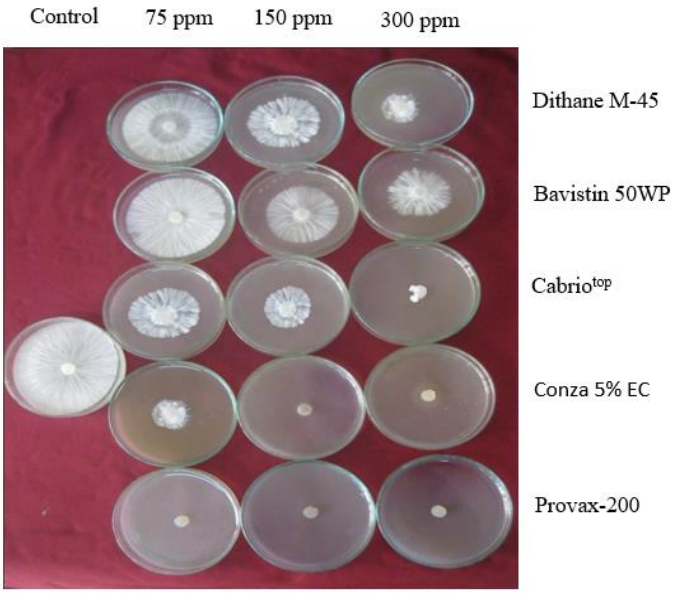

Fig. 4. Effect of fungicides at different concentrations on radial growth of *S. rolfsii* isolate SSC-1 (3 days old cultures).

***In-vitro* evaluation of organic amendments against test pathogen:**

Mustard oil cake at the highest concentration (3%) was significantly superior to all other amendments in preventing the radial growth of *S. rolfsii* by 65.92%. Mustard oil cake (at a concentration of 2%) and sesame oil cake (at a concentration of 3%) were both found to be highly inhibitive, with respective values of 47.40 and 38.51%. Soybean oil cake at 1% concentration showed the lowest inhibition (14.81%), which was statistically equivalent to that of tea waste at same concentration. Maximum mycelial dry weight 73.78% was reduced by 3% mustard oil cake. Mycelial dry weight inhibition was also found to be at its lowest with the lowest concentrations of soybean oil cake and tea waste. Inhibition of sclerotia formation was the highest (80.62%) when 3% mustard oil cake was added, far exceeding that of any other amendment. Mustard oil cake (2% concentration) and sesame oil cake (3% concentration) both showed moderately higher sclerotia inhibition (Table 5 and Fig. 5).

Table 5. *In vitro* effect of organic amendments on *S. rolfsii* isolate SSC-1

Organic amendments	Conc. (%)	% inhibition in *S. rolfsii*		
		radial growth	mycelial dry weight	Sclerotia
Mustard oil cake	1	32.59 ^{de} (34.80)*	39.65 ^{d} (39.02)	44.51 ^{e} (41.85)
	2	47.40 ^{b} (43.51)	52.37 ^{b} (46.36)	59.59 ^{b} (50.53)
	3	65.92 ^{a} (54.28)	73.78 ^{a} (59.19)	80.62 ^{a} (63.89)
Sesame oil cake	1	23.70 ^{gh} (29.12)	31.71 ^{fg} (34.26)	35.91 ^{f} (36.81)
	2	30.74 $^{d-f}$ (33.67)	34.05 ^{ef} (35.70)	42.34 ^{e} (40.60)
	3	38.51 ^{c} (38.36)	44.46 ^{c} (41.82)	54.57 ^{c} (47.63)
Soybean oil cake	1	14.81 ^{i} (22.60)	19.02 ^{h} (25.84)	21.93 ^{g} (27.91)
	2	23.33 ^{gh} (28.87)	29.69 ^{g} (33.01)	36.80 ^{f} (37.35)
	3	35.92 ^{cd} (36.82)	38.69 ^{d} (38.47)	49.87 ^{d} (44.93)
Coconut oil cake	1	20.37 ^{h} (26.82)	22.32 ^{h} (28.18)	23.72 ^{g} (29.14)
	2	27.40 ^{fg} (31.55)	31.29 ^{fg} (33.94)	35.83 ^{f} (36.24)
	3	33.70 ^{cd} (35.48)	35.74 ^{de} (36.71)	42.52 ^{e} (40.70)
Tea waste	1	16.29 ^{i} (23.61)	19.70 ^{h} (26.29)	20.92 ^{g} (27.11)
	2	27.40 ^{fg} (31.55)	28.40 ^{g} (32.20)	32.07 ^{f} (34.49)
	3	28.14 $^{e-g}$ (32.04)	30.10 ^{fg} (33.28)	34.93 ^{f} (36.22)
Control		90.00	1.018 g	573

* The converted arcsine values are indicated by the figures in parenthesis. **It has been determined by using the Duncan Multiple Range Test that there is no statistically significant difference (*P>0.05*) between the means of the same column followed by common letter(s).

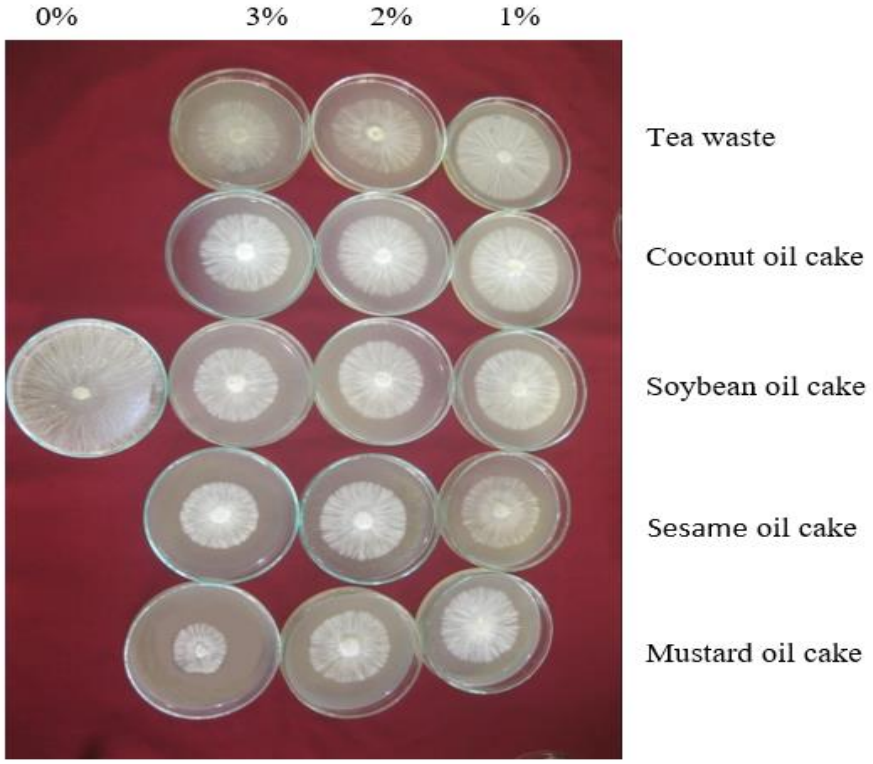

Fig. 5. Effect of organic amaendment at different concentrations on radial growth of *S. rolfsii* isolate SSC-1 (3 days old cultures).

Integrated effect of bio-agent, fungicide, and organic amendment:

Effect on soybean collar rot:

The treatment T_1, where no pathogen was added, showed the lowest pre- and post-emergence seedling mortality as well as the lowest overall seedling mortality at 7.89%, 1.75%, and 9.64%, respectively. However, the treatment T_9 resulted in the highest reduction of seedling mortality at 80.24%. On the other hand, treatment T_5 showed the lowest reduction of seedling mortality, which was similar to the reduction observed in treatment T_3 (Table 6). The application of bio-agents, fungicides, and organic amendments either alone or in combination also affected the incidence and severity of collar rot disease in soybean. The treatment T_9 had the lowest disease incidence at 7.40% and severity at 6.67%, followed by treatments T_6 and T_7 (Table 7 and Fig. 6).

Table 6. Integrated effect of bio-agent, fungicide, and organic amendment on seedling mortality of soybean

Treatments	% Seedling mortality			% reduction
	Pre-emergence	Post-emergence	Total	
T_1 [Seeds sown in sterilized soil (Control-1)]	7.89 (16.31)	1.75 (7.61)	9.64 ^{g**} (18.08) *	-
T_2 [Inoculated Soil (IS) + Fresh seeds (Control-2)]	53.50 (47.01)	21.92 (29.92)	75.42 ^{a} (60.29)	-
T_3 [IS + Seeds Treated with Fungicide (STF)]	28.69 (32.39)	13.15 (21.26)	41.84 ^{c} (40.31)	44.52
T_4 [IS + Colonizd Wheat Grains *T. harzianum* (CWGT) + Fresh seeds]	24.56 (29.70)	12.28 (20.51)	36.84 ^{cd} (37.37)	51.15
T_5 [IS+ Organic compound (OC) + Fresh seeds]	35.08 (36.32)	17.54 (24.76)	52.62 ^{b} (46.50)	30.23
T_6 (IS+ CWGT + STF)	15.35 (23.06)	8.77 (17.22)	24.12 ^{f} (29.41)	68.01
T_7 (IS+ CWGT + OC + Fresh seeds)	18.85 (25.73)	10.52 (18.93)	29.37 ^{ef} (32.82)	61.05
T_8 (IS+ OC + STF)	21.05 (27.31)	12.28 (20.51)	33.33 ^{de} (35.26)	55.80
T_9 (IS+ CWGT + OC + STF)	9.64 (18.09)	5.26 (13.26)	14.90 ^{g} (22.71)	80.24

* The converted arcsine values are indicated by the figures in parenthesis. **It has been determined by using the Duncan Multiple Range Test that there is no statistically significant difference (*P>0.05*) between the means of the same column followed by common letter(s).

Table 7. Integrated effect of bio-agent, fungicide and organic amendment on collar rot disease of soybean

Treatments	% disease incidence	PDI	% disease reduction	
			Incidence	PDI
T_1 [Seeds sown in sterilized soil (Control-1)]	0.00 ^{j} (1.28) *	0.00 ^{g} (1.28) *	100	100
T_2 [Inoculated Soil (IS) + Fresh seeds (Control-2)]	53.09 ^{a} (46.77)	44.00 ^{a} (41.55)	-	-
T_3 [IS + Seeds Treated with Fungicide (STF)]	29.41 ^{c} (32.84)	23.33 ^{bc} (28.88)	44.60	46.98
T_4 [IS + Colonizd Wheat Grains *T. harzianum* (CWGT) + Fresh seeds]	25.92 ^{d} (30.61)	21.33 ^{cd} (27.49)	51.17	51.52
T_5 [IS+ Organic compound (OC) + Fresh seeds]	37.03 ^{b} (37.48)	26.67 ^{b} (31.08)	30.25	39.38
T_6 (IS+ CWGT + STF)	16.98 ^{g} (24.33)	14.67 ^{e} (22.47)	68.01	66.65
T_7 (IS+ CWGT + OC + Fresh seeds)	20.64 ^{f} (27.02)	17.33 ^{de} (24.57)	53.58	60.61
T_8 (IS+ OC + STF)	23.44 ^{e} (28.96)	18.67 ^{de} (25.57)	55.85	57.57
T_9 (IS+ CWGT + OC + STF)	7.40 ^{h} (17.76)	6.67 ^{f} (14.80)	86.06	84.84

* The converted arcsine values are indicated by the figures in parenthesis. **It has been determined by using the Duncan Multiple Range Test that there is no statistically significant difference (*P>0.05*) between the means of the same column followed by common letter(s).

Fig. 6. Symptoms of collar rot disease at soybean field

Effect on yield and yield components of soybean:

The highest increases in plant height, pod yield, and seed weight were observed with the combination of treatments T_9 and T_7. The lowest level of growth promotion was found with treatment T_3, which was statistically indistinguishable from the control-2 condition. Significant differences in total yield from treatment to control-2 were also observed. The highest seed yield (2.14 t ha^{-1}) was achieved with the T_9 treatment, which included colonized *T. harzianum*, Provax 200 treated seed, and mustard oil cake. All three T_6–T_8 treatments produced more seeds than the control–2 treatment. The lowest yield, 0.47 t ha^{-1}, came from treatment T_2, which involved sowing fresh seeds into soil already inoculated with *S. rolfsii* isolate SSC-1 and then did not take any preventative measures (Table 8).

Table 8. Effect of different treatments on yield and yield components of soybean

Treatments	Plant height (cm)	No. of pod/ plant	100 seed weight (g)	Yield (t ha^{-1})	% increased
T_1 [Seeds sown in sterilized soil (Control-1)]	72.70 def	51.11 d	10.38 bc	1.98 b	-
T_2 [Inoculated Soil (IS) + Fresh seeds (Control-2)]	69.90 f	46.33 e	8.85 d	0.47 g	-
T_3 [IS + Seeds Treated with Fungicide (STF)]	71.03 ef	50.34 de	9.48 cd	1.35 e	187.23
T_4 [IS + Colonizd Wheat Grains *T. harzianum* (CWGT) + Fresh seeds]	72.70 def	51.47 d	10.25 bc	1.65 cd	251.09
T_5 [IS+ Organic compound (OC) + Fresh seeds]	75.16 cd	56.00 bc	9.63 cd	1.15 f	144.68
T_6 (IS+ CWGT + STF)	74.06 cde	52.22 cd	10.52 bc	1.81 c	285.10
T_7 (IS+ CWGT + OC + Fresh seeds)	79.26 b	58.44 ab	10.98 ab	1.69 cd	259.57
T_8 (IS+ OC + STF)	76.26 bc	57.46 b	10.30 bc	1.63 d	246.80
T_9 (IS+ CWGT + OC + STF)	83.26 a	62.33 a	11.87 a	2.14 a	355.32

* The converted arcsine values are indicated by the figures in parenthesis. **It has been determined by using the Duncan Multiple Range Test that there is no statistically significant difference (*P>0.05*) between the means of the same column followed by common letter(s).

DISCUSSION

The cultural characterization of *Sclerotium rolfsii* based on the observations of Sharma *et al.* (2002) highlights specific morphological traits that aided in its identification. The fungus exhibits fluffy mycelium and forms sclerotia ranging from light brown to dark brown, with diameters typically falling within the range of 1.0 to 1.2 mm. Notably, the sclerotia possess a well-defined structure consisting of a rind, cortex, and medulla, which distinguishes them from sclerotia produced by other fungi.

In terms of pathogenicity, *S. rolfsii* has been extensively studied for its detrimental effects on various crops, particularly soybeans. Isolate SSC-1, characterized as highly virulent, has been shown to cause significant mortality in soybean seedlings due to foot and root rot. Pathogenicity tests conducted by Akther (2000) and Hasnat (2004) confirmed the pathogen's responsibility for 100% mortality in soybean seedlings under controlled conditions. Furthermore, similar tests conducted by other researchers have highlighted the broad spectrum of crops susceptible to *S. rolfsii*, including potato, lentil, bush bean, and carrot (Rubayet and Bhuiyan, 2016; Bhuiyan and Rubayet, 2023, Das *et al.,* 2019, Liton *et al.,* 2019, Ahmed *et al., 2019*; Rubayet *et al.,* 2020).

The efficacy of Trichoderma isolates as biocontrol agents against *S. rolfsii* has been demonstrated through *in vitro* screening assays. These isolates exhibited antagonistic behavior towards *S. rolfsii*, forming a clear zone of interaction on PDA media within 24 hours. This interaction leads to the inhibition of *S. rolfsii* growth, as evidenced by the formation of small tufts. This biocontrol mechanism has also been reported by Sen (2010), Liton *et al.* (2019), and Das *et al.* (2019), indicating the consistent efficacy of *Trichoderma* spp. against *S. rolfsii* and other phytopathogens such as *Colletotrichum capsici* (Simi *et al.,* 2019), *Fusarium oxysporum* (Rahman *et al.,* 2020), *Macrophomina phaseolina* (Rahman *et al.,* 2021; Rubayet and Bhuiyan, 2023).

Regarding chemical control measures, fungicides such as Provax-200 and Conza 5%EC have been identified as effective options for managing *S. rolfsii* infections, even at lower concentrations. This is supported by previous studies conducted by Verma and Dohroo (2002), Pant and Mukhopadhyay (2001), and Manu *et al.* (2012). In addition to chemical treatments, organic amendments like mustard oil cake have been shown to inhibit the growth and sclerotia formation of *S. rolfsii in vitro* (Sen, 2010; Rubayet *et al.*, 2011). The suppression of *S. rolfsii* by mustard oil cake may be attributed to the release of fungal toxic substances during decomposition, as suggested by Gautam and Kolte (1979).

Furthermore, the use of *Trichoderma harzianum* as a bio-agent has been found to promote plant growth and reduce disease incidence, leading to increased crop yield. *T. harzianum* enhances iron availability in the soil by dissolving essential minerals, contributing to improved plant health (Altomare *et al.,* 1999). Seed treatment with *T. harzianum* spore suspension has been shown to be more effective than foliar spray in protecting against southern blight disease and enhancing carrot yield (Ahmed *et al.,* 2019). Additionally, *T. harzianum*-based treatments effectively control anthracnose disease in chili (Simi *et al.,* 2019) and reduce the prevalence of potato stem rot caused by *S. rolfsii* under open field conditions (Rubayet and Bhuiyan 2016).

The comprehensive understanding of the cultural characterization, pathogenicity, management strategies, and impact of *S. rolfsii* presented in this study highlights the importance of integrated pest management approaches for effective disease control and sustainable crop production.

CONCLUSION

The findings of the current investigation demonstrated that the combined application of bio-agent (*T. harzianum* isolate ISR-26), seed treatment with fungicide (Provax 200), and organic amendment (Mustard oil cake) provided a successful approach for controlling collar rot disease in soybean induced by *S. rolfsii* isolate SSC-1. Furthermore, this strategy could be considered as a viable and sustainable substitute for decreasing the pathogen population density and enhancing soybean yield in the agricultural field.

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