Research in: Agricultural & Veterinary Sciences
Vol.3, No.3, 2019, pp.113-125

EFFICACY OF CHITOSAN AND BIO-AGENT IN CONTROLLING SOUTHERN BLIGHT DISEASE OF CARROT CAUSED BY *Sclerotium rolfsii* AND IMPROVEMENT THE CROP PRODUCTION

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Abstract. An experiment was conducted for management of southern blight disease of carrot caused by *Sclerotium rolfsii* isolate BS-5 using chitosan and *Trichoderma harzianum* isolate PB-7 spore suspension by means of seed treatment and foliar spray. Before setting experiments in the field, a series of preliminary laboratory trials were carried out to select a virulent isolate of *S. rolfsii* (BS-5) by pathogenicity test, an effective antagonist isolate of *Trichoderma harzianum* (PB-7) by screening against *S. rolfsii* isolate BS-5 and a suitable concentration of chitosan (600 ppm) by the radial growth test against *S. rolfsii* isolate BS-5. Seed treatment and foliar spray with *T. harzianum* isolate PB-7 spore suspension (5×10^5 spores ml^{-1}) and chitosan 600 ppm was applied both and/or individual according to the design of the experiment. Significantly, the highest 90.91% reduction of post-emergence seedling mortality was recorded with treatment T_6. Similarly, the highest 81.41% reduction of the southern blight incidence and Percent Disease Index (PDI) 81.97% was observed in the treatment T_6 followed by the treatments T_7, T_8 and T_9, respectively. All the treatments significantly reduced the disease incidence and increased the yield compared to the untreated control treatment T_1. The highest yield 22.60 t ha^{-1} with the maximum increasing of yield 53.33% was observed in T_6 over control treatment T_1 followed by the treatments T_7, T_8 and T_9, respectively. Seed treatment was found more effective than foliar spray in case of individual or integrated application of chitosan and *T. harzianum* spore suspension for the protection of southern blight disease and also increases the yield of carrot.

Keywords: *Southern blight, S. rolfsii, Chitosan, T. harzianum, Yield of Carrot.*

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Received: *29 September 2019;* ***Accepted:*** *4 November 2019;* ***Published:*** *30 December 2019.*

1. Introduction

Carrot (*Daucus carrota* L.) belongs to the family Apiaceae which is one of the most popular vegetable in Bangladesh. It is grown all over the world in spring, summer and autumn in temperate countries and during winter under tropical and subtropical climate. It is the best source of carotene; a precursor of Vitamin A (Zeb & Mahmood, 2004). Moreover, carrot contain also abundant quantities of nutrients and minerals (Nicolle *et al*., 2004). The average yield of this crop in Bangladesh is 8.90 t ha^{-1} (Anon, 2017). The yield of carrot is very low in Bangladesh due to various reasons, where disease is considered as important one. Among them, southern blight caused by *Sclerotium rolfsii* is one of the most common soil-borne disease of carrot in Bangladesh.

This disease not only loss of yield in the field condition but also damage during transportation and storage. Infection of *S. rolfsii* usually begins at base point of main stem. As a result, leaf tissues become brown in color and wilted. A white color cushion like mycelial growth may appear on the soil surface. As the root rot gradually progress downwardly and small spherical tan fungal bodies called sclerotia develop within the damaged root tissues. *S. rolfsii* reduces the crop stand by causing pre- and post-emergence damping off of seedling. Contamination of carrot root with *S. rolfsii* in the field lead to the development of rot in the storage and during transportation (Sen, 2010). The *S. rolfsii* is a soil-borne pathogens and very difficult to manage due to it wide host range. Sclerotia can be disseminated through planting materials, water, wind etc. Soil-borne plant pathogens cause heavy losses to all major crops, leading to reductions in both yield and quality. Effective control of soil-borne plant pathogens is a serious challenge to farmers. Applications of seed treating fungicides, solarization and use of antagonistic microorganisms, deep ploughing, crop rotation, and incorporation of organic and inorganic residues can be used to minimize the population density of *S. rolfsii* (Punja, 1985). But application of chemical fungicides resulting in continuous damage of natural ecosystems, ground water and other environmental degradation.

Biological control is thus being considered as an important component either individual or integrated management. Different species of *Trichoderma* have been reported to be effective antagonist against S. rolfsii of different crops (Islam & Bhuiyan, 2006).

Chitosan (Parke *et al*., 2002) with antagonist fungi like *Trichoderma* isolates are important components of Integrated Disease Management (IDM) that help minimize chemical control. To achieve economic control of sclerotia forming pathogen with single method alone may not be effective. Chitosan can also enhance number of fruits or leaves (Trotel-Aziz *et al*., 2006) and reduce disease caused by fungal pathogens (El-Mohamedy *et al*., 2013).

Considering the deleterious effect of synthetic pesticides, alternative measures should be taken for the control of plant pathogenic microorganisms. Therefore, economic and eco-friendly effective control methods are now recommended to be pursued worldwide. That's why day by day increasing the interest of crop production towards the organic cultivation. Information regarding the control measures of southern blight of carrot with biocontrol agents and their integrated approach is scanty. So far, very few works have been published on the soil-borne disease management of carrot in Bangladesh. Development of integrated disease management technology against the soil-borne pathogens of carrot will be highly beneficial for economic viability of carrot production in the country. Therefore, the present study was undertaken to evaluate the effect of seed treatment and foliar spray with chitosan 600 ppm and *T. harzianum* spore suspension on management of southern blight disease of carrot at artificially inoculated field condition.

2. Materials and Methods

Collection, isolation and preservation of S. rolfsii isolates

Five isolates of *S. rolfsii* designated as BS-1 to BS-5 were isolated from the rhizosphere and rhizoplane of potato (*Solanum tuberosum*), tomato (*Solanum lycopersicon*), chilli (*Capsicum frutesence*), Carrot (*Daucus carrota)* and Soybean (*Glycine max* L.). The specimens which had typical symptoms of southern blight was

selected from infected fields. The fungal isolates were isolated following standard method (Mian, 1995). The fungal colonies were grown on PDA and identified by following standard key (Barnet & Hunter, 1972). The pure culture of *S. rolfsii* was preserved by using PDA slants at 10℃ in refrigerator for future research purpose.

Inoculum preparation of test pathogen

Inoculum of the *S. rolfsii* was prepared and stored following the standard method (Rubayet & Bhuiyan, 2016).

Pathogenicity test of S. rolfsii isolates on seedling

The pathogenicity of *S. rolfsii* isolates were done according to Rubayet *et al.*, 2017.

Pathogenicity of S. rolfsii isolates on carrot

Pathogenicity test with five selected isolates of the pathogen *S. rolfsii* was conducted by inoculating harvested storage taproot of carrot. Each isolates of *S. rolfsii* was grown on PDA plates separately. Collected healthy carrots were rinsed with sterile water and air dried. Inoculation was made by placing a mycelial disc of 2 mm diameter from 3 days old culture near the crown of the carrot storage taproot. Three carrots for each replication and three replications for each isolates (treatments) were maintained. After inoculation, carrots of each replication were kept in a separate transparent polyethylene bag to avoid drying. Inoculated carrot was incubated for 4 days at room temperature (25±2℃). Carrot taproot similarly prepared but received only PDA disk, served as control. After four days, disease severity was rated following 0-5 scale, where 0=No visible sign or symptoms, 1=<15%, 2=15-35%, 3=36-49%, 4=50-75% and 5=>75% lesion or mycelium on carrot storage organ circumference covered with.

Collection, isolation and preservation of T. harzianum isolates

A total of 20 isolates of *T. harzianum*, whereas that 12 isolates were isolated from the rhizosphere and rhizoplane of different crops such as Potato, Brinjal, Tomato, Carrot, Cauliflower, Cabbage, Chilli and Broccoli field following the soil dilution plate technique (Mian, 1995). And 8 isolates were collected directly from the plant pathology laboratory, BSMRAU, Bangladesh. All the isolated *Trichoderma* spp. were identified as *T. harzianum* based on the different characteristics like hyphal growth, spore formation and color (Shaiesta *et al.*, 2012.). The pure culture of *T. harzianum* was preserved following standard method (Rubayet *et al.*, 2011) for future use.

Collection of Chitosan

Chitosan was collected from Bangladesh Atomic Energy Commission (BAEC), Dhaka, Bangladesh. It was derived from the shell of sea shrimp and then irradiated with γ-ray (20 kD). This product can play a significant role on plant growth promotion (Akter *et al.*, 2018).

In-vitro evaluation of chitosan against S. rolfsii isolate BS-5

The inhibitory effect of chitosan against *S. rolfsii* isolate BS-5 was tested *in-vitro* by using three concentrations i.e 200, 400 and 600 ppm. Chitosan concentrations were prepared according to (Benhamou *et al.*, 1994). Chitosan was added to conical flasks containing sterilized PDA before solidification and rotated gently then poured into

sterilized petri dishes (9 cm diameter). Plates were individually challenged at the center with equal agar plugs (5 mm diameter) taken from *S. rolfsii* 5 days-old cultures then incubated at 25 ± 2°C. Mean colony diameter was measured when the control plates (PDA free of chitosan) reached full growth.

Screening of T. harzianum isolates against S. rolfsii isolate BS-5

Screening was conducted to evaluate the antagonistic effect of selected 20 isolates of *T. harzianum* against *S. rolfsii* isolate BS-5 on potato dextrose agar (PDA) medium by Dual Plate Culture technique (Dhingra & Sinclair, 1985). After 7 days of incubation the inhibition percentage of radial growth of *S. rolfsii* were calculated following the formula (Sundar *et al.*, 1995).

$$\% \text{ Inhibition of growth} = \frac{X - Y}{X} \times 100$$

where, X = Mycelial growth of pathogen in absence of *T. harzianum* (control)
Y= Mycelial growth of pathogen in presence of *T. harzianum*

Preparation of spore suspension of T. harzianum isolate PB-7

A spore suspension of the test isolate of *T. harzianum* was prepared according to the method of Das *et al.*, 2019. The spore concentration of the filtrate was adjusted to 5×10^5 spore ml^{-1} using sterilized distilled water.

Field experiment

The experiment was laid out in the Randomized Complete Block Design (RCBD) with 3 replications. The plot size was 2.0 m×1.5 m. distance between block to block was 1.0 m and that of plot to plot in a block was 0.50 m. Drains were made surrounding the each unit plots and the excavated soil was used for raising plots 15 cm high from the soil surface. Nine different treatments were allotted randomly to nine unit plots per blocks. After preparation seeds were sown in the field. Before sowing, seeds were soaked for 24 h to facilitate the germination. Seed were air dried before sowing to avoid excess water. For respective seed treatment seeds were treated with chitosan solution or spore suspension of *T. harzianum.* Seeds were sown in lines uniformly by hand at the rate 4.0 kg/ha keeping the row to row distance of 25 cm. Sowing of seeds were done in rows at a depth of one centimeter for easy emergence and covered with pulverized soil just after sowing and gently pressed with hands. After sowing, the soil was mulched with rice straw for preservation of moisture and to facilitate germination. Mulches were removed after 6 days when seeds were started to germinate. Intercultural operations, irrigation and other management were done when it was required.

Treatments and their application methods

The inoculum of *S. rolfsii* isolate BS-5 was thoroughly mixed with soil according to the design and layout @ 94 gm^{-2} soil as suggested by Yuen *et al.* (1994) for sclerotia forming fungal pathogens.

Following treatments were applied in the field experiment:
T_1= Uninoculated field (Control)
T_2= Inoculated with *S. rolfsii* field (ISF) + Seed treated with *T. harzianum* (STT)
T_3= ISF + Seed treated with chitosan 600 ppm (STC)
T_4= ISF + Foliar spray with *T. harzianum* (FST)
T_5= ISF + Foliar spray with chitosan 600 ppm (FSC)

T_6= ISF + STC + STT
T_7= ISF + STC + FST
T_8= ISF + FSC + STT
T_9= ISF + FSC + FST

Nine treatments were tested in the field under artificially *S. rolfsii* isolate BS-5 inoculated plots. Spore suspension (5×10^5 spores ml^{-1}) of *T. harzianum* (PB-7) and chitosan at 600 ppm were applied either as seed treatment or as foliar spray. For the treatments of foliar spray chitosan 600 ppm solution and *T. harzianum* isolate spore suspension were prepared in the same way as described earlier and applied on experimental carrot plants in accordance to the treatment plan after germination at 10, 20 and 35 days interval at morning using a hand sprayer. Seed treatment was done with spore of *T. harzianum* (3 g Kg-1 seed) and chitosan at 600 ppm following standard methods (Arefin *et al.*, 2019, Mahdavi & Rahimi, 2013, Jannat *et al.*, 2018).

Data collection

Germination and seedling disease development was observed regularly and recorded at 10, 20, and 30 days after sowing to estimate the effect of pathogen on pre- and post-emergence seedling mortality. The causal agent of mortality was confirmed after re-isolation of the pathogen from the ungerminated seeds and diseased seedlings. Southern blight disease incidence at different growth stage was recorded. Disease severity of southern blight caused by *S. rolfsii* was appraised by indexing on five degree of rating scale where 0=No visible sign or symptoms, 1=<15%, 2=15-35%, 3=36-49%, 4=50-74% and 5=>75% of carrot storage taproot circumference covered with lesion or mycelium. Then, the disease incidence and disease severity were measured by the following formula (Rahman *et al.*, 2013).

$$\text{Disease Incidence (DI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \text{ x } 100$$

$$\text{Percent Disease Index (PDI)} = \frac{\text{Summation of all ratings}}{\text{Total number of rating x Maximum disease grade (5)}} \text{ x } 100$$

After harvested, fresh taproot weight in each plot was recorded and taproot yield per hectare was calculated by following formula (Razaq *et al.*, 2015).

$$\text{Total taproot yield } (\text{tha}^{-1}) = \frac{\text{Yield per plot (kg)}}{\text{Area of plot } (\text{m}^2) \text{ x } 1000 \text{ (Kg)}} \text{ x } 10000 \text{ m}^2$$

Data analysis

Statistically data were analyzed by using the Statistix-10 program. The treatment means were compared following Duncan's Multiple Range Test (Gomez & Gomez, 1984).

3. Results and Discussion

Isolation of S. rolfsii isolates

Five isolates of *S. rolfsii* named as BS 1 to BS 5 were isolated from different infected crops such as Potato, Brinjal, Tomato, Carrot, Cauliflower etc.

Pathogenicity test of S. rolfsii isolates in pot culture

Pathogenicity test of 5 selected isolates of *S. rolfsii* against carrot variety 'New Kuroda' were conducted in earthen pots containing sterilized soil to find out the virulent isolate. The *S. rolfsii* isolate BS-5 was appeared to be the most virulent causing the highest 83% total seedling mortality followed by isolate BS-4 caused 80.00% total seedling mortality. Significantly, the lowest 65.67% total seedling mortality was observed with the isolate BS-1. Identical total seedling mortality of the isolates BS-2 and BS-3 were 75.67 and 73.00%, respectively was observed. No pre- and post-emergence seedling mortality was observed in the uninoculated control pot. All the tested isolates of *S. rolfsii* are found to virulent ranging from 65.67-83% total seedling mortality, mostly caused at pre-emergence seedling mortality while post-emergence mortality ranging from 6.67-9.33% (Table 1). The results of the present study indicated that all the isolates are pathogenic to seed and seedlings of carrot but the virulence of the isolates were variable and are in agreement with several investigators (Chowdhury & Ahmed, 1989; Naher *et al.*, 2007; Sen, 2010).

Table 1. Pathogenicity test of *S. rolfsii* isolates on seedling of carrot in pot culture

Isolates of *S. rolfsii*	% mortality		
	Pre-emergence	Post-emergence	Total
BS-1	59.00	6.67	65.67 ^{e}
BS-2	68.00	7.67	75.67 ^{cd}
BS-3	66.00	7.00	73.00 ^{d}
BS-4	71.67	8.33	80.00 ^{bc*}
BS-5	73.67	9.33	83.00 ^{a}
Control	0.00	0.00	0.00

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

Pathogenicity of S. rolfsii isolates on carrot

The highest PDI was observed in the carrot inoculated with the isolates BS-5 (PDI=89.00) followed by BS-4 (PDI=76.56) (Fig. 1).

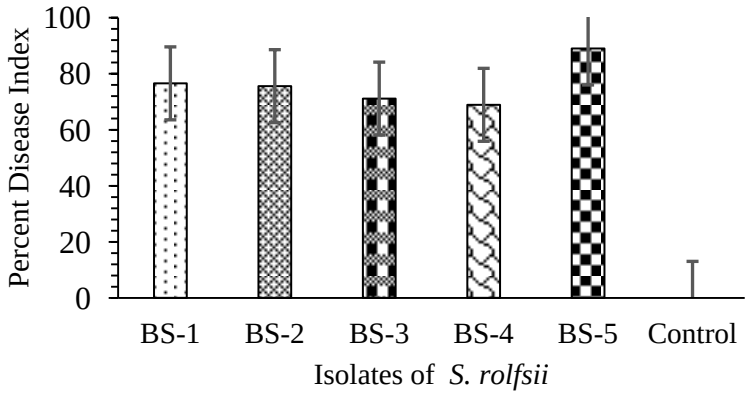

Fig. 1. Percent disease index of different isolates of *S. rolfsii* on carrot

Mycelium from the inoculated disc develops very rapidly over the carrot storage root surface causing no depressed lesion. The virulence of the isolates supports the

virulence of the same isolate in the earlier pot culture experiment. Tissue of the carrot was destroyed. Response to rapid mycelial growth associate with damaging tissue of the carrot storage taproot confirmed the isolate as potential pathogen (Sen, 2010). From the results of the two pathogenicity tests, it might be concluded that the isolate BS-5 of *S. rolfsii* was most virulent to carrot and therefore, it was selected as test pathogen for inoculation in the pot and field experiments.

In-vitro screening of T. harzianum isolates against S. rolfsii isolate BS-5

A total of 20 selected isolates of *T. harzianum* were tested against *S. rolfsii* isolate (BS-5) on Potato Dextrose Agar (PDA) by dual culture technique to observe the antagonistic effect of *T. harzianum*. All the tested 20 isolates of *T. harzianum* showed more than 50% inhibition of radial growth of the tested pathogen *S. rolfsii* as compared with the control. Among the tested *T. harzianum* isolates, total 7 and 12 isolates showed Bell's rating 1 and 2 antagonism, respectively. The only isolate PB-4 showed Bell's rating 3 antagonism against *S. rolfsii*. The highest 89.80% inhibition of the radial growth of *S. rolfsii* was observed with the isolates *T. harzianum* (PB-7) and isolate Pb-4 was appeared as lowest 56.67% radial growth inhibition against the tested *S. rolfsii* isolate BS-5 (Table 2). The study of the screening of *T. harzianum* isolates against the soil-borne pathogen *S. rolfsii* by dual plate culture technique were observed by several investigators throughout the world including Bangladesh (Raihan *et al.*, 2003; Khandakar *et al.*, 2005; Rubayet & Bhuiyan, 2016, Liton *et al.*, 2019).

Table 2. Screening of *T. harzianum* isolates against *S. rolfsii* isolate BS-5 in dual culture technique

T. harzianum	% inhibition *of S. rolfsii*	Bell's scale *
PB-1	63.32	R_2
PB -2	67.38	R_2
PB -3	66.67	R_2
PB -4	56.66	R_3
PB -5	79.52	R_1
PB -6	77.52	R_1
PB -7	89.80	R_1
PB -8	65.55	R_2
PB -9	76.12	R_1
PB -10	79.33	R_1
PB -11	65.58	R_2
PB -12	73.57	R_2
PB -13	78.48	R_1
PB 14	65.92	R_2
PB -15	61.47	R_2
PB -16	60.73	R_2
PB -17	62.24	R_2
PB -18	72.99	R_2
PB-19	71.50	R_2
PB-20	78.83	R_1
Control	00.00	-

* Bell's scale: R_1=100% Overgrowth of *T. harzianum*, R_2=75% Overgrowth of *T. harzianum*, R_3= 50% Overgrowth of *T. harzianum*, R_4=Block at point of contact and R_5=Pathogen overgrows against antagonist.

In-vitro evaluation of chitosan against S. rolfsii isolate BS-5

All the selected three concentrations viz, 200, 400 and 600 ppm of chitosan amended with PDA plate significantly were reduced the radial growth of *S. rolfsii*

isolate BS-5. The reduction of the radial growth of *S. rolfsii* significantly increased with increasing the amended concentration of chitosan. Significantly, the highest 72.97% reduction of the radial growth of *S. rolfsii* over the control PDA plate was observed at the highest 600 ppm of chitosan amended with PDA plate followed by the second highest 400 ppm of chitosan with 59.26% reduction of radial growth. On the contrary, the lowest 32.97% reduction of the radial growth of *S. rolfsii* was observed at the lowest 200 ppm concentration of chitosan amended with the PDA plate (Table 3). Based on the *in-vitro* evaluation 600 ppm chitosan was selected for the field trial.

Table 3. The radial growth of *S. rolfsii* isolate BS-5 in PDA plate with different doses of chitosan

Treatments	Radial growth of *S. rolfsii* (mm)	% reduction over control
Control	90.0 [a]	-
Chitosan 200 ppm	60.33 [b]	32.97
Chitosan 400 ppm	36.67 [c]	59.26
Chitosan 600 ppm	24.33 [d]	72.97

Effect of T. harzianum isolate PB-7 and chitosan 600 ppm on seedling mortality and southern blight disease incidence

Post-emergence seedling mortality, southern blight disease incidence and PDI was recorded at different growth stages from infected roots and leaves of carrot in the field experiment. In the field experiment, significantly the highest 14.33% post-emergence seedling mortality was observed in the control plot (T_1) where only pathogen was inoculated in the soil without *T. harzianum* and chitosan either individually or in combination was applied. The highest 90.91% reduction of seedling mortality was found with the treatment T_6 where seed treatment was done in combination of chitosan 600 ppm and *T. harzianum* spore suspension and significantly superior in comparison to all other treatment. In case of post-emergence seedling mortality the treatment T_7, T_8 and T_9 were identical. On the other hand, post-emergence seedling mortality was found to be identical in the treatment T_2, T_3, T_4 and T_5 where chitosan and *T. harzianum* were applied individually either in the form of seed treatment or spray on the growing plant.
The present study reveals that the best method of application to control post-emergence seedling mortality of carrot is the seed treatment in combination of chitosan and *T. harzianum* (Table 4). Significantly, the highest 66.33% disease incidence was recorded in the treatment T_1 where only pathogen was inoculated without chitosan and *T. harzianum*. On contrast, the lowest disease incidence and PDI 12.35 and 11.35% was recordedwith the treatment T_6 which was significantly the highest 81.41% and 89.97%, reduction of southern blight disease incidence and PDI, respectively in comparison to the all other treatments. The lowest 23.61% disease reduction and the lowest PDI reduction 28.61% was observed with treatment T_5 where only chitosan was applied as foliar spray in comparison to the all other treatments. The second highest 72.36 and 73.01% reduction of disease incidence and PDI, respectively were observed with treatment T_7 followed by the treatments T_8 and T_9 where integrated application of chitosan and *T. harzianum* either as seed treatment or foliar spray. Identical reduction of diseases was observed with the treatments T_3 and T_4 but statistically inferior to all other treatments except treatment T_5 which appeared to be the most inferior compare to the all other treatments except for control T_1. Comparing between foliar spray and seed treatment with chitosan and *T. harzianum* spore suspension, the maximum performance was observed in controlling post-emergence seedling mortality and southern blight

disease of carrot was observed through the application method of seed treatment instead of foliar spray either individual or integrated method. The study reveals that application of chitosan in combination with *T. harzianum* appeared to be superior to individual application.

The results of the present study supports the observation of several investigators (Begum, 2003; Raihan *et al*., 2003; Harman *et al*., 2004; Sen, 2010) who reported that *T. harzianum* is used as a successful biological control agent to control different soil-borne plant pathogens such as *Pythium* spp. *Rhizoctonia solani*, *Fusarium* spp., *S. rolfsii* in many vegetable crops including carrot. The results of the current study also justify the statements of Benhamou *et al*. (1994) and O'Herlihy *et al*. (2003) that chitosan has been considered as an alternative to chemical fungicides.

Table 4. Effect of the treatments on disease incidence of southern blight caused by *S. rolfsii* isolate BS-5 in carrot under field condition

Treatments	% Post emergence seedling mortality	% reduction	% Disease Incidence	% reduction	PDI	% reduction
T_1	14.33 [a]	-	66.33 [a]	-	62.87 [a]	-
T_2	6.33 [b]	55.83	38.00 [d]	42.71	34.11 [d]	45.74
T_3	6.67 [b]	53.81	42.00 [c]	36.68	39.19 [c]	37.67
T_4	7.33 [b]	48.85	45.33 [c]	31.66	42.20 [bc*]	32.87
T_5	7.67 [b]	46.12	50.67 [b]	23.61	44.88 [b]	28.61
T_6	1.33 [d]	90.91	12.33 [h]	81.41	11.33 [g]	81.97
T_7	3.67 [c]	74.39	18.33 [g]	72.36	16.97 [f]	73.01
T_8	4.44 [bc]	69.25	31.67 [e]	52.26	26.07 [e]	58.53
T_9	4.67 [bc]	67.41	26.00 [f]	60.80	23.69 [e]	62.31

* Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT. [Where, T_1= Uninoculated field (Control), T_2= Inoculated with *S. rolfsii* field (ISF) + Seed treated with *T. harzianum* (STT), T_3= ISF + Seed treated with chitosan 600 ppm (STC), T_4= ISF + Foliar spray with *T. harzianum* (FST), T_5= ISF + Foliar spray with chitosan 600 ppm (FSC), T_6= ISF + STC + STT, T_7= ISF + STC + FST, T_8= ISF + FSC + STT, T_9= ISF + FSC + FST]

Effect of T. harzianum isolate PB-7 and chitosan 600 ppm on yield contributing characters of carrot

The main economic part of the carrot plant is taproot. Southern blight of carrot hampers the optimum vegetative growth and development and reduces the biomass. To get maximum output of carrot, it is necessary to control the southern blight pathogen *S. rolfsii*. The field experiment was laid out with nine treatments including one untreated control treatment T_1 inoculated with *S. rolfsii* without chitosan and *T. harzianum* isolate and eight treatments were comprising *S. rolfsii* along with chitosan and *T. harzianum* applied either individual or in combination applied as seed treatment or foliar spray. Yield contributing characters measured as taproot weight, root length and root diameter were also recorded and the results are presented in the Table 5. The growth promotion component root weight was increased 2.40-12.42% over the control treatment T_1, while the highest 12.42% increase root weight was recorded at the treatment T_6, where seeds were treated with chitosan and *T. harzianum* isolate PB-7. The lowest root weight (59.89 g) was observed at the control treatment T_1 where no chitosan and *T. harzianum* were applied. Similarly the root length was also increased at all the treatments ranged from 2.31-20.64% over the control treatment. The highest 20.64% root length was increased in treatment T_6, where seeds were treated with chitosan and *T. harzianum*. The increased percent of root length was higher in all the treatments while chitosan and

T. harzianum were applied in combination compare to the individual application of *T. harzianum* or chitosan. In case root diameter, similar trend of the results were also observed. The lowest 0.69% root diameter was increased in the treatment T_5, while the highest 13.19% root diameter was increased in the treatment T_6 followed by the treatment T_7, T_8 and T_9. In case of all the growth promotion components, the foliar spray treated plots performed lower than plots those were managed with seed treatment *T. harzianum* (Elad *et al*., 2006).

Furthermore, several species of *T. harzianum* promoted growth and development of seedlings of vegetable and non-vegetable crops (Bal & Altintas, 2008; Rubayet & Bhuiyan, 2016). Similarly, there are observations and published reports that chitosan increased the growth promotion components of rice, vegetables including carrot. Vasudevan *et al*. (2002) suggested that application of chitosan formulations can serve as increase in root and shoot length, and grain yield.

Table 5. Effect of chitosan 600 ppm and *T. harzianum* isolate PB-7 on yield contributing characters of carrot

Treatments	Taproot Wt. (g)	% increased	Taproot length (cm)	% increased	Taproot Diameter (cm)	% increased
T_1	59.89	-	12.11	-	2.88	-
T_2	64.00	6.87	12.50	3.22	3.15	9.38
T_3	63.22	5.57	12.89	6.44	3.00	4.12
T_4	62.22	5.57	12.83	5.95	2.90	0.69
T_5	61.33	2.40	12.39	2.31	2.99	3.82
T_6	67.33	12.42	14.61	20.64	3.26	13.19
T_7	66.11	10.39	13.89	14.67	3.22	11.81
T_8	64.33	7.41	13.55	11.89	3.22	11.81
T_9	64.78	8.16	13.00	7.35	3.15	9.38

[Where, T_1= Uninoculated field (Control), T_2= Inoculated with *S. rolfsii* field (ISF) + Seed treated with *T. harzianum* (STT), T_3= ISF + Seed treated with chitosan 600 ppm (STC), T_4= ISF + Foliar spray with *T. harzianum* (FST), T_5= ISF + Foliar spray with chitosan 600 ppm (FSC), T_6= ISF + STC + STT, T_7= ISF + STC + FST, T_8= ISF + FSC + STT, T_9= ISF + FSC + FST]

Effect of T. harzianum isolate PB-7 and chitosan 600 ppm on yield of carrot

Southern blight disease caused a substantial loss both in the field condition and at post-harvest stage of carrot. The effect of *T. harzianum* and chitosan on the yield of carrot in the field experiment was also recorded. The results of the effect of *T. harzianum* and chitosan on the total marketable yield are presented in the Table 6. The lowest 14.74 t ha^{-1} carrot was recorded in the control plot treatment T_1 where *S. rolfsii* was inoculated without applying any *T. harzianum* and chitosan. Marketable taproot yield of carrot was significantly influenced by the application of different treatments against *S. rolfsii* isolate BS-5. The root yield increased over control (S. *rolfsii* inoculated only) ranged from 19.36 to 53.33% of different treatments. The highest 22.60 ton root yield per hectare was recorded in the plots of the treatment T_6 followed by treatment T_7, T_8 and T_9. The lowest yield was recorded in treatment T_5 over the control treatment T_1 where no *T. harzianum* and chitosan was applied. Result indicated that yield per hectare was influenced by the application of different treatments in the field. The findings of the current study supports the findings of Sen (2010) who observed that southern blight disease of carrot has abruptly reduce yield of carrot and application of *T. harzianum* not only reduce the disease rather increased the yield substantially. The effect of chitosan in

increasing yield of many crops are also in agreement with results of the present study. Martinez *et al*. (2007) observed that seed treated with chitosan increased the plant height, stem diameter and root length in tomato. Tomar and Ramgiry (1997) found that tomato plant treated with chitosan showed significantly greater number of fruits/plant than untreated control. Boonlertnirun *et al*. (2008) showed that application of chitosan by seed soaking and soil application four times throughout cropping season significantly increased rice yield over the other treatments.

Table 6. Effect chitosan 600 ppm and *T. harzianum* isolate PB-7 on yield of carrot

Treatments	Yield (t ha^{-1})	% increased yield
T_1= Uninoculated field (Control)	14.74 ^{h}	-
T_2= Inoculated with *S. rolfsii* field (ISF) + Seed treated with *T. harzianum* (STT)	18.13 ^{f}	23.00
T_3= ISF + Seed treated with chitosan 600 ppm (STC)	18.44 ^{ef*}	25.10
T_4= ISF + Foliar spray with *T. harzianum* (FST)	18.77 ^{de}	27.32
T_5= ISF + Foliar spray with chitosan 600 ppm (FSC)	17.59 ^{g}	19.36
T_6= ISF + STC + STT	22.60 ^{a}	53.33
T_7= ISF + STC + FST	21.44 ^{b}	45.45
T_8= ISF + FSC + STT	19.73 ^{c}	33.85
T_9= ISF + FSC + FST	19.22 ^{d}	30.41

[Where, T_1= Uninoculated field (Control), T_2= Inoculated with *S. rolfsii* field (ISF) + Seed treated with *T. harzianum* (STT), T_3= ISF + Seed treated with chitosan 600 ppm (STC), T_4= ISF + Foliar spray with *T. harzianum* (FST), T_5= ISF + Foliar spray with chitosan 600 ppm (FSC), T_6= ISF + STC + STT, T_7= ISF + STC + FST, T_8= ISF + FSC + STT, T_9= ISF + FSC + FST]

4. Conclusion

Based on the present study it could be concluded that southern blight disease caused by *S. rolfsii* isolate BS-5 of carrot can be effectively controlled by seed treatment or foliar spray with chitosan 600 ppm and *T. harzianum* isolate PB-7 spore (5×10^5 spores ml^{-1}) suspension. Combine effect of chitosan 600 ppm and *T. harzianum* isolate PB-7 is superior for controlling disease, increasing growth promoting components and yield of carrot than an individual one. However, considering the application method of chitosan and *T. harzianum* spore suspension seed treatment could be the best technique compared to foliar spray either an individual or integrated form of application for minimizing the southern blight disease and augmentation of carrot production.

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