Role of Chitosan in Disease Suppression, Growth and Yield of Carrot

M. A. Rahman, R. Jannat, A. M. Akanda, M. A. R. Khan, and M. T. Rubayet

ABSTRACT

An attempt was made for controlling of Rhizoctonia canker caused by *Rhizoctonia solani* and southern blight caused by *Sclerotium rolfsii* in pot and field experiments under inoculated condition and also to increase the growth promoting factors and yield of carrot through the application of chitosan. Before setting the experiments in the field, laboratory experiments were carried out to select virulent isolates of *R. solani* and *S. rolfsii* and effective dose of chitosan on mycelial growth inhibition of virulent isolates of test pathogens. In the pathogenicity test, *R. solani* isolate R-1 and *S. rolfsii* isolate S-1 were found to be the most virulent against carrot seedlings. *In vitro* application of 1.0% chitosan was found to inhibit 100% mycelial growth of both tested pathogens. The field experiment was laid out following randomized complete block design with four treatments, where no treatment was done in T1, pathogen was inoculated in T2 and seed treatment and soil amendment with 1.0% chitosan was done in T3 and T4, respectively, in pathogen inoculated condition. Application of 1.0% chitosan as seed treatment or soil amendment significantly reduced post-emergence seedling mortality, incidence of diseases and enhanced seedling growth and also yields of carrot. On the contrary, post-emergence seedling mortality, incidence of Rhizoctonia canker and southern blight of carrot were highest in treatment T1 where soil was inoculated with pathogens. Chitosan could be used as an alternative of fungicide to suppress *Rhizoctonia* canker and southern blight in sustainable agriculture and improvement the yield of carrot.

Keywords: Chitosan, Rhizoctonia root rot, Southern blight, Growth, and Yield.

I. INTRODUCTION

Carrot (*Daucus carota* L.) belongs to the family Apiaceae and grown all over the world in spring, summer and autumn in temperate and during winter under tropical and subtropical regions. In Bangladesh mid-November to early December (rabi season) is the best time for its cultivation [1]. Carrot roots are valued as food mainly for its high carotene content. Additionally, it is an excellent source of iron and contains good qualities of vitamin B and C and rich in sugar [2].

However, there are several factors ascribed for the low production of carrot such as climatic conditions, variation in rainfall pattern, and an out-break 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 Rhizoctonia crown rot and canker, southern blight, Pythium rot, cottony rot, black rot, scab, bacterial soft rot, etc. But information regarding diseases of carrot and their management is very scarce in Bangladesh.

Rhizoctonia fungus which causes damping off when seedlings are small. On carrot roots, early symptoms are horizontal dark brown lesions; as the crop matures the tops may die in patches in the field. Pre-and post-emergence damping off were observed in the greenhouse for approximately 30 days after planting. Crown rot symptoms were predominant in inoculated plants at 30-60 days after seeding and in naturally infected plants of similar age in the field. Rhizoctonia canker was the predominant symptom in infected plant 60 or more days old. The incidence and severity of canker increased through the growing season. Rhizoctonia crown rot and canker can severely downgrade carrot pack out and profitability.

On the other hand, infection of *S. rolfsii* usually begins at or near the soil surface. Rotting begins at the top of the taproot and the base of the leaf petioles. Leaf tissues turn brown and may wilt. A white mycelial growth may appear on the soil surface. As the root rot progresses downward, small spherical tan fungal bodies called sclerotia may develop within the rotted root tissues. Moreover, *S. rolfsii* reduces the crop stand by causing pre- and post-emergence damping off of seedlings. Contamination of carrot root with *S. rolfsii* in the field lead to the development of rot in the storage and during conveyance.

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Despite the importance of these pathogens in carrot fields, limited information has been available on the disease epidemiology and control. Currently, there is no effective control strategy for the control of serious diseases of carrot. Control of soil borne diseases especially caused by *Rhizoctonia* and *Sclerotium* are nearly impossible if crop is grown under wet condition. Planting on ridges, crop rotation, careful handling during harvest, storage sanitation and extremely good storage conditions might reduce losses to the disease. Seed treatment with fungicide reduces damping off and seedling mortality caused by soil borne fungal pathogens. Indiscriminate use of chemical pesticides and fertilizers in modern agriculture has resulted in the development of several problems such as pesticide resistance in pests, resurgence of target and non-target pests, destruction of beneficial organisms and chemical residues in food, feed and fodder.

Considering the deleterious effect of synthetic pesticides on life supporting system, there is an urgent need for alternative agents for the control of pathogenic microorganisms. Therefore, in the absence of resistant cultivars, bio-pesticides, such as chitosan offer a more sustainable approach for the control of diseases in fruits and vegetables [3], [4]. Chitosan is a linear polysaccharide composed of randomly distributed – β-(1→4)-linked D-glucosamine (deacetylated unit). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, like sodium hydroxide. It is considered as a biodegradable and biocompatible material with no toxicity or side effects [5]. Over the last decade, chitosan polysaccharide has taken on enormous importance in the control of pathogenic microorganisms. The presence of amino groups (-NH₂) in its chemical structure gives chitosan unique and ideal food conservation and security properties which are exploited through the development of biodegradable edible coatings and films containing natural antimicrobials; it also has elicitor properties that enhance the natural defenses of fruit, vegetables and grains [6]. Vasudevan et al. [7] suggested that application of chitosan formulation can increase root and shoot length and grain yield. It also increases the growth of nursery-raised plants such as cucumber, pepper and tomato etc. Defense mechanism in plant has been accelerated by using chitosan. Ortega-Ortiz et al. [8] reported that chitosan could increase catalase (CAT) and peroxidase (POD) enzymes activity in *Lycopersicon esculentum*. Therefore, the research aim is to evaluate the effect of chitosan in controlling the diseases and progress growth and yield of carrot.

II. MATERIALS AND METHODS

A. Experimental Site

A field experiment was carried out at Bangabandhu Sheikh Mujibur Rahman Agricultural University from 2018 to 2019. Geographically the experimental area is located at 24° 09’ N latitude and 90° 26’ longitudes at the elevation of 8.2 m from sea level [9], [10]. The soil type of the experimental site belongs to the shallow red-brown terrace type under Salma series of Madhupur tract of Agroecological zone (AEZ) 28 which is characterized by silty clay with a pH value of 6.5. The experimental site is under the subtropical climatic zone which is characterized by less rainfall, almost clear sunshine and moderate temperature. The average temperature and annual rainfall in Gazipur are 25.8 °C and 2036 mm, respectively.

B. Experimental Materials

Seeds sample of carrot variety “New Kuroda” was collected from the Lal teer seed limited, Dhaka, Bangladesh.

C. Collection, Isolation, and Preservation of *R. solani* and *S. rolfsii*

Three individual isolates of *R. solani* and *S. rolfsii* were collected from the infected tomato, carrot, and potato fields at BSMRAU, Gazipur, Bangladesh. The specimens which had typical symptoms of root rot and blight were selected from the infected fields. The fungal isolates were isolated according to standard method [11]. Then, the fungal colonies were grown on PDA and identified according to Barnet and Hunter [12]. Finally, the isolates were purified following hyphal tip technique and stored in PDA slants at 10 °C.

D. Pathogenicity Test for the Selection of Virulent Isolates of the Test Pathogens in Pot Culture

The pathogenicity test of *R. solani* and *S. rolfsii* isolates were conducted in pot culture on carrot seedling according to the Akter et al. [3], Rubuyet al. [13], Liton et al. [14].

E. Inoculum Preparation of the Test Pathogens

Inocula of the *R. solani* and *S. rolfsii* isolates were prepared and stored according to the standard method [15].

F. Collection and Preservation of Chitosan

Chitosan was collected from Sisco Research Laboratories Pvt. Ltd. (SRL), India. It was derived from the cell of quick growing sea shrimp. The solution was extracted from sea shrimp and then it was irradiated with γ-ray (20 KD) which acts as a plant growth promoter.

G. In vitro Screening of Chitosan

Different concentrations of chitosan such as 0.6, 0.8, and 1.0% were evaluated on PDA plate against *R. solani* and *S. rolfsii* following the methods of Akter et al. [3], Jannat et al. [4]. Finally, the percent inhibition of the radial growth was calculated as described by the formula as given below:

\[
\% \text{ inhibition of growth} = \frac{X-Y}{X} \times 100
\]

where, X=Mycelial growth of pathogen without chitosan (control), and Y=Mycelial growth of pathogen with chitosan (*R. solani* and *S. rolfsii*).

H. Treatments

Two field experiments were laid out for *R. solani* and *S. rolfsii* separately with the following treatments:

- **T。<sub>1</sub>**= Control;
- **T。<sub>2</sub>**= Soil inoculated with pathogen (*S. rolfsii R. solani*);
- **T。<sub>3</sub>**= Soil inoculated with pathogen (*S. rolfsii R. solani*); and seed treated with 1.0% chitosan;
- **T。<sub>4</sub>**= Soil inoculated with pathogen (*S. rolfsii R. solani*); and amendment with 1.0% chitosan.

I. Land Preparation and Design of Experiment

Land was prepared for well tilth using a tractor driven disc plough, rotavator and harrow. The experiment was laid out in
the Randomized Complete Block Design (RCBD) with three replications. After land preparation the whole experimental area was divided into three blocks, which represent three replications. The unit plot size was 2.0 m × 2.0 m. Distance between block to block was 1.0 m and that of plot to plot in a block was 0.5 m. Drains were made surrounding each unit plots and the excavated soil was used for raising plots 15 cm high from the general soil surface. Four different treatments were allotted randomly to four-unit plots per block.

J. Use of Manure and Fertilizer

Well decomposed cow dung @ 5 t/ha was applied during the land preparation. For medium soil NPKS applied @ 60-20-45-12 kg per hectare, respectively. All fertilizer except urea were applied two splits at 3rd and 5th weeks after sowing. Top dressing of fertilizer was followed by irrigation.

K. Seed Treatment and Soil Amendment

Before sowing, seeds were treated with 1.0% chitosan for 12 h as per the required treatment. Seeds were air dried before sowing to avoid excess water. Soil was amended with 1.0% chitosan just 7 days before of seed sowing.

L. Sowing of Seeds in Field

Seeds were sown in the field on 17 November, 2018. Seeds were sown in lines uniformly by hand at the rate of 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. Two hundred seeds were required for one square meter area. 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.

M. Intercultural Operations

Weeding, mulching and gap filling were done 3 times up to 45 days of sowing to keep the crop free from weeds and to pulverized the soil crust for better aeration and conserve soil moisture. Seedlings were thinned in continuous row maintaining about 7.0 cm plant to plant spacing. Light irrigation was provided over the plant at an interval of 14 days (after every alternate week) with the hose pipe until the soil was properly wet.

N. Data Collection

Data on the % seedling mortality, % disease incidence, % disease severity, root and shoot length (cm), fresh and dry weight (g), root diameter (cm), root weight/ plant (g), yield (t/ha) were collected.

O. Observation of Disease Development

Germination and seedling disease development were observed regularly and seedling growth recorded at 10, 20 and 30 days after sowing to estimate the effect of chitosan on pre- and post-emergence seedling mortality and growth of carrot. The causal agent of Rhizoctonia root rot and southern blight were confirmed after re-isolation of the pathogens from the ungerminated seeds and diseased seedlings. Rhizoctonia root rot and southern blight disease incidence at different growth stages were recorded. Disease severity of Rhizoctonia root rot was appraised by indexing on five degrees of rating scale in which 0= no symptoms, 1= 1-25%, 2= 26-50%, 3= 51-75%, and 4= 76-100% of carrot root covered with lesions. For, southern blight, severity was assessed based on the rating scale 0-5, where 0= no visible sign or symptoms, 1 = less than15%, 2 =15-35%, 3 = 36-49%, 4 = 50-74% and 5 = more than 75% of carrot rot circumference covered with lesion or mycelium.

P. Disease Assessment

Disease incidence and percent disease index (PDI) were assessed by the following formula.

\[
\text{PDI} = \left( \frac{\sum \text{rating of plants observed}}{\text{No. of plants observed} \times \text{Max. score of the scale used}} \right) \times 100
\]

Percent disease control (PDC) was calculated by the following formula:

\[
PDC = \left( \frac{(\% \text{ disease in check}) - (\% \text{ disease in treatment})}{(\% \text{ disease in check})} \right) \times 100
\]

Q. Harvesting

Harvesting was done on 18th March 2019 that was three months after sowing. By this time, all the tap roots reached at their desired size and shape. After harvesting weight and diameter of the tap roots were recorded.

R. Statistical Analysis

Data recorded on diseases and yield component parameters and transformed whenever necessary. Finally, data were analyzed using the Statistix 10 statistical computer program. The means were compared following LSD (Least Significant Difference) test.

III. RESULTS AND DISCUSSION

A. Pathogenicity Test of R. solani and S. rolfsii Isolates against Carrot Seedlings in Pot Culture

The pathogenicity test of the three selected isolates of R. solani and S. rolfsii against carrot seedlings were conducted in pot containing sterilized soil to find out most virulent isolates of the test pathogens. All the isolates of the test pathogens were virulent but variable in causing total seedling mortality of carrot (Table I and Fig. 1). The isolate R1 and S1 were appeared to be the most virulent causing the highest 88.34% and 92.47% total seedling mortality followed by isolate R2 and S2 caused 75.23% and 71.14% mortality in R. solani and S. rolfsii, respectively. In R. solani significantly the lowest (57.94%) total seedling mortality was observed with the isolate R3 and in S. rolfsii significantly the lowest (56.71%) total seedling mortality was observed with the isolate S3. No pre-emergence and post-emergence seedling mortality was observed in the untreated control pot. All the three isolates of each pathogen were able to develop characteristics symptom of Rhizoctonia crown rot and canker and southern blight in carrot seedlings. The variability of these test pathogens were also found by several investigators in different crops [16]-[19].
In addition, to know the effect of chitosan on germination and post-emergence seedling mortality seed treatment and soil amendment were done with 1.0% of chitosan and there was no chitosan in untreated control seeds. These seeds were sown in plastic tray after required treatments and data were recorded up to complete germination. All treatments increased the germination percentage compared to the treatment where soil was inoculated with the pathogens (Table III). In case of R. solani inoculated condition, the range of germination percentage was 50.42-71.71%. The highest germination 71.71% was in the T3 treatment where soil amendment was done with 1.0% chitosan followed by T1 treatment where seed treatment was done with 1.0% chitosan and significantly the lowest germination percentage 50.42% was in the T2 treatment where soil was inoculated with R. solani without chitosan. In case of S. rolfsii, the range of germination percentage was 52.47-71.83%. The highest germination 71.83% was in the T1 treatment where soil amendment was done with 1.0% chitosan followed by T3 treatment where seed treatment was done with 1.0% chitosan and significantly the lowest germination percentage 52.47% was in the T2 treatment where soil was inoculated with S. rolfsii without chitosan. The highest germination increased 42.23% and 36.90% over pathogen inoculation in R. solani and S. rolfsii were recorded in the T3 treatment followed by T1 treatment, respectively. In case of seedling mortality, application of chitosan as seed treatment or soil amendment reduced post-emergence seedling mortality compared to the treatment T2 where soil was inoculated with the pathogens. The highest reduction of post emergence seedling mortality 68.20% and 50.06% over pathogen inoculation (T2) were recorded in T1 treatment where seed treatment was done with 1.0% chitosan in R. solani and S. rolfsii, respectively followed by T4 treatment where soil amendment was done with 1.0% chitosan. This experiment showed that chitosan was effective to increase germination and to control post-emergence seedling mortality of carrot.

Seed priming with chitosan improved the germination of ajowan under salt stress [22]. It is well known that chitosan application is beneficial in order to enhance the germination and decrease seedling mortality of many crops [4], [23].

### C. Effect of Chitosan on Germination and Post-emergence Seedling Mortality

![Fig. 1 Virulent isolates of R. solani (A) and S. rolfsii (B).](http://dx.doi.org/10.24018/ejfood.org)

**Table I. Pathogenicity Test of R. solani and S. rolfsii Isolates against Carrot Seedlings in Pot Culture**

| Isolates | Pre-emergence Mortality (%) | Post-emergence Mortality (%) | Total Mortality (%) |
|----------|-----------------------------|-----------------------------|---------------------|
| R1       | 54.34 a                     | 34.00 a                     | 88.34 a*            |
| R2       | 44.83 b                     | 30.40 b                     | 75.23 b             |
| R3       | 34.14 c                     | 23.80 c                     | 57.94 c             |
| Untreated control | 0.00 d                  | 0.00 d                      | 0.00 d              |

*Means within the same column having a common letter (s) do not differ significantly (P=0.05) by LSD.

**TABLE II. Mycelial Growth Inhibition of R. solani and S. rolfsii by Chitosan on PDA**

| Treatments | Mycelial growth after 7 days of incubation | % mycelial growth inhibition over control |
|------------|------------------------------------------|----------------------------------------|
| R. solani  |                                          |                                        |
| Control (no chitosan) | 79.20 a                  | 80.00 a*                              |
| 0.6% chitosan | 25.12 b                  | 30.67 b                               |
| 0.8% chitosan | 20.10 c                  | 25.96 c                               |
| 1.0% chitosan | 0.00 d                  | 0.00 d                                |
| S. rolfsii |                                          |                                        |
| Control (no chitosan) |                            |                                        |
| 0.6% chitosan |                            |                                        |
| 0.8% chitosan |                            |                                        |
| 1.0% chitosan |                            |                                        |

*Means within the same column having a common letter (s) do not differ significantly (P=0.05) by LSD.

**TABLE III. Effect of Chitosan on Germination Percentage and Post-emergence Seedling Mortality in R. solani and S. rolfsii Inoculated Condition**

| Treatments | Percentage |
|------------|------------|
| germination | increase over T2 | post-emergence mortality | reduction over T2 |
| R. solani   |                                        |
| T1         | 69.62 ab     | 5.79 ab                 | -                  |
| T2         | 50.42 c      | 9.78 a*                 | -                  |
| T3         | 66.17 b      | 31.24                   | 3.11 b             |
| T4         | 71.71 a      | 42.23                   | 4.44 b             | 54.60 |
| S. rolfsii |                                        |
| T1         | 71.97 a      | 4.88 b                  | -                  |
| T2         | 52.47 c      | 8.89 a                  | -                  |
| T3         | 61.20 b      | 16.63                   | 4.44 b             | 50.06 |
| T4         | 71.83 a      | 36.90                   | 4.88 b             | 45.11 |

*Means within the same column having a common letter (s) do not differ significantly (P=0.05) by LSD. Note: T1= Seed without any treatment (control), T2= Soil inoculation with pathogen, T3= T1+seed treatment with 1.0% chitosan, T4= T1+soil amendment with 1.0% chitosan.
D. Effect of Chitosan on Growth of Carrot Seedling at Different Days after Sowing (DAS)

To know the effect of chitosan on growth different related parameters such as shoot length, root length, fresh weight, and dry weight were measured at 30, 40, and 50 DAS, respectively of carrot seedlings in pathogens inoculated condition. Chitosan as seed treatment or soil amendment increased growth of carrot seedlings at 30, 40, and 50 DAS, respectively (Table IV and Fig. 2).

By the application of chitosan, growth promoting characters were significantly increased in all the treatments over the treatment T3 where soil was inoculated with *R. solani* and *S. rolfsii*. Thirty days after sowing, the highest root length (3.69 cm), shoot length (5.20 cm), fresh weight (0.48 g) and dry weight (0.09 g) were recorded in T3 treatment where soil amendment was done with 1.0% chitosan in *R. solani* inoculated condition followed by T1 where soil treatment was done with 1.0% chitosan. In *S. rolfsii* inoculated condition, the highest root length (2.78 cm), shoot length (5.21 cm), fresh weight (0.37 g) and dry weight (0.065 g) were recorded in T3 treatment where soil treatments was done with 1.0% chitosan followed by T4 treatment where soil amendment was done with 1.0% chitosan in *R. solani* inoculated condition followed by T3 where seed treatment was done with 1.0% chitosan in *S. rolfsii* inoculated condition.

At 50 DAS, the highest root length (9.32 cm) and shoot length (18.45 cm) were recorded in T3 treatment where seed treatment was done with 1.0% chitosan and highest fresh weight (13.93 g) and dry weight (3.86 g) were recorded in T4 treatment where soil amendment was done with 1.0% chitosan in *R. solani* inoculated condition followed by T3 where seed treatment was done with 1.0% chitosan in pathogen inoculated condition followed by T3 where seed treatment was done with 1.0% chitosan. In case of *S. rolfsii* inoculated condition, the highest root length (6.63 cm), shoot length (9.94 cm) and fresh weight (3.18 g) were recorded in T3 treatment where seed treatment was done with 1.0% chitosan followed by T4 treatment where soil amendment was done with 1.0% chitosan in pathogen inoculated condition. The highest dry weight (0.45 g) was recorded in both T3 and T4 treatments.

At 50 DAS, the highest root length (9.32 cm) and shoot length (18.45 cm) were recorded in T3 treatment where seed treatment was done with 1.0% chitosan and highest fresh weight (13.93 g) and dry weight (3.86 g) were recorded in T4 treatment where soil amendment was done with 1.0% chitosan in *S. rolfsii* inoculated condition followed by T3 where seed treatment was done with 1.0% chitosan. The lowest root length, shoot length, fresh weight and dry weight were recorded in T2 treatment where soil was inoculated with pathogen in both *R. solani* and *S. rolfsii* inoculated fields. There was no statistical difference between the T1 and T2 treatments.

These results confirmed from the findings of Akter et al. [3], and Mondal et al. [24] in which they reported that application of chitosan enhanced seedling growth of different crops. It is also reported that phenolic compounds, flavonoids and antioxidant power increased with increasing concentrations of chitosan as an elicitor [25].

E. Effect of Chitosan on Rhizoctonia Crown Rot and Canker and Southern Blight Disease Incidence (DI) and Percent Disease Index (PDI)

Application of chitosan reduced disease incidence (DI) and percent disease index (PDI) in all treatments over pathogens inoculated plots (Table V and Fig. 3). In *R. solani* inoculated field, the highest DI (36.79%) and PDI (27.75%) at harvest were recorded in the treatment T3 where field was inoculated with pathogen. The lowest DI (22.37%) and PDI (16.77%) was recorded in the treatment T3 where soil was treated with 1.0% chitosan and the lowest PDI (27.75%) was recorded in the treatment T3 where soil amendment was done with 1.0% chitosan. The highest reduction of DI (39.20%) was recorded in T4 where soil amendment was done with 1.0% chitosan.

![Fig. 2. Effect of chitosan on growth of carrot seedlings in *R. solani* (A) and *S. rolfsii* (B) inoculated field at 30 DAS (T1-T4).](image)

*Means within the same column having a common letter (s) do not differ significantly (*P*≤0.05) by LSD. Note: T1= Seed without any treatment (control), T2= Soil inoculation with pathogen, T3= T1+seed treatment with 1.0% chitosan, T4= T3+soil amendment with 1.0% chitosan.

| TABLE IV: EFFECT OF CHITOSAN ON GROWTH CHARACTERS OF CARROT AT 30, 40, AND 50 DAS |
|---------------------|---------------------|---------------------|---------------------|
| Treatments          | Root length (cm)    | Shoot length (cm)   | Fresh weight (g)   |
|---------------------|---------------------|---------------------|---------------------|
|                      | R. solani          |                      | R. solani          |
|                      | 30 DAS              |                      | 40 DAS              |
| T1                   | 3.35 ab             | 4.90 a              | 0.31 ab             |
| T2                   | 2.90 b              | 3.84 b              | 0.20 b              |
| T3                   | 3.35 ab             | 4.81 a              | 0.37 ab             |
| T4                   | 3.69 a              | 5.20 a              | 0.48 a              |
|                      | 0.06 bc*            | 0.06 bc             | 0.09 ab             |
|                      |                     |                     |                     |
|                      | 30 DAS              |                      | 50 DAS              |
| T1                   | 6.22 b              | 8.85 ab             | 3.30 ab             |
| T2                   | 5.22 c              | 6.59 b              | 2.24 b              |
| T3                   | 6.70 ab             | 9.29 a              | 3.30 ab             |
| T4                   | 7.08 a              | 9.73 a              | 3.54 a              |
|                      | 0.33 ab             | 0.43 ab             | 0.58 a              |
|                      |                     |                     |                     |
|                      | 30 DAS              |                      | 40 DAS              |
| T1                   | 8.02 a              | 18.28 a             | 9.90 ab             |
| T2                   | 6.12 b              | 9.53 b              | 4.30 b              |
| T3                   | 9.32 a              | 18.45 a             | 13.87 a             |
| T4                   | 8.98 a              | 18.20 a             | 13.93 a             |
|                      | 2.28 b              | 1.11 c              | 3.74 a              |
|                      |                     |                     | 3.86 a              |
|                      |                     |                     |                     |
|                      | 30 DAS              |                      | 50 DAS              |
| T1                   | 2.53 ab             | 4.54 a              | 0.27 b              |
| T2                   | 2.15 b              | 3.81 d              | 0.17 c              |
| T3                   | 2.78 a              | 5.21 ab             | 0.37 a              |
| T4                   | 2.62 a              | 4.71 bc             | 0.34 a              |
|                      | 0.047 b             | 0.040 b             | 0.065 a             |
|                      |                     |                     | 0.057 ab            |
|                      |                     |                     |                     |
|                      | 50 DAS              |                      |                     |
| T1                   | 6.27 a              | 8.67 a              | 2.29 ab             |
| T2                   | 4.69 b              | 6.05 b              | 1.52 b              |
| T3                   | 6.63 a              | 9.94 a              | 3.18 a              |
| T4                   | 6.60 a              | 9.39 a              | 3.03 a              |
|                      | 0.31 ab             | 0.22 b              | 0.45 a              |
|                      |                     |                     |                     |
|                      |                     |                     |                     |
|                      |                     |                     |                     |
|                      |                     |                     |                     |
1.0% chitosan and the highest reduction of PDI (39.57%) was recorded in T3 where seed was treated with 1.0% chitosan over pathogen inoculated plot. In S. rolfsii inoculated condition, the highest DI (24.40%) and PDI (17.10%) at harvest were recorded in the treatment T2 where field was inoculated with S. rolfsii. The lowest DI (14.14%) was recorded in T4 where seed were treated with 1.0% chitosan followed by T4 treatment where soil amendment was done with 1.0% chitosan. The lowest PDI (8.96%) was recorded in the treatment T4 treatment where soil amendment was done with 1.0% chitosan. The highest reduction of DI (42.10%) was recorded in T3 where seed were treated with 1.0% chitosan over pathogen inoculated plot and the highest reduction of PDI (47.60%) was recorded in T4 treatment where soil amendment was done with 1.0% chitosan over pathogen inoculated plot. No statistical difference was found between T3 and T4 treatments. Chitosan has antifungal properties and thus may protect plant against fungal pathogens.

Chitosan is often used in plant disease control as a powerful elicitor rather than a direct antimicrobial or toxic agent [26]. Akter et al. [3] and Jannat et al. [4] reported that chitosan effectively reduces DI and PDI of chili and eggplant. Management of many fungal pathogens in different pathosystems through the application of Trichoderma or chitosan individually or in combination is well documented [27], [28].

**F. Effect of Chitosan on the Yield**

By the application of chitosan, yield and yield contributing components were significantly increased in all the treatments over the treatment T2 where soil was inoculated with pathogens (Table VI-VII). In R. solani inoculated condition, significantly highest yield (23.17 t/ha), root length (16.20 cm) and root diameter (15.10 cm) were recorded in T3 treatment where soil was treated with 1.0% chitosan followed by T2 treatment where soil amendment was done with 1.0% chitosan. Significantly lowest yield (12.00 t/ha), root length (13.44 cm) and root diameter (10.98 cm) were recorded in T2 Treatment where soil was inoculated with R. solani. The highest increase of yield (92.67%), root length (20.54%) and diameter (37.52%) were observed in T3 followed by T4 over T2.

In S. rolfsii inoculated field, the highest yield was (19.91 t/ha) was observed in T4 treatment where soil amendment was done with 1.0% chitosan and root length (16.81 cm) and root diameter (13.55 cm) were recorded in T3 treatment where seed treatment was done with 1.0% chitosan followed T4 treatment where soil amendment was done with 1.0% chitosan. Significantly lowest yield (11.48 t/ha), root length (14.86 cm) and root diameter (10.36 cm) were recorded in T2 treatment where soil was inoculated with S. rolfsii. Application of chitosan showed the highest increase in root length (13.12%) and diameter (30.79%) in T3 treatment followed by T4 treatment over T2 treatment and the highest increase yield (73.43%) in T4 treatment followed by T3 treatment over T2 treatment. No statistical difference was found between T3 and T4 treatments.

Akter et al. [3], Jannat et al. [4], and Ahmed et al. [29] reported that chitosan significantly increased growth and yield of chilli, eggplant, and carrot.

**TABLE V: Effect of Chitosan on Rhizoctonia Canker and Southern Blight Disease Incidence (DI) and Percent Disease Index (PDI)**

| Treatments | % DI | % Reduction of DI over T2 | % Reduction of PDI over T2 |
|------------|------|---------------------------|----------------------------|
| **R. solani** |      |                           |                            |
| T1         | 26.94 b | -                         | 16.60 b                    |
| T2         | 36.79 a  | -                         | 27.75 a*                   |
| T3         | 25.66 bc | 30.25                     | 16.77 b                    |
| T4         | 22.37 c  | 39.20                     | 18.10 b                    |
| **S. rolfsii** |      |                           |                            |
| T1         | 17.07 b  | -                         | 10.52 b                    |
| T2         | 24.40 a  | -                         | 17.10 a                    |
| T3         | 14.14 bc | 42.10                     | 10.97 b                    |
| T4         | 17.11 b  | 29.88                     | 8.96 b                     |

*Means within the same column having a common letter (s) do not differ significantly (P=0.05) by LSD. Note: T1= Seed without any treatment (control), T2= Soil inoculation with pathogen, T3= T1+seed treatment with 1.0% chitosan, T4= T1+soil amendment with 1.0% chitosan.

**TABLE VI: Effect of Chitosan on Yield Contributing Characters of Carrot**

| Treatments | Root length (cm) | % increase over T2 | Root diameter (cm) | % increase over T2 |
|------------|------------------|--------------------|--------------------|-------------------|
| **R. solani** |                  |                    |                    |                   |
| T1         | 14.57 b          | -                  | 12.15 b            | -                 |
| T2         | 13.44 b          | -                  | 10.98 c            | -                 |
| T3         | 16.20 a          | 20.54              | 15.10 a*           | 37.52             |
| T4         | 16.07 a          | 19.57              | 14.36 a            | 30.78             |
| **S. rolfsii** |                |                    |                    |                   |
| T1         | 15.69 bc         | -                  | 12.59 c            | -                 |
| T2         | 14.86 c          | -                  | 10.36 d            | -                 |
| T3         | 16.81 a          | 13.12              | 13.55 b            | 30.79             |
| T4         | 16.15 ab         | 8.68               | 13.51 bc           | 30.41             |

**TABLE VII: Effect of Chitosan on Yield of Carrot**

| Treatments | Weight of single root (g) | No. of root per plot | Yield (t/ha) | % increase over T2 |
|------------|---------------------------|----------------------|--------------|--------------------|
| **R. solani** |                              |                      |              |                   |
| T1         | 113.48 a                  | 57.33 b              | 21.59 a*     | -                 |
| T2         | 86.38 b                   | 41.67 c              | 12.05 b      | -                 |
| T3         | 111.85 a                  | 62.00 h              | 23.17 a      | 92.67             |
| T4         | 106.92 a                  | 64.00 b              | 22.81 a      | 90.08             |
| **S. rolfsii** |                             |                      |              |                   |
| T1         | 102.14 a                  | 56.67 a              | 19.29 a      | -                 |
| T2         | 83.29 c                   | 41.33 b              | 11.48 b      | -                 |
| T3         | 95.83 a                   | 62.00 a              | 19.81 a      | 72.56             |
| T4         | 94.81 b                   | 63.00 a              | 19.91 a      | 73.43             |

*Means within the same column having a common letter (s) do not differ significantly (P=0.05) by LSD. Note: T1= Seed without any treatment (control), T2= Soil inoculation with pathogen, T3= T1+seed treatment with 1.0% chitosan, T4= T1+soil amendment with 1.0% chitosan.

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Fig. 3. Rhizoctonia crown rot and canker of carrot caused by R. solani (A-B) and southern blight of carrot caused by S. rolfsii (C-D).
IV. CONCLUSION

The study revealed that use of chitosan as seed treatment or soil amendment appeared to be effective in increasing germination percentage, controlling pre- and post-emergence seedling mortality, decreasing disease incidence and severity of Rhizoctonia canker and southern blight with the significant increase of growth and yield of carrot. Farmers may adopt eco-friendly control measures against Rhizoctonia canker and southern blight of carrot through the application of seed treatment by 1.0% chitosan as an alternative to chemical pesticides.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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