Research Article

Bangladesh Journal of Plant Pathology, 39, 25-38
ISSN : 1012-9279 (Print)
ISSN : 2412-7558 (Online)

EVALUATION OF CHITOSAN FOR THE MANAGEMENT OF RHIZOCTONIA ROOT ROT AND SOUTHERN BLIGHT OF TOMATO

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How to cite this article
Lubna, N., Jannat, R., Hossain, M. M., Rubayet, M. T., Tasnim, F. and Kamal, M. Z. 2023. Evaluation of chitosan for the management of Rhizoctonia root rot and Southern blight of tomato. Bangladesh J. Plant Pathol. 39 (1 & 2): 25-38

ABSTRACT

An attempt was made to manage Rhizoctonia root rot and Southern blight of tomato, caused by *Rhizoctonia solani* and *Sclerotium rolfsii*, using chitosan as a seed treatment and soil amendment to promote growth and enhance yield. Virulent isolates of *R. solani* and *S. rolfsii* were identified, and an effective concentration of chitosan was determined through in vitro trials before conducting field experiments. Isolates R1 of *R. solani* and S1 of *S. rolfsii* were found to be the most virulent, causing severe disease in tomato seedlings. Preliminary screening showed that chitosan at 1.0% was the most inhibitory against these pathogens. Seed treatment with 1.0% chitosan for 6 hours effectively improved germination and seedling growth. In field experiments, chitosan was applied either as a seed treatment or through soil incorporation. This application significantly reduced disease incidence (DI) and percent disease index (PDI), while enhancing growth parameters and yield of tomato compared to pathogen-inoculated controls. Moreover, chitosan treatment notably improved plant growth and yield, indicating its potential as an effective seed treatment and soil amendment for managing Rhizoctonia root rot and Southern blight of tomato to maximize yield.

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

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most popular and nutritious vegetables across the world under the Solanaceae family. Tomato and its processed products are gaining more popularity nowadays due to their various uses including salad, soup, sauce, flavor in crackers, and biscuits. It is grown not only in Bangladesh but also in many countries of the world for its nutritional status. Tomato is an excellent source of minerals, carboxylic acids, citric, malic, fumaric, and oxalic acids (Hernandez-suarez *et al*., 2007), antioxidants such as lycopene, β-carotene, vitamins such as vitamin C, E, folic acid, niacin and trace elements for instance, selenium, copper, manganese, iron and zinc (Molla *et al*., 2012). Moreover, the consumption of tomato could reduce the risk of cardiovascular disease and certain types of cancer such as cancers of the prostate, lung, and stomach (Canene - Adams *et al*., 2005).

However, a lot of problems has been identified for the cultivation of tomato in open field conditions such as heavy rainfall, wind, high temperature, storms, fertilizer, pure seeds, soil acidity- alkalinity, poor organic matter, irrigation, and after all plant diseases. Plant diseases represent a critical problem to the successful production of tomato.Major diseases of tomato include Damping-off, Fusarium wilt, Southern blight, Rhizoctonia root rot, etc. Soil-borne pathogens predominantly *Sclerotium rolfsii, Rhizoctonia solani, Fusarium oxysporum*f. sp. *lycopersici, and Pythium* spp. are important biological constraints in tomato production both seedling stage and mature stage in the field (Jones *et al*., 1991). Several viral, bacterial, and root-knot diseases caused by *Meloidogyne* spp. are also cause of substantial losses of tomato in Bangladesh (Ahmed, 1994). Among them, Rhizoctonia root rot and southern blight are the most devastating diseases of tomato caused by *R. solani* and *S. rolfsii. R. solani* causes damping-off and can damage the root in the early stage. *S. rolfsii* infects the collar region of the plant and shows lesions on the stem at or near the soil line **(**Mullen, 2001**).** It can occur on tomato plants at early and later stages and can reduce the yield of tomato. Soil-borne diseases are complicated to control

in field-grown tomatoes because the pathogen rapidly colonizes soil and persists for long periods (Ma *et al*., 2023).

At present diseases are mainly managed by chemical pesticides despite their harm to human health and the environment (Ahmad *et al*., 2024). The extensive use of pesticides may cause problems by targeting beneficial organisms and the continuous use of these chemical pesticides leads to loss of biodiversity (Yasmin and D'Souza, 2010). Although the application of fungicides is primarily considered the most effective method in controlling different soil-borne fungi, it can be involved in many problems due to health risk concerns and environmental pollution. Thus, there is a growing need to develop alternative approaches for the management of soil-borne diseases.

Among the alternatives that are currently under investigation to avoid the use of chemical products to control plant diseases and increase crop productivity are biopolymer-based materials (Malerba and Cerana, 2018). An acceptable approach that is being actively investigated involves the use of bioactive substances like chitosan in controlling soil-borne fungi (Akram and Anjum, 2011; El-Mohamedy *et al*., 2013). Therefore, biopesticides, such as chitosan are a more dependable way for controlling the diseases of fruits and vegetables in the absence of resistant cultivars (Ali *et al*., 2010; Maqbool *et al*., 2010). Chitosan is a natural, safe, and cheap biopolymer produced from chitin, the major constituent of arthropod's exoskeleton and fungus cell walls and the second renewable carbon source after lignocellulosic biomass. According to Rodriguez-Pedroso *et al*. 2009, chitosan is a linear polysaccharide composed of randomly distributed –β-(1→ 4)-linked D-glucosamine (deacetylated unit), biodegradable and biocompatible material with no toxicity or side effects. It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, like sodium hydroxide (NaOH). Chitosan and its derivatives display antibiotic activity against microorganisms including bacteria and fungi as well as it can also increase growth and yield (Russell, 2013; Anusuya and Sathiyabama, 2016). Chitosan displays antimicrobial activity against microorganisms and it can also increase growth and yield (Sharp, 2013; Anusuya and Sathiyabama, 2016). It can also enhance fruit size or branching (Trotel-Aziz *et al*., 2006) and reduce disease caused by fungal pathogens (El-Mohamedy *et al*., 2013). There is strong evidence that the mycelial growth of fungi in the medium can be inhibited when the medium is amended with chitosan. The mechanism by which chitosan affects the growth of several phytopathogenic fungi has not been fully elucidated, but several hypotheses have been postulated. Because of its polycationic nature, it is believed that chitosan interferes with negatively charged residues of macromolecules exposed to the fungal cell surface. The main properties of chitosan are biocompatibility, nontoxicity, and biodegradability (Rinaudo, 2006).

Chitosan has some benefits over other bio-control agents as it has the potential to control plant diseases along with its ability to induce resistance in the host plants (Yin *et al*., 2010). Over the last decade, chitosan has taken on enormous importance in controlling pathogenic microorganisms. Induction of resistance can be obtained against many pathogens by chitosan coatings (Benhamou *et al*., 1998). Management of many fungal pathogens in different pathosystems through the application of chitosan individually is well documented (Abd-El-Kareem *et al*., 2006). There are very few reports about the use of chitosan for controlling soil-borne diseases of tomato. Considering the above-mentioned facts, the study has been undertaken to optimize the most effective dose of chitosan against the virulent isolates of *R. solani* and *S. rolfsii* and examined the effect of chitosan in reducing Rhizoctonia root rot and Southern blight diseases of tomato as well as observe the effect of chitosan on the growth and yield of tomato.

MATERIALS AND METHODS

Experimental site

A field experiment was carried out at Bangabandhu Sheikh Mujibur Rahman Agricultural University (24° 09' N latitude and 90° 26' longitudes) from 2018 to 2019. The soil type of the experimental site belongs to the shallow red-brown terrace type under Salna series of Madhupur tract of Agroecological zone (AEZ) 28 which is characterized by silty clay with a pH value of 6.5.

Experimental material

Seed samples of tomato variety “Raton” (BARI Tomato-2) were collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh.

R. solani and *S. rolfsii* collection, isolation and preservation

Three individual isolates of *R. Solani* and *S. rolfsii* were collected from the infected tomato, carrot, and potato fields at BSMRAU, Gazipur, Bangladesh, and fungal isolates were isolated according to the standard method (Mian, 1995). Then, the fungal colonies were grown on PDA and identified according to Barnet and Hunter, 1972. Finally, it was kept under 10°C in PDA slants for further use.

Pathogenicity test

The pathogenicity test of selected isolates of *R. solani* and *S. rolfsii* was conducted in pot culture on tomato seedlings by soil infestation method according to the Rubayet *et al*., 2017; Akter *et al*., 2018; Liton *et al*., 2019. Two experiments, one for *R. solani* and another

for *S. rolfsii* were conducted. Each earthen pot was filled with 2.0 kg of sterilized soil. Eleven seeds of tomato were sown in each pot and before seed sowing, the soil was mixed with inocula of isolates of two pathogens. But in control pot seeds were sown without any treatment. Disease development was observed and recorded at 10 to 20 days after sowing to estimate the effect of pathogens in causing pre-emergence and post-emergence seedling mortality. The causal agents of seedling mortality were confirmed after re-isolation of the pathogen from ungerminated seeds and infected seedlings.

Inoculum preparation of the test pathogens

Inocula of the *R. solani* and *S. rolfsii* isolates were prepared on autoclaved moist wheat bran in a polybag (Rubayet & Bhuiyan, 2016). Ten mycelial discs (5 mm) were transferred into 200g autoclaved wheat bran and incubated at 25±2 ^{0}C for 15 days. They were mixed well after 2-3 days intervals for even growth. The colonized wheat bran was air-dried on brown paper for 2 days and stored at 4°C for future use.

Collection of chitosan

Chitosan was collected from Bangladesh Atomic Energy Commission (BAEC), Dhaka, Bangladesh which was derived from the shell of quick-growing sea shrimp. After processing, the extracted solution was irradiated with γ-ray (20 kD) which also acts as a plant growth promoter.

In vitro screening of chitosan

Akter *et al*., 2018; Jannat *et al*., 2018 methods were followed for the evaluation of different concentrations of chitosan such as 0.6, 0.8, and 1.0% on PDA plate against *R. solani and S. rolfsii.* Finally, the percent inhibition of the radial growth was calculated as described by the formula given below-

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

Where,

X = Mycelial growth of pathogen without chitosan (control)

Y = Mycelial growth of pathogen with chitosan (*R. solani* and *S. rolfsii)*

Seed treatment with chitosan

Seeds of tomato were surface sterilized by immersion of 1.0% sodium hypochlorite, thoroughly rinsed with sterile distilled water, and immersed into each of the chitosan solutions (pH 5.5 -6). Seeds were treated with 1.0% chitosan for 3, 6, 12, and 24 hrs. After immersion, the wetted seeds were air-dried in a sterile cabinet and kept in a desiccator until use. Seed treatment with 1.0% chitosan for 6 hrs was selected for field experiment based on better germination and growth performance.

Soil amendment with chitosan

About 45 cm length × 35 cm diameter sized pits were prepared for seedling transplanting. At the bottom of the pits, polythene sheets were placed then the soil was poured into the pits. The soil was mixed with 1% chitosan for each pit per plot as a soil amendment. Soil amendment was done before 3 days of transplanting of tomato seedlings and after 7 days of inoculum application in the field.

Raising of seedlings

For the raising of tomato seedlings, the soil was prepared by mixing fertilizers and cow dung. The seeds of the tomato variety "Raton" were sown in the tray in October 2018. After sowing, the seeds were covered with light soil. Proper care was taken to raise healthy seedlings.

Land preparation and design of the experiment

The land was prepared for good tilth and conducted two separate field experiments for *R. solani* and *S. rolfsii*. The experiments were laid out in the Randomized Complete Block Design (RCBD) with three replications (Plot size was 2.25 m × 1.5 m).

Transplanting of seedlings

Twenty days old healthy tomato seedlings variety "Raton" was collected from the tray for plantation. Distance between plant to plant was 75 cm and row to row was 75 cm. A total of six seedlings were planted in each plot in November 2018. Weeding, irrigation, and intercultural operations were done when necessary, until the maturity of plants.

Treatments

T_1: Seed and soil without any treatment

T_2: Soil inoculation with the pathogen (*R. solani* / *S. rolfsii*)

T_3: T_2 + seed treatment with 1.0% chitosan

T_4: T_2 + soil amendment with 1.0% chitosan

Intercultural operations

Weeding was done 4 weeks after planting to get a competitive advantage over the weeds. Irrigation was supplied when it was necessary. Earthing up was done to keep the soil loose and destroy weeds. Two or three earthing up were done at an interval of 15-20 days. Thirty days after transplanting (DAT) each plant was staked with bamboo sticks to keep them erect and to protect them from damage by storm and high speedy winds.

Use of manure and fertilizer

Well-decomposed cow dung @ 10 tons/ ha was applied during the land preparation. Urea, Triple Super Phosphate (TSP), and Muriate of Potash (MP) were applied @550, 450, and 250kg respectively per hectare. The entire quantity of TSP, one-third of the MP, and

one-third of Urea were applied at the time of final land preparation. The rest of the Urea and MP were applied in an equal installment of 21, 35, and 50 days after transplanting.

Data collection

Data on the germination percentage, seedling mortality (%), root length (cm), shoot length (cm), fresh weight (g), dry weight (g), plant height (cm), number of branches, (%) disease incidence (DI), (%) disease severity/ percent disease index (PDI), and yield (t/ha) were collected.

Observation of disease development

Data were recorded at 10, 20, and 30 days after sowing to estimate the effect of chitosan on pre-emergence and post-emergence seedling mortality and growth of tomato. The causal agent of Rhizoctonia root rot and Southern blight was confirmed after re-isolation and the disease incidence and disease severity were recorded. 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% of tomato root covered with lesions (Grisham and Anderson, 1983). For southern blight, severity was assessed based on the rating scale 0-5, where 0= no visible signs or symptoms, 1 = less than15%, 2 =15-35%, 3 = 36-49%, 4 = 50-74% and 5 ≥75% of tomato root circumference covered with lesion or mycelium (Sundar *et al.*, 1995).

Disease assessment

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

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{PDI} = \frac{\Sigma \text{ of rating of plants observed}}{\text{Number of plants observed} \times \text{Highest score of the scale used}} \times 100$$

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

$$\text{PDC} = \frac{(\text{\% disease in check}) - (\text{\% disease in treatment})}{(\text{\% disease in check})} \times 100$$

Harvesting

The first harvesting of ripe tomato was started at 75 days after transplanting. At the initial ripening stage, the tomato was harvested at 5-day intervals two times and after a few days, tomatoes were harvested at 2-day intervals five times. All the harvests were completed by 18 March 2019.

Data analysis

Data recorded on various parameters of diseases and yield components were analyzed statistically using the Statistix 10 statistical computer program after transformation whenever necessary. The mean was compared following the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Pathogenicity test of *R. solani* and *S. rolfsii* isolates against tomato seedlings in pot culture

The pathogenicity test of the three selected isolates of *R. solani* and the three selected isolates of *S. rolfsii* against tomato seedlings was conducted in the pot containing sterilized soil. The isolate R1 and S1 appeared to be the most virulent causing the highest 92.39% and 94.50% total seedling mortality followed by isolate R2 and S2 caused 69.83% and 65.74% mortality in *R. solani* and *S. rolfsii*, respectively (Table 1 and Fig.1). The lowest (57.18% and 52.09%) total mortality was found in the isolates R3 and S3. No pre-emergence and post-emergence seedling mortality was observed in the untreated control pot. The isolates R1 and S1 were selected based on the pathogenicity test for field trials. The pre-emergence and post-emergence mortality of tomato and other vegetables caused by *R. solani* and *S. rolfsii* were also confirmed by many researchers (Nitu *et al*., 2016; Das *et al*., 2019; Rahman *et al*., 2020).

Effect of chitosan on the *in vitro* mycelia growth of *R. solani* and *S. rolfsii*

The mycelial growth of the test pathogens was significantly reduced with all three selected concentrations of chitosan as compared to untreated control where only solvent was used (Table 2 and Fig. 2). All three concentrations of chitosan (viz., 0.6, 0.8, and 1.0%) were significantly variable in reducing the mycelial growth of *R. solani* and *S. rolfsii*. The highest 100.00% reduction of the mycelial growth of *R. solani* and *S. rolfsii* over the control PDA plate was observed at 1.0% of chitosan amended with PDA plate followed by 0.8% of chitosan with 92.22% and 94.44% reduction of mycelial growth, respectively. On the contrary, the lowest 85.56% and 90.00% reduction of the mycelial growth of *R. solani* and *S. rolfsii* were observed at the lowest 0.6% concentration of chitosan amended with the PDA plate. Similar results in reducing the mycelial growth of different pathogens by chitosan were found in the reports of Sunpapao *et al*., 2014; Nitu *et al*. 2016; Akter *et al*., 2018; Jannat *et al*., 2018. Chitosan has a positive charge that attracts and binds to the negatively charged cell walls of pathogens, disrupting their cell membranes and causing cell death (Andrews, 2001). Microscopic observation reported that chitosan oligomers diffuse inside hyphae interfering with the enzyme activity responsible for the fungus growth (Eweis *et al*., 2006).

Standardization of time for seed treatment with chitosan

Tomato seeds were soaked with 1.0% chitosan at different times for standardization of the duration of seed soaking. The effect of soaking time was evaluated based on seed germination and seedling growth-related

parameters. Germination percentage, root length, shoot length, fresh weight, and dry weight of seedlings were measured from randomly taken five seedlings for each replication of all treatments at 10 DAS and 20 DAS, respectively. Seeds were soaked with chitosan for 0, 3, 6, 12, and 24 hrs. Germination percentages and growth of tomato seedlings were increased at 3 and 6 hrs soaked seeds and after that decreased gradually for 12 and 24 hrs soaked seeds. The significantly highest germination percentage, root length, shoot length, fresh weight, and dry weight were observed where 6hrs soaked seeds were sown for raising seedlings at 10 and 20 DAS (Table 3 and Fig. 3). In the case of 10 DAS, the highest germination percentage was 95.53% and the root length, shoot length, fresh weight, and dry weight were 2.07 cm, 5.80 cm, 2.21 g, and 1.13 g, respectively and the lowest germination percentage was60.00% and the root length, shoot length, fresh weight, and dry weight were 1.10 cm,2.50 cm,0.58 g and 0.02 g, respectively. In the case of 20 DAS, the highest root length, shoot length, fresh weight, and dry weight were 3.70 cm, 9.30 cm, 3.0 g, and 1.97 g, respectively and the lowest root length, shoot length, fresh weight, and dry weight were 2.50 cm, 5.97 cm, 0.69 g, and 0.06 g, respectively. Finally, seed treatment for 6 hrs with chitosan was selected for the field experiment.

Effect of chitosan on germination and seedling mortality

To know the effect of chitosan on germination, pre- and post-emergence seedling mortality of tomato, seed treatment, and soil amendment were done with 1.0% of chitosan in pathogen inoculated condition. These seeds were sown in the plastic tray after the required treatments and data were recorded up to complete germination. All the treatments increased the germination percentage compared to treatment T_2 where the soil was inoculated with the pathogen (Table 4).

The range of germination percentage was 58.33-90.00% and 56.64-93.33% in *R. solani* and *S. rolfsii* inoculated fields, respectively. The highest germination percentages 90.00% and 93.33% were in the treatment T_3 where seeds were treated with 1.0% chitosan followed by T_4 where the soil was amended with 1% chitosan both in *R. solani* and *S. rolfsii* inoculated fields, respectively and significantly the lowest germination percentage 58.33% and 56.64% were in the treatment T_2 where the soil was inoculated with the pathogen. This experiment showed that seeds treated with 1.0% chitosan can increase the germination percentage. A similar result in increasing germination percentages by chitosan was found in the reports of Photchanachai *et al*., 2012. Chitosan can improve water absorption and nutrient uptake in seeds, thereby promoting seed germination and early seedling growth (Riseh *et al*., 2024). In the case of seedling mortality, all treatments reduced seedling mortality compared to treatment T_2 where the soil was inoculated with the pathogen (Table 4). The highest total mortality percentages were 86.49% and 89.83%, respectively in T_2 and the lowest total mortality percentage were found 30.69% and 29.33% in *R. solani* and *S. rolfsii* inoculated plots, respectively. This experiment showed that seeds treated with 1.0% chitosan was effective in reducing total seedling mortality of tomato. This result is supported by several authors like were Islam, 2006; Nitu *et al*., 2016; Akter *et al*., 2018 in tomato, chilli, etc. Chitosan reduces seedling mortality by interfering with pathogen development, such as hyphal growth, spore formation, spore viability, germination, and fungal virulence factor production (Badawy *et al*., 2011).

Effect of chitosan on the growth of tomato seedlings

To know the effect of chitosan on growth different related parameters such as root length, shoot length, fresh weight, and dry weight were measured randomly taking five plants from each replication of all treatments at 10, 20, and 35 DAS, respectively of tomato seedlings in pathogens inoculated condition. Application of chitosan as a seed treatment or soil amendment increased the growth of tomato seedlings at 10, 20, and 35 DAS, respectively. The highest root length, shoot length, fresh weight, and dry weight of tomato seedlings at 10, 20, and 35 DAS were found by chitosan application as seed treatment (T_3) followed by T_4 where soil amended with chitosan (Fig. 4). But T_3 and T_4 were statistically identical. The lowest root length, shoot length, fresh weight, and dry weight were found in (T_2) pathogen inoculated condition. At 10 DAS, the highest root length, shoot length, fresh weight, and dry weight were 2.46 cm, 8.14 cm, 1.22 g, and 0.30 g, respectively in *R. solani* inoculated plot and 2.00 cm, 7.88 cm, 1.42 g and 0.26 g, respectively in *S. rolfsii* inoculated plot and the lowest root length, shoot length, fresh weight and dry weight were 1.24 cm, 5.64 cm, 0.62 g and 0.05 g, respectively in *R. solani* inoculated plot and 1.34 cm, 5.71 cm, 0.53 g and 0.06 g, in *S. rolfsii* inoculated plot, respectively (Table 5). At 20 DAS, the highest root length, shoot length, fresh weight, and dry weight were 2.80 cm, 12.75 cm, 1.76 g, and 0.36 g, respectively in *R. solani* inoculated plot and 2.98 cm, 13.50 cm, 1.54 g, and 0.27 g in *S. rolfsii* inoculated plot, respectively and the lowest root length, shoot length, fresh weight and dry weight were 1.44 cm, 8.13 cm, 0.88 g and 0.06 g, respectively in *R. solani* inoculated plot and 1.54 cm, 7.15 cm, 0.69 g and 0.09 g in *S. rolfsii* inoculated plot, respectively (Table 6). At 35 DAS, the highest root length, shoot length, fresh weight, and dry weight were 6.02 cm, 25.13 cm, 3.79 g, and 0.59 g, respectively in *R. solani* inoculated plot and 4.49 cm, 23.88 cm, 4.07 g and 0.62 g in *S. rolfsii* inoculated plot, respectively and the lowest root length, shoot length, fresh weight and dry weight were 2.83 cm, 17.25 cm, 3.79 g and 2.24 g in *R. solani* inoculated plot and 2.98 cm, 18.50 cm, 2.49 g and 0.38 g in *S. rolfsii* inoculated plot, respectively (Table 7). The reason for this is that

chitosan plays a direct role in supplying amino acids to the plant, which was reflected positively in the increase in vegetative growth indicators such as plant height, number of total leaves, leaf area, and percentage of dry matter in the leaves, which was positively reflected in the yield of one plant (Colman *et al*., 2019). It is reported that chitosan increased Indolacetic acid (IAA) and Phenol content which may help the seedling growth of tomato. Similar reports were found in Benhamou *et al*., 1994, O· Herlihy *et al.,* 2003, and Akter *et al.,* 2018 who observed the enhancement of growth-promoting components (root length, shoot length, etc.) of brinjal, chili, etc. by chitosan.

Effect of chitosan on plant height and number of branches of tomato

To know the effect of chitosan on plant height and number of the branches of tomato seedlings in field conditions, data were taken from four plants for each replication of all treatments at 15 DAT and 30 DAT, respectively. Application of chitosan increased the plant height and number of branches of tomato seedlings compared to the pathogen-inoculated condition. The highest plant heights and number of branches of tomato plants were observed in chitosan-treated plots T_3 followed by T_4 where soil was amended with chitosan over T_2 at 15 and 30 DAT in both *R. solani* and *S. rolfsii* inoculated fields. But T_3 and T_4 were statistically identical. At 15 DAT, the highest plant heights were 27.00 cm and 28.42 cm and the highest number of branches were 8.17 and 7.08, the lowest plant heights were 20.67 cm and 20.83 cm and the number of branches was 3.75 and 3.00 in *R. solani* and *S. rolfsii* inoculated fields (Table 8). At 30 DAT, the highest plant heights were 46.92 cm and 41.78 cm, the highest number of branches was 14.17 and 12.50 and the lowest plant heights were 32.25 cm and 28.17 cm and the number of branches was 8.58 and 6.72, respectively in *R. solani* and *S. rolfsii* inoculated fields (Table 9). Among the key minerals, the highest levels of Ca, Mg, Na, K, S, and P, as well as the majority of tomato biochemical characteristics, increase with chitosan application, having a substantial effect on growth and yield (Parvin *et al*., 2019). Mondal *et al*., 2013, Nitu *et al*. 2016, Akter *et al.,* 2018 reported that chitosan increased plant height and the number of branches in tomato, chilli, etc. The results of the study are in agreement with these reports.

Effect of chitosan on disease incidence (DI) and percent disease index (PDI) of tomato

Applying chitosan reduced disease incidence (DI) and percent disease index (PDI) in all treatments over pathogen-treated plots. The highest DI (45.25% and 46.25%) and PDI (35.50% and 37.00%) at harvest were recorded in the treatment T_2 where the field was inoculated with *R. solani* and *S. rolfsii* (Table 10 and Fig. 5). The lowest DI (10.15% and 9.33%) and PDI (7.25% and 7.50%) were recorded in the treatment T_3 where chitosan was used as a seed treatment followed by T_4 where soil amended with chitosan. But T_3 and T_4 were statistically identical. The highest reduction in disease incidence (77.57% and 79.82%) and severity (79.58% and 79.73%) were found in T_3 treatment over T_2 treatment in *R. solani* and *S. rolfsii*, respectively. Chitosan is often used in plant disease control as a powerful elicitor rather than a direct antimicrobial or toxic agent and also effectively reduces DI and PDI of anthracnose of chilli and eggplant (El-Mohamedy *et al*., 2013; Akter *et al*. 2018; Jannat *et al*. 2018). Nitu *et al*. 2016 reported that chitosan was effective in reducing southern blight and dry rot of tomato. The results suggested that seed treatment with chitosan is effective in controlling Rhizoctonia root rot and Southern blight diseases of tomato. Chitosan controls plant pathogens through several methods, including antimicrobial activity, induction of plant defense mechanisms, increased plant resistance, and physical barrier (Andrews, 2001). The use of chitosan can help boost pathogen defense systems by enhancing gene transcripts, the manufacture of lytic enzymes through growth hormone upregulation, and the expression of defense-related proteins. Glucanase and chitinase were upregulated and the expression of these enzymes in plants or certain plant parts was associated with the plant's defense system (Suwanchaikasem *et al*., 2023).

Effect of chitosan on the yield of tomato

Results of the present study indicate that by the application of different treatments of chitosan yield and yield contributing components were significantly increased in all the treatments over the treatment T_2 where pathogens were inoculated in the fields. The highest yield was recorded in treatment T_3 where seed treatment was done with 1.0% chitosan followed by T_4 where soil amended with 1% chitosan in pathogen-inoculated fields. The lowest yield was recorded in the treatment T_2 where fields were pathogen inoculated without chitosan. Moreover, applications of chitosan significantly increased the number of fruits and weight of fruits at harvest compared to pathogen-inoculated untreated fields. The highest number of fruits per plant (86.83 and 83.17) and yield (23.50 t/ha and 22.50 t/ha) were recorded in T_3 in *R. solani and S. rolfsii* inoculated fields, respectively, and the lowest number of fruits per plants (61.33 and 61.00) and weight of fruits (13.83 t/ha and 14.38 t/ha) recorded in the treatment T_2 (Table 11). The highest increase in yield (69.92 % and 56.47 %) was observed in T_3 where the seed was treated with 1.0% chitosan followed by T_4 where soil was amended with 1.0% chitosan over T_2 treatment where soil was inoculated with *R. solaniand S. rolfsii*, respectively (Fig. 6). It is reported that chitosan can increase soil fertility and enhance nutrient uptake, induced phytohormone which may contribute to increasing yield of tomato (Nitu *et al*., 2016). It promotes plant development by altering plant physiological processes such as food intake, cell division, cell elongation, enzyme activation, and protein synthesis, which can ultimately lead to

enhanced yield. Akter *et al*., 2018, Jannat *et al*., 2018 and Rahman *et al*., 2021 reported that chitosan increases the growth and yield of chili, eggplant, and carrot. Liu *et al.* (2007) found similar results, observing that tomato yield increased with chitosan application.

Table 1. Pathogenicity test of *R. solani* and *S. rolfsii* isolates against tomato seedlings

Isolates	Mortality (%)		
	Pre-emergence	Post- emergence	Total
	R. solani		
R1	52.09 a	40.30 a	92.39 a*
R2	37.33 b	32.50 b	69.83 b
R3	32.18 c	25.00 c	57.18 c
Untreated control	0.00 d	0.00 d	0.00 d
CV (%)	3.15	3.92	2.35
SE (±)	0.7817	0.7817	1.0541
	S. rolfsii		
S1	60.00 a	34.50 a	94.50 a
S2	45.18 b	20.56 b	65.74 b
S3	42.09 c	10.00 c	52.09 c
Untreated control	0.00 d	0.00 d	0.00 d
CV (%)	3.84	3.07	1.80
SE (±)	1.1547	0.4082	0.7817

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

Table 2. Mycelial growth inhibition of *R. solani* and *S. rolfsii* by chitosan on PDA

Treatments	Mycelial growth (mm) after 7 days of incubation		% mycelial growth inhibition over control	
	R. solani	*S. rolfsii*	*R. solani*	*S. rolfsii*
Control (no chitosan)	90.00 a	90.00 a*	-	-
0.6% chitosan	13.00 b	9.00 b	85.56	90.00
0.8% chitosan	7.00 c	5.00 c	92.22	94.44
1.0% chitosan	0.00 d	0.00 d	100	100
CV (%)	2.78	3.14	-	-
SE(±)	0.6236	0.6667	-	-

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

Table 3. Effect of the time duration of seed soaking with chitosan on germination and growth parameters of tomato seedlings at 10 and 20 DAS

Time duration (hrs) for seed soaking with chitosan	% germination	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
		10 DAS			
0	66.67 d	1.10 c	2.50 e	0.65 c	0.02 d
3	77.80 b	1.70 b	5.17 b	1.14 b	0.35 b
6	95.53 a	2.07 a	5.80 a	2.21 a	1.13 a*
12	73.33 c	1.60 b	3.20 c	1.20 b	0.28 b
24	60.00 e	1.20 c	2.83 d	0.58 c	0.10 c
CV (%)	1.20	6.41	4.07	11.87	19.64
SE (±)	0.7286	0.0803	0.1295	0.1121	0.0600
		20 DAS			
0		2.67 c	5.97 e	0.69 c	0.06 d
3		3.33 b	8.23 b	2.10 b	0.70 c
6		3.70 a	9.30 a	3.0 a	1.97 a
12		3.20 b	7.70 c	2.03 b	1.30 b
24		2.50 c	6.73 d	1.80 b	1.18 b
CV (%)		4.25	3.50	24.86	10.95
SE (±)		0.1070	0.2165	0.3780	0.0934

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

Table 4. Effect of chitosan on germination and seedling mortality of tomato in *R. solani* and *S. rolfsii*inoculated soil

Treatments	%germination	% increased germination over T_2	Seedling mortality (%)		
			Pre- emergence	Post- emergence	Total
		R. solani			
T_1	73.33 c	25.71	32.52 b	18.35 b	50.87 b
T_2	58.33 d	-	48.24 a	38.25 a*	86.49 a
T_3	90.00 a	54.29	18.44 d	12.25 d	30.69 d
T_4	88.33 b	51.43	20.34 c	15.50 c	35.84 c
CV (%)	0.74	-	1.91	5.48	3.50
SE (±)	0.4714	-	0.4714	0.9428	0.2165
		S. rolfsii			
T_1	83.33 b	47.12	33.40 b	16.60 b	50.00 b
T_2	56.64 c	-	52.50 a	37.33 a	89.83 a
T_3	93.33 a	64.78	16.50 d	13.43	29.33 c
T_4	90.00 a	59.48	18.18 c	14.40 c	32.90 c
CV (%)	2.14	-	1.66	9.78	4.57
SE (±)	1.4142	-	0.4082	1.6330	1.8856

*Means within the same column having a common letter (s) do not differ significantly (*P=0.05*) by LSD. Note: T_1= Seed without any treatment (control-1), T_2= Soil inoculation with pathogen (control-2), T_3= T_2+seed treatment with 1.0% chitosan, T_4= T_2+soil amendment with 1.0% chitosan.

Table 5. Effect of chitosan on growth parameters of tomato seedlings at 10 DAS

Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
	R. solani				*S. rolfsii*			
T_1	1.36 b	6.06 b	0.79 b	0.10 b	1.57 b	6.02 b	0.63 c	0.09 c
T_2	1.24 b	5.64 b	0.62 c	0.05 c	1.34 b	5.71 b	0.53 d	0.06 d
T_3	2.46 a	8.14 a	1.22 a	0.30 a	2.00 a	7.88 a	1.42 a	0.26 a*
T_4	2.14 a	7.95 a	1.06 a	0.26 a	1.94 a	7.58 a	1.36 b	0.20 a
CV (%)	17.14	7.83	10.18	13.92	11.21	5.96	17.03	16.11
SE (±)	0.2179	0.3844	0.0664	0.0176	0.1358	0.2863	0.0443	0.0130

*Means within the same column having a common letter (s) do not differ significantly (*P=0.05*) by LSD. Note: T_1= Seed without any treatment (control-1), T_2= Soil inoculation with pathogen (control-2), T_3= T_2+seed treatment with 1.0% chitosan, T_4= T_2+soil amendment with 1.0% chitosan.

Table 6. Effect of chitosan on growth parameters of tomato seedlings at 20 DAS

Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
	R. solani				*S. rolfsii*			
T_1	2.15 b	10.63 b	0.89 c	0.13 c	1.90 b	11.63 b	0.82 b	0.12 b
T_2	1.44 c	8.13 c	0.88 c	0.06 d	1.54 b	7.15 c	0.69 b	0.09 b
T_3	2.80 a	12.75 a	1.76 a	0.36 a	2.98 a	13.50 a	1.54 a	0.27 a*
T_4	2.70 a	11.88 ab	1.27 b	0.29 b	2.73 a	11.88 b	1.28 a	0.24 a
CV (%)	14.97	10.21	6.98	11.78	14.34	8.16	20.05	19.71
SE (±)	0.2422	0.7827	0.0594	0.0176	0.2292	0.6362	0.1534	0.0249

*Means within the same column having a common letter (s) do not differ significantly (*P=0.05*) by LSD. Note: T_1= Seed without any treatment (control-1), T_2= Soil inoculation with pathogen (control-2), T_3= T_2+seed treatment with 1.0% chitosan, T_4= T_2+soil amendment with 1.0% chitosan.

Table 7. Effect of chitosan on growth parameters of tomato seedlings at 35 DAS

Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
	R. solani				*S. rolfsii*			
T_1	3.68 b	22.13 b	2.88 b	0.41 b	3.14 b	21.13 b	3.04 b	0.40 b
T_2	2.83 c	17.25 c	2.24 c	0.39 b	2.98 b	18.50 c	2.49 c	0.38 b
T_3	6.02 a	25.13 a	3.79 a	0.59 a	4.49 a	23.88 a	4.07 a	0.62 a*
T_4	5.81 a	24.88 a	3.39 a	0.52 a	4.28 a	23.25 a	4.02 a	0.51 a
CV (%)	9.85	5.45	8.60	5.54	4.38	6.26	5.19	11.26
SE (±)	0.2863	0.8421	0.1899	0.0187	0.1006	0.9592	0.1249	0.0381

*Means within the same column having a common letter (s) do not differ significantly (*P=0.05*) by LSD. Note: T_1= Seed without any treatment (control-1), T_2= Soil inoculation with pathogen (control-2), T_3= T_2+seed treatment with 1.0% chitosan, T_4= T_2+soil amendment with 1.0% chitosan.

Table 8. Effect of chitosan on plant height and number of branches of tomato at 15 DAT

Treatments	*R. solani*		*S. rolfsii*	
	Plant height (cm)	Number of branch/ plant	Plant height (cm)	Number of branch/ plant
T_1	24.17 ab	6.17 b	23.92 b	5.50 b
T_2	20.67 b	3.75 c	20.83 c	3.00 c
T_3	27.00 a	8.17 a	28.42 a	7.08 a*
T_4	25.33 a	7.32 a	26.92 a	7.00 a
CV (%)	8.44	12.33	6.60	13.01
SE (±)	1.6746	0.6142	1.3744	0.6264

*Means within the same column having a common letter (s) do not differ significantly (P=0.05) by LSD. Note: T_1= Seed without any treatment (control-1), T_2= Soil inoculation with pathogen (control-2), T_3= T_2+seed treatment with 1.0% chitosan, T_4= T_2+soil amendment with 1.0% chitosan.

Table 9. Effect of chitosan on plant height and number of branches of tomato at 30 DAT

Treatments	*R. solani*		*S. rolfsii*	
	Plant height (cm)	Number of branch/ plant	Plant height (cm)	Number of branch/ plant
T_1	34.33 c	9.83 c	27.39 b	8.16 b
T_2	32.25 c	8.58 d	28.17 b	6.72 b
T_3	46.92 a	14.17 a	41.78 a	12.50 a*
T_4	42.50 b	12.17 b	39.50 a	11.33 a
CV (%)	3.64	5.25	6.40	15.54
SE (±)	1.1602	0.4799	1.7871	1.2280

*Means within the same column having a common letter (s) do not differ significantly (*P=0.05*) by LSD. Note: T_1= Seed without any treatment (control-1), T_2= Soil inoculation with pathogen (control-2), T_3= T_2+seed treatment with 1.0% chitosan, T_4= T_2+soil amendment with 1.0% chitosan.

Table 10. Effect of chitosan on Rhizoctonia root rot and Southern blight disease incidence (DI) and percent disease index (PDI) of tomato

Treatments	*R. solani*			
	% DI	% decrease of DI over T_2	% PDI	% decrease of PDI over T_2
T_1	16.50 b	-	13.25 b	-
T_2	45.25 a	-	35.50 a*	-
T_3	10.15 c	77.57	7.25 c	79.58
T_4	11.00 c	75.69	8.50 c	76.06
CV (%)	6.82	-	6.20	-
SE (±)	1.1547	-	0.8165	-
	S. rolfsii			
T_1	15.60 b	-	12.50 b	-
T_2	46.25 a	-	37.00 a	-
T_3	9.33 c	79.82	7.50 c	79.73
T_4	10.50 c	77.30	8.00 c	78.38
CV (%)	6.16	-	7.74	-

SE (±)	1.0274	-	1.0274	-

*Means within the same column having a common letter (s) do not differ significantly (*P=0.05*) by LSD. Note: T_1= Seed without any treatment (control-1), T_2= Soil inoculation with pathogen (control-2), T_3= T_2+seed treatment with 1.0% chitosan, T_4= T_2+soil amendment with 1.0% chitosan.

Table 11. Effect of chitosan on the yield of tomato

Treatments	*R. solani*		
	No. of fruits/plant	Weight of fruits (g/plant)	Yield (t/ha)
T_1	64.69 c	6203.6 b	15.50 c
T_2	61.33 d	5533.7 c	13.83 d
T_3	86.83 a	9397.0 a	23.50 a*
T_4	79.50 b	9297.1 a	23.25 b
CV (%)	0.02	2.80	0.49
SE (±)	0.0145	174.21	0.0757
	S. rolfsii		
T_1	62.67 c	6440.0 c	15.33 c
T_2	61.00 d	5748.5 d	14.38 d
T_3	83.17 a	9050.3 a	22.50 a
T_4	82.42 b	8596.4 b	21.24 a
CV (%)	0.40	2.33	0.94
SE (±)	0.2355	141.96	0.0757

*Means within the same column having a common letter (s) do not differ significantly (*P=0.05*) by LSD. Note: T_1= Seed without any treatment (control-1), T_2= Soil inoculation with pathogen (control-2), T_3= T_2+seed treatment with 1.0% chitosan, T_4= T_2+soil amendment with 1.0% chitosan.

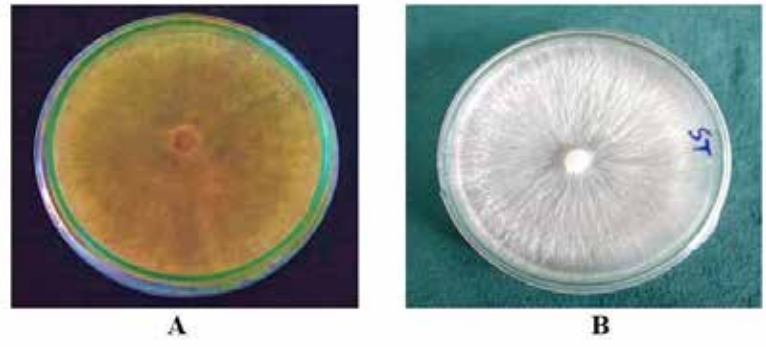

Figure 1. Virulent isolates of *R. solani (*A*)* and *S. rolfsii*(B*)*

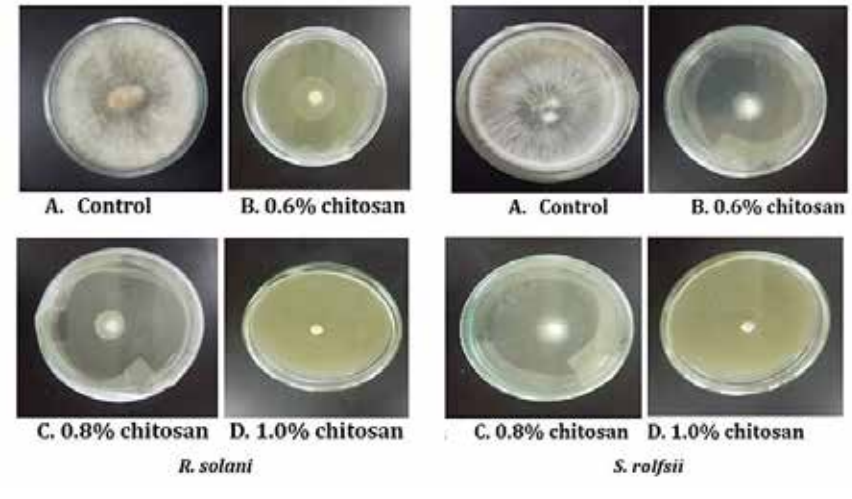

Figure 2. Mycelial growth inhibition of *R. solani* and *S. rolfsii* by chitosan on PDA

Figure 3. Effect of time duration (0-24 hrs) on seed soaking with chitosan on growth parameters of tomato seedlings at 10 and 20 DAS

Figure 4. Seedling growth of tomato with chitosan at 10, 20, and 35 DAS in *R. solani* (A) and *S. rolfsii* (B) inoculated condition

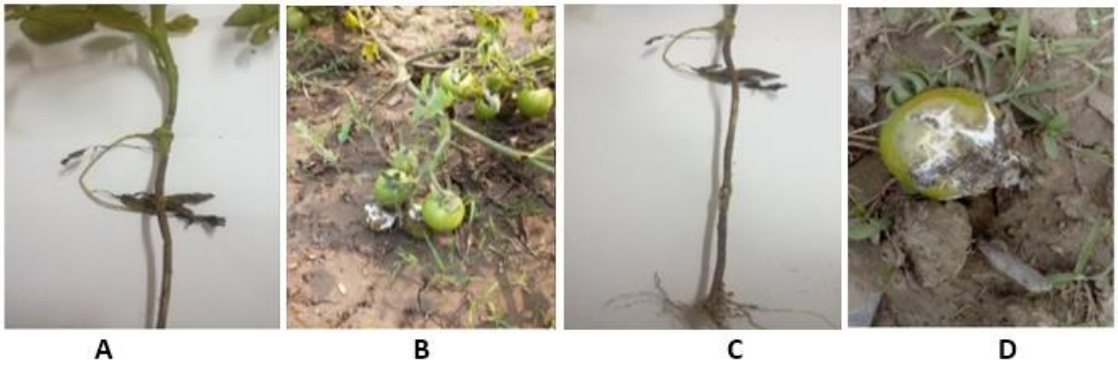

Figure 5. Disease symptoms of Rhizoctonia root rot and Southern blight caused by *R. solani* (A-B) and *S. rolfsii* (C-D)

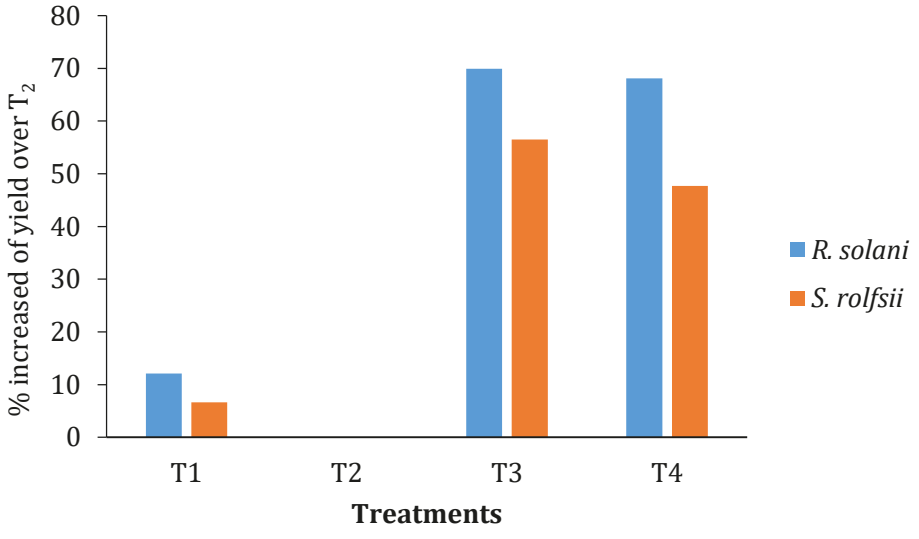

Figure 6. Effect of chitosan on the yield of tomato in *R. solani* and *S. rolfsii* inoculated fields

CONCLUSIONS

The present study reveals that Rhizoctonia root rot and southern blight of tomato can be effectively controlled by the application of chitosan. Seed treatment with 1.0% chitosan appeared to be excellent in increasing germination percentage, root length, shoot length, fresh weight, dry weight, plant height, branch number, and controlling pre- and post-emergence seedling mortality of tomato. Chitosan applied as seed treatment or soil amendment reduced disease incidence, and disease severity and increased seedling growth and yield of tomato. Farmers can adopt eco-friendly control measures for Rhizoctonia root rot and Southern blight of tomato through the application of chitosan in the field at a lower cost as an alternative to chemical fungicides. Further study is required to investigate the mechanism and what signaling pathways lead to growth and disease control.

Acknowledgment

The authors express their gratefulness to the Ministry of Science and Technology, Dhaka, Bangladesh for providing financial support to accomplish this research work.

Conflict of Interest: Authors declare that there is no conflict of interest.

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