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Research Article

Use of non-chemical methods for the management of southern blight disease of carrot incited by *Sclerotium rolfsii*

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Abstract

This experiment was conducted to assess the effect of *Trichoderma harzianum*, soil solarization, and biofumigant on southern blight disease of carrot incited by *Sclerotium rolfsii* isolate CS 5. A series of *in-vitro* and *in-vivo* trials laid out to select a virulent isolate of *S. rolfsii* against the carrot variety *New Kuroda* and evaluated the mustard, cabbage, cauliflower, and broccoli leaf extracts for choosing an effective biofumigant against test pathogen. Mustard was the most effective in inhibiting the radial growth, and sclerotia formation (80.37 and 83.37%) of *S. rolfsii* (isolate CS 5) at 40% level of concentration followed by cabbage leaf extract (62.22 and 68.69%). On the contrary, a total of 10 isolates of *T. harzianum* were screened against the test pathogen on Potato Dextrose Agar (PDA) medium for choosing a dominant isolate of *T. harzianum*. The isolate Th-6 was found as the most active in inhibiting the radial growth (84.44%) of *S. rolfsii* followed by Th-1 (75.56%). In the application of *T. harzianum*, biofumigation, and soil solarization, the treatment appeared to be the most superior in reducing pre- and post-emergence mortality of carrot during secondary field trials. The lowest southern blight disease incidence (10.77%), and disease severity (12.78%) were found at the same treatment. Subsequently, the yield of carrot was increased 155.18% which might be due to the reduction of carrot disease as well as the addition of organic materials in the soil.

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Introduction

Carrot *(Daucus carrota* L.) belongs to the family Apiaceae which is cultivating all over the world in spring, summer, and autumn in temperate countries and during winter under the tropical and subtropical regions. It is greatly nutritious as well as delicious vegetable. Additionally, carrot contains ample quantities of nutrients such as carotene and minerals. However, there are several factors attributed to low production of carrot such as climatic conditions, variation in rainfall pattern, and an outbreak of diseases and pests. Among these factors, plant diseases play a major role in yield reduction of carrot. The most important diseases of carrot roots are southern blight, *Rhizoctonia* crown rot and canker, brown rot, damping off, *Phytophthora* root rot, *Sclerotinia* rot, *Pythium* rot, cottony rot, black rot, scab, bacterial soft rot, root knot and lesion nematodes. The southern blight disease caused by *Sclerotium rolfsii*, is a serious fungal disease affecting carrot production around the world, especially in tropical and subtropical regions.

This disease does not only reduce yield in the field condition but also deteriorates the yield quality during transportation and storage condition. Furthermore, the infection of *S. rolfsii* generally starts at base point of main stem. As a result, leaf tissues become brown in color and wilted. A white color cushion like mycelial growth appear on the collar region. The root rot gradually progresses downwardly and small spherical tan fungal bodies like sclerotia develop within the damaged root tissues. Moreover, pathogen reduces the crop stand by causing pre- and post-emergence damping off of seedling stage (Ahmed et al., 2019).

On the other hand, *S. rolfsii* is a soil-borne pathogen and overwinters as sclerotia, and mycelia in or on infected plants and debris. Sclerotia can be disseminated through transplant seedlings, water, wind, or any cultural practices. Consequently, soil-borne plant pathogens cause heavy losses to all major crops, leading to reduction in both yield and quality. An effective control of soil-borne plant pathogens are remain a serious challenge to the farmers and home gardeners. Chemical compounds have been used as seed treating fungicide and sometimes effective to control soil-borne pathogens but their exploitation has favored the development of pathogens resistance to fungicides and polluted the environment.

Considering the deleterious effect of synthetic pesticides, alternative measures should be taken for the control of plant pathogenic microorganisms. Therefore, economic and effective eco-friendly control methods are now recommended worldwide.

Now-a-days, organic cultivation such as application of biofumigation, soil solarization, and biological control alone or combinations are demanding issues for safe, low-cost, antitoxic, and effective control of many devastating soil-borne pathogens and pests (Saygı et al., 2019). In biocontrol method, *Trichoderma* spp. is excellent effective agent against plant diseases (Rubayet & Bhuiyan, 2012). According to Khattabi et al. (2001), combination of fungicide application (Hymexazol) and *T. harzianum* is highly effective against *S. rolfsii*. In addition, these procedures not only suppress–the soil-borne pathogens of carrot but also control weed, improve soil health and provide essential plant nutrients which could be significantly augmented the crop production. However previous studies showed that, the information about southern blight disease management through non chemical methods are very rare and needs to be precise. Keeping this view, the present study has been undertaken to use non-chemical methods such as biocontrol agent, soil solarization, and biofumigation for the management of southern blight disease of carrot incited by *S. rolfsii* and sustainable crop production.

Materials and Methods

Laboratory trials

A series of laboratory experiments were conducted by using Completely Randomized Design with 3 replications for each treatment. The treatments were leaves extract of mustard, cabbage, cauliflower, and broccoli at four concentrations such as 10, 20, 30, and 40%.

Isolation and preservation of S. rolfsii isolates

Six isolates of *S. rolfsii* designated as CS 1 to CS 6 were isolated from infected root, and stem

tissues of potato, tomato, chickpea, chilli, carrot, and eggplant. The specimens which had distinctive symptoms of root rot were selected from the infected fields. The fungal isolates were isolated. Then, the fungal colonies were developed on PDA and identified by following the standard method. The isolates were purified following hyphal tip method and stored in PDA slants at 10 °C.

Cultural characterization of S. rolfsii isolates

The selected isolates of CS 1 to CS 5 were individually inoculated into three replicated PDA plates using mycelial disks (Ø = 5 mm) which taken from 3 days old of PDA cultures. Then, all PDA culture plates were wrapped with parafilm paper tightly and incubated at room temperatures (25±2 °C) for 7 days. After 7 days of incubation, observation on cultural characteristics such as colony color, colony type, sclerotial population, and sclerotial distribution were recorded. All colonies were whitish, radiate and fluffy and dark brown in color. The sclerotia population density was counted by magnifying glass.

Inoculum preparation of test pathogen

Inoculum of the *S. rolfsii* isolates were prepared and stored according to the established method (Rubayet & Bhuiyan, 2016).

Pathogenicity test

The pathogenicity test of *S. rolfsii* isolates (CS 1– CS 6) were conducted in pot culture on carrot seedling according to the standard method (Rubayet et al., 2017). Nine seeds of carrot were sown in each earthen pot. Three replications for each treatment (CS 1 – CS 6) were maintained and arranged following Completely Randomized Design. Plant disease development was observed regularly and recorded at 10, 15, and 21 days after sowing for assessment the effect of the pathogen in causing pre- and post-emergence seedling mortality. The causal agent of pre- and post-emergence seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds as well as infected roots (Liton et al., 2019).

Collection, isolation and preservation of T. harzianum isolate

A total of 10 isolates of *Trichoderma* spp., whereas 7 isolates were isolated from the different crop fields of Gazipur, Bangladesh following the soil dilution plate technique (Sinclair and Dhingra, 1995). And rest of 3 isolates were collected directly from the plant pathology laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. All the isolated *Trichoderma* spp. were identified as *T. harzianum* based on the different morphological characteristics like hyphal growth, spore formation and color. The pure culture of *T. harzianum* was preserved following a regular method for future application (Das et al., 2019).

Screening of Trichoderma spp. isolates against test pathogen

The in-vitro screening was conducted to evaluate the antagonistic effect of selected 10 isolates of *Trichoderma* spp. against *S. rolfsii* isolate CS 5 on PDA medium by dual culture technique (Sinclair & Dhingra, 1995). After 7 days of incubation the inhibition percentage of radial growth of *S. rolfsii* isolate CS 5 was calculated using the following formula (Sundar et al., 1995).

$$\% \text{ inhibition of growth } = \frac{A - B}{A} \times 100$$

Where, A = Mycelial development of pathogen in absence of *Trichoderma* sp. (control) and B= Mycelial development of pathogen in presence of *Trichoderma* sp.

Preparation of biocontrol inoculum

Wheat grain colonized inoculum for the selected isolate of *T. harzianum* isolate Th-6 was prepared by following the standard procedures (Rubayet & Bhuiyan, 2016).

Preparation of Brassica spp. leaves extract

Fresh parts of the test plants such as *Brassica oleracea* var. *capitata* (cabbage), *Brassica oleracea* var. *botrytis* (cauliflower), *Brassica nigra* (mustard), and *Brassica oleracea* var. *italica* (broccoli) were collected and washed thoroughly with tap water. Then, the extract was

collected and stored according to the standard method (Rubayet et al., 2018).

In-vitro screening of Brassica spp. against S. rolfsii isolate CS 5

All the plant extracts were tested at 10, 20, 30, and 40% concentrations under *in-vitro* conditions by using food poison technique to study the inhibitory effect of these botanicals against mycelial growth of test pathogen (Rubayet et al., 2018). The radial growth of the test pathogen from culture plate was measured by scale and the number of sclerotia per plate was counted by magnifying glass. The percent inhibition of the radial growth was calculated as described above by Sundar et al. (1995).

Field experiments

Repeated field experiments were conducted in the research field of Plant Pathology department at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. The experimental site was located at 24°09' N latitude and 90°26' E longitude center of Madhupur tract (agroecological zone), Gazipur, Bangladesh which characterized by deep to shallow red-brown terrace soils (entisols based on US soil taxonomy), silty clay soil (pH 6.5) with poor nutrients, moderate rainfall, almost clear sunshine, and moderate temperature.

Land preparation and design of experiment

The cultivated land was prepared by using a tractor driven disc plough, rotavator and harrow. At the meantime, standard dose of organic and chemical fertilizer was applied (Cow dung @ 5 ton ha^{-1}, Urea, Triple Super Phosphate (TSP), and Muriate of Potash (MP) @ 200, 100, 175 kg ha^{-1}). The field experiment was designed by using a Randomized Complete Block Design with 8 treatments and 3 replications. The unit plot size was made at 4 m × 3 m where row to row distance 25 cm.

Treatments of the experiment

The experiment consisted of eight treatments: T_1= Healthy seeds sown in uninoculated soil (Control), T_2= Soil inoculated with test pathogen + Healthy seeds, T_3= T_2 + Wheat grain colonized *T. harzianum* inoculum, T_4= T_2 + Soil solarization, T_5= T_2 + Bio-fumigant, T_6= T_3 + Soil solarization, T_7= T_5 + Soil solarization, T_8= T_3 + Bio-fumigant + Soil solarization.

Treatments application methods

Among the 8 treatments, 7 treatments were tested in the field under artificially inoculated condition and rest one treatment was tested in uninoculated soil. Inoculum of the selected isolate of test pathogen and biocontrol agent were thoroughly mixed with soil according to design and layout @ 90 g m^{-2}, moistened to about 50% water holding capacity before 21 days carrot seed sowing.

Assessment of solar heat against S. rolfsii isolate CS 5

Another experiment was accompanied for evaluation of the effect of soil solarization on declining of the soil pathogen population density. The individual plot size was made at 4 m x 3 m and arranged in Randomized Complete Block Design with 3 replications. The selected treatments (T_4-T_8) were inoculated with the test pathogen. Then, the plots were kept 3 weeks within sufficient soil moisture and undisturbed for proper growth of the test pathogen population. After that, each plot in term of the treatment was covered with 100 µm thickness transparent polyethylene sheet to rise soil temperature for four weeks before carrot seeds sowing.

Assessment of biofumigation against S. rolfsii isolate CS 5

The *Brassica nigra* (mustard) was grown for determination of biofumigant effect against test pathogen. When the mustard crop was about 4 inches height, it was cut and incorporated into the soil properly then either covered or exposed according to the treatment with polythene sheet for 5 weeks before carrot seeds sowing.

Collection of seeds

Carrot seed sample variety *New Kuroda* was collected from open market, Dhaka, Bangladesh.

Seed sowing

Seeds were soaked for 24 hours to facilitate the germination before sowing. Then, seeds

were sown in lines uniformly by hand @ 3 kg ha^{-1}. After seeds sowing the soil was mulched with rice straw for preservation soil moisture. Mulches were removed after 6 days when seeds were started to germinate. Weeding, mulching, and irrigation were done in the experimental field whenever necessary.

Data recording and analysis

The typical symptom of southern blight was observed and data recorded from growing to harvesting period. Data recorded on germination, number of healthy plants and number of infected plants. Forty-five plants in each plot were randomly selected and uprooted carefully from soil, washed with running water then checked individually and disease severity was rated as 0-4 scale in which 0= No symptoms, 1=1-25%, 2=26-50%, 51-75% and 4=76-100% of carrot tap root covered with lesions. The disease incidence and disease severity were assessed by the following formula (Rahman et al., 2013; Razaq et al., 2015).

$$\text{DI} = \frac{\text{No. of infected plants}}{\text{Total No. of plants assessed}} \times 100$$

$$\text{PDI} = \frac{\text{Summation of all ratings}}{\text{Total No. of rating} \times \text{Max. disease grade (4)}} \times 100$$

$$\text{Total yield (ton ha}^{-1}) = \frac{\text{Yield per plot (kg)}}{\text{Area of plot (m}^2) \times 1000 \text{ (kg)}} \times 10000 \text{ m}^2$$

Data analysis

Statistically, data were analyzed using the Statistix 10 computer program after proper transformation whenever it was necessary. The treatment means were compared following Duncan's Multiple Range Test ($\alpha = 0.05$).

Results and Discussion

Isolation and cultural characterization of S. rolfsii isolates

The six isolates of *S. rolfsii* were isolated from the different crops field at Gazipur district, Bangladesh (Table 1). Mycelium of all the isolates were found whitish fluffy with radiate growth on PDA. Sclerotia of relatively uniform size produced on mycelium. Sclerotia were round and white when immature and then became dark brown at mature stage. Moreover, a mature sclerotium resembled mustard seed-like. All the isolates produced large number of small sclerotia which ranged from 402-585. The highest number of sclerotia formation in culture plate was observed in isolate CS 5 (585) and the lowest number was recorded in isolate CS 4 (402) (Table 1).

Table 1. Cultural characterization of S. rolfsii isolates

Isolates	Sources	Colony type	No. of sclerotia/plate	Pattern of sclerotia formation	Sclerotia color
CS 1	Chilli	Whitish, Radiate and Fluffy	463	Spread all over the plate, preferably in margin side	Dark brown
CS 2	Potato	Whitish, Radiate and very Fluffy	552	Spread all over the plate	Dark brown
CS 3	Tomato	Whitish, Radiate and Fluffy	518	spread all over the plate, preferably in margin side	Dark brown
CS 4	Eggplant	Whitish, Radiate and Fluffy	402	Mostly in the periphery	Dark brown
CS 5	Carrot	Whitish, Radiate and Fluffy	585	Mostly in the periphery	Dark brown
CS 6	Chickpea	Whitish, Radiate and very Fluffy	428	Spread all over the plate, preferably in margin side	Dark brown

Pathogenicity test of S. rolfsii isolates

Total six selected isolates of *S. rolfsii* were evaluated in the pot culture experiment for selecting the most virulent isolate causing seedlings mortality of carrot. All the tested isolates of *S. rolfsii* were found highly pathogenic against carrot seedlings causing 37.50 to 100% seedling mortality. The highest 100% seedling mortality for *S. rolfsii* was observed with the isolate CS 5 followed by the isolate CS 2 (81.25%) and CS 3 (68.75%) (Table 2). Pre- and post- emergence mortality of seedling caused by *S. rolfsii* was also reported by several studies (Akhter et al., 2015; Rubayet et al., 2017; Ahmed et al., 2019). The result of this present study was also agreed with the aforesaid studies.

Table 2. Pathogenicity test of S. rolfsii isolates against carrot seedlings

Isolates of *S. rolfsii*	Seedling mortality (%)		Total mortality (%)
	Pre-emergence	Post- emergence	
CS 1	31.25	31.25	62.50 bc[1]
CS 2	62.50	18.75	81.25 ab
CS 3	43.75	25.00	68.75 b
CS 4	25.00	12.50	37.50 c
CS 5	68.75	31.25	100.00 a
CS 6	37.50	18.75	56.25 bc

[1]Means within same column followed by common letter(s) are not significantly different (α = 0.05)

Screening of Trichoderma spp. against test pathogen

Ten isolates of *Trichoderma* spp. were tested against the isolate of CS 5 of *S. rolfsii* on PDA medium by dual culture technique. All the tested isolates of *Trichoderma* spp. showed more than 50% inhibition of the radial growth of the test pathogen over control treatment. Among the tested isolates, Th-6 showed the highest (84.44%) reduction of the radial growth against *S. rolfsii* followed by Th-1 (75.56 %). On the other hands, the lowest radial growth inhibition of *S. rolfsii* was observed by the isolate T-17 (50.00%) (Table 3). Similarly, significant reduction of mycelial growth of *S. rolfsii* in presence of *T. harzianum* was also reported by several previous studies (Bell et al., 1982; Rubayet et al., 2011; Liton et al., 2019; Simi et al., 2019).

Table 3. Screening of Trichoderma spp. against S. rolfsii isolate CS 5 in dual culture

Trichoderma isolates	Percent of inhibition	Bell's scale[1]
Th-6	84.44	R_1
Th-1	75.56	R_1
Th-3	71.11	R_2
Th-5	65.56	R_2
Th-4	64.44	R_2
Th-2	63.33	R_2
Th-8	62.22	R_2
Th-9	61.11	R_2
Th-7	58.89	R_2
Th-10	50.00	R_3
Control	90.00 mm	

[1]The lysed mycelium of *S. rolfsii* by *Trichoderma* sp. was scoring by the modified Bell's scale (Bell et al., 1982), R_1 ≥75% overgrowth of *Trichoderma* sp., R_2 ≥55% overgrowth of *Trichoderma* sp., R_3 ≥50% of *Trichoderma* sp., R_4 ≤50% and blocked at the point of contact, and R_5= Pathogen overgrowth against antagonist.

Effect of Brassica spp. leaves extract on S. rolfsii isolate CS 5

The highest 80.37% inhibition of mycelial growth of *S. rolfsii* isolate CS 5 was observed at the highest 40% concentration of mustard leaves extract followed by 62.59% inhibition at 30% concentration. Additionally, cabbage 62.22%, cauliflower 55.56%, broccoli leaves extract 50.37% inhibition of mycelium radial growth of *S. rolfsii* isolate CS 5 at 40% concentration level. On the other hands, the lowest inhibition of *S. rolfsii* isolate CS 5 was recorded 17.41% at 10% concentration level of broccoli leaves extract (Table 4). In case of sclerotia formation of test pathogen, the highest 83.37% inhibition were achieved at 40% mustard leaves extract followed by 68.69% and 60.74% cabbage and broccoli leaves extract at same concentration. Cauliflower leaves extract was appeared ineffective in reducing sclerotia formation. However, the highest 83.37% reduction of sclerotia formation of *S. rolfsii* isolate CS 5 was observed at the highest 40% concentration of mustard leaves extract which indicated that mustard leaves extract was significantly superior to all others leaves extract in reducing the radial colony growth and sclerotia formation. Significant inhibition in radial growth and sclerotia formation were also observed on cabbage and broccoli leaves extract but statistically inferior than mustard leaves extract. Similar result was also reported in reduction of radial growth of *S. rolfsii* in various plant extracts by Dwivedi and Prasad, 2016.

Table 4. Preliminary laboratory evaluation of leaves extract in inhibition of radial growth and sclerotia formation of test pathogen

Leaves extract	Concentration (%)	% inhibition	
		Radial growth	Sclerotia formation
Mustard	10	32.22 f-h[1]	39.87 i
	20	43.33 de	48.42 g
	30	62.59 b	65.32 c
	40	80.37 a	83.37 a
Cabbage	10	18.89 ij	24.58 k
	20	30.37 gh	35.76 j
	30	49.26 cd	52.73 f
	40	62.22 b	68.69 b
Cauliflower	10	17.41 j	21.62 l
	20	26.30 hi	33.94 j
	30	39.63 ef	44.31 h
	40	55.56 bc	57.58 e
Broccoli	10	18.15 j	25.19 k
	20	28.52 h	35.62 j
	30	37.41 e-g	42.36 h
	40	50.37 cd	60.74 d
Control	90mm		495

[1]Means within same column followed by common letter(s) are not significantly different (α = 0.05)

Integrated effect of biofumigant, soil solarization and bio-agent on southern blight

Effect on pre-and post-emergence mortality

Significantly, the highest pre-and post-emergence seedling mortality 32.31 and 22.56% were recorded in the treatment T_2 where seeds were sown in the inoculated soil without any other amendment, followed by treatment T_1 (control) where seeds were sown in uninoculated field. On the contrary, the lowest total seedling mortality in comparison with all other treatments were observed at treatment T_8 (16.41%) followed by T_6 (21.54%), T_7 (25.13%), and T_5 (27.18%) (Table 5). Among

the different treatments including biofumigation, soil solarization and biocontrol agent either individual or in combination, the treatment T_8 was appeared to be the most superior in reducing the pre-and post-emergence mortality of carrot caused by *S. rolfsii* isolate CS 5. The treatment performance of T_6, T_7, and T_5 were identical with T_8 in term of reducing total seedling mortality. The results of the current study suggest the superiority of integrated approach for management of *S. rolfsii* isolate CS 5 either individual treatment by biofumigant and/soil solarization or biocontrol agent. Seedling mortality and other seedling diseases of different crops were controlled through the integration of antagonist with diverse organic amendments by different studies (Rahman et al., 2012; Rubayet et al., 2018) which support the findings of this present study.

Table 5. Effect of integrated use of soil solarization, biofumigation and biocontrol agent on carrot seedling mortality caused by test pathogen

Treatments	Mortality %		
	Pre-emergence	Post-emergence	Total
T_1	29.74	15.38	45.13 b
T_2	32.31	22.56	54.87 a
T_3	29.23	8.21	37.44 c[1]
T_4	23.59	8.21	31.79 cd
T_5	20.00	7.18	27.18 de
T_6	15.90	5.64	21.54 ef
T_7	18.46	6.67	25.13 e
T_8	13.33	3.08	16.41 f
CV			10.38

[1]Means within same column followed by common letter(s) are not significantly different (α = 0.05)

Effect on disease incidence and severity

The disease incidence and severity of southern blight of carrot were significantly influenced by single or combination of biofumigant, biocontrol agent, and solarized soil (Figure 1). The lowest disease incidence (10.77%) and severity (12.78%) as well as maximum reduction (75.86 and 78.30%) of southern blight disease of carrot were found in T_8 followed by T_6 (Table 6). On the contrary, significantly highest disease incidence (74.87%) and severity (79.44%) of southern blight disease of carrot were observed in the T_2 treatment where seeds were sown in the test pathogen inoculated soil without any other amendment. The results indicated that all the treatments except T_1 and T_2 were significantly effective in plummeting disease incidence and severity of carrot seedlings which is also agreed with several previous studies in different crops by Rahman et al., 2013; Chandel & Sharma, 2014; Rubayet et al., 2018.

Table 6. Effect of integrated use of soil solarization, biofumigation and biocontrol agent on incidence and severity of southern blight disease in carrot under open field condition

Treatments	% disease incidence	% disease incidence reduction/ increase (-) over control	% disease severity	% disease severity reduction/ increase (-) over control
T_1	44.62 b	-	58.89 b	-
T_2	74.87 a	-67.79	79.44 a	-34.90
T_3	33.33 c	25.30	47.78 c	18.87
T_4	28.21 cd	36.78	39.44 cd[1]	33.03
T_5	25.64 cd	42.54	31.11 de	47.17
T_6	14.36 ef	67.82	15.19 fg	74.21
T_7	20.51 de	54.03	24.44 ef	58.50
T_8	10.77 f	75.86	12.78 g	78.30
CV	14.10	-	15.28	-

[1]Means within same column followed by common letter(s) are not significantly different (α = 0.05)

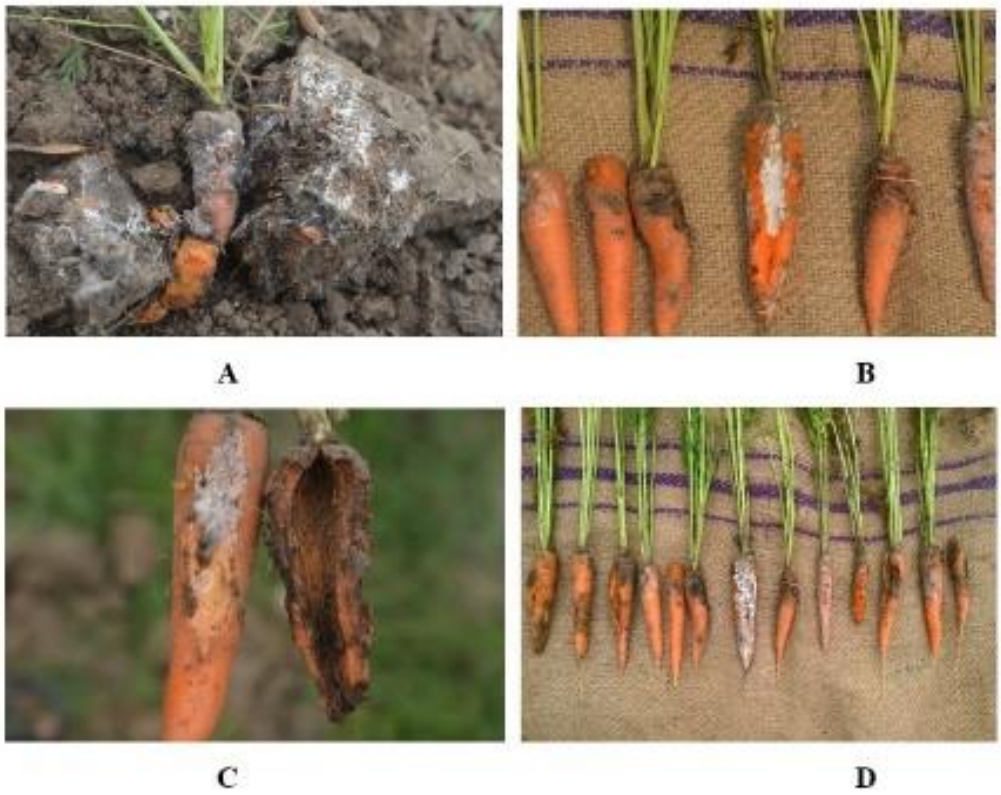

Figure 1. Southern blight disease of carrot caused by S. rolfsii isolate CS 5 (A-D)

Effect on the yield of carrot in field

In this experiment, the application of biofumigant, soil solarization and bio-agent not only compacted the disease development caused by *S. rolfsii* isolate CS 5 but also significantly improved the yield of carrot (Table 7). The highest 41.11 ton ha^{-1} yield was recorded in the plot where soil solarized by polythene mulch, used

mustard as biofumigant and wheat grain colonized *T. harzianum* in the treatment T_8 followed by T_6 and T_7. On the contrary, significantly the lowest yield in control plot T_1 (16.11 ton ha^{-1}) was recorded where seed sown uninoculated field without any other amendment and in the treatment T_2 (11.67 ton ha^{-1}) where seeds sown in test pathogen inoculated field without any other amendment. Moreover, the maximum yield (155.18%) was increased in treatment T_8 followed by T_6. On the other hands, around 27.56% yield was declined in treatment T_2 because of hefty pathogen population density as well as devastating disease infestation.

Table 7. Effect of integrated use of soil solarization, biofumigation and biocontrol agent on yield response in carrot under open field condition

Treatments	Yield (ton ha^{-1})	% yield increase/ reduction (-) over control
T_1	16.11 ef[1]	-
T_2	11.67 f	-27.56
T_3	22.22 de	37.93
T_4	25.56 cd	58.66
T_5	26.67 cd	65.55
T_6	33.89 b	110.37
T_7	31.11 bc	93.11
T_8	41.11 a	155.18
CV	18.86	-

[1]Means within same column followed by common letter(s) are not significantly different ($\alpha = 0.05$)

Therefore, biofumigation is used as a means to control many soil-borne diseases by biocidal compounds (mainly isothiocyanates) released from glucosinolates in mustard seed meal which is hydrolyzed during degradation in soil (Shaban et al., 2011). In addition, soil solarization practice can rise soil temperature to such level that may kill many disease causing pathogens such as nematodes, fungi, bacteria and weed seeds and seedlings. It also helps to disintegrate the organic materials in the soil and upsurges the amount of soluble nutrients such as Ammonium (NH_4^+), Nitrate (NO^{3-}), Potassium (K), Calcium (Ca), and Magnesium (Mg) in the soil which are energetic for the growth and development of plants. Accordingly, several volatile bio-toxic compounds are discharged when organic matter is heated and they may expand the biocidal activity in soil. It has been found that plants grow quicker when grown in solarized soil. Integrated use of solarization with biofumigation and compost amendments the re-introduction of biocontrol agents such as *Trichoderma* spp. and *Bacillus* spp. may be more effective than either treatment alone in controlling soil-borne disease. Population density of these two microbial antagonists increase relatively higher than other microorganisms under the solarized soil. On the other hands, *T. harzianum* produces a large number of chemical substances to solubilize rock Phosphate (PO_4^{3-}), Cupper ions (Cu^{2+}), Manganese ions (Mn^{4+}) and Zinc (Zn^{2+}) increase iron (Fe) availability in the soil (Altomare et al., 1999). Thus, the solubilization and chelating abilities of *T. harzianum* may also influenced the cumulative yield of carrot. The findings of the present investigation was in good agreement with the previous studies (Arefin et al., 2019; Rahman et al., 2020a; Rahman et al., 2020b).

Conclusion

The study revealed that *T. harzianum* isolate Th-6 as biocontrol agent and mustard leaves extract as natural biofumigant appeared highly effective in terms of inhibiting the radial growth and sclerotia formation of *S. rolfsii* isolate CS 5 in *in-vitro* trials. In the field condition, integrated use of biofumigation, solarization,

and biocontrol agent provided the active control measure against southern blight of carrot caused by *S. rolfsii* isolate CS 5.

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Conflict of Interest

Authors declare that there is no conflict of interest.

Authors Contribution

M. T. Rubayet and M. K. A. Bhuiyan conceived the presented idea. They also conducted both laboratory and field experiments and recorded the observations accurately. F. Prodhan performed the computations and made the draft manuscript. M. S. Hossain, M. Ahmed and M. A. A. Mamun verified the statistical data analysis and updated the references. Finally, all authors read the manuscript carefully and approved the final version.

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