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Research Article

Chitosan for suppression of fusarium wilt and plant growth promotion of brinjal

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Abstract

Chitosan is a biodegradable natural compound that has a great potentiality in agriculture for controlling plant diseases. An attempt was made to control Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melongenae* under inoculated field condition and increase the growth and yield of brinjal by chitosan. Before setting the experiments in the field, preliminary laboratory experiments were carried out to select virulent isolate and effective dose of chitosan against the mycelial growth of the selected pathogen. *F. oxysporum* f. sp. *melongenae* isolate F-1 was found to be the most virulent on brinjal in pathogenicity test. Chitosan @ 1.0% concentration was appeared to be the highest inhibitory to the test pathogen at *in vitro* condition. Additionally, seed treatment with 1.0% chitosan for 12 hrs resulted in the highest increased in germination and seedling growth of brinjal. The field experiment was conducted following Randomized Complete Block Design (RCBD) with four treatments. No treatment was given in T_1, the pathogen was inoculated in T_2 and seed treatment and soil amendment with 1.0% chitosan was done in T_3 and T_4, respectively, in test pathogen inoculated condition. Application of 1.0% chitosan as a seed treatment (T_3) or soil amendment (T_4) significantly reduced pre- and post-emergence seedling mortality, incidence and severity of Fusarium wilt as well as enhanced germination percentage, plant growth and yield of brinjal. On the contrary, pre-emergence and post-emergence seedling mortality, disease incidence and severity of Fusarium wilt were highest in treatment T_2 where the soil was inoculated with pathogen without chitosan. Therefore, chitosan could be used against this vascular disease as an alternative to inorganic fungicides and augment yield.

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Introduction

Brinjal (*Solanum melongena* L.) belongs to the family Solanaceae, an important and popular vegetable in Bangladesh. It is grown in almost all agroclimatic zones with more than 100 different varieties under cultivation, offering fruits of different colors, sizes, shapes, and tastes. It may ovoid to long club shaped and colors such as yellow, white, green and purple skin coloring to almost black. It has great importance as a vegetable in the human diet because of its worth in vitamins, proteins and fundamental minerals. It is the 2nd most important vegetable grown in Bangladesh, by about 1.5 lakh resource poor farmers on 50,955 ha of land with a total production of 5.07 lakh metric tons in 2018 (Bangladesh Bureau of Statistics, 2018).

However, there are many factors remain beyond the low production of brinjal for instance, aberrant climatic conditions, soil fertility, seeds quality, and plant diseases. Among these factors, disease is one of the main constraints for the low yield and quality of this crop. Brinjal is susceptible to several diseases such as Fusarium wilt, Verticillium wilt and Bacterial wilt. The most dreadful disruption in sound brinjal production is the Fusarium wilt caused by *F. oxysporum* f. sp. *melongenae*. *F. oxysporum* f. sp. *melongenae* is a soil inhabiting fungus responsible for Fusarium wilt of brinjal is very common in the brinjal growing regions. It can decline the crop yield severely. Firstly, wilting symptoms appear in isolated patches containing less or more circular outlines whose enlargement proceeds as the disease progresses. Curling starts from the lower surface of leaflets and progresses upward. Thus, the crown may start bending and plants die. The root system of plants cannot develop properly and thus discoloring or browning occurs. Partial or whole discoloration of roots may occur. In early cases, the tapering root system is destroyed from tips and shows abnormal stunted growth. The growth of secondary roots occurs rapidly in clusters above level of disease. Affected portions of roots showed discoloration or browning of some walls and fungal hyphae are noticed in some vessels near the walls (Abdel-Monaim et al., 2014). The fungus chokes the xylem-phloem of plant and plant dies from water and nutritional deficiency.

Controlling this devastating disease is very difficult as the pathogen is primarily soil borne and produces macroconidia, microconidia and chlamydospores. These viable and thick walled chlamydospores, filled with lipid like material, survive long time in the soil (Agrios, 2006). The suggested management methods against the wilts are the use of crop rotation of crops, use of resistant varieties, solarization, soil sterility and use of fungicides. Although the use of *Fusarium* resistant brinjal variety can deliver some degree of control of this disease, the occurrence and development of new pathogenic race is a great challenge. Recently, there are no commercially acceptable variety with adequate resistance to *F. oxysporum* f. sp. *melongenae*.

On the other hand, use of chemical fungicides is costly and hazardous to health and environment. The broad-spectrum pesticides may cause problems by targeting beneficial organisms and the recurrent use of these synthetic pesticides leads to loss of biodiversity (Yasmin & D'Souza, 2010). It is now well documented that treatment of plants with various eliciting agents leads to an induced resistance against subsequent pathogen attacks, both locally and systemically. Therefore, in order to enhance plant resistance, the use of elicitors is becoming very attractive. In agriculture, ecofriendly tool that could provide efficient alternatives to the usage of chemical pesticides for managing plant diseases, thus reducing their environmental negative impacts. Chitosan is a linear amino polysaccharide obtained by deacetylation of chitin (poly-N-acetyl glucosamine). It is a cationic polymer. It possesses primary amino groups in its structure and acts as an antimicrobial agent due to the presence of these amino groups (Ramirez et al., 2010). It gives a satisfactory level of crop protection against *Fusarium* spp. as compared to chemical pesticide (Benhamou et al., 1998).

Chitosan induces phenolic compounds such as lignin, phytoalexins, PR proteins (pathogenesis-related proteins), callose, and modulate the activity of critical enzymes of metabolic pathways involved in the defensive response, for instance PAL, peroxidases and chitinase (Li & Zhu, 2013; Orzali et al., 2014). However, a

few studies have been done on the management of Fusarium wilt disease of brinjal but there is no report on chitosan-based management of the above-mentioned disease of brinjal in Bangladesh. Considering the aforesaid facts, the present research was undertaken to evaluate the effectiveness of chitosan in reducing Fusarium wilt of brinjal and enhancing growth and yield of brinjal.

Materials and methods

Experimental site

A field experiment was laid out at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) from 2018 to 2020 Geographically the experimental area is located at 24° 09' N latitude and 90° 26' longitudes at the elevation of 8.2m from sea level. 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. 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.

Experimental materials

Seeds sample of brinjal variety "BARI Begun-4 (Kajla) was collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh.

Collection, isolation and preservation of F. oxysporum f. sp. melongenae isolates

Three isolates of *F. oxysporum* f. sp. *melongenae* designated as F-1 to F-3 were isolated from infected root, stem and pod tissues of brinjal, pea, and tomato. The specimens which had characteristic symptoms of root rot, as well as wilt, were selected from the infected fields. The fungal isolates were isolated. Then, the fungal colonies were grown on PDA and identified by following the standard method. The isolates were purified following the hyphal tip method and stored in PDA slants at 10°C.

Inoculum preparation of test pathogen

Inoculum of the *F. oxysporum* f. sp. *melongenae* isolates were made and preserved according to Rubayet and Bhuiyan (2016). In field experiments, 40 g wheat grain colonized inoculum were applied per plot as per the required treatments before sowing the seeds.

Pathogenicity test

Three isolates, namely F-1, F-2 and F-3 of *F. oxysporum* f. sp. *melongenae* were tested for their virulence against pre-emergence and post-emergence mortality of brinjal by soil infestation method in pot culture experiment as stated by Datar (2011). Each earthen pot was filled with 3.0 kg sterilized soil. Fifteen seeds were sown in every pot and before sowing the soil was mixed with inoculum tested pathogen. But in control pot seeds were sown without any inoculum. Disease development was observed and recorded to estimate the effect of pathogen in causing pre-emergence and post-emergence seedling mortality of brinjal. Disease incidence and severity were taken 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 brinjal stem covered with lesions (El-Bramawy & Wahid, 2007). The causal agent was confirmed after re-isolation of pathogen from infected roots and seedling.

Selection of effective dose of chitosan against F. oxysporum f. sp. melongenae

Chitosan was collected from Sisco Research Laboratories Pvt. Ltd. (SRL), India. It was derived from the cell of quick growing sea shrimp. A series of preliminary evaluations of chitosan were done with different concentrations of chitosan such as 0.6, 0.8, and 1.0% on PDA plate against *F. oxysporum* f. sp. *melongenae.* Plates were individually tested at the center with equal agar plug (5 mm in diameter) taken from seven days old culture of the pathogen and incubated at 25°C. Mean colony diameter was measured when the control plate reached full growth. The radial growth of *F. oxysporum* f. sp. *melongenae* in 3 replications were recorded distinctly and their means were taken. The percent inhibition of the radial growth was calculated as described by Sundar et al. (1995).

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

Where,
X = Mycelial growth of pathogen without chitosan (control)
Y = Mycelial growth of pathogen with chitosan

Treatments

The treatment combinations were as follows:
T_1: Seed and soil without any treatment (without chitosan, only fertilizers)
T_2: Soil inoculation with pathogen *F. oxysporum* f. sp. *melongenae*
T_3: T_2 + seed treatment with 1.0% chitosan
T_4: T_2 + soil amendment with 1.0% chitosan

Seed treatment with chitosan at different time

Seeds of brinjal were surface sterilized by immersion of 1.0% NaOCl, thoroughly cleaned with sterile distilled water and were deep into each of the chitosan solution (pH 5.5 -6) at 1.0% concentration. Seeds were treated with 1.0% chitosan for 3, 6, 9, 12, and 24 hrs. After immersion the wetted seeds were air dried in a sterile cabinet and kept in desiccator until use. Seed soaked with 1.0% chitosan for 12 hrs was selected for field experiment.

Raising of seedlings

The soil was prepared through mixing fertilizers and cowdung for raising brinjal seedlings. Seeds were sown in the tray on September 2019 after the required treatments. After sowing, the seeds were covered with light soil. Proper care was taken to raise healthy seedlings.

Land preparation and design of experiment

The field was made with well tilth using a tractor driven disc plough, rotavator and harrow. The experiment was laid out in the RCBD with three replications. After land preparation the experimental area was divided into three blocks, representing 3 replications. The unit plot size was 1.5 m × 1.5 m.

Use of manure and fertilizer

During the land preparation well decomposed cow dung was applied. Urea (150 kg), Triple Super Phosphate (130 kg), and Muriate of Potash (55 kg) per ha were applied according to the recommended dose.

Soil amendment with chitosan

Soil (3kg) was mixed with 1.0% chitosan for each pit per plot as soil amendment. Soil amendment was done before 3 days of transplanting of brinjal seedlings and after 7 days of inoculum applied in the field.

Transplanting of seedlings

Twenty-five days aged healthy brinjal seedlings variety "Kajla" were collected from the tray for plantation. Distance between plant to plant was 75 cm and row to row was 75 cm. A total of four seedlings were planted in each plot after 25 days of seed sowing in October 2019. The intercultural activates as for example weeding, irrigation etc. were done whenever it was necessary until the maturity of plants.

Data collection

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

Observation of disease development

Germination and seedling disease development was observed regularly and seedling growth was recorded at 20, 30, and 40 days after sowing to estimate the effect of chitosan on pre-emergence and post-emergence seedling mortality and growth of brinjal. Plant After 95 DAS disease incidence and severity were taken 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 brinjal stem covered with lesions (El-Bramawy & Abd-AlWahid, 2007). The causal agent of Fusarium wilt (*F. oxysporum* f. sp. *melongenae*) was confirmed after re-isolation of the pathogens from the ungerminated seeds and diseased (wilted) seedlings.

Disease assessment

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

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

$$PDI = \frac{\Sigma \text{ of rating of plants observed}}{\text{No. of plants observed} \times \text{Max. score of the scale used}} \times 100$$

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

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

Harvesting

Brinjal harvesting was started at 120 days after transplanting. The 2nd and 3rd harvesting were done after 15 days interval, respectively. All the harvests were completed by 23 March 2020.

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.

Results and discussion

Pathogenicity test of F. oxysporum f. sp. melongenae isolates against brinjal seedlings

The pathogenicity test of the 3 selected isolates of *F. oxysporum* f. sp. *melongenae* against brinjal seedlings was done in the pot containing sterilized soil to find out the most virulent isolate of the test pathogen. All the test pathogen isolates were virulent but showed variability, causing total seedling mortality of brinjal (Table 1). The isolate F-1seemed to be the most virulent causing the highest (81.98%) total mortality followed by isolate F-2(53.89%). The lowest mortality was observed (35.73%) by F-3isolate. Non-inoculated control plants exhibited no mortality.

At 45 DAS, seedlings shown leaf chlorosis and minor vein clearing on the outer leaflets followed by yellowing and dropping of leaves, discoloration of the stem and death of the above-ground plant parts of whole plant (Table 2). Pathogenicity was confirmed by re-isolation and identification of the pathogen from the infected plants. All isolates obtained were identified as *F. oxysporum f. sp. melongenae* due to the production of morphological characteristics like 3-5 septate, sickle shaped macroconidia with a foot shaped basal cell, ellipsoid microconidia borne in false heads on short monophialides and chlamydospores. Only the *Fusarium* species was isolated. The disease severity of wilt symptoms on the stems was assessed using a wilt disease scale. All the isolates tested were pathogenic to brinjal with different degrees of disease severity. All tested isolates caused 100% DI. The highest PDI (52.00%) was recorded in F-1 inoculated plants. Based on this pathogenicity test, the isolate F-1 was chosen for the following studies. There are strong evidences found where pre-emergence and post-emergence mortality occurred due to virulent isolate of *F. oxysporum* f. sp. *melongenae* (Goswami et al., 2012; Adhikary et al., 2017).

Table 1. Pathogenicity test of F. oxysporum f. sp. melongenae isolates against brinjal seedlings in pot culture

Isolates	Mortality (%)		
	Pre-emergence	Post-emergence	Total
F-1	46.67 a	35.32 a	81.98 a
F-2	30.96 b	22.93 b	53.89 b
F-3	23.05 c	12.68 c	35.73 c
Untreated control	0.00 d	0.00 d	0.00 d
CV (%)	0.93	0.92	-
SE (±)	1.32	1.31	-

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

Table 2. Fusarium wilt disease incidence (DI) and percent disease index (PDI) of brinjal at 45 DAS in pot culture

Isolates	% DI	PDI
F-1	100.00 a*	52.00 a
F-2	100.00 a	45.00 b
F-3	100.00 a	36.00 c
Untreated control	00.00 b	00.00 d
CV (%)	-	6.48

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

Effect of chitosan on mycelial growth of virulent isolate of F. oxysporum f. sp. melongenae

Chitosan @ 0.6, 0.8, and 1.0%were tested against the hyphal growth retarded of *F. oxysporum* f. sp. *melongenae* on potato dextrose agar medium. The inhibitory effect was found to increase while rising the chitosan concentration level. The highest (100.00%) reduction of the mycelial growth of *F. oxysporum* f. sp. *melongenae* over the control PDA plate was observed at 1.0% of chitosan amended with PDA plate followed by 0.8% of chitosan with 83.33% reduction of mycelial growth respectively. Significantly the lowest (61.11%) reduction of the mycelial growth of the pathogen was examined at 0.6% of chitosan mixed PDA plate (Table 3 and Figure 1). Considering the *in vitro* evaluation, the most effective 1.0% chitosan concentration was selected for further study. There are huge number of evidences of mycelial growth reduction by chitosan (Saharan et al., 2013; Silva et al., 2014; Nitu et al., 2016; Akter et al., 2018; Jannat et al., 2018).

Table 3. Effect of chitosan on mycelial growth of F. oxysporum f. sp. melongenae

Treatments	Radial mycelial growth (mm) at 10 days after incubation	% mycelial growth inhibition over control
Control	90.00 a	-
0.6% chitosan	35.00 b	61.11
0.8%chitosan	15.00 c	83.33
1.0% chitosan	0.00 d	100.00
CV (%)	0.95	-
SE (±)	0.19	-

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

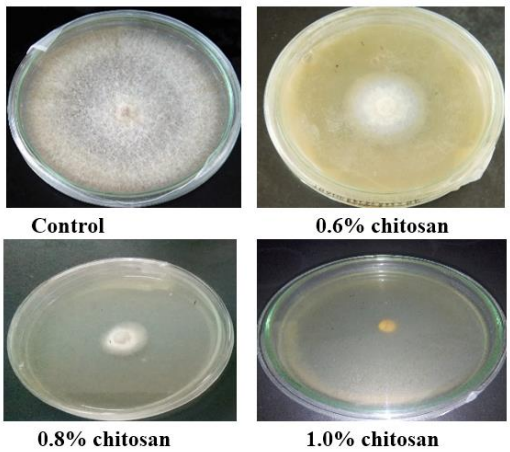

Figure 1. Colony of F. oxysporum f. sp. melongenae under chitosan treament on PDA

Morphological change was also noticed among the plates treated with chitosan and the untreated plate with the light microscope (Figure 2). The changed hyphae of *F. oxysporum* f. sp. *melongenae* were agglomeration of hyphae, abnormal in shape, swelling of cells, hyphae size reduction and empty cells devoid of cytoplasm in the mycelia were observed in chitosan treated plate compared to untreated plate or normal PDA plate which strongly follow the articles (Al-Hetar, 2011).

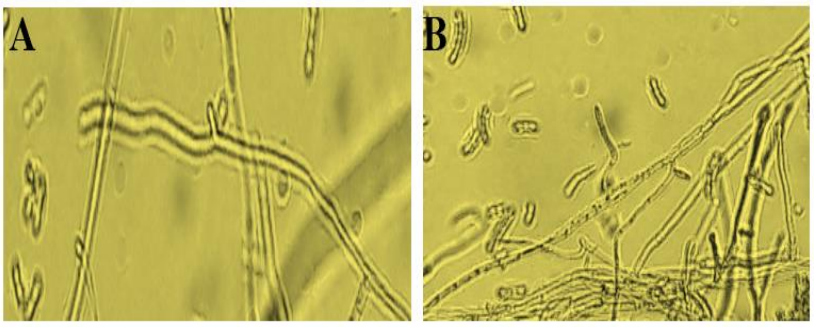

Figure 2. Mycelial change in chitosan mixed PDA plates. Here, normal hypha (A), deformed hypha (B)

Standardization of time for seed treatment with chitosan

Brinjal 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 and shoot length of seedlings were measured from randomly taken five seedlings for each replication of all treatments at 20 DAS respectively. Seeds were soaked with chitosan for 0, 3, 6, 9, 12, and 24 hrs. Germination percentages and growth of brinjal seedlings were increased gradually at 0, 3, 6, 9, and 12 hrs soaked seeds and after that decreased at 24 hrs soaked seeds (Table 4 and Figure 3). The significantly maximum germination percentage (80.33%), root length (8.30 cm) and shoot length (10.83 cm) were noticed at 12 hrs seed treatment. The lowest germination percentage (38.11%), root length (6.23 cm) and shoot length (9.20 cm) were observed at 24 hrs seed treatment. Finally, 12 hrs duration of seed treatment was selected for field experiment. This experiment is highly supported by Lizárraga-Paulín et al. (2013).

Table 4. Effect of the time duration of seed soaking with chitosan on germination and growth parameters of brinjal seedlings at 20 DAS

Time duration (hrs) for seed treatment with 1.0% chitosan	% germination	Root length (cm)	Shoot length (cm)
0	48.42 e	4.20 e	5.10 e
3	53.22 d	5.58 d	6.43 d
6	55.56 c	6.51 c	8.26 c*
9	64.42 b	7.10 b	8.70b c
12	80.33 a	8.30 a	10.83 a
24	38.11 f	6.23 c	9.20 b
CV (%)	1.68	3.84	4.21
SE (±)	0.55	0.14	0.19

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

Figure 3. Effect of time duration (0-24 hrs) on seed soaking with 1.0% chitosan on growth of brinjal seedlings at 20 DAS

Effect of chitosan on germination percentage, pre-emergence and post-emergence seedling mortality of brinjal

To know the effect of chitosan on germination, pre- and post-emergence seedling mortality of brinjal, 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 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 5). The highest germination percentage (66.86%), and reduction of total seedling mortality (65.87%) were observed in T_3 followed by T_4. But, the highest total seedling mortality (74.78%) and lowest germination (43.75%) were found in T_2 where soil was inoculated with the test pathogen. This data supported by Nitu et al. (2016); Akter *et al.* (2018) where application of chitosan was found to increase germination and decrease seedling mortality of tomato and chili, respectively. Chitosan has an outstanding substantial of forming a semi-permeable film on the seed surface that can retain the moisture of the seed and absorb extra moisture from the soil, thereby promoting seed germination (Zeng & Luo, 2012).The potential contributions are higher seed germination, enhanced seedling growth and development, and activation of antioxidant enzymes to prevent the potential damage by the reactive oxygen species (ROS) at the time of seed germination (Saharan et al., 2013; Anusuya et al., 2016). Chitosan as seed treatment concerns the elicitation of the systemic resistance in plants.

Table 5. Effect of chitosan on germination percentage, pre-emergence and post-emergence seedling mortality of brinjal in F. oxysporum f. sp. melongenae inoculated field

Treatments	% germination	% increased	Seedling Mortality (%)			
			Pre-emergence	Post-emergence	Total	% decreased
T_1	84.50 a	-	0.00 d	0.00 d	0.00 d	-
T_2	43.75 d	-	40.75 a	36.03 a	74.78 a	-
T_3	73.00 b	66.86	11.50 c	14.00 c	25.52 c	65.87
T_4	61.50 c	40.57	23.00 b	17.67 b	40.67 b	45.57
CV (%)	2.46	-	8.60	8.97	3.60	-
SE (±)	0.81	-	0.80	0.87	0.10	-

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.

Effect of chitosan on growth of brinjal seedling at different days after sowing

To know the effect of chitosan on growth related parameters such as root and shoot length, fresh and dry weight were measured from randomly taken five plants from each replication of all treatments at 10, 20, and 30 DAS, respectively of brinjal seedlings in pathogen inoculated condition. Application of chitosan as seed treatment or soil amendment increased growth of brinjal seedlings at 10, 20, and 30 DAS, respectively (Table 6 and Figure 4). At 10 DAS, the highest value of root length (5.75 cm) was observed both in T_3 and T_4 treatment where seed treatment and soil amendment were done, respectively. The highest shoot length (5.60 cm), fresh weight (0.24 g) and dry weight (0.040 g) were recorded in T_4 treatment followed by T_3. Where the lowest value of root length (2.63 cm), shoot length (3.97 cm), fresh weight (0.10 g) and dry weight (0.015 g) were recorded in treatment T_2. At 20 DAS, the highest value of root length (7.32 cm), shoot length (9.22 cm), fresh weight (0.79 g) and dry weight (0.26 g) were observed in T_3 followed by T_4. Significantly the lowest value of root length (5.10 cm), shoot length (6.52cm), fresh weight (0.50 g) and dry weight (0.06 g) were observed in pathogen inoculated plot T_2. At 30 DAS, the highest root length (9.75 cm), fresh weight (1.01), dry weight (0.43 g) was observed T_3, where seed treatment was done with 1.0% chitosan followed by T_4 where soil amendment was done with 1.0% chitosan. The highest shoot length (11.07 cm) was recorded T_4 followed by T_3. The lowest root length (6.45 cm), shoot length (7.85 cm), fresh weight (0.70 g) and dry weight (0.20 g) were recorded in T_2 (Table 6 and Figure 4). Treatments T_3 and T_4 were statistically identical.

The improvement of growth-promoting components (root and shoot length, etc.) of brinjal, chili, etc. by chitosan was also found in Li et al. (2013); Akter et al. (2018). Chitosan helped to activate the hydrolytic enzymes needed to degrade and mobilize reserve food materials including starch and protein (Hameed et al., 2013). It can also promote the division of root cells by activating plant hormones including auxin and cytokinin that further lead to increased nutrient intake (Dzung et al., 2011). Based on recent findings, chitosan when applied as a seed treatment, behaves as a resistance elicitor, inducing a physiologically enriched defensive ability in seedlings and plants, whereby the plant's innate defences are potentiated (Orzali et al., 2014). In addition, soil amendments with chitosan were reported to induce callose formation, proteinase inhibitors and phytoalexin biosynthesis in many dicot plant species. This includes the modulation of several enzyme activities, involved in detoxification processes as well as the increasing of the activity of enzymes involved in plant defense barriers (Mishra et al., 2014).

Table 6. Effect of chitosan on growth of brinjal seedling at different days after sowing

Treatments	Root length (cm)	% increase over T_2	Shoot length (cm)	% increase over T_2	Fresh weight (g)	% increased over T_2	Dry weight (g)	% increase over T_2
				10 days after sowing				
T_1	4.53 b	-	5.02 a	-	0.28 a	-	0.032 b	-
T_2	2.63 c	-	3.97 b	-	0.10 b	-	0.015 c	-
T_3	5.75 a	118.63	5.45 a	37.27	0.20 a	100	0.034 ab	126.67
T_4	5.75 a	118.63	5.60 a	41.11	0.24 a	140	0.040 a*	166.67
CV (%)	11.97	-	8.41	-	28.43	-	14.33	-
SE (±)	0.27	-	0.21	-	0.02	-	2.18	-

... continued Table 6

Treatments	Root length (cm)	% increase over T_2	Shoot length (cm)	% increase over T_2	Fresh weight (g)	% increased over T_2	Dry weight (g)	% increase over T_2
				20 days after sowing				
T_1	6.65 a	-	7.50 b	-	0.66 c	-	0.20 c	-
T_2	5.10 b	-	6.52 c	-	0.50 d	-	0.06 d	-
T_3	7.32 a	43.52	9.22 a	41.41	0.79 a	58	0.26 a	333.33
T_4	7.07 a	38.63	8.72 a	33.74	0.74 b	48	0.23 b	283.33
CV (%)	9.05	-	7.14	-	3.37	-	7.05	-
SE (±)	0.29	-	0.28	-	0.01	-	6.80	-
				30 days after sowing				
T_1	9.25 a	-	10.30 a	-	0.86 b	-	0.41 a	-
T_2	6.45 b	-	7.85 b	-	0.71 c	-	0.20 b	-
T_3	9.75 a	51.16	10.50 a	33.76	1.01 a	42.25	0.43 a	115
T_4	9.10 a	41.08	11.07 a	41.01	1.00 a	40.84	0.42 a	110
CV (%)	10.52	-	5.89	-	2.48	-	5.09	-
SE (±)	0.45	-	0.29	-	0.01	-	9.35	-

*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 4. Seedling growth of brinjal with chitosan at 10 DAS (A), 20 DAS (B), and 30 DAS (C) in F. oxysporum f. sp. melongenae inoculated condition

Effect of chitosan on plant height and number of branches of brinjal at different days after transplanting

To know the effect of chitosan on plant height and number of branches of brinjal plant in field condition, data were taken from four plants for each replication of all treatments at 30, 45, and 60 DAT, respectively (Table 7). Application of chitosan as seed treatment or soil amendment increased the plant height and number of branches of brinjal plant compared to the pathogen inoculated condition. At 30 DAT, the highest value of plant height (17.09 cm) and number of branches (6.41) were observed in T_3 followed by T_4. At 45 DAT, the highest value of plant height (27.16 cm) was recorded in T_3 followed by T_4 and the highest branch number per plant (9.00) was recorded

in both T_3 and T_4. At 60 DAT, highest plant height (48.50 cm) was found in T_4 followed by T_3 and highest number of branches per plant (10.25) was recorded in T_3 followed by T_4. T_3 and T_4 were statistically similar. The lowest value of plant height (11.90 cm, 18.67 cm, 33.58 cm) and number of branches (4.50, 7.00, 7.33) at 30, 45, 60 DAT, respectively were recorded in T_2 where pathogen was inoculated in the field. This results also correlated with Rehman et al. (2014); Nitu et al. (2016); Akter et al. (2018). Application of chitosan improves plant growth, yield and induces synthesis of secondary metabolites such as polyphenolics, lignin, phytoalexins as well as flavonoids in plants (Xoca-Orozco, 2017).

Table 7. Effect of chitosan on plant height and number of branches of brinjal seedling at 30, 45, and 60 days after transplanting in field condition

Treatments	30 DAT		45 DAT		60 DAT	
T_1	14.27 ab	4.75 b	19.25 b	8.25 a	34.67 b	8.25 bc
T_2	11.90 b	4.50 b	18.67 b	7.00 b	33.58 b	7.33 c
T_3	17.09 a	6.41 a	27.16 a	9.00 a	46.91 a	10.25 a*
T_4	16.02 a	6.08 a	25.12 a	9.00 a	48.50 a	9.33 ab
CV (%)	13.03	8.57	5.37	6.95	3.83	8.10
SE (±)	1.13	0.26	0.69	0.33	0.90	0.58

*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.

Effect of chitosan on Fusarium wilt disease incidence and percent disease index of brinjal

Application of chitosan reduced disease incidence (DI) and percent disease index (PDI) in all treatments over pathogen treated plots. Significantly the highest value of DI (45.54 %) and PDI (25.40 %) were observed in T_2 and the lowest value of DI (14.25 %) and PDI (9.23 %) were recorded in T_3 followed by T_4 treatment (Table 8 and Figure 5).

Chitosan is often used in plant disease control as a powerful elicitor rather than a direct antimicrobial or toxic agent (El-Mohamedy et al., 2013). It can effectively reduce DI and PDI of anthracnose of chili and eggplant (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. Chitosan as soil amendment was found to successfully decrease Fusarium wilt in several plant species.

The disease severity was reduced by seed treatment in sunflower and wheat (Orzali et al., 2014). These evidences are in strong agreement of current study and proved that either seed treatment (T_3) or soil amendment (T_4) with chitosan reduce disease severity and incidence of Fusarium wilt of brinjal.

Table 8. Effect of chitosan on Fusarium wilt disease incidence and percent disease index of brinjal in the field

Treatment	DI (%)	% reduction of DI over T_2	PDI	% reduction of PDI over T_2
T_1	17.75 b	-	17.55 b	-
T_2	45.54 a	-	25.40 a	-
T_3	14.25 c	68.71	9.23 c	63.66
T_4	17.33 b	61.94	10.67 c	57.99
CV (%)	4.93	-	19.68	-
SE (±)	0.67	-	1.78	-

*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 5. Disease symptoms of Fusarium wilt of brinjal caused by F. oxysporum f. sp. melongenae in field (A-B) and cross section of infected stem (C-D)

Effect of chitosan on yield and yield contributing characters of brinjal

The yield contributing components were significantly increased in all the treatments over the treatment T_2 where pathogen was inoculated in the field (Table 9). Significantly the highest number of fruits (84.89) per plant, weight of per fruit (153.79 g) and yield (43.41 t/ha) were recorded in T_4 treatment where soil amendment was done with 1.0% chitosan followed by T_3 treatment where seed treatment was done at same concentration. On the other side significantly the lowest number of fruits (52.08) per plant, weight of per fruit (114.92 g) per plant and yield (19.91 t/ha) were found in T_2 treatment where pathogen was inoculated without chitosan.

The present study revealed that the application of chitosan as seed treatment or soil amendment increased yield of brinjal as compared to pathogen inoculated plots, which is supported by Nitu et al. (2016); Akter et al. (2018); Jannat et al. (2018); Ahmed et al. (2019) who studied the efficacy of chitosan in enhancing yield of tomato and brinjal. Chitosan prompts plant growth by influencing plant physiological processes like nutrient uptake, cell elongation, cell division, enzymatic activation and synthesis of protein that can eventually lead to increased yield.

Table 9. Effect of chitosan on yield and yield contributing characteristics of brinjal

Treatments	No. of fruits/plot	No. of fruits /plant	Weight of fruits (g/plot)	Weight of per fruit (g)	Yield (t/ha)	% increase yield over T_2
T_1	279.98 c	69.98 b	8810 c	126.06 b	29.36 c	-
T_2	208.27 d	52.06 c	5970 d	114.92 b	19.90 d	-
T_3	302.77 b	73.50 b	10997 b	148.16 a*	36.66 b	84.22
T_4	358.73 a	84.89 a	13023 a	153.79 a	43.41 a	118.14
CV (%)	3.70	3.88	4.40	6.77	4.39	-
SE (±)	1.15	0.42	246.24	5.30	0.61	-

*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.

Conclusion

Based on the findings of this study, it could be concluded that chitosan at 1.0% concentration was found to be effective against the virulent isolate F-1 of *F. oxysporum* f. sp*. melongenae*. Seed treatment or soil amendment with same dose not only reduced seedling mortality, disease incidence and severity but also increased the germination percentage, seedling growth and yield of brinjal. Farmers can adopt chitosan @ 1.0% for plant growth promotion and suppression of Fusarium wilt of brinjal as ecofriendly and low-cost alternative to chemical pesticides.

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Author's declaration and contribution

Authors declare that there is no conflict of interest. S. C. and R. J. conceived the presented idea. They also conducted both laboratory and field experiments and recorded the observations accurately. M.T.R. performed the computations and made the draft manuscript. M.M.H. and M. R. A. verified the statistical data analysis and updated the references. Finally, all authors read the manuscript carefully and approved the final version.

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