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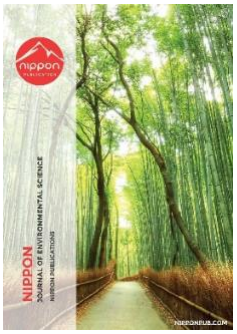

Full Length Research Paper

Integrated management of rhizoctonia root rot disease of soybean caused by *Rhizoctonia solani*

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Abstract. Integration of bio-agent, fungicide and organic amendment was applied for the management of rhizoctonia root rot disease of soybean caused by *Rhizoctonia solani*. Before going to the field experiment, *in vitro* trials were conducted to select a virulent isolate of *R. solani*, an effective antagonistic isolate of *Trichoderma harzianum*, suitable fungicide and organic amendment. Among the ten isolates of *R. solani*, RS-5 isolate was selected as a test pathogen by the pathogenicity test. On the other hand, among the eighty-seven isolates of *T. harzianum*, ISR-26 isolate depicted the highest (77.37%) inhibition of radial growth of test pathogen. In the case of fungicidal evaluation trial, Conza 5% EC, Bavistin 50 WP and Provax 200 WP were found the most effective fungicide at all concentration such as 75, 150 and 300 ppm. Additionally, *in vitro* trial of different organic amendments, mustard oil cake was found the most active organic amendment for reducing the radial growth (66.29%) and mycelial dry weight (73.46%) at the level of 3% concentration. In the field trial, integrated use of *T. harzianum* with Provax 200 WP and mustard oil cake under the treatment T_9 was appeared the effective treatment in reducing seedling mortality (73.34%), disease incidence (84.72%) as well as disease severity (84.21%) caused by the pathogen of *R. solani*. Moreover, treatment T_9 was the best treatment not only for management of the soybean root rot disease but also increased the significant quantity of yield (126.53%).

Keywords: *R. solani*, *T. harzianum*, Fungicides, Organic amendments and Soybean.

1. INTRODUCTION

Soybean (*Glycine max* L.) can contribute not only around 25% of the global edible oil but also about two-thirds of the world's protein concentrate for livestock feeding. Moreover, it is comparatively cheaper than the animal sources of protein such as meat, fish, milk, egg etc. It has an average 42.5% protein, 19% edible oil, 35% carbohydrate and vitamins (Kaul and Das, 1986). Now-a-days, soybean is becoming a popular winter crop especially in the southern part districts of Bangladesh. Farmers are becoming attracted to cultivate soybean instead of other legume crops due to market demand. But, there are some problems for the production of soybean such as quality seeds, climatic conditions, differences in rainfall pattern, an outbreak of diseases and pests etc. Among these features, plant disease is one of the major factors for low production of soybean all over the world. Meanwhile, around hundred of devastating plant pathogens has been identified against soybean diseases where 66 fungi, 6 bacteria, 8 viruses and 7 nematodes (Sinclair, 1978).

R. solani is one of the notorious soil-borne fungus which can cause *Rhizoctonia* damping-off and root rot disease of soybean. The prominent symptoms of this disease are usually remarked by early summer, where wilted or dead seedlings are found scattered throughout the field or in small concentrated areas. This fungus can cause seed rot, root rot and lesions on hypocotyls. The damping-off disease occurs when germinating seedlings are infected prior to emergence. Additionally, reddish-brown, sunken lesions form on hypocotyls of young seedlings. The resulting firm, dry canker can tie the seedling and cause it to demise. On the other hand, older plants or seedlings that may survive even exhibit the similar symptoms such as characteristic sunken, reddish-brown cankers on the lower stem near the soil surface and irregular or stunted growth. Older diseased plants ultimately become chlorotic and closely resemble plants with a nitrogen deficiency.

However, management of this pathogen is very difficult because its wide host range. Sometimes seed treatment with chemical fungicide is effective against this fungus but these chemicals are costly and detrimental for human and animals. Moreover, indiscriminate use of chemical pesticides and fertilizers in modern agriculture has resulted in the enlargement of several complications such as pesticide resistance in pests, the resurgence of target and non-target pests, destruction of beneficial organisms like honey bees, ladybird bettle and chemical residues in food, feed and fodder. On the other hand, Integrated Disease Management (IDM) strategy is comparatively safe, environment friendly and durable. Integration of chemical, cultural and biological approaches to control *R. solani* may be most effective rather than individually application. Effective and proficient use of chemicals, bio-control agents and organic amendments therefore may be potential to control the rhizoctonia root rot disease of soybean caused by *R. solani.* Several reporters (Rubayet and Bhuiyan, 2016; Arefin et al., 2019; Liton et al., 2019; Ahmed et al., 2019) found that combined application of fungicide, organic amendment (mustard oilcake), biocontrol agent (*Trichoderma* sp.) are highly effective for controlling the soil-borne as well as seed-borne plant pathogens during crops cultivation. This combined package not only minimizes the plant diseases but also improves the soil health and ultimately crop production. However, a few studies have been done on management of rhizoctonia root rot disease of soybean but there is no report on integrated management of above mentioned disease of soybean in Bangladesh.

Considering the aforesaid facts, the present research was undertaken to evaluate the effectiveness of integrated disease management strategies consisted of bio-agent, fungicide and organic amendment against rhizoctonia root rot disease of soybean caused by *R. solani*.

2. MATERIALS AND METHODS

2.1. Isolation and preservation of *R. solani* isolates

Ten isolates of *R. solani* designated as RS-1 to RS-10 were isolated from infected root, and pod tissues of soybean, bush bean, pea and carrot. The specimens which had typical symptoms of root rot were selected from the infected fields. The fungal isolates were isolated according to standard method (Mian, 1995). Then, the fungal colonies were grown on PDA and identified according to Barnet and Hunter, 1972. The isolates were purified following hyphal tip technique and stored in PDA slants at 10 °C.

2.2. Cultural characterization of *R. solani* isolates

The selected isolates RS-1 to RS-10 were individually inoculated into three replicated PDA plates using 5 mm diameter mycelial disk which taken from 3 days old PDA cultures. Then, all PDA culture plates were sealed with parafilm paper tightly and incubated at room temperatures (25±2°C) for 7 days. After 7 days of incubation, the cultural characteristics such as colony color, colony type, zonation, sclerotia formaton and types were observed and recorded. The isolates colony type and colony color were mostly compact and dark brown in color.

2.3. Inoculum preparation of test pathogen

Inoculum of the pathogen isolates were made and stored following standard method (Rubayet and Bhuiyan, 2016).

2.4. Pathogenicity test

The pathogenicity test of *R. solani* isolates were conducted in pot culture on soybean plant according to the standard method (Rubayet et al., 2017; Liton et al., 2019).

2.5. Collection, isolation and preservation of *T. harzianum* isolates

A total of 87 isolates of *T. harzianum* whereas 37 isolates were isolated from the different crop fields of Gazipur, Chuadanga and Meherpur districts of Bangladesh following the soil dilution plate technique (Dhingra and Sinclair, 1985). And rest of 50 isolates were collected directly from the plant pathology laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh. All the isolated *Trichoderma* spp. were identified as *T. harzianum* based on the diverse morphological features like hyphal growth, spore formation and color. The pure culture of *T. harzianum* was preserved for future application (Das et al., 2019).

2.6. Screening of *T. harzianum* isolates against *R. solani* isolate RS-5

In vitro screening was accompanied to assess the antagonistic effect of 87 selected isolates of *T. harzianum* against test pathogen on PDA medium by dual plate culture technique (Dhingra and Sinclair, 1985). After 7 days of incubation the inhibition percentage of radial growth of test pathogen was calculated using the following formula (Sundar et al., 1995).

% inhibition of growth = (X-Y/X) ×100. Where, X = Mycelial growth of pathogen in absence of *T. harzianum* (control) and Y= Mycelial growth of pathogen in presence of *T. harzianum*.

2.7. Evaluation of fungicides and organic amendments against *R. solani* isolate RS-5

2.7.1. Effect on radial colony growth

Five fungicides with three different concentrations viz., 75, 150 and 300 ppm were assessed their effect on radial colony growth following poison food technique (Dhingra and Sinclair, 1985) (Table 1). Three replicated plates with Complete Randomize Design (CRD) were used for each dose of every fungicide. The inoculated plates were incubated in the laboratory having an ambient temperature of 28±3 °C (Rubayet et al., 2011). Data on radial colony diameter were recorded after 3-days of incubation when the check plate was enclosed with the growth of test pathogen. The diameter of colonies on PDA with and without fungicide were measured from the bottom side of the petri dishes. The inhibition of radial colony growth in amended plates were calculated based on colony diameter of check plate following the formula as suggested by Sundar et al. (1995) mentioned earlier.

Another an *in vitro* experiment was conducted to determine the effect of organic amendments (mustard oil cake, sesame oil cake, soybean oil cake, coconut oil cake and tea waste) at 3 different concentrations such as 1, 2 and 3% on the growth of *R. solani* isolate RS-5 following standard techniques (Dhingra and Sinclair, 1985; Rubayet et al., 2011; Rubayet et al., 2018). Three days after incubation the inhibition of radial colony growth in the amended plates were computed based on colony diameter of control plate using the same formula as stated above by Sundar et al. (1995).

Table 1. List of fungicides and their active ingredients

Fungicides	Active ingredients	Mode of actions
Conza 5% EC	Hexaconazole 5% EC	Systemic
Cabrio*^{Top}	Pyroclostribin 5% + Metirum 55% WP	Systemic and contact
Provax 200 WP	Carboxin 37.5% + Thiram 37.5% WP	Systemic and contact
Bavistin 50 WP	Carbendazim 50% WP	Systemic
Dithane M-45	Mancozeb 80% WP	Systemic and contact

2.7.2. Effect on mycelial dry weight

The effect of aforesaid fungicides on mycelial dry weight of *R. solani* isolate RS-5 were determined by Rahman et al. (2020). On the other hand, the effect of organic amendments on mycelial dry weight of *R. solani* isolate RS-5 were also determined by growing fungi in the potato dextrose broth amended with individual organic amendments at the concentration of 1, 2 and 3% (v/v) following the same technique as described earlier (Dhingra and Sinclair, 1985). Inhibition of mycelial dry weight in the amended broth was calculated based on dry weight in control treatment following the aforesaid formula.

2.8. Compatibility of *T. harzianum* isolate ISR-26 with fungicides and organic amendments

The compatibility test of *T. harzianum* isolate ISR-26 with fungicides and organic amendments were justified according to the Rubayet and Bhuiyan (2012), and Rahman et al. (2020).

2.9. Preparation of bio-agent inoculum

The selected isolate of *T. harzianum* isolate ISR-26 was used for production of wheat grain colonized inoculum following the standard procedures (Rubayet and Bhuiyan, 2016).

2.10. Integrated effect of *Trichoderma*, fungicide and organic amendment on *R. solani* isolate RS-5

A field experiment was conducted to find out the effect of integrated use of *T. harzianum* isolate ISR-26, Provax-200 WP and mustard oil cake against the rhizoctonia root rot disease of soybean caused by *R. solani* isolate RS-5 and response on yield. The test pathogen was artificially inoculated in the respective experimental field before sowing the seeds.

2.11. Cultivation of soybean

Cultivable land was prepared and made the plot according to Rahman et al. (2018). Nine different treatments were allotted randomly to nine unit plots per block. Before sowing, seeds were soaked for 24 hours to facilitate the germination and also dried for avoiding excess water. For the respective treatment of trial, seeds were treated with Provax 200 WP @ 0.20 g 100^{-1}g seeds. Then, seeds were sown in lines uniformly by hand (45 kgha^{-1}) and maintain

the row to row distance 25 cm. Weeding, mulching and irrigation were done in the experimental field whenever it was necessary.

2.12. Treatments and application methods

The treatments were T_1 = Untreated healthy seeds sown in sterilized soil (control-1), T_2 = Soil inoculated with *R. solani* isolate RS-5 + Fresh seeds (control-2), T_3 = Soil inoculated with *R. solani* isolate RS-5 + Provax 200 WP treated seeds, T_4 = Soil inoculated with *R. solani* isolate RS-5 + Wheat grain colonized *T. harzianum* isolate ISR-26 + Fresh seeds, T_5 = Soil inoculated with *R. solani* isolate RS-5 + Mustard oil cake + Fresh seeds, T_6 = Soil inoculated with *R. solani* isolate RS-5 + Wheat grain colonized *T. harzianum* isolate ISR-26 + Provax 200 WP treated seeds, T_7 = Soil inoculated with *R. solani* isolate RS-5 + Wheat grain colonized *T. harzianum* isolate ISR-26 + Mustard oil cake + Fresh seeds, T_8 = Soil inoculated with *R. solani* isolate RS-5 + Mustard oil cake + Provax 200 WP treated seeds, T_9 = Soil inoculated with *R. solani* isolate RS-5 + Wheat grain colonized *T. harzianum* isolate ISR-26 + Provax 200 WP treated seeds + Mustard oil cake.

Nine treatments were tested in the open field under artificially inoculated condition. Control-1 was sterilized with 1% formaldehyde by drenching the soil properly. After treating with formaldehyde the soil was covered with transparent polyethene sheets. Polyethene sheets were removed after 48 hours and exposed to air 7 days before sowing. Inoculum of a selected isolate of *R. solani* was thoroughly mixed with soil according to design and layout @ 90 gm^{-2} soil as suggested by Yuen et al. (1994). Water soaked, sterilized and air dried wheat grains were inoculated at the same rate in the control plots. Mustard oil cake was mixed with soil of concerned treatments plot @ 5 tha^{-1}. After 21-days, wheat grains colonized *T. harzianum* isolate ISR-26 was mixed thoroughly with the soil of selected treatments @ 50 gm^{-2} (Abd-El-Khair et al., 2010). Then after 3-days, soybean seeds were sown in the plots of all treatments. In the case of seeds treatment with fungicide, around 100 g seeds were taken in a conical flask then added 0.2 g Provax 200 WP and mixed properly before sowing.

2.13. Data recording

The number of emerged seedling was recorded after 15-days of sowing and converted into percent pre- and post-emergence mortality of seedlings. Diseased seedlings were counted every alternate day and continued up to 30 days after sowing (Rahman et al., 2018*)*. Germination and seedling mortality were expressed in percentage based on total number of seeds planted. The disease incidence (DI), percent disease index (PDI) and total yield were assessed by the following formulas (Rahman et al., 2013; Razaq et al., 2015).

DI=(No. of infected plants/ Total No. of plants assessed) × 100

PDI=[Summation of all ratings/ {Total No. of rating × Max. disease grade (4)}] × 100

Total pod yield (tha^{-1})=[Yield per plot (kg)/{Area of plot (m^2) × 1000 (kg)}] × 10000 m^2

2.14. Data analysis

Statistically, data were analyzed using the MSTAT-C computer program after proper transformation whenever it was necessary. The treatment means were compared following Duncan's Multiple Range Test (Gomez and Gomez, 1984).

3. RESULTS AND DISCUSSION

3.1. Isolation and cultural characterization of *R. solani* isolate RS-5

All the isolates were identified based on the morphological characteristics such as colony color, type, structure and sclerotia formation etc. which produced on PDA medium (Butler and Braker, 1970). Initially all the isolates produced hyaline color when they were young on PDA plates, but during the maturity colony of three isolates became brown and seven isolates became light brown in color. Colony structures were varied compact and slightly fluffy. On the country, five isolates produced concentric zones on mycelial colony and another five isolates had no concentric zonation. Number of zonation also varied from isolate to isolate. (Table 2). Moreover, a slightly constriction at the point of hyphal branching and right angle branching were observed under compound microscope in matured hyphae.

3.2. Pathogenicity test of *R. solani* isolates in pot culture

The pathogenicity test was done of ten selected isolates of *R. solani* designated as RS-1 to RS-10 against soybean seedlings in the pot culture experiment. The highest pre-emergence mortality (74.06%) was caused by the isolate RS-5 followed by isolate RS-7 (70.36%). On the other hand, the lowest pre-emergence mortality (7.41%)

was found in control treatment which was followed by the isolate RS-6 (37.03%). In the meantime, RS-2 and RS-4 isolates dipcted the highest post-emergence mortality (18.52%) of soybean seedling which was followed by RS-1, RS-5 and RS-6, respectively (Table 3). While considering total seedling mortality, the highest total mortality (88.87%) was observed due to infection of RS-5 isolate followed by RS-1 (77.77%). All other isolates caused more than 50% total mortality due to dry root rot of soybean. The results of the present study indicated that all the isolates are pathogenic to seed and seedlings of soybean but varied to their virulency. Based on the present findings isolate RS-5 was selected for further study. The results of the pathogenicity test are supported by Haider (2005) who found 96.67% seedling mortality due to dry root rot of soybean.

Table 2. Cultural characterization of *R. solani* isolates on PDA medium

Isolates	Sources	Colony types	No. of zonations	Colony colors	Sclerotial population
RS-1*	Soybean	Compact	2	Light brown	+++
RS-2*	Carrot	Slightly fluffy	No	Brown	++
RS-3*	Soybean	Slightly fluffy	No	Brown	++
RS-4*	Bush bean	Slightly fluffy	2	Brown	++
RS-5º	Soybean	Compact	4	Light brown	++
RS-6Ö	Soybean	Compact	2	Light brown	++
RS-7Ø	Soybean	Compact	No	Light brown	++
RS-8*	Soybean	Compact	No	Light brown	+++
RS-9*	Pea	Compact	2	Light brown	++
RS-10*	Carrot	Slightly fluffy	No	Light brown	+++

Location: *= BSMRAU, º = Meherpur, Ö = BARI, Ø = Chuadanga. Sclerotium of *R. solani* was measured on eye observation such as +++, ++, + and -, representing abundant, moderate, minimum and no sclerotial crust, respectively.

Table 3. Pathogenicity test of *R. solani* isolates in pot culture

Isolates	% seedling mortality		
	Pre-emergence	Post-emergence	Total
RS-1	62.96	14.81	77.77 ^{ab} (62.38)*
RS-2	44.44	18.52	62.96 $^{b-e}$ (52.55)
RS-3	59.25	11.11	70.36 $^{b-d}$ (57.11)
RS-4	40.74	18.52	59.26 $^{c-e}$ (50.37)
RS-5	74.06	14.81	88.87 ^{a**} (70.52)
RS-6	37.03	14.81	51.84 ^{e} (46.06)
RS-7	70.36	3.70	74.06 ^{bc} (59.49)
RS-8	59.26	7.40	66.66 $^{b-d}$ (54.93)
RS-9	48.14	11.11	59.25 ^{cd} (50.37)
RS-10	51.85	3.70	55.55 ^{de} (48.24)
Control	7.41	0.00	7.41 ^{f} (13.53)

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

3.3. Screening of *T. harzianum* isolates against *R. solani* isolate RS-5

An *in vitro* experiment was conducted to evaluate the antagonistic efficiency of 87 isolates of *T. harzianum* against one selected isolate of *R. solani* isolate RS-5 following dual plate culture technique.

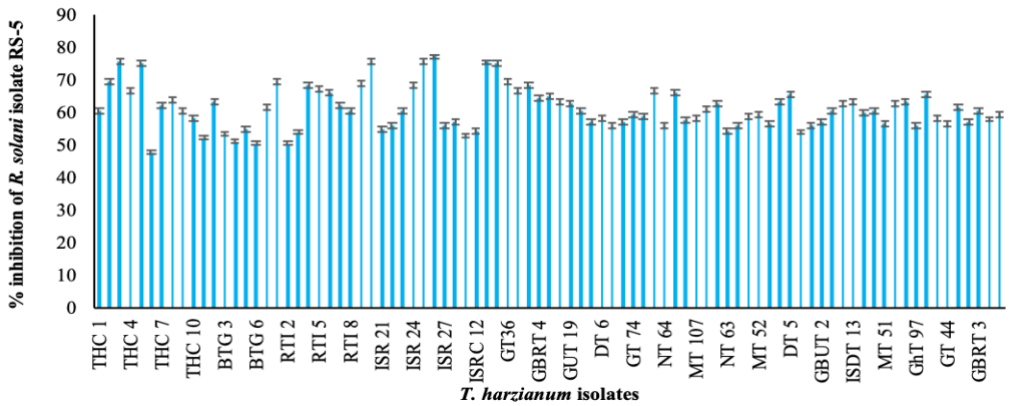

Figure 1: Percent inhibition of *R. solani* isolate RS-5 mycelial growth by *T. harzianum* isolates in dual culture on PDA

T. harzianum isolate ISR-26 showed the highest 77.37% inhibition of radial growth followed by the isolate ISR-25 (75.82%) (Fig. 1 & 2). The lowest 47.93% inhibition in radial growth was observed with the isolate THC-6 against *R. solani* isolate RS-5. *T. harzianum* showed a great variation in their degree of antagonism which differ from isolate to isolate against *R. solani*. Among the isolates, only seven isolates (8.04%) were appeared as antagonism class 1 and 11 isolates (12.64%) showed antagonism class 2 while 63 isolates (72.43%) showed antagonism class 3 and rest six isolates (6.89%) showed antagonism class number 4 (Table 4). There was no isolate recorded under the antagonism of class 5. Moreover, the variation of growing pattern in dual culture plate was found with different isolates of *T. harzianum*. Similar observations were also reported by other investigators (Das et al., 2019; Rahman et al., 2020).

Figure 2: Antagonism of *T. harzianum* against *R. solani* isolate RS-5 on PDA (A = ISR-25, B = ISR-26)

Table 4. Antagonism of *T. harzianum* isolates against *R. solani* isolate RS-5 in dual culture on PDA

Classes	*T. harzianum* isolates	Number of isolates	% isolates
1	ISR-26, ISR-25, GT-71,THC-5, RTI-10, THC-3, MYT-75	7	8.04
2	GT-76, RTI-1, THC-2, RTI-9, ISR-24, RTI-4, RTI-5, THC-4, GT-36, MT -59, NT-65,	11	12.64
3	NT-66, RTI-6, DT-5, TT-112, GT-35, GBRT-4, THC-8, GSET-11, ISDT-13, GT-23, MT-55, BTG-2, GBUT-1, CT-102, GUT-19, MT-53, THC-7, RTI-7, GT-93, BTG-7, MT-58, MT-104, THC-9, THC-1, GBRT-31, ISR-23, RTI-8, GBRT-3, DT-7, CT-99, MT-52, GT-74, CT-100, MT-57, MT-106, MT-107, GT-80, THC-10, DT-6, RT-90, MT-105, ISR-28, ISDT-15, GBUT-2, CT-101, GHT-98, GT-44, GSET-10, MT-51, ISDT-16, GT-34, ISR-22, GT-77, ISR-27, GHT-97, NT-64, BTG-5, ISR-21, ISRC-12, NT-63, GT-20, RTI-3, BTG-3,	63	72.43
4	ISRC-11, BTG-1, BTG-4, RTI-2, BTG-6, THC-6	6	6.89
5	-	-	-
Total	--	87	100

3.4. Efficacy of fungicides against *R. solani* isolate RS-5

An *in vitro* test was conducted to evaluate the efficacy of five different fungicides with three different concentrations on radial colony growth, mycelial dry weight and sclerotial population of *R. solani* isolate RS-5. The complete inhibition of *R. solani* was achieved with all the three (75, 150 and 300 ppm) concentrations of Conza 5% EC, Provax 200 WP and Bavistin 50 WP fungicides. The second highest inhibition (65.92%) of linear mycelial growth was obtained with 300 ppm of Dithane M-45 followed by 150 ppm. Cabrio^{*Top} was found significantly inferior in inhibiting the growth of *R. solani* where only 10.21% inhibition of mycelial growth was observed with 300 ppm and lower doses had no effect on the tested pathogen (Table 5). The complete inhibition of sclerotial population was also detected with all concentration of Conza 5% EC, Bavistin 50 WP and Provax 200 WP. Plentiful sclerotial crust was noticed on the PDA plates which were amended with 75 and 150 ppm of Dithane M-45. Cabrio^{*Top} had no efficacy against *R. solani* except a little effect only with the highest concentration. Results of the present study suggested that even at low concentration Conza 5% EC, Bavistin 50 WP and Provax 200 WP were the most effective fungicides against *R. solani* isolate RS-5. Dubey and Patel (2001) also reported that Bavistin 50 WP and Provax 200 WP were effective against *R. solani.*

Table 5. *In vitro* evaluation of fungicides against the radial growth and mycelial dry weight of test pathogen

Fungicides	Conc. (ppm)	% inhibition in *R. solani* isolate RS-5		Sclerotia***
		radial growth	mycelial dry weight	
Conza 5% EC	75	100 ^{a**} (88.35)	100 ^{a} (88.35) *	-
	150	100 ^{a} (88.35)	100 ^{a} (88.35)	-
	300	100 ^{a} (88.35)	100 ^{a} (88.35)	-
Cabrio*^{Top}	75	0.00 ^{f} (1.65)	0.00 ^{f} (1.65)	++
	150	0.00 ^{f} (1.65)	0.00 ^{f} (1.65)	++
	300	10.21^{e} (19.06)	21.02 ^{e} (27.29)	++
Bavistin 50 WP	75	100 ^{a} (88.35)	100 ^{a} (88.35)	-
	150	100 ^{a} (88.35)	100 ^{a} (88.35)	-
	300	100 ^{a} (88.35)	100 ^{a} (88.35)	-
Provax 200 WP	75	100 ^{a} (88.35)	100 ^{a} (88.35)	-
	150	100 ^{a} (88.35)	100 ^{a} (88.35)	-
	300	100 ^{a} (88.35)	100 ^{a} (88.35)	-
Dithane M-45	75	17.40 ^{d} (24.56)	26.35 ^{d} (30.89)	++
	150	37.03 ^{c} (37.11)	55.47 ^{c} (48.15)	++
	300	65.92 ^{b} (53.92)	81.25 ^{b} (64.38)	+
Control		90 mm	0.563 g	

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT. *** Sclerotium of *R. solani* was measured on eye observation as ++, + and -, representing moderate, minimum, and no sclerotial crust, respectively.

3.5. Effect of organic amendments on *R. solani* isolate RS-5

The highest concentration (3%) of mustard oil cake provided maximum inhibition (66.29%) of mycelial growth followed by sesame oil cake (48.51%) and 2% mustard oil cake (44.81%). On the contrary, soybean oil cake, coconut oil cake and tea waste at 3% concentration showed 42.59, 37.03 and 40.74% inhibition of mycelium radial growth *R. solani* respectively. The lowest inhibition was recorded with 1% tea waste. Morover, the highest inhibition of mycelial dry weight was also observed with 3% mustard oil cake (73.46%) followed by sesame oil cake (55.58%) and 2% mustard oilcake (53.96%), respectively (Table 6). Additionally, the lowest number of sclerotium formation was also found with 3% mustard as well as sesame oil cake. Result of the present investigation revealed that mustard oilcake was the most effective in inhibiting the radial growth, mycelial dry weight and sclerotium formation of *R. solani*. The finding of experiment is similar to the results of several authors (Mazzola et al., 2001; Dhingra et al., 2004).

Table 6. *In vitro* evaluation of organic amendments against test pathogen

Ogranic amendments	Conc. (%)	% inhibition in *R. solani* isolate RS-5		Sclerotia***
		radial growth	mycelial dry weight	
Mustard oil cake	1	39.62 $^{c-e}$ (39.01)*	46.15 ^{c**} (42.79)	++
	2	44.81 ^{bc} (42.02)	53.96 ^{b} (47.27)	++
	3	66.29 ^{a} (54.51)	73.46 ^{a} (49.01)	+
Sesame oil cake	1	36.66 $^{d-f}$ (37.27)	38.03 ^{e} (38.07)	++
	2	40.00 $^{c-e}$ (39.23)	43.33 ^{cd} (41.16)	++
	3	48.51 ^{b} (44.15)	55.58 ^{b} (48.21)	+
Soybean oil cake	1	30.37 ^{gh} (33.42)	32.89 ^{fg} (34.97)	++
	2	34.81 ^{efg} (36.15)	39.95 ^{de} (39.20)	++
	3	42.59 ^{c} (40.74)	52.09 ^{b} (46.20)	++
Coconut oil cake	1	27.03 ^{h} (31.42)	29.70 ^{gh} (33.03)	++
	2	32.22 ^{fg} (34.57)	36.55 ^{ef} (37.20)	++
	3	37.03 $^{d-f}$ (37.47)	40.51 ^{de} (39.53)	++
Tea waste	1	20.37 ^{i} (26.82)	21.66 ^{i} (27.71)	++
	2	27.03 ^{h} (31.30)	28.33 ^{h} (32.15)	++
	3	40.74 ^{cd} (39.64)	44.82 ^{cd} (42.03)	++
Control		90.00 mm	0.552 g	

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT. *** Sclerotium of *R. solani* was measured on eye observation as ++, + and -, representing moderate, minimum, and no sclerotial crust, respectively.

3.6. Integrated effect of bio-agent, fungicide and organic amendment

3.6.1. Effect on soybean root rot disease

The lowest pre- and post- emergence as well as total seedling mortality was found in the treatment T_1 (7.92, 3.50 and 11.42%) where no pathogen was inoculated. But, the highest 73.34% reduction of seedling mortality was recorded in the treatments T_9. On the contrary, significantly the lowest (22.95%) reduction of seedling mortality was observed in the treatment T_5 but identical with T_3 (Table 7). Moreover, disease incidence and severity of root rot disease of soybean were also influenced by the application of bio-agent, fungicide and organic amendment either alone or in combination. The lowest disease incidence 5.33% and severity 4.63% were observed in the treatment T_9 followed by the treatments T_6 and T_7 (Table 8). More or less same result also found in vegetable crops disease management which is caused by *Alternaria*, *R. solani*, *S. rolfsii* and *F. oxysporum* (Rubayet and Bhuiyan, 2016; Das et al., 2019; Arefin et al., 2019).

Table 7. Effect of bio-agent, fungicide, and organic amendment on seedling mortality of soybean

Treatments°	% Seedling mortality			% reduction
	Pre-emergence	Post-emergence	Total	
T_1	7.92 (16.34)	3.50 (10.79) *	11.42 ^{f} ** (19.76)	-
T_2	40.35 (39.43)	18.85 (25.73)	59.20 ^{a} (50.30)	-
T_3	21.92 (27.92)	13.15 (21.26)	35.07 ^{c} (36.32)	40.76
T_4	16.22 (23.75)	11.40 (19.73)	27.62 ^{de} (31.71)	53.34
T_5	27.63 (31.71)	17.98 (25.09)	45.61 ^{b} (42.48)	22.95
T_6	14.03 (22.00)	9.64 (18.09)	23.67 ^{e} (29.12)	60.01
T_7	17.10 (24.43)	10.08 (18.51)	27.18 ^{de} (31.43)	54.08
T_8	20.17 (26.69)	11.40 (19.73)	31.57 ^{cd} (34.19)	46.67
T_9	9.21 (17.66)	6.57 (14.86)	15.78 ^{f} (23.41)	73.34

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

Table 8. Effect of bio-agent, fungicide and organic amendment on dry root rot disease of soybean

Treatments°	% disease incidence	PDI	% disease reduction	
			Incidence	PDI
T_1	0.00 ^{h} (1.28) *	0.00 ^{f} (1.28)	100	100
T_2	34.98 ^{a} (36.25)	29.33 ^{a**} (32.79)	-	-
T_3	21.07 ^{c} (27.29)	21.33 ^{ab} (27.47)	39.76	27.28
T_4	16.00 ^{de} (23.58)	18.00 ^{bc} (25.08)	54.26	38.62
T_5	26.42 ^{b} (30.93)	22.00 ^{ab} (27.92)	24.47	24.99
T_6	13.69 ^{f} (21.71)	12.67 ^{c} (20.84)	60.86	56.80
T_7	15.19 ^{ef} (22.93)	16.67 ^{bc} (24.09)	56.58	43.16
T_8	18.29 ^{d} (25.31)	17.33 ^{bc} (24.57)	47.71	40.91
T_9	5.33 ^{g} (13.35)	4.63 ^{d} (10.59)	84.72	84.21

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

3.6.2. Effect on yield and yield components of soybean

The highest plant height (84.07 cm) and average number of pod per plant (62.89) were observed in the treatment T_9 followed by T_1 and T_6. The highest (2.22 tha^{-1}) seed yield was obtained from the treatment T_9, where colonized *T. harzianum*, Provax 200 WP treated seed and mustard oil cake were used together. Considerably higher and statistically similar seed yield over control-2 was obtained in the treatment T_1, T_6, T_7 and T_8, respectively. On the other hand, the lowest yield (0.98 tha^{-1}) was obtained in the treatment T_2 where fresh seeds were sown in *R. solani* isolate RS-5 inoculated soil without application of any means of control (Table 9). Results of the present study indicated that the application of different treatments in the field; seed yield and yield contributing components were significantly augmented by all the treatments over treatment T_2. Statistically, the maximum yield was amplified with the treatment T_9 (126.53%). The yield increased not only because of declining plant disease but also might be due to the secretion of different growth promoting substances in the soil by bio-agent *T. harzianum*. According to Altomare *et al*. (1999), *Trichoderma* produced various chemical substances which are accelerated to solubilize minerals for instance, rock phosphate, Zn, Mn^{4+}, Fe^{3+}, Cu^{2+} etc. and improved iron availability. These nutrient substances might be endowed in swelling crop growth and development as well as yield. The present study is supported by many researchers, for instance Simi et al. (2019), Ahmed et al. (2019) and Rahman et al. (2020).

Table 9. Effect of different treatments on yield and yield components of soybean

Treatments°	Plant height (cm)	No. of pod plant^{-1}	Yield (tha^{-1})	% yield increased
T_1	75.13 ^{bc}	53.89 ^{cd}	2.00 ^{b}	-
T_2	65.10 ^{d}	44.11 ^{f}	0.98 ^{h}	-
T_3	72.57 ^{c}	48.44 ^{e}	1.33 ^{f}	35.71
T_4	76.47 ^{abc}	52.11 ^{d}	1.52 ^{e}	55.10
T_5	79.10 ^{abc}	55.11 ^{cd}	1.23 ^{g}	25.51
T_6	77.33 ^{abc}	52.78 ^{cd}	1.83 ^{c}	86.73
T_7	83.70 ^{a}	59.67 ^{b}	1.71 ^{d*}	74.49
T_8	82.53 ^{ab}	55.78 ^{c}	1.64 ^{d}	67.35
T_9	84.07 ^{a}	62.89 ^{a}	2.22 ^{a}	126.53

*Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

° T_1 = Untreated healthy seeds sown in sterilized soil (control-1), T_2 = Soil inoculated with *R. solani* isolate RS-5 + Fresh seeds (control-2), T_3 = Soil inoculated with *R. solani* isolate RS-5 + Provax 200 WP treated seeds, T_4 = Soil inoculated with *R. solani* isolate RS-5 + Wheat grain colonized *T. harzianum* isolate ISR-26 + Fresh seeds, T_5 = Soil inoculated with *R. solani* isolate RS-5 + Mustard oil cake + Fresh seeds, T_6 = Soil inoculated with *R. solani* isolate RS-5 + Wheat grain colonized *T. harzianum* isolate ISR-26 + Provax 200 WP treated seeds, T_7 = Soil inoculated with *R. solani* isolate RS-5 + Wheat grain colonized *T. harzianum* isolate ISR-26 + Mustard oil cake + Fresh seeds, T_8 = Soil inoculated with *R. solani* isolate RS-5 + Mustard oil cake + Provax 200 WP treated seeds, T_9 = Soil inoculated with *R. solani* isolate RS-5 + Wheat grain colonized *T. harzianum* isolate ISR-26 + Provax 200 WP treated seeds + Mustard oil cake.

4. CONCLUSION

The result of present study exposed that integrated use of bio-agent (*T. harzianum* isolate ISR-26), fungicide (Provax 200 WP) and organic amendment (Mustard oil cake) provided the effective control measure against rhizoctonia root rot disease of soybean caused by *R. solani* isolate RS-5. Moreover, this management technique may be a sustainable alternative to reduce the pathogens population density as well as increasing the soybean production at field level.

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