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Integrated options for the management of black root rot of strawberry caused by *Rhizoctonia solani* Kuhn

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ABSTRACT

An investigation was made to manage strawberry black root rot caused by *Rhizoctonia solani* (*R. solani*) through the integration of *Trichoderma harzianum* (*T. harzianum*) isolate STA7, mustard oil cake and Provax 200. A series of preliminary experiments were conducted to select a virulent isolate of *R. solani*, an effective isolate of *T. harzianum*, a suitable organic amendment, and a suitable fungicide before setting the experiment for integration. The pathogenicity of the selected four isolates of *R. solani* was evaluated against strawberry and isolate SR1 was selected as the test pathogen due to its highest virulent (95.47% mortality) characteristics. Among the 20 isolates of *T. harzianum*, isolate STA7 showed maximum inhibition (71.97%) against the test pathogen (*R. solani*). Among the fungicides, Provax-200 was found to be more effective at lowest concentration (100 ppm) and highly compatible with *Trichoderma* isolates STA7. In the case of organic amendments, maximum inhibition (59.66%) of *R. solani* was obtained through mustard oil cake at the highest concentration (3%), which was significantly superior to other amendments. Minimum percentages of diseased roots were obtained with pathogen (*R. solani*) + *Trichoderma* + mustard oil cake + Provax-200 treatment, while the highest was observed with healthy seedlings with a pathogen-inoculated soil. In the case of leaf and fruit rot diseases, significantly lowest infected leaves as well as fruit rot were observed with a pathogen + *Trichoderma* + mustard oil cake + Provax-200 treatment in comparison with the control. A similar trend of high effectiveness was observed by the integration of *Trichoderma*, fungicide and organic amendments in controlling root rot and fruit diseases of strawberry. Single application of *Trichoderma* isolate STA7, Provax 200 or mustard oil cake did not show satisfactory performance in terms of disease-free plants, but when they were applied in combination, the number of healthy plants increased significantly. The result of the current study suggests the superiority of our integrated approach to control the sclerotia forming pathogen *R. solani* compared to the individual treatment either by an antagonist or by a fungicide or by mustard oil cake.

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1. Introduction

One of the best-loved fruits in many parts of the world is strawberry. Indeed, people from all walks of life truly enjoy the venerable strawberry. Strawberry is a major fruit of temperate regions, but with the advent of day-neutral cultivars, it grows profitably well in the subtropical regions also [1]. Its commercial cultivation could not become popular in Bangladesh due to the lack of adequate knowledge on its cultivation. Strawberry provides vitamin A, C, E, folic acid (selenium), calcium polyphenol, ellagic acid, cumeric acid querciten, xanthomycen, and phytosterol. Strawberry also contains significant levels of antioxidants that fight against free radicals (the elements that can damage cells and are thought to contribute to the formation of many kinds of cancer) [2].

Even though Bangladesh is located in subtropical regions, a nutritious and delicious exotic strawberry can be adapted well in Bangladesh by both individual and institutional effort. Strawberry suffers from a number of disesases caused by different pathogenic organisms, particularly fungi and bacteria, and their economic importance is often determined by the cultural systems and the varieties used, the local environment and the limitations on their availability, as well as by the effectiveness of management strategies. Among the different diseases of strawberry, black root rot is most common in most of the strawberry growing countries, including Bangladesh. Strawberry root rots have been reported from Great Britain, France, Holland, Australia, and other parts of the world [3]. Black root rot has been found in every strawberry growing area of the United States of America [4]. Researchers have isolated many species of fungi of black root rot from strawberries, but some are more often involved than other. *Rhizoctonia solani*, *R. fragraria*, *Pythium* spp. are all isolated frequently, often in various combination [5,6]. Wilhelm and Nelson [7] stated their belief that *R. fragariae* or its perfect stage, *Ceratobastidium*, and *P. ultimum* were the "primary factor responsible" for black root rot. Most of the fungi involved in black root rot are weak parasites, living saprophytically in the soil and becoming pathogenic only when strawberry roots are stressed by a condition of water logging, low oxygen, or root injury.

In Bangladesh, only *R. solani* has been isolated from black root rot-infected strawberry plants from the different strawberry growing areas of the country. Black root rot is a complex disaese of strawberry that can reduce the plant's vigour and productivity [8]. The diseases may be introduced into transplant and field production systems by infested plant material [9,10]. In addition, the causal agent of this disease, *R. solani*, can also cause crown rot, root rot, and plant death [11]. As black root rot is favored by poorly drained soil, where possible, strawberries are grown only in fields having a sloping topography to avoid settling water, and having light, sandy soil. Currently management of strawberry black rot relies nearly exclusively on chemicals, particularly fumigation with methyl bromide [12]. Although there is a substantial progress in devoloping chemical and organic ammendment-based alternatives to methyl bromide [13,14], infested transplants are still undergoing a serious risk of black rot.

Modern fungicides are urgently needed to control this disease and at the same time management practises that favour biocontrol activity and reduce the risk of root rot development during the growing season are also needed. *Trichoderma* spp. are well known for their biocontrol activity against a number of soil-borne pathogens [15]. For example, the mycoparasitic fungus *T. harzianum* has been used under field condition to control *R. solani* in beans and in cotton. Disease control was improved when *T. harzianum* was applied after soil solarization or fumigation with methyl bromide in potatoes and carnations. In Israel, Elad et al. [15] reported a 92% reduction of black root rot incidence and a 37% increase of fruit yield in strawberry when the infested nursery soil and infected strawberry plants were treated with *T. harzianum*. As strawberry fruits are consumed directly, the use of chemicals is not desirable. Alternate management of this disease is most expected for avoiding health hazard. Keeping the above facts in mind, experiments were conducted to provide (a) suitable management option(s) against the black root rot disease of strawberry caused by *R. solani* by using biocontrol agent *T. harzianum* and organic amendment in addition to fungicides.

2. Materials and methods

The field experiment was conducted at the experimental farm of the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) during the winter season in 2008 and 2009. Integration of *T. harzianum* with Provax 200 and mustard oil cake were used both in cultures out of pot as well as in field experiments to control the black root rot disease of strawberry caused by *R. solani*. A series of preliminary experiments were conducted to select a virulent isolate of *R. solani*, an effective isolate of *T. harzianum*, a suitable fungicide, and an effective organic amendment.

2.1. Isolation of R. solani

Four isolates of the test pathogen were isolated from infected root, leaf, stem tissues of strawberry, bush bean, and pea. The infected plant specimens were washed with tap water and were cut into small pieces (5 mm). The pieces were surface sterilized with 1.0% chlorox for 5 min and then rinsed in sterilized water for three times. The sterilized pieces of the diseased tissue were placed on 1.5% water agar amended with streptomycin sulfate. After 3 days, hyphal tips of isolates were transferred into Potato Dextrose Agar (PDA) plates, which were incubated in the laboratory at room temperature (25 °C). Sclerotia were removed by elutriation and sieving onto screens with 600- and 150-micrometer openings [16]. Isolates from sclerotia were obtained by germinating sclerotia on water agar and transferring hyphal tips to PDA. The isolates were purified following hyphal tip technique [17], identified using standard key [18], and stored in PDA slants in test tubes at 10 °C.

2.2. Preparation of inocula of R. solani

Inocula of the four isolates of *R. solani* were prepared on autoclaved-moist wheat grain. Wheat grain was soaked in

water for 12 h. After soaking, excess water was drained off and water-soaked wheat grains were poured into 500-mL Erlenmeyer flasks. The inoculum of each isolate was prepared in a separate flask. Five-millimeter-diameter mycelial discs were cut from the edge of 3-day-old PDA cultures of the pathogen. Ten to 12 mycelial discs from each isolate of the pathogen were added to the flasks containing autoclaved wheat grains in the flasks and incubated at 25 °C for 21 days. They were shaken by hand at 2–3 days interval for even colonization. The colonized wheat grain was air dried for 1 week and stored at 10 °C before being used as an inoculum of the pathogen.

2.3. *Pathogenicity of the isolates of* R. solani

The isolates of *Rhizoctonia* spp. were evaluated and compared with regard to their pathogenicity by the soil inoculation technique using strawberry plants in cultures out of pot. Earthen pots were filled with sterilized soil (500 g/pot). The inocula of *R. solani* isolates were thoroughly mixed with the soil at the rate of 20 g/kg soil. The treatments consisted of four isolates including an uninoculated control where only sterilized soil was used and were replicated three times. Three seedlings of the 'BARI strawberry-1' variety were sown in each earthen pot. The plant-containing pots were arranged in a completely random design. Disease development was observed regularly and recorded 15, 30, 45 and 60 days after sowing to estimate the lethal effect of pathogens at the vegetative and reproductive stages. The causal agent of mortality was confirmed after re-isolation of the pathogen from infected roots, leafs, and fruits of strawberry.

2.4. *Selection of* T. harzianum *isolates*

A total of 20 isolates of *T. harzianum* were screened against *R. solani* to select the most effective biocontrol agent. All the isolates were isolated from the rhizosphere and rhizoplane soils of bean, rice, chilly, tomato, sunflower, strawberry, and wheat. The soil dilution plate technique and root washing methods were used for isolation of the fungus isolates [17]. In the dilution plate technique, 10 g of composite soil collected from the rhizosphere and the rhizoplane of the selected plant species were taken in a 250-mL Erlenmeyer flask. Sterilized water was added to flasks (100 mL/flask). The flasks were agitated on a vortex for 2 min for thorough mixing and 1-mL sub-samples were transferred from each flask to another one containing 9 mL of sterile water. In this way, a 5-fold serial dilution of the soil suspension was prepared. Then, 0.1 mL of each dilution was incorporated into the PDA plate. The isolates of *T. harzianum* were purified in acidified water agar using hyphal tip culture technique; the cultures were maintained in PDA slants at 10 °C for further use.

2.5. *Preliminary laboratory evaluation of* T. harzianum *isolates against* R. solani

An *in vitro* test was conducted to find out the comparative antagonistic potential of 20 selected *Trichoderma* isolates against *R. solani* isolate SR1 on PDA by dual culture technique [19]. Discs of mycelium (5-mm diameter) of each of the selected fungal isolates were cut from the edge of an actively growing fungal colony with a cork borer (5-mm diameter). One mycelial disc of individual isolates of *Trichoderma* and one disc of a test fungal pathogen were placed simultaneously on the edge of each PDA Petri plate at opposite directions. Three replicate plates were used for each isolate of *Trichoderma* and a test pathogen. The plates were arranged on the laboratory desks following a completely randomized design. The plates receiving only discs of *R. solani* served as controls. The plates were incubated in the laboratory at an ambient temperature of 25 ± 3 °C. Thereafter, inhibition percentages of the *R. solani* were calculated based on the growth of the pathogen on PDA plates following the formula suggested by Sundar et al. [20].

2.6. *Preliminary laboratory evaluation of selected fungicides against* R. solani

Three fungicides, namely Bavistin 50 WP (Carbendazim), Ridomil (Metalaxyl) and Provax-200 (Carboxin), were tested *in vitro* to evaluate their effect on colony growth and sclerotia formation of *R. solani* following the poison food technique on PDA Petri plates [19]. All fungicides were used at 100, 250, and 500 ppm. The details of the fungicides are presented in Table 1.

2.7. *Effect of fungicides on radial growth and sclerotia formation*

The effect of fungicides on radial growth and sclerotia formation of *R. solani* isolate SR1 was determined on a PDA medium. PDA was prepared by mixing infusion of

Table 1
Details of fungicides used in the present study.

Trade name	Common name	Chemical name	Mode of action	Active ingredient (%)
Bavistin 50 WP	Carbendazim	Methyl-benzimidazol-2-yl carbamate	Systemic fungicide	50 WP
Ridomil	Metalaxyl	*N*-(2,6-Dimethylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester	Systemic fungicide	25 EC
Provax-200	Carboxin	5,6-Dihydro-2-methyl-1,4-oxathin-3-carboxanilide	Systemic fungicide	75 WP

WP: wettable powder; EC: emulcifiable concentrate.

200 g of peeled potato, 20 g dextrose and 17 g of agar in 1000 mL of distilled water. The medium was cooked properly and poured into conical flasks at 100 mL per flask. Before solidification, the requisite quantity of individual fungicide was added to the medium to have concentrations of 100, 250, and 500 ppm. After thorough mixing with the fungicide, the medium was autoclaved at 121 °C under a pressure of 1 kg/cm for 20 min. Approximately 20 mL of melted PDA mixed with fungicides was poured into each Petri dish. After solidification, the plates were inoculated by 5-mm-diameter discs of 3-day-old PDA cultures of *R. solani*. Three replicated plates were used for each dose of every fungicide. Three replicated PDA plates received no fungicide and were also inoculated as controls. The inoculated plates were incubated at 28 ± 1 °C till the fungus covered the PDA in control plates, and sclerotia formation was recorded after 7 days of inoculation. The inhibition of radial growth was computed based on colony diameter on control plate using the formula shown below [20]. To determine the sclerotia formation at different doses of each fungicide, pathogens were evaluated through visual rating as no sclerotial crust, minimum sclerotial crust, moderate sclerotial crust, and maximum number of sclerotial crust by taking a 1-cm^2 area of the plate. The evaluation was replicated thrice.

2.8. Effect of fungicide on mycelial dry weight

To determine the effect of the fungicides on mycelial dry weight of *R. solani*, potato dextrose (PD) broth was used. Requisite quantity of each fungicide was added to the broth to have concentrations of 100, 250 and 500 ppm. Three replicated flasks were used for each dose of the three fungicides. The flasks were inoculated with mycelial discs of 5-day-old *R. solani* cultured on PDA. These mycelial disc were cut and put into the flask. Additional three flasks containing the PD broth receiving no fungicides were used as controls. The inoculated flasks were incubated at room temperature (25–28 °C) for 14 days. At the end of the incubation, the cultures in all flasks were filtered separately through pre-weighted filter paper. The dry weight of mycelium was determined after drying the mycelium on filter paper in an oven at 70 °C for 12 h. The dry weight of mycelium was obtained by subtracting the weight of the filter paper from the combined weight of the filter paper and of mycelium. Inhibition of mycelia dry weight was measured comparing with the control flasks.

2.9. Laboratory evaluation of organic amendments on the radial growth of R. solani

Another *in vitro* test was conducted to determine the effect of organic amendment on the growth of *R. solani* following the poison food technique [19]. Hundred grams of each organic amendments viz. mustard oilcake (*Brassica napus*), coconut oilcake (*Cocos nucifera*), til oilcake (*Sesame indicum*), and soybean oilcake (*Glycine max*) were added in 1000 mL of water and preserved in earthen pots for 2 weeks. A requisite quantity of individual oilcake extracts was added to 100-mL conical flasks with PDA medium to have concentrations of 1, 2 and 3% (v/v). After thorough mixing of oilcake extracts, the medium was autoclaved and approximately 15 mL of melted PDA with extracts were poured into each Petri dish. After solidification, the plates were inoculated by placing 5-mm discs of 3-day-old PDA cultures of *R. solani*. Inhibition of radial growth was computed on colony diameter on control plate using the same formula [20].

The field experiment was conducted in Randomized Block Design with nine treatment combinations replicated three times. The size of the individual plots was 1 m × 1.5 m and the plot–plot distance was 0.5 m. Recommended doses of fertilizers (30:50:20 for N-P-K) were applied. Muriate of potash was sprayed @ 3% solution at the flower bud initiation stage between 30 and 35 days after sowing with a hand sprayer. Field application of *T. harzianum* isolate STA7 was done by broadcasting on the surface of plots on the day before planting @ 2.0% (WAV) by growing on autoclaved wheat grain. The procedure for the preparation of inocula of *Trichoderma* was the same as that described earlier for the preparation of inocula of *R. solani*. Seedlings of 'BARI-1' strawberry variety were collected from the Bangladesh Agricultural Research Institute and three seedlings were transplanted in each plot. Three replications were used for each treatment. Provax-200 50WP was applied as foliar spray at 10 days after transplanting using a hand sprayer. The *Trichoderma* isolate STA7, mustard oil cake and Vitavax-200 was applied in different combinations in nine different treatments viz. T_1 = pathogen + *Trichoderma* isolate STA7; T_2 = pathogen + Provax-200; T_3 = pathogen + mustered oil cake; T_4 = pathogen + *Trichoderma* isolate STA7 + Provax-200; T_5 = pathogen + *Trichoderma* isolate STA7 + mustered oil cake; T_6 = pathogen + mustered oil cake + Provax-200; T_7 = pathogen + *Trichoderma* isolate STA7 + mustered oil cake + Provax-200; T_8 = healthy seedling in a sterilized soil (healthy control); T_9 = healthy seedlings in a pathogen-inoculated soil (diseased control). Data on the mortality of seedlings were recorded at the vegetative and reproductive stages. Diseased seedlings were counted every alternate day and continued for 28 days after planting. To determine the cause of death of the seedlings, the dead seedlings were uprooted gently. The causal pathogens associated with the dead seeds and seedlings were isolated and identified for the confirmation of the causal agents. The data were analyzed for ANOVA using MSTAT-C program, and mean separations were performed by DMRT.

3. Results

3.1. Isolation and identification of R. solani from a strawberry field

Four isolates of *R. solani* were isolated from infected root, leaf, and stem tissues of strawberry. The isolated *R. solani* were mostly brown in color, with dense mycelial growth with zonation, but the number of sclerotia formation varied. Based on the number of sclerotia, *R. solani* isolates were rated as +, ++, +++ and ++++

Table 2
Pathogenicity test of four isolates of *R. solani* against BARI strawberry-1.

Isolates of *R. solani*	Rating of the number of sclerotia	% Mortality of strawberry plants		
		Vegetative stage	Reproductive stage	Total plant mortality
SR1	+	82.48[a]	12.66[c]	95.47[a]
SR16	++	52.48[d]	20.11[a]	73.15[c]
SR7	+++	65.72[c]	17.23[b]	83.74[b]
SR13	++++	69.61[b]	17.47[b]	86.63[b]
Control		0.00[e]	0.00[d]	0.00[d]
LSD(0.01)		2.08[f]	1.47[f]	3.33[f]

R. solani: *Rhizoctonia solani*; LSD: least significant difference.
Mean values within a column having a common letter do not differ significantly ($P = 0.01$).
[f] Indicates significance at 1% level of probability.

sclerotial groups, and four *R. solani* isolates, namely SR1, SR16, SR7 and SR13 representing the lower number of sclerotia to the maximum number of sclerotia, respectively, were randomly selected for the pathogenicity test against strawberry plants in cultures out of pot.

3.2. Pathogenecity test

The pathogenecity test of *R. solani* isolates was done against strawberry plants in cultures out of pot (Table 2). All the isolates developed black root rot disease, causing more than 70% of mortality in strawberry plants. The isolates SR1 showed the highest root rot disease development, causing 95.47% plant mortality followed by SR13, SR7 and SR16, showing 86.63, 83.74 and 73.15% plant mortality, respectively. Based on the present findings, isolates SR1 was selected for further study.

3.3. *Screening of* T. harzianum *isolates against* R. solani

To observe the antagonistic effect of *T. harzianum* against *R. solani*, both were tested on PDA using the dual culture technique (Table 3). All 20 isolates of *T. harzianum* showed more than 50% of inhibition of the radial growth of the test pathogen compared to control. Among the isolates, STA7 showed significantly highest inhibition (71.97%) of the radial growth, followed by STA9 (68.91%) and STA5 (66.85%). The lowest 51.24% radial growth inhibition was observed at STA17. The isolate STA7 was selected for further study.

3.4. *Laboratory evaluation of selected fungicides against* R. solani

All the selected concentrations of Provax-200 inhibited above 85% of radial growth and mycelia dry weight of *R. solani*, even at the lowest concentration (Table 4). The number of sclerotia was also negligible at the lowest concentration of Provax-200. Fungicide Ridomil appeared to be highly ineffective in inhibiting radial growth, mycelia dry weight, and sclerotia formation. Bavistin appeared as moderately effective against *R. solani*. Complete inhibition of the radial growth of *R. solani* was observed at the higher concentrations, i.e. 250 and 500 ppm, of Provax-200. The highest concentration of Bavistin was found to inhibit about 90.37% of the radial growth and 95.00% of the mycelia dry

Table 3
Screening of *Trichoderma* isolates against the radial growth of *R. solani* in dual culture technique.

Trichoderma harzianum isolates	% Inhibition of radial growth of *R. solani*
STA1	56.93[e,f]
STA2	56.46[f,g]
STA3	63.19[c,d]
STA4	63.06[c,d]
STA5	66.85[a,b,c]
STA6	62.77[c,d]
STA7	71.97[a]
STA8	62.17[c,d,e]
STA9	68.91[a,b]
STA10	65.59[b,c]
STA11	64.20[b,c]
STA12	58.46[d,e,f]
STA13	55.33[f,g]
STA14	56.85[e,f]
STA15	66.37[b,c]
STA16	53.27[f,g]
STA17	51.24[g]
STA18	65.96[b,c]
STA19	61.87[c,d,e]
STA20	54.52[f,g]
Control	9.0[h]
LSD(0.01)	4.95[i]

R. solani: *Rhizoctonia solani*; LSD: least significant difference.
Mean values within a column having a common letter do not differ significantly ($P = 0.01$).
[i] Indicates significance at 1% level of probability.

Table 4
Preliminary laboratory evaluation of fungicides on inhibition of the radial growth, mycelial dry weight and sclerotia formation of *R. solani*.

Fungicides	Concentration (ppm)	% Inhibition		Formation of sclerotial crust
		Radial growth	Mycelial dry wt.	
Provax-200	100	86.55[b]	90.95[c]	+
	250	100.00[a]	100.00[a]	–
	500	100.00[a]	100.00[a]	–
Bavistin 50 WP	100	72.96[g]	90.69[c]	++
	250	84.44[d]	93.83[b]	++
	500	90.37[c]	95.00[b]	+
Ridomil 75 EC	100	3.77[f,g]	38.26[f]	++
	250	6.33[f]	41.21[e]	++
	500	7.20[f]	44.88[d]	++
Control		9.0[e]	0.44[g]	+++
LSD(0.01)		1.285[h]	1.73[h]	

R. solani: *Rhizoctonia solani*; LSD: least significant difference; WP: wettable powder; EC: emulcifiable concentrate.
Mean values within a column having a common letter do not differ significantly ($P = 0.01$).
[h] Indicates significance at 1% level of probability.

weight of the test pathogen, with a minimum number of sclerotia.

3.5. Laboratory evaluation of organic amendments on the radial growth of R. solani

The effects of organic amendments on the inhibition of the hyphal growth of *R. solani* are presented in Table 5. The study revealed that the maximum inhibition (59.66%) of the hyphal growth of *R. solani* was obtained in mustard oil cake at the highest concentration (3%), which is significantly superior to all other amendments. At the highest 3% concentration of till oil cake and 2% concentration of mustard oil cake, inhibition rates of the radial growth of *R. solani* of 56.85% and 54.55%, respectively, were obtained.

Table 5
Preliminary *in vitro* evaluation of organic amendments in inhibition of the radial growth of *Rhizoctonia solani*.

Organic amendment	Concentration (%)	Inhibition (%)
Mustard oil cake	1	43.72[e]
	2	54.55[c]
	3	59.66[a]
Til oil cake	1	50.30[d]
	2	51.67[d]
	3	56.85[b]
Coconut oil cake	1	7.85[h,i]
	2	15.17[g]
	3	23.67[f]
Soybean oil cake	1	0.55[k]
	2	3.28[j]
	3	6.44[i]
Control (mm)		90.00
LSD(0.01)		1.62[k]

Mean values within a column having a common letter do not differ significantly ($P = 0.01$). LSD: least significant difference
[k] Indicates significance at 1% level of probability.

3.6. Integration of Trichoderma, fungicides and mustard oil cake in controlling black root rot

An integrated control approach has been made to control strawberry fruit rot. A total of three pathogens, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, and *R. solani*, were isolated from the infected leaves, out of which C. *gloeosporioides* were the most predominant in T_9, T_3 and T_8. The second highest 26.33% *C. gloeosporioides* was isolated from infected leaves in T_3, where only healthy seedlings were sown without any control measure. A significant number of the least infected leaves and of the healthiest leaves were observed at T_7. Mustard oil cake appeared to be less effective in controlling the leaf diseases and significantly inferior to other treatments. The integration of different component in the treatment T_7 also appeared significantly superior in controlling leaf diseases of strawberry in comparison to any individual or mixed component of other treatments. Integrated management options (T7) produced healthy leaves and fruits (Fig. 1), while in the disease control treatment, dead plants (Fig. 2) were observed.

The effect of different combinations of *Trichoderma*, fungicide and mustard oil cake on strawberry leaf and fruit health in field conditions are given in Tables 6 and 7. Among the three pathogens, associated with fruit rot disease of strawberry, *C. gloeosporioides* was found as the most predominant in each case. The highest 87.00% fruit rot was observed in T_9 followed by T_3 (75.03%). In T_8, only healthy seedlings were grown, significant anthracnose symptoms developed on the fruit. A significantly highest percentage of healthy fruits (76.33%, Fig. 1) was obtained in T_7 followed by T_4. In fact, mustard oil cake alone seems to be less effective in controlling fruit rot disease.

4. Discussion

A good number of fungi were isolated from infected stems and roots of strawberry field near BSMRAU

Fig. 1. (Color online.) Showing healthy plant with fruits in integrated mangement treatment.

Fig. 2. (Color online.) Showing dead plant infected by black root rot disaese in control treatment.

campus, Salna, Gazipur, Bangladesh. The isolated fungi were identified as *C. gloeosporioides*, causing anthracnose, *F. oxysporum* causing black root rot and wilt, *R. solani* causing black root rot and *Sclerotium rolfsii* causing southern stem blight and root rot diseases of strawberry. Among the isolated fungi, *R. solani* was found to be the most predominant. Based on the number of sclerotia, *R. solani* isolates were rated as +, ++, +++ and ++++ sclerotial groups, and four *R. solani* isolates, namely SR1, SR16, SR7, and SR13, representing the lowest number of sclerotia to the maximum number of sclerotia, respectively. The isolates SR1 showed the highest root rot disease development, causing 95.47% of plant mortality, and were selected for further study. Disease development was much higher in the vegetative stage than in the reproductive stage of the growth. The virulence of the tested isolates differed significantly and the influence of the number of sclerotia of the isolates had no correlation on disease development. Muyolo et al. [21] also found 100% mortality of strawberry plants due to black root rot and extensive root decay caused by *R. solani* in the culture out of pot experiment.

To observe the antagonistic effect of *T. harzianum* against *R. solani*, isolate STA7 was selected, which inhibited 71.97% of the radial growth of *R. solani* in culture out of pot. Significant reductions of the mycelial growth of *R. solani* in the presence of *Trichoderma* spp. were also reported by many other workers [22–25], and

Table 6
Effect of different combinations of *Trichoderma*, fungicide and mustard oil cake on leaf health of Strawberry.

Treatments	Healthy leaf	Diseased leaf			
		F. oxysporum	*C. gloeosporioides*	*R. solani*	Total
T_1 = Pathogen + *Trichoderma*	59.77^{e}	7.30^{c}	10.97^{c}	21.93^{c}	40.23^{c}
T_2 = Pathogen + Provax-200	63.03^{d}	6.70^{d}	10.07^{d}	20.17^{d}	36.97^{d}
T_3 = Pathogen + Mustered oil cake	33.33^{f}	16.20^{b}	24.27^{b}	26.23^{b}	66.67^{b}
T_4 = Pathogen + *Trichoderma* + Provax-200	69.37^{d}	5.60^{f}	8.367^{f}	16.70^{e}	30.63^{f}
T_5 = Pathogen + *Trichoderma* + Mustered oil cake	66.37^{c}	6.10^{e}	9.167^{e}	18.33^{d,e}	33.63^{e}
T_6 = Pathogen + Mustered oil cake + Provax-200	63.37^{e}	6.66^{d}	10.00^{d}	20.00^{d}	36.63^{d}
T_7 = Pathogen + *Trichoderma* + Mustered oil cake + Provax-200	91.47^{a}	1.567^{g}	2.33^{g}	4.63^{f}	8.53^{g}
T_8 = Healthy seedling in field soil (Healthy control)	71.33^{b}	1.57^{g}	1.57^{h}	25.33^{b}	28.67^{f}
T_9 = Healthy seedling in pathogen-inoculated soil (Diseased control)	12.00^{g}	19.87^{a}	29.80^{a}	38.37^{a}	88.00^{a}
LSD	2.537[h]	.54[h]	0.66[h]	1.763[h]	2.537[h]

F. oxysporum: *Fusarium oxysporum*; *C. gloeosporioides*: *Colletotrichum gloeosporioides*; *R. solani*: *Rhizoctonia solani*; LSD: least significant difference.
Mean values within a column having a common letter do not differ significantly (P = 0.05).
[h] Indicates significance at 5% level of probability.

Table 7
Effect of different combinations of *Trichoderma*, fungicide and mustard oil cake on fruit.

Treatments	Healthy fruit	Diseased fruit			
		F. oxysporum	*C. gloeosporioides*	*R. solani*	Total
T_1 = Pathogen + *Trichoderma*	38.00[d,e]	18.67[b,c]	30.97[c,d]	12.30[b,c,d]	62.00[c,d]
T_2 = Pathogen + Provax-200	32.37[e]	18.07[b,c]	33.80[c]	15.73[a]	67.63[c]
T_3 = Pathogen + Mustered oil cake	24.97[f]	21.07[b]	37.53[b]	16.40[a]	75.03[b]
T_4 = Pathogen + *Trichoderma* + Provax-200	53.67[c]	12.97[d]	23.17[e]	10.17[d]	46.33[e]
T_5 = Pathogen + *Trichoderma* + Mustered oil cake	37.60[d,e]	19.30[b]	31.17[c,d]	11.90[c,d]	62.40[c,d]
T_6 = Pathogen + Mustered oil cake + Provax-200	39.67[d]	15.90[c,d]	30.17[d]	14.30[a,b,c]	60.33[d]
T_7 = Pathogen + *Trichoderma* + Mustered oil cake + Provax-200	76.33[b]	4.933[e]	11.83[f]	6.933[e]	23.67[f]
T_8 = Healthy seedling in field soil (Healthy control)	50.67[c]	15.90[c,d]	20.43[e]	13.00[a,b,c]	49.33[e]
T_9 = Healthy seedling in pathogen-inoculated soil (Diseased control)	13.00[g]	28.23[a]	43.50[a]	15.23[a,b]	87.00[a]
LSD	5.97[h]	3.06[h]	2.97[h]	2.95[h]	5.78[h]

F. oxysporum: *Fusarium oxysporum*; *C. gloeosporioides*: *Colletotrichum gloeosporioides*; *R. solani*: *Rhizoctonia solani*; LSD: least significant difference.
Mean values within a column having a common letter do not differ significantly ($P = 0.05$).
[h] Indicates significance at 5% level of probability.

the results of the present investigation are in full agreement with the above-mentioned investigators. The variation among the different isolates of *Trichoderma* spp. may be due to a genetic make-up of the antagonists for their antagonistic activity.

Among the three fungicides, Provax-200 inhibited radial growth and mycelia dry weight of *R. solani* above 85 and 90%, respectively, even at the lowest concentration, and they were selected for further study. Among the fungicides, Ridomil appeared to be highly ineffective, while Bavistin appeared as moderately effective against *R. solani.* These results are also in agreement with several other investigators [23,26,27].

This study also reveals that mustard oil showed a maximum 59.66% inhibition of *R. solani*, which was significantly superior to other amendments. Soybean oil cake appeared to be the most ineffective, followed by coconut oil cake. Rai and Singh [28] also observed that mustard oil cake significantly reduced the radial growth of *R. solani*. Similar results were also observed by Sen [27].

An integrated control approach has been made to control strawberry black root rot disease by integration of wheat grain colonized *Trichoderma* isolate STA7 with Provax-200 and mustard oil cake, based on the preliminary *in vitro* trial in different treatment combinations. *Trichoderma* isolate STA7 was compatible with carboxin as well as mustard oil cake [29]; 73.33% of mortality of strawberry plants was found when the soil was inoculated with *R. solani* without Provax-200 or mustard oil cake or antagonist. Strawberry plant mortality was most predominant at the vegetative stage than at the reproductive stage. The current study suggests a superiority of the integrated approach to control *R. solani* over the individual treatments either by antagonist or by fungicide or by mustard oil cake, which fully supports the observation of other researchers [26,27,30,31] in cases of other different crops. There is a distinct possibility of combining biological, botanical, and chemical control for improving their performance further.

5. Conclusion

Based on the findings of the present investigation, the isolate STA7 of *T. harzianum*, Provax-200 and mustard oil cake was effective against the suppression of radial growth, sclerotia formation and mycelial dry weight of *R. solani*. The efficacy of the above three individual component of biocontrol agents along with a fungicide was more effective when they were applied in integrated approach. Therefore, Provax-200 in combination with wheat grain colonized with *T. harzianum* isolate STA7 and mustard oil cake gave the best control against black root rot of strawberry caused by *R. solani* and can be recommended for the control of strawberry black root rot.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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