

Chitosan for Plant Growth Promotion and Disease Suppression against Anthracnose in Chilli

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Abstract— Chitosan is naturally occurring compound potentially used in sustainable agriculture to control plant diseases and enhance growth. An attempt was made to control anthracnose or ripen fruit rot of chilli caused by *Colletotrichum capsici* in the field under inoculated condition and to increase the growth and yield of chilli by different concentrations of chitosan as seed treatment and foliar application methods. *C. capsici* isolate “So” was found to be the most virulent against chilli at the time of pathogenicity test. Chitosan at 1% concentration was found to be most effective against the radial growth of *C. capsici*. Subsequently, seed treatment or foliar spray was done with *C. capsici* spore suspension ($5 \times 10^6 \text{ ml}^{-1}$) and different concentrations of chitosan as per requirement of the treatments. Anthracnose or ripen fruit rot of chilli and post-harvest disease incidence (DI) and percent disease index (PDI) were significantly lowest in the treatment T₈, where seeds were treated with 1% chitosan combination with foliar spray of chitosan (0.5%) in pathogen inoculated condition. On the contrary, anthracnose or ripen fruit rot of chilli and post-harvest DI and PDI were significantly highest in the treatment T₁, where seeds were treated with *C. capsici*. Germination percentage, growth promoting components, yield and thousand seed weight (TSW) were also highest in treatment T₈ compared to all other treatments. As a result, the combined use of chitosan as seed treatment (1%) and foliar spray (0.5%) appeared to be most effective in controlling anthracnose of chilli and increased yield and yield contributing characters.

Keywords— Anthracnose, biological control, chitosan, *Colletotrichum capsici*, yield.

I. INTRODUCTION

Chilli (*Capsicum frutescense* L.) is an important spice and cash crop that is grown throughout tropical, subtropical and temperate regions. In Bangladesh, 102.06 thousand hectares of cultivable land is under chilli cultivation and the country produced 1.30 lakh tons of chilli in 2015-2016 with an average yield 1.27 t/ha (Annon., 2017). Diseases are one of the main constraints for the low yield and quality of this crop (Poonpolgun and Kumphai, 2007). Anthracnose or ripen fruit rot of chilli caused by *Colletotrichum capsici* is one of the most devastating disease of chilli. Anthracnose is mainly a problem on mature fruits, causing severe losses due to both pre-and post-harvest fruit decay (Hadden and Black, 1989).

At present diseases are mainly managed by the use of chemicals such as fungicides. The continuous and indiscriminate use of chemicals to manage the crop diseases results in accumulation of harmful chemical residues in the soil, water and grains. The indiscriminate use of chemicals is not only hazardous to living being but also breaks the natural ecological balance by killing the beneficial and antagonistic microorganisms. In addition, over usage of synthetic fungicide has facilitated the development of fungicide resistance among some pathogenic population (Rhouma *et al.*, 2009). Therefore, in the absence of anthracnose resistant cultivars, bio-pesticides, such as chitosan offer a more sustainable approach for the control of diseases in fruits and vegetables (Bautista-Baños *et al.*, 2006; Ali *et al.*, 2010; Maqbool *et al.*, 2010). Chitosan is a linear polysaccharide composed of randomly distributed β -(1 \rightarrow 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 (Rodriguez-Pedroso *et al.*, 2009). 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 (Bautista-Baños *et al.*, 2016). Vasudevan *et al.*, (2002) 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. Chitosan coatings can control several fungal diseases in plants such as table grapes (Romanazzi *et al.*, 2006), delay the ripening mechanisms in banana fruits (Maqbool *et al.*, 2010) and induce resistance against many pathogens (Wilson *et al.*, 1994; Benhamou, 1998). It has been hypothesized that the interaction of chitosan with negatively charged molecules on the fungal cell surface causes leakage of proteinaceous compounds (Leuba and Stossel, 1986). It is also believed that the interaction between chitosan and fungi leads to the inhibition of mRNA and protein synthesis (Hadwiger, 1999). However, using chitosan very little is known about disease control and enhancement of growth and yield of chilli in Bangladesh. For that reason, this study was undertaken to select the most effective dose of chitosan in reducing the mycelial growth of *C. capsici* and to evaluate the effect of chitosan in controlling the disease and progress growth and yield of chilli.

II. MATERIALS AND METHODS

2.1 Experimental site and materials

A field experiment was carried out at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, during the period from March 2016 - August 2017. Seeds sample of chilli variety "BARI Morich 3" was collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh.

2.2 Collection, isolation and identification of *C. capsici*

Three isolates of *C. capsici* were collected from infected region of chilli fruits following standard phytopathological procedures (Dasgupta, 1981; Agostini and Timmer, 1992). Disease samples were collected from the field and carried to the laboratory in polythene bags. The infected plant parts were cut into small pieces (1 cm²) in advance lesion margin

where healthy and disease tissues remain together. The cut plant parts and fruit tissues were surface sterilized by 1% sodium hypochloride (NaOCl) solution for 2-3 minutes then washed three times with sterile deionized water to remove the NaOCl. The cut pieces were then placed onto sterilized agar (20 ml) in glass petridishes and incubated at room temperature (25 ± 2 °C) until acervuli formation. Conidia produced in acervuli came out in the form of ooze and were placed onto PDA and incubated at room temperature for seven days. The fungal pathogen was identified by observing the morphological feature, acervuli formation, presence or absence of setae, cultural and conidial characters under compound microscope (Barneet and Hunter, 1972).

2.3 Preparation of spore suspension

Ten days old culture grown on PDA was flooded with 10 ml of sterilized distilled water. Acervuli and conidia along with mycelial mass were separated from the substratum by scrapping with a narrow edge sterilized glass slide (Sharif and Bhuiyan, 2005). The suspension was sieved through double layer cheese cloth to discard acervuli and mycelial mass. The spore suspension was adjusted to 5×10⁶ ml⁻¹ by adding sterilized distilled water and counting under compound microscope using counting slide (haemocytometer). Seeds were submerged in spore suspension with gentle stirring for 5 minutes. The wetted seeds were air dried in a sterile cabinet and prepared for further treatment.

2.4 Pathogenicity test for the selection of virulent isolate of the test pathogen in pot culture

Three isolates of *C. capsici* named as So, Mo and Ba were evaluated for their pathogenicity test in the pot culture experiment under the shade condition. Each earthen pot was filled with 2.0 kg sterilized soil. Twenty-five pieces of chilli seeds for each pot were inoculated with spore suspension of So, Mo and Ba isolate of *C. capsici*. Then, the inoculated seeds were sown in each pot and in control pot healthy seeds were sown without inoculation. Disease development was observed regularly 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 agent of seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds.

2.5 Pathogenicity test of the test pathogen on detached chilli fruit

Pathogenicity test with three selected isolates of the pathogen *C. capsici* was conducted by inoculating harvested chilli fruit. Each isolate of *C. capsici* was grown on PDA plates separately. Inoculation was made by puncturing the tissue with small sterilized needle and then spore suspension (5×10⁶ spore ml⁻¹) of *C. capsici* sprayed over the chilli fruit. After

inoculation, chilli fruits were kept in a separate transparent polyethylene bag to avoid drying. Inoculated chillies were incubated for four days at room temperature. After four days, percent disease index (PDI) was rated following 0-4 scale, where 0=No visible sign or symptoms, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% infection on chilli fruits (Abraham *et al.*, 1996).

2.6 Collection of chitosan

Chitosan was collected from Bangladesh Atomic Energy Commission (BAEC), Dhaka, Bangladesh. Chitosan was derived from the shell 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.

2.7 In-vitro evaluation of chitosan for their inhibitory effect on the radial growth of *C. capsici*

Several preliminary evaluations of chitosan were done with different concentration of chitosan such as 0.4%, 0.6%, 0.8%, 1% and 1.2% on PDA plate against the *C. capsici*. Plates were individually challenged at the center with equal agar plug (5 mm in diameter) taken from 7 days old culture of the pathogen and incubated at 25°C. Mean colony diameter was measured when the control plate (without chitosan) reached full growth. The radial growths of *C. capsici* in three replications were recorded separately and there averages were taken. The percent inhibition of the radial growth was calculated as described by Sundaret *et al.*, (1995).

$$\% \text{ inhibition over control} = \frac{X - Y}{X} \times 100 \text{ (equation 1)}$$

Where,

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

Y = Mycelial growth of pathogen with chitosan

2.8 Seed treatment with chitosan and rising of seedlings

Seeds of chilli were surface sterilized by immersion of 1% NaOCl. Then seeds were thoroughly rinsed in sterile distilled water and immersed into each of the chitosan solution (p^H 5.5-6.0) at concentrations ranging from 0.6-1%. After gentle stirring, seeds were submerged for 1 hour. Finally, the wetted seeds were air dried in a sterile cabinet and sown in the tray containing soil that was well prepared through mixing fertilizers and cow dung. Chitosan (0.5%) was applied as foliar spray at three times such as 10 days after transplanting, at vegetative stage and fruiting stage.

2.9 Land preparation and transplanting of seedlings

Land was prepared with well tilth using a tractor driven disc plough and harrow. After land preparation the whole experimental area was divided into three blocks, representing three replications. The unit plot size was 3.0 m X 2.0 m.

Distance between block to block was 1.0 m and that plot to plot in a block was 0.50 m. Drains were made surrounding the each unit plots and the excavated soil was used for raising plots 15 cm high from the general soil surface. Ten different treatments were allotted randomly to each block. Thirty-five days aged apparently healthy chilli seedlings of variety 'BARI Morich 3' was collected from the tray. A total of 12 seedlings were planted in each plot on November 2016. Weeding, irrigation and other intercultural operations were done as and when necessary until the maturity of plants.

2.10 Treatments of the experiment

The treatments of the field experiment were as follows:

T₀= Untreated healthy seeds without pathogen (Control 1)

T₁= Seed treated with *C. capsici* (Control 2)

T₂= Seed treated with *C. capsici* + 0.6% chitosan

T₃= Seed treated with *C. capsici* + 0.8% chitosan

T₄= Seed treated with *C. capsici* + 1% chitosan

T₅= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan

T₆= T₂ + Foliar spray (0.5%) of chitosan

T₇= T₃ + Foliar spray (0.5%) of chitosan

T₈= T₄ + Foliar spray (0.5%) of chitosan

T₉= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

2.11 Observation of disease development

Chilli plants were observed regularly after transplanting of seedlings to record the incidence of post emergence seedling mortality, anthracnose diseases on fruits. The disease incidence was recorded two times at 90 and 120 day after transplanting (DAT). Observations were made by selecting six plants randomly from each plot. Flowerings of chilli plants were started at 40-45 DAT. Plants were produced flowers and fruits continuously up to 3-4 months after transplanting. But all fruits didn't mature and ripe at a time. The ripened fruits were harvested and weighed and it was continued up to 4 months after transplanting. Post-harvest disease incidence and PDI were recorded in the laboratory at 135 days after harvesting (DAH).

2.12 Data recording and disease assessment

Data were taken on germination percentage, mortality percentage, root length and shoot length, root fresh weight and shoot fresh weight, root dry weight and shoot dry weight, plant height and number of branch, disease incidence and percent disease index (PDI) and yield. The following formulae were used for calculation (Rahman *et al.*, 2013).

Disease incidence

$$= \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100 \text{ (equation 2)}$$

$$\text{Disease index} = \frac{\text{Summation of all rating of fruits observed}}{\text{Number of fruits observed} \times \text{Maximum rating}} \times 100 \text{ (equation 3)}$$

Percent disease index (PDI) was rated by following 0-4 scale, where 0=No visible sign or symptoms, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% infection on chilli fruits.

2.13 Experimental design and data analysis

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Data recorded on various parameters were analyzed statistically using the Statistix 10 statistical computer programme after proper transformation whenever necessary. The means were compared following LSD (Least significance Differences) test (Gomez and Gomez, 1984).

III. RESULTS

3.1 Pathogenicity test of *C. capsici* isolates against chilli seedlings in pot culture and detached chilli fruits

The pathogenicity test of the three selected isolates of *C. capsici* against chilli seedlings variety BARI Morich-3 were conducted in pot containing sterilized soil to find out most virulent isolate of the test pathogen. All the isolates of the test pathogen were virulent but variable in causing total seedling mortality of chilli ranged from 28.32-63.00% (Table 1 and Fig. 1-2). The *C. capsici* isolate So was appeared to be the most virulent causing the highest 63.00% total seedling mortality followed by isolate Mo caused 43.00% mortality. Significantly the lowest 28.32% total seedling mortality was observed with the isolate Ba. No pre-emergence and post-emergence seedling mortality was observed in the untreated control pot. Furthermore, every isolates were sowed pathogenic reaction to the detached chilli fruit (Table 1 and Fig. 3). All the three isolates were able to develop characteristic symptom such as sunken necrotic lesion with concentric ring of acervuli in chilli fruits. Chilli fruit which was inoculated with the isolate So showed highest PDI (83.33%). The virulence of the isolates in detached fruits was supported by the virulence of the same isolates in the earlier pot culture.

3.2 Effect of chitosan on mycelial growth of virulent isolate of *C. capsici*

The mycelial growth of the test pathogen was significantly reduced with all the five selected concentrations viz., 0.4%, 0.6%, 0.8%, 1% and 1.2% of chitosan as compared to untreated control (Table 2 and Fig. 4). All the five concentrations of chitosan are significantly variable in reducing the mycelial growth of *C. capsici*. Significantly the highest 100.00% reduction of the mycelial growth of *C. capsici* over the control PDA plate was observed at both 1%

and 1.2% of chitosan amended with PDA plate followed by the second highest 0.8% of chitosan with 87.79% reduction of mycelial growth. Significantly the lowest 28.56% reduction of the mycelial growth of *C. capsici* was observed at the lowest 0.4% concentration of chitosan amended with the PDA plate. Based on the *in-vitro* evaluation 0.6%, 0.8% and 1% chitosan concentrations were selected for the field trial.

3.3 Effect of chitosan on germination percentage and post-emergence seedlings mortality

In addition, to know the effect of chitosan on germination and post-emergence seedling mortality chilli seeds were treated with 0.6%, 0.8% and 1% of chitosan. For positive control Bavistin (0.1%) 50 WP was used and there was no treatment 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 seeds treated with *C. capsici* (Table 3). The range of germination percentage was 56.37-92.10%. Significantly the highest germination percentage 92.10% was in the treatment where seeds were treated with *C. capsici* and 1% chitosan and significantly the lowest germination percentage 56.37% was in the treatment where seeds were treated with *C. capsici* without chitosan or any fungicides such as Bavistin (0.1%). In case of seedling mortality all treatments reduced post-emergence seedling mortality compared to the treatment where seeds were treated with *C. capsici* (Table 3). Significantly the lowest 7.67% post-emergence seedling mortality was found in the treatment where seeds were treated with *C. capsici* and 1% chitosan. No statistical difference was found among the treatments where seeds were treated with 0.6% chitosan, 0.8% chitosan and 0.1% Bavistin. This experiment showed that seed treatment with 1% chitosan was most effective to increase germination and to control post-emergence seedling mortality of chilli.

3.4 Effect of chitosan and Bavistin on growth of chilli seedlings

Thirty-five days after sowing growth promoting components such as shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight were increased in the treatments where seeds were treated with chitosan in pathogen inoculated condition comparison to the treatment where seeds were treated with *C. capsici* (Table 4). Seed treatment with 1% chitosan showed the highest shoot length (14.84 cm), root length (8.09 cm), fresh shoot weight (0.63 g), fresh root weight (0.045 g), dry shoot weight (0.084 g) and dry root weight (0.0098 g) in pathogen inoculated condition. The lowest shoot length (9.48 cm), root length

(4.68 cm) and fresh shoot weight (0.46 g) were recorded in the treatment where seeds were untreated and the lowest fresh root weight (0.019 g), dry shoot weight (0.055 g) and dry root weight (0.0020 g) were recorded in the treatment where seeds were treated with *C. capsici*. In the field, plant height and number of branches were observed at mature stage of chilli plant. All the applications of chitosan as seed treatment or foliar spray increased the plant height and number of branches in comparison to the treatment T₁ where seeds were treated with *C. capsici* (Table 5). The highest plant height (58.57 cm) at flowering stage (45 DAT) and (60.83 cm) at fruiting stage (60 DAT) were recorded in the treatment T₈ where seeds were pathogen inoculated condition and treated with 1% chitosan and foliar spray (0.5%) of chitosan. The lowest plant height (45.52 cm) at flowering stage and (47.93 cm) at fruiting stage were recorded in the treatment T₁ where seeds were treated with *C. capsici*. In the same way, the highest number of branches (17.55) at flowering stage and (21.59) at fruiting stage were recorded in the treatment T₈ where seeds were pathogen inoculated condition and treated with 1% chitosan and foliar spray (0.5%) of chitosan. The lowest number of branches (11.30) at flowering stage and (13.18) at fruiting stage were recorded in the treatment T₁ where seeds were treated with *C. capsici*.

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

Disease incidence and percent disease index (PDI) were reduced in the treatments where chitosan or Bavistin were used as seed treating agent or foliar spray of chitosan over pathogen treated plots. Disease incidence and PDI were recorded two times before first and second harvesting of fruits. Significantly the highest DI (64.67%) at 90 days after transplanting (DAT) and (90.33%) at 120 DAT and PDI (3.34%) at 90 DAT and (4.96%) at 120 DAT were recorded in the treatment T₁ where seeds were treated with *C. capsici* (Table 6). Significantly the lowest DI (10.08%) at 90 DAT and (22.63%) at 120 DAT and PDI (0.51%) at 90 DAT and (1.29%) at 120 DAT were recorded in the treatment T₈ where seeds were pathogen inoculated condition and treated with 1% chitosan and foliar spray (0.5%) of chitosan.

3.6 Effect of chitosan and Bavistin on the yield and thousand seed weight (TSW) of chilli

Results of the present study indicates that by the application of different treatments of chitosan yield and yield contributing components were significantly increased in all the treatments over the treatment T₁ where seeds were treated with *C. capsici* (Table 7). Significantly the highest yield (7.03 t/ha) was recorded in the treatment T₈ where seeds were treated with *C. capsici*, 1% chitosan in combination with foliar spray (0.5%) of chitosan. Significantly the lowest yield

(2.86 t/ha) was recorded in the treatment T₁ where seeds were treated with *C. capsici*. Total three harvests were done at different times as chilli is an indeterminate crop and fruits are not mature and ripen at the same time. Moreover, applications of chitosan significantly increased TSW compare to pathogen inoculated condition. Significantly highest TSW (5.04 g) was recorded in the treatment T₈ where seeds were pathogen inoculated condition and treated with 1% chitosan and foliar spray (0.5%) of chitosan. On the contrary, significantly the lowest TSW (4.08 g) was recorded in the treatment T₁ where seeds were treated with *C. capsici*.

3.7 Effect of chitosan and Bavistin on post-harvest disease incidence (DI) and percent disease index (PDI)

Post-harvest disease incidence and PDI were recorded in the laboratory at 135 days after harvesting (DAH). Post-harvest disease incidence and PDI were reduced in those treatments where chitosan or Bavistin were used as seed treating agent or foliar spray (0.5%) of chitosan in the field compare to pathogen inoculated condition (Table 8). Significantly the highest post-harvest DI (32.61%) and PDI (0.165%) were recorded in the treatment T₁ where seeds were treated with *C. capsici*. Significantly the lowest post-harvest DI (8.58%) and PDI (0.026%) were recorded in the treatment T₈ where seeds were treated with 1% chitosan combined with foliar spray of 0.5% chitosan in pathogen inoculated condition.

IV. DISCUSSION

The anthracnose or ripen fruit rot of chilli is a seed borne as well as soil borne disease caused by *Colletotrichum capsici* that occurs every year with intensities and imposes considerable quantitative and qualitative losses of the crop in the field as well as in the storage. This disease is mostly difficult to control as *Colletotrichum* species naturally produce micro-sclerotia to allow dormancy in the soil during the winter or when subjected to stressful conditions and these micro-sclerotia can survive for many years (Pring *et al.*, 1995) and subsequently attack the crop resulting poor yield. In recent times, chitosan has been extensively used to control fungal diseases in many fruits and vegetables. In this study, all the tested concentrations of the chitosan were found effective against *C. capsici* which was a dose dependent manner. The complete reduction of the mycelial growth of *C. capsici* was got at 1% of chitosan. The result is supported by the reports of Chookhongkga *et al.*, 2013 where chitosan was found to reduce the mycelial growth of *Rhizopus* sp., *C. capsici*, *C. gloeosporioides* and *Asperillus niger*. Chitosan shows direct toxicity to pathogens, can form physical barriers around the penetration sites of pathogens, preventing them from spreading to healthy tissues and is known to induce reactions locally and systemically that entail signaling

cascades, and the activation and accumulation of defenses-related antimicrobial compounds and proteins. It is also reported to induce a decline in malondialdehyde content, alter the relative permeability of the plasma membrane and increase the concentrations of soluble sugars and proline, and of peroxidase and catalase activities. Chitosan is often used in plant disease control as a powerful elicitor rather than a direct antimicrobial or toxic agent (Algam *et al.*, 2010).

We observed highest seedling mortality, disease incidence (DI) and percent disease index (PDI) and lowest seed germination, growth, yield and thousand seed weight (TSW) of chilli in pathogen inoculated condition. Application of chitosan increased seed germination, growth promoting components, yield and TSW and reduced seedling mortality, DI and PDI. The highest post-emergence mortality was found in chilli caused by *C. capsici*. The pre-emergence and post-emergence mortality of different vegetable crops including chilli caused by the tested pathogen are also reported by Simi, 2017. Significantly the highest germination percentage and the lowest post-emergence seedling mortality were found in the treatment where seeds were treated with *C. capsici* and 1% chitosan. The results are supported by Photchanachai *et al.*, 2012 and Nitu *et al.*, 2016 that chitosan increased germination percentage and reduced post-emergence seedling mortality of different crops. Moreover, we have observed highest shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight in the treatments where seeds were treated with chitosan in pathogen inoculated condition compared to the treatment where seeds were treated with *C. capsici*. The results of the present study supported by the observation of Benhamou *et al.*, 1994, O'Herlihy *et al.*, 2003; Guan *et al.*, 2009 who observed the enhancement of growth promoting components including shoot length, root length etc. of different crops by chitosan. Chitosan has also been extensively utilized as a foliar treatment to control the growth, spread and development of fungal diseases and to increase yield and quality in many crops. Consequently, we found that foliar application of chitosan increased the plant height and number of branches. The highest plant heights and number of branches and the lowest DI and PDI were recorded in the treatment T₈ where seeds were treated with 1% chitosan combination with foliar spray of 0.5% chitosan in pathogen inoculated condition. These results are supported by Mondal *et al.*, (2013) that foliar application of chitosan increased most of the morphological characters such as plant height, leaf number per plant and growth such as total dry mass per plant, absolute growth rate, relative growth rate with increasing concentration of chitosan in okra. Moreover, Faoro *et al.*, (2008) showed that the use of chitosan applied

as a foliar spray on barley reduced locally and systemically the infection by powdery mildew pathogen *Blumeria graminis* f. sp. *hordei*. The results of the current study justify the statement of Benhamou *et al.*, (1994); O'Herlihy *et al.*, (2003) and Edirisinghe *et al.*, 2014 that chitosan has been considered as alternate to chemical fungicides to control diseases in different crops. However, significantly highest yield and TSW of chilli were recorded in the treatment T₈ and these results are supported by Walker *et al.*, (2004) and Mondal *et al.*, (2013) that chitosan increased yield and yield attributes with increasing concentration of chitosan in different crops. Edirisinghe *et al.*, (2014) and Sivakumar *et al.*, (2016) also examined that chitosan could become a promising substance to control postharvest diseases in different crops. Accordingly, post-harvest DI and PDI were reduced by all the chitosan treatments over the treatment T₁ and significantly lowest post-harvest DI and PDI were found by combined use of chitosan as seed treatment and foliar spray. Edirisinghe *et al.*, (2014), Berumen-Varela *et al.*, (2015), Romanazzi & Feliziani (2016) and Sivakumar *et al.*, (2016) reported that chitosan increases phenyl ammonia lyase, chitinase (endo and exochitinases) and β -1,3-glucanase activities in numerous treated tropical and temperate fruits. They also found that chitosan application induces fruit disease resistance during fungal infection through regulation of reactive oxygen species (ROS) levels, antioxidant enzymes, and the ascorbate-glutathione cycle. The present study revealed that combined use of chitosan as seed treatment and foliar spray could provide excellent protection against anthracnose or ripen fruit rot of chilli, had influenced on growth promoting components, yield, TSW and reduced post-harvest DI and PDI.

V. CONCLUSION

The present study showed that seed treatment with 1% chitosan combined with foliar spray of 0.5% chitosan was appeared to be excellent in controlling post-emergence seedling mortality, anthracnose or ripen fruit rot of chilli, post-harvest disease incidence (DI) and percent disease index (PDI) with the significant increase of growth, yield and thousand seed weight. Moreover, Chitosan at 1% concentration completely inhibited the mycelial growth of *C. capsici* in *in vitro* evaluation. Growers can adopt eco-friendly control measures against anthracnose or ripen fruit rot of chilli through the seed treatment with 1% chitosan combined with foliar spray of 0.5% chitosan as an alternative to chemical fungicides.

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Table.1. Pathogenicity of *Colletotrichum capsici* isolates on seedlings and fruits of chilli

<i>Colletotrichum capsici</i> isolates	Seedlings			Fruits
	Pre-emergence mortality (%)	Post-emergence mortality (%)	Total mortality (%)	PDI
So	17.00	46.00	63.00 a	83.33 a
Ba	9.66	18.66	28.32 c	56.25 c
Mo	8.30	34.70	43.00 b	62.50 b
Untreated Control	0.00	0.00	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.2. Effect of chitosan on mycelial growth of *C. capsici*

Treatment	Average mycelial growth after 10 days (mm)	% mycelial growth inhibition over control
Control	90.00 a	-
0.4% chitosan	64.30 b	28.56
0.6% chitosan	36.70 c	59.22
0.8% chitosan	11.00 d	87.78
1% chitosan	0.00 e	100.00
1.2% chitosan	0.00 e	100.00

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

Table 3. Effect of chitosan and Bavistin on germination percentage and post-emergence seedling mortality

Treatment	% germination	% increase over control	% post-emergence mortality	% reduction over control
Control	73.00 c	29.50	13.28 b	46.88
Seed treated with <i>C. capsici</i>	56.37 d	-	25.00 a	-
Seed treated with <i>C. capsici</i> + 0.6% chitosan	82.00 b	45.00	10.50 bc	58.00
Seed treated with <i>C. capsici</i> + 0.8% chitosan	85.00 b	45.47	9.47 bc	62.12
Seed treated with <i>C. capsici</i> + 1% chitosan	92.10 a	63.38	7.67 d	67.32
Seed treated with <i>C. capsici</i> + Bavistin (0.1%) 50 WP	81.57 b	44.70	11.21bc	55.16

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

Table.4: Effect of chitosan and Bavistin on growth parameters of chilli seedlings at 35 days after sowing (DAS)

Treatments	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g/plant)	Fresh root weight (g/plant)	Dry shoot weight (g/plant)	Dry root weight (g/plant)
Control	9.48 c	4.68 c	0.46 cd	0.029 b	0.063 c	0.0064 b
Seed treated with <i>C. capsici</i>	10.12 c	5.13 c	0.53 bc	0.019 b	0.055d	0.0020 c
Seed treated with <i>C. capsici</i> +	12.90 b	6.59 b	0.43 d	0.025 b	0.066 c	0.0067 b

0.6% chitosan						
Seed treated with <i>C. capsici</i> + 0.6% chitosan	13.20 b	7.14 ab	0.57 ab	0.032 ab	0.078 b	0.0092 a
Seed treated with <i>C. capsici</i> + 0.8% chitosan	14.84 a	8.09 a	0.63 a	0.045 a	0.084 a	0.0098 a
Seed treated with <i>C. capsici</i> + 1% chitosan	12.85 b	6.46 b	0.44 d	0.031 b	0.067 c	0.0071 b
Seed treated with <i>C. capsici</i> + Bavistin (0.1%) 50 WP						

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

Table.5: Effect of chitosan and Bavistin on plant height and number of branches of chilli

Treatment	45 DAT		60 DAT	
	Plant height (cm)	No. of branching	Plant height (cm)	No. of branching
T ₀	46.47 h	13.58 h	49.68 ef	16.16 g
T ₁	45.52 i	11.30 i	47.93 f	13.18 h
T ₂	47.30 g	14.50 f	49.46 ef	17.40 e
T ₃	48.70 f	14.57 f	50.83 e	17.65 e
T ₄	53.78 d	15.05 e	56.52 c	17.72 e
T ₅	52.26 e	16.68 c	54.04 d	19.42 c
T ₆	53.93 c	15.64 d	57.71 bc	18.41 d
T ₇	57.54 b	16.90 b	59.49 ab	19.79 b
T ₈	58.57 a	17.55 a	60.83 a	21.59 a
T ₉	47.76 g	13.76 g	51.18 e	16.74 f

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

T₀= Untreated healthy seeds without pathogen (Control 1), T₁= Seed treated with *C. capsici* (Control 2), T₂= Seed treated with *C. capsici* + 0.6% chitosan, T₃= Seed treated with *C. capsici* + 0.8% chitosan, T₄= Seed treated with *C. capsici* + 1% chitosan, T₅= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan, T₆= T₂ + Foliar spray (0.5%) of chitosan, T₇= T₃ + Foliar spray (0.5%) of chitosan, T₈= T₄ + Foliar spray (0.5%) of chitosan, T₉= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

Table 6. Effect of chitosan and Bavistin on disease incidence (DI) and percent disease index (PDI) of chilli

Treatment	90 DAT				120 DAT			
	% DI	% Reduction over T ₁	PDI	% Reduction over T ₁	% DI	% Reduction over T ₁	PDI	% Reduction over T ₁
T ₀	39.37 b	39.12	1.30 b	59.68	67.83 b	24.90	2.6 b	47.58
T ₁	64.67 a	-	3.34 a	-	90.33 a	-	4.96 a	-
T ₂	28.09 c	56.56	1.20 c	64.00	58.95 c	34.74	2.36 cd	52.42
T ₃	27.07 c	58.14	1.13 c	66.07	48.67 d	46.12	2.25 d	54.64
T ₄	16.06 e	75.15	0.77 e	77.04	40.33 e	55.35	1.94 e	60.89
T ₅	16.20 e	74.95	0.79 e	76.45	34.22 fg	62.12	1.90 e	61.69
T ₆	15.13 ef	76.60	0.74 e	77.75	34.44 f	61.87	2.02 e	59.27
T ₇	13.33 f	79.60	0.71 e	78.74	31.49 g	65.24	1.79 f	63.91
T ₈	10.08 g	84.41	0.51 f	84.83	22.63 h	74.95	1.29 g	73.40
T ₉	20.37 d	68.50	0.92 d	72.46	43.13 e	52.25	2.24 d	54.84

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

T₀= Untreated healthy seeds without pathogen (Control 1), T₁= Seed treated with *C. capsici* (Control 2), T₂= Seed treated with *C. capsici* + 0.6% chitosan, T₃= Seed treated with *C. capsici* + 0.8% chitosan, T₄= Seed treated with *C. capsici* + 1% chitosan, T₅= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan, T₆= T₂ + Foliar spray (0.5%) of chitosan, T₇= T₃ + Foliar spray (0.5%) of chitosan, T₈= T₄ + Foliar spray (0.5%) of chitosan, T₉= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

Table 7. Effect of chitosan and Bavistin on yield and thousand Seed weight (TSW) of chilli

Treatment	Yield t/ha	% increased yield over T ₁	TSW (g)	% increased TSW over T ₁
T ₀	3.62 i	26.57	4.28 g	4.90
T ₁	2.86 j	-	4.08 h	-
T ₂	4.06 g	41.91	4.33 g	6.13
T ₃	4.36 f	52.45	4.49 f	10.05
T ₄	4.80 e	67.83	4.61 de	12.10
T ₅	5.60 d	95.80	4.56 e	11.76
T ₆	5.98 c	109.09	4.75 c	16.42
T ₇	6.20 b	116.78	4.88 b	19.61
T ₈	7.03 a	145.80	5.04 a	23.52
T ₉	3.94 h	37.76	4.67 d	14.46

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

T₀= Untreated healthy seeds without pathogen (Control 1), T₁= Seed treated with *C. capsici* (Control 2), T₂= Seed treated with *C. capsici* + 0.6% chitosan, T₃= Seed treated with *C. capsici* + 0.8% chitosan, T₄= Seed treated with *C. capsici* + 1% chitosan, T₅= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan, T₆= T₂ + Foliar spray (0.5%) of chitosan, T₇= T₃ + Foliar spray (0.5%) of chitosan, T₈= T₄ + Foliar spray (0.5%) of chitosan, T₉= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

Table 8: Effect of chitosan and Bavistin on post-harvest disease incidence (DI) and percent disease index (PDI) of chilli at 135 days after harvesting

Treatment	% DI	% reduction over T ₁	PDI	% reduction over T ₁
T ₀	24.83 b	23.86	0.140 b	15.15
T ₁	32.61 a	-	0.165 a	-
T ₂	17.08 c	47.62	0.065 c	60.60
T ₃	15.62 d	52.10	0.061 d	63.03
T ₄	12.08 f	62.96	0.055 e	66.67
T ₅	14.54 e	55.41	0.057 e	65.45
T ₆	12.78 f	60.81	0.045 f	72.73
T ₇	12.33 f	62.19	0.042 f	74.55
T ₈	8.58 g	73.69	0.026 g	84.24
T ₉	14.89 de	54.34	0.057 e	65.45

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

T₀= Untreated healthy seeds without pathogen (Control 1), T₁= Seed treated with *C. capsici* (Control 2), T₂= Seed treated with *C. capsici* + 0.6% chitosan, T₃= Seed treated with *C. capsici* + 0.8% chitosan, T₄= Seed treated with *C. capsici* + 1% chitosan, T₅= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan, T₆= T₂ + Foliar spray (0.5%) of chitosan, T₇= T₃ + Foliar spray (0.5%) of chitosan, T₈= T₄ + Foliar spray (0.5%) of chitosan, T₉= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP



Fig. 1: Pathogenicity test of *C. capsici* isolates against chilli seedling

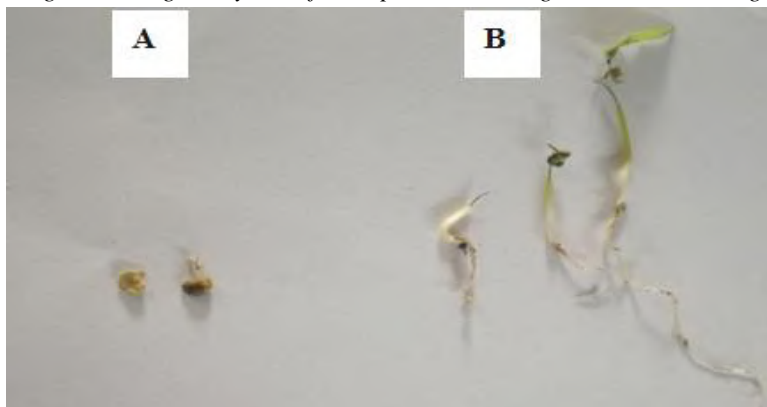


Fig. 2: Pre-emergence (A) and post-emergence (B) seedling mortality



Fig. 3: Pathogenicity test of *C. capsici* isolates on chilli fruit

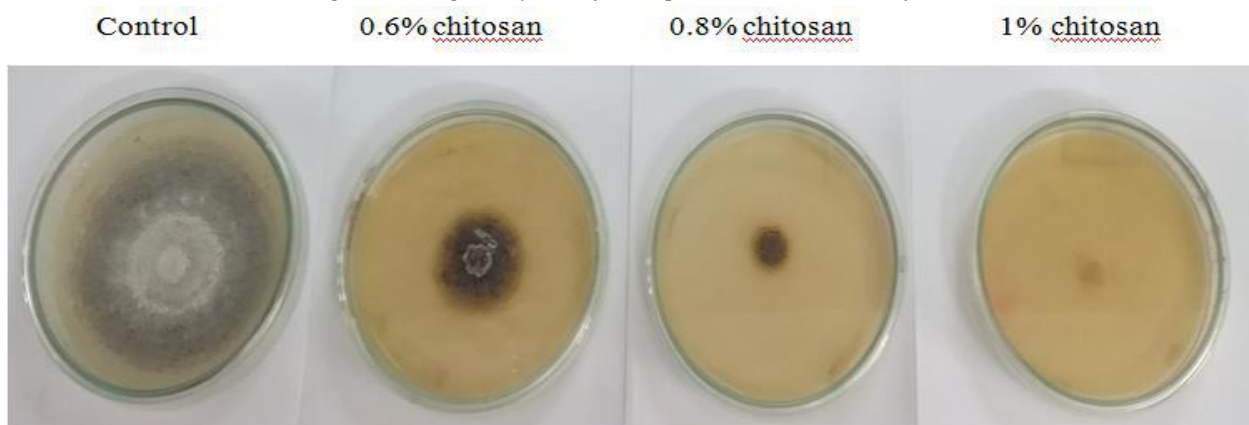


Fig. 4. Mycelial growth inhibition of *C. capsici* by chitosan on PDA