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Role of Chitosan in Induction of Defense Response against *Phomopsis vexans* and Augmentation of Growth and Yield of Eggplant

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Role of Chitosan in Induction of Defense Response against *Phomopsis vexans* and Augmentation of Growth and Yield of Eggplant

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Abstract- Phomopsis blight and fruit rot disease caused by the fungus *Phomopsis vexans* is one of the most devastating diseases of eggplant that causes great economic losses especially in tropical and subtropical climates. An experiment was conducted to evaluate the antifungal properties of chitosan against *P. vexans* by inducing defense-related enzymes and increase the growth and yield contributing characters of eggplant. *P. vexans* isolate VS-2 was identified as most virulent against eggplant by pathogenicity test. The concentrations of chitosan 0, 0.1, 0.2, 0.3, 0.4 and 0.5% were used to control the fungus in *in-vitro* trial. There was an effect on mycelial growth reduction of *P. vexans* by chitosan. The highest mycelial growth reduction was found with 0.5% chitosan at seven days after incubation. Consequently, seeds were treated with chitosan @ 0.5% concentration. Chitosan enhanced the germination percentage and seedling growth such as shoot length, root length, fresh weight and dry weight and reduced post-emergence seedling mortality. In the field condition, chitosan reduced leaf blight and fruit rot disease incidence and severity, simultaneously increased plant height and number of branching in eggplant after certain maturity. It was also found to increase the yield and yield contributing characters of eggplant. The defense enzymes catalase (CAT) and peroxidase (POD) activities were also increased several folds by this elicitor. Chitosan contains antifungal properties against *P. vexans* as well as growth promoting substances to induce resistance of eggplant.

Keywords: chitosan, defense, eggplant, growth, phomopsis blight and fruit rot, P. vexans, yield.

I. Introduction

Eggplant (*Solanum melongena* L.) belongs to the family Solanaceae is a popular and widely grown year-round vegetable of Bangladesh. In Bangladesh, eggplant is the second most important vegetable crop with respect to total acreage (50181.02 ha) and production (475,000 MT annually) (BBS, 2017). Eggplant suffers heavy yield losses due to many diseases like bacterial wilt, little leaf, Phomopsis blight and fruit rot, Verticillium wilt, Sclerotinia blight, Fusarium wilt, root-knot and leaf spots, etc. Among them, Phomopsis blight and fruit rot caused by *Phomopsis vexans* have been treated as one of the constraints to eggplant cultivation in the country (Khan, 1999). This disease appears as damping off, tip over and seedling blight in the nursery and fruit rot in the harvesting crop (Singh, 1992).

Seed is the infection source of *P. vexans* and may serve as a substrate for pathogen survival (Pan and Acharya, 1995). The pathogens remain on the seed coat and the cotyledons of eggplant seeds which causes various degrees of seed discoloration and tiny black pycnidial bodies (Karuna *et al.*, 1994). Fresh seeds can be separated easily by discarding the infected, abnormal and discolored seed.

Nowadays new approaches and practices are being developed for sustainable crops and vegetable production in Bangladesh. Chitosan is one of the most abundant natural amino polysaccharides extracted from the exoskeleton of crustaceans, insect, fungal cell walls, etc. Chitosan has a wide variety of applications in agricultural and biotechnological industries (Brine *et al*., 1992; Majeti and Kumar, 2000). The antimicrobial activity of chitosan was recognized and considered as important natural properties which can be used directly for plant disease suppression (Zhao and Xia, 2006).

Management of many fungal pathogens in different pathosystems through the application of chitosan is well documented (Abd-El-Kareem *et al*., 2006; Chittenden and Singh, 2009; El-Mohamedy *et al*., 2014).

Defense mechanisms in plant has been accelerated by using chitosan. Ortega-Ortiz *et al.,* (2007) reported that chitosan had increased catalase (CAT) and peroxidase (POD) enzymes activity in *Lycopersicon esculentum*. An induction of resistance in plants by application of elicitor (chitosan) is becoming a promising approach for management of plant diseases. The introduction of chitosan into agricultural practice could minimize the scope of chemical control, thus contributing to the development of sustainable agriculture. However, there have been no published reports on induction of resistance in eggplant against *P. vexans* by chitosan. Therefore, the present experiment was designed to investigate the role of chitosan against *P. vexans* in inducing biochemical defense enzyme

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(Catalase and peroxidase) activation and increase the growth and yield of eggplant.

II. Materials and Methods

a) Experimental Site and Plant Material

The experiment was conducted in the Plant Pathology laboratory and research field in the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. Seeds of eggplant variety BARI Begun 4 (Kajla) were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Eggplant seedlings were grown in a fertile and irrigated plot an open ambient climate and net house. Leaves were collected from uniformly grown plants at seedling stage for using in enzyme extraction.

b) Isolation and Identification of P. vexans

The fungus was isolated from infected eggplants following the tissue planting method (Mian, 1995). The fungal colonies were grown on Potato Dextrose Agar (PDA) and identified by following standard key (Barneet and Hunter, 1980). The pure culture of *P. vexans* isolates was named individually with English capital letter and numerical number codes then preserved by using PDA slants at 10°C in the refrigerator as a stock culture for further study.

c) Preparation of Spore Suspension of P. vexans

The ten-days-old culture was grown on PDA plate and flooded with 10 ml of sterilized distilled water. Pycnidia and conidia along with mycelial mass were separated from the substratum by scrapping with a narrow edge glass slide (Jahanara *et al*., 2018) and blended in an electric blender at high speed for 5-7 minutes. The suspension was sieved through double layer cheesecloth to discard pycnidia and mycelial mass. The spores were counted under the compound microscope by using counting slide haemocytometer. The spore suspension was adjusted to $2x10^5$ ml^{-1} by adding sterilized distilled water (Ashrafuzzaman, 1976). Seeds were submerged in spore suspension with gentle stirring for 5 minutes, the wetted seeds were air dried in a sterilized cabinet, and then further treatments were done.

d) Pathogenicity Test for the Selection of Virulent Isolate of the Test Pathogen

Pathogenicity test was done against eggplant seedlings as well as detached fruits following the methods as stated by Jahanara *et al*., 2018. Four isolates of the test pathogen named as VS-1, VS-2, VS-3, and VS-4 were evaluated for their pathogenicity test in the tray under the shade condition. Tray was filled by sterilized soil. For inoculation purpose, seeds were treated with the spore suspension of the four isolates of *P. vexans* separately and sown in an individual tray. In control tray seeds were sown without any treatment. Disease development was observed timely and recorded at 20 days after sowing (DAS) to estimate the effect of the pathogen in causing pre-emergence and post-emergence seedling mortality. The causal agent of pre-emergence and post-emergence seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds and death seedlings respectively. For detached fruits, at first eggplant fruits were rinsed with sterilized water and air dried. The inoculation process was made by puncturing the tissue with small sterilized needle. Spore suspension (2×10^5 spore ml^{-1}) of *P. vexans* was sprayed over the eggplant fruits. The un-inoculated fruit was selected as control treatment. After inoculating, eggplant fruits were kept in a transparent polyethylene bag separately to maintain humidity for seven days at room temperature (25 ± 2°C). Disease symptoms and pycnidia formation were recorded, and 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 eggplant fruits (Abraham *et al*., 1996).

e) In Vitro Evaluation of Chitosan for their Inhibitory Effect on the Radial Growth of P. vexans

A series of preliminary evaluation of chitosan was done by using the lower concentration such as 0.1, 0.2, 0.3, 0.4 and 0.5% on PDA plate against the *P. vexans*. Chitosan was added to conical flasks containing sterilized PDA before solidification and rotated gently then poured into sterilized petri dish (9 cm diameter). Individual plate was challenged with 7 days-old culture of the pathogen at the center by equal agar plug (5 mm in diameter), and incubated at room temperature (25 ± 2°C) for 72 hours. The colony diameter was measured when the control plate (without chitosan) reached full growth. The radial growths of *P. vexans* in three replicates were recorded separately, and their average data were taken. Based on the laboratory results, the most antagonistic effect of chitosan @ 0.5% conc. was selected for seed treating purpose. The percent inhibition of the radial growth was calculated as described by Jahanara *et al.,* (2018).

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

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

f) Seed Treatment with Chitosan

Chitosan was collected from Bangladesh Atomic Energy Commission, Dhaka, Bangladesh. It 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). Seed treatment was done by following the standard procedure described by

Mahdavi and Rahimi, 2013; Mishra *et al*., 2014. Seeds of eggplant were surface sterilized by immersion of 0.1% NaOCl, thoroughly rinsed in sterilized distilled water and were immersed into the 0.5% chitosan solution (pH 5.5-6.0). After gentle stirring submersed for 3 h, the wetted seeds were air dried in a sterilized cabinet and kept in a desiccator until use. For positive control Bavistin (0.1%) 50 WP was used, and there was no treatment in untreated control seeds. In a plastic tray, seeds were sown after required treatments for confirming the effect of chitosan on germination and post-emergence seedling mortality. Finally, data were recorded up to the complete emergence of the seedlings.

g) Land Preparation and Rising of Seedlings

The land was prepared with good tilth using a tractor driven disc plow and harrow. After land preparation the whole experimental area was divided into three blocks, representing three replications. The unit plot size was 3 m × 2 m where row to row distance 75 cm and plant to plant 75 cm. Distance between block to block was 1.0 m and that of plot to plot in a block was 0.5 m. Drains were made surrounding each unit plot and the excavated soil was used for raising plots 15 cm high from the general soil surface. Six different treatments were allotted randomly to each block. Thirty-five-days aged healthy eggplant seedlings of variety 'BARI Begun 4' were collected from the tray. Weeding, irrigation and other intercultural operations were done as and when necessary until the maturity of plants.

Treatments of the Experiment

The treatments of the field experiment were as follows:

T_1=Untreated seed (control-1)

T_2=Seed treated with *P. vexans* (control-2)

T_3=Seed treated with 0.5 % chitosan

T_4=Seed treated with *P. vexans* + 0.5% chitosan

T_5=Seed treated with 0.1% Bavistin

T_6=Seed treated with *P. vexans* + 0.1% Bavistin

h) Data Recording and Disease Assessment

Data were taken on germination percentage, mortality percentage, root length, shoot length, fresh seedling weight, seedling dry weight, enzyme activities (CAT, POD), plant height, number of branches, disease incidence, percent disease index (PDI), number of fruits, fruit weight and yield both in the absence and presence of *P. vexans*. Seedling growth was measured at 28 days after sowing (DAS). Plants were dried for dry weight measurement in an oven at 60°C for 3 days, and weight was evaluated for each treatment. Eggplants were observed regularly after transplanting of seedlings to record the incidence of post-emergence seedling mortality, Phomopsis leaf blight and fruit rot diseases on fruits. Disease incidence and PDI were recorded based on a scale of 0 to 4 as described by Abraham *et al*., (1996). Then, the following formulae were used for calculation (Rahman *et al*., 2013).

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

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

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 eggplant fruits.

i) Extraction of Protein and Defense Enzymes

The eggplant leaves were homogenized in 10 ml of chilled 0.1 M phosphate buffer (pH 7.0). The homogenate materials were centrifuged at 4°C for 30 min at 10000 rpm. After centrifugation, the supernatant portion was used as enzyme extract for the determination of enzyme activity.

j) Protein Assay

The protein content in the extracts was estimated by the dye-binding method of Bradfort (1976) using Bovine Serum Albumin (BSA) as standard.

k) Catalase (CAT) Activity Assay

The activity was assessed by measuring the rate of disappearance of H_2O_2 at 240 nm using a BioMate TM 3 spectrophotometer. The reaction mixture (2 ml) was consisting of 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.2 ml enzyme extract. Enzyme activity was calculated by using the molar extinction of co-efficient $36x10^3$ mM^{-1} min^{-1} and expressed as µmol H_2O_2 oxidized per g fresh weight per min (g^{-1} FW min^{-1}). One unit was defined as a changed in absorbance of 0.1 under the assay conditions (Cakmak and Marschner, 1992).

l) Peroxidase (POD) Assay

POD activity was assessed by following standard method (Chance and Maehly, 1955). The enzyme activity was determined by measuring with increasing in absorbance at 470 nm due to oxidation of guaiacol to tetraguaiacol. The reaction mixture was consisted of 20 mM guaiacol (0.5 ml), 0.1 mM acetate buffer (pH 5.0; 2.1 ml), 40 mM H_2O_2 (0.2 ml) and enzyme extract (0.2 ml) with a final volume of 3 ml. The linear portion of the activity curve was used to calculate enzyme activity. One POD unit was defined as the change of 1.0 absorbance unit per ml enzymatic extract and expressed as units of enzyme activity per g fresh weight per min (UA g^{-1} FW min^{-1}). One unit of enzyme activity was represented as the amount of enzyme catalyzing the oxidation of 1 µmol of guaiacol in 1 min.

m) Experimental Design and Data Analysis

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

III. Results And Discussion

a) Pathogenicity Test for the Selection of Virulent Isolate of P. vexans

The pathogenicity tests of the randomly selected four isolates of the pathogen were done against eggplant seedlings as well as in the detached fresh fruits. Seedlings were grown in the plastic tray containing sterilized soil to select the most virulent isolate of the test pathogen for evaluation the effect of chitosan. The most virulent isolate was selected based on highest seedling mortality and disease symptoms and pycnidial formation of eggplant caused by the isolate. The *P. vexans* isolate VS-2 was appeared to be the most virulent isolate. Significantly, the highest 91.83% seedling mortality was caused by VS-2 isolate followed by VS-1 (70.93%) (Table 1 and Fig. I). On the contrary, significantly the lowest (53.90%) seedling mortality was observed with the isolates VS -3. No pre-emergence and post-emergence seedling mortality was observed in the control tray. All the tested isolates of *P. vexans* were found to be virulent and seriously causing seedling mortality. Pre-emergence seedling mortality was appeared higher at ranging from 42.17-78.53% than post-emergence mortality at ranging from 11.73-14.53%. Furthermore, every isolates were showed a pathogenic reaction to the detached eggplant fruits (Table 1 and Fig. 2). Among the eggplant fruits, which were inoculated with the isolate VS-2 showed highest PDI (88.90%). The virulence of the isolates in detached fruits supports the virulence of the same isolates in the earlier tray culture experiment. These results approved by the observation of Islam and Meah, 2011. The results of the present study indicated that all the isolates were pathogenic to eggplant seedling but the virulence of the isolates were variable. The VS-2 isolate of *P. vexans* was the most virulent to eggplant, and therefore, it was selected as test pathogen for inoculation in the field trial.

Table 1: Pathogenicity Test of *P. Vexans* Isolates on Seedlings and Fruits of Eggplant

P. vexans Isolates	Seedlings			Fruits PDI
	Pre-emergence mortality (%)	Post-emergence mortality (%)	Total mortality (%)	
VS-1	58.33	12.6	70.93 b	75.25 b
VS-2	78.53	13.3	91.83 a	88.90 a
VS-3	42.17	11.73	53. 90 d	59.56 d
VS-4	45.87	14.53	60.40 c	66.11 c
Untreated Control	0.00	0.00	0.00 e	0.00 e

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

Fig. 1: Pathogenicity Test of *P. vexans* Isolates against Eggplant Seedlings in Tray Culture.

Fig. 2: Pathogenicity Test of *P. vexans* Isolates on Eggplant Fruits.

b) Effect of Chitosan on the Mycelial Growth of P. vexans

The mycelial growth of most virulent isolate VS-2 of *P. vexans* in PDA plate were amended with five different concentrations viz, 0.1, 0.2, 0.3, 0.4, and 0.5% of chitosan (Table 2 and Fig. 3). All the selected concentrations of chitosan amended with PDA plate significantly reduced the mycelial growth of *P. vexans*

over control PDA plate (without chitosan). But all the five concentrations of chitosan were significantly varied in reducing the mycelial growth of *P. vexans*. Significantly, the highest 84.58% reduction of the mycelial growth of *P. vexans* over the control PDA plate was observed at the highest 0.5% concentration of chitosan amended with PDA plate, followed by the second highest (73.75%) at 0.4% concentration of chitosan. On the other hand, significantly the lowest 22.5% reduction of the mycelial growth of *P. vexans* was observed at the lowest 0.1% concentration of chitosan amended with the PDA plate. Based on the *in-vitro* evaluation, 0.5% chitosan was selected for seed treatment. These results are supported by El-Mohamedy *et al*., 2014 who reported that chitosan applied at different concentrations (from 0.5 to 4 g/L) had decreased the mycelial growth of *Fusarium.* But the complete inhibition was obtained at the highest concentration @ 4 g/L.

Table 2: Effect of Chitosan on Mycelial Growth of *P. vexans*

Treatment	Average mycelial growth after 7 Days (mm)	% mycelial growth inhibition over control
No Chitosan (Control)	80.00 a	-
0.1% Chitosan	62.00 b	22.50
0.2% Chitosan	49.67 c	37.92
0.3% Chitosan	37.67 d	52.92
0.4% Chitosan	21.00 e	73.75
0.5% Chitosan	12.33 f	84.58

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

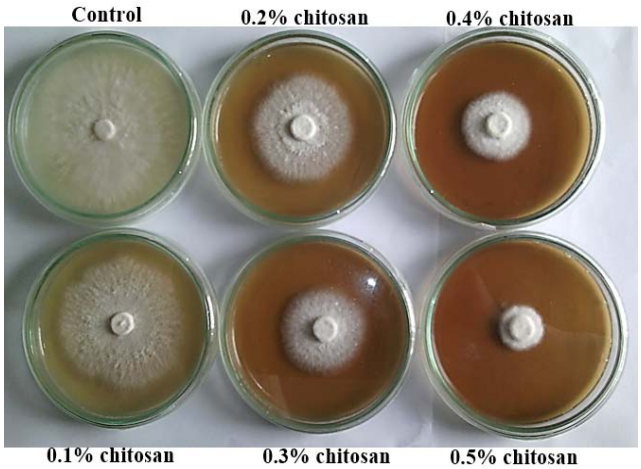

Fig. 3: Mycelial Growth Inhibition of *P. vexans* by Chitosan on PDA Plate.

c) *Effect of Chitosan and Bavistin on Germination Percentage and Post-emergence Seedling Mortality*

The germination percentage was increased in all treatments over the treatment T_2 where seeds treated with *P. vexans* (Table 3). The range of germination percentage was 67.33-93.33%. The highest (93.33%) germination was found in the treatment T_5 followed by the treatment T_3 (92.00%), T_4 (89.33%) and T_6 (88.67%) but these were statistically identical. In contrast, significantly the lowest germination (67.33%) was found in the treatment T_2 where seeds were treated with *P. vexans*. In case of seedling mortality, all treatments reduced post-emergence seedling mortality compared to the treatment T_2, where seeds were treated with *P. vexans* (Table 3). Significantly, the lowest (3.33%) post-emergence seedling mortality was found in the treatment T_3 followed by T_4 (4.44%), T_5 (5.56%), and T_6 (6.67%) but these were statistically identical. Chitosan increased 36.64% and 32.67% germination and decreased post-emergence seedling mortality by 85.73% and 80.97% in natural and inoculated condition of the pathogen respectively. This experiment showed that seed treatment with 0.5% chitosan was effectively to increase germination and control post-emergence seedling mortality of eggplant like Bavistin 50 WP. The similar results in increasing germination percentage and decreasing post-emergence seedling mortality with chitosan were reported by Mahdavi and Rahimi, 2013 and Jahanara *et al*., 2018.

Table 3: Effect of Chitosan and Bavistin on Seed Germination and Post-emergence Seedling Mortality of Eggplant

Treatments	% Seedling Emergence	% Increase Over T_2	% Post Emergence Seedling Mortality	(%) Reduction Over T_2
T_1=Untreated Seed (Control-1)	80.00 b	18.82	16.67 b	28.55
T_2=Seed Treated With *P. vexans* (Control-2)	67.33 c	-	23.33 a	-
T_3=Seed Treated With 0.5% Chitosan	92.00 a	36.64	3.33 c	85.73
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	89.33 a	32.67	4.44 c	80.97
T_5=Seed Treated With 0.1% Bavistin	93.33 a	38.62	5.56 c	76.12
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	88.67 a	31.69	6.67 c	71.41

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

d) Effect of Chitosan and Bavistin on the Growth of Eggplant Seedlings

Growth promoting factors like shoot length, root length, fresh weight and dry weight were recorded randomly from five plants of each treatment at the seedling stage (28 DAS). Seed treatment with chitosan, as a result, increased the growth promoting components in comparison to the treatment T_2 where seeds were treated with *P. vexans* spore suspension (Table 4). The highest shoot length (6.16 cm), root length (4.54 cm), fresh weight (0.2728 g) and dry weight (0.0364 g) of seedlings were found in the treatment T_4, where seeds were treated with chitosan (0.5%) in the pathogen inoculated condition. On the other hand, the lowest shoot length (4.72 cm) and lowest fresh weight (0.058 g) were recorded in T_1, and lowest root length (2.44 cm) and dry weight (0.0082 g) were recorded in T_6. In seedling growth, the concentration of 0.5 % chitosan increased shoot length, root length, fresh weight and dry weight by 16.23, 29.96, 116.85, and 139.47% respectively in T_4 over T_2 treatment after 28 DAS. Plant height and number of branches were recorded randomly from three plants after certain maturity (75 days after transplanting) of eggplant in the field. Chitosan as seed treatment increased the plant height and number of branches in comparison to the treatment T_2, where seeds were treated with *P. vexans* (Table 5). The highest plant height (83.33 cm) and number of branches (23.00) were achieved by chitosan in the treatment T_3 followed by plant height (78.17 cm) and number of branches (22.00) in T_4 but no statistical difference was found among them. In contrast, the lowest plant height (50.40 cm) and number of branches (10.33) were recorded in the treatment T_2 where seeds were treated with *P. vexans*. These results are agreed with several investigators (Harada *et al*., 1995; Shao *et al*., 2005; Algam *et al*., 2010; Mahdavi and Rahimi, 2013; Mishra *et al*., 2014) where seed treatment with chitosan increased growth parameters of seedling as well as mature plant like shoot growth, branch length, node number per plant, seed yield, total root length per plant in different crops.

Table 4: Effect of Seed Treatment with Chitosan and Bavistin on Growth Parameters at the Seedling Stage (28 DAS) of Eggplant

Treatments	Shoot Length (cm)	Root Length (cm)	Fresh Weight of Seedling (g/plant)	Dry Weight of Seedling (g/plant)
T_1=Untreated Seed (Control-1)	4.72 c	3.00 b	0.0580 d	0.0104 d
T_2=Seed Treated With *P. vexans* (Control-2)	5.30 bc	3.18 b	0.1258 c	0.0152 c
T_3=Seed Treated With 0.5% Chitosan	5.92 ab	4.46 a	0.2302 b	0.0302 b
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	6.16 a	4.54 a	0.2728 a	0.0364 a
T_5=Seed Treated With 0.1% Bavistin	5.16 c	3.10 b	0.0658 d	0.0102 d
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	4.82 c	2.44 c	0.0746 d	0.0082 d

* Mean values 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 Eggplant

Treatments	Plant Height (cm)	% Increase Over T_2	Number of Branching / Plant	% Increase Over T_2
T_1=Untreated Seed (Control-1)	57.17 c	13.43	11.67 c	12.97
T_2=Seed Treated With *P. vexans* (Control-2)	50.40 d	-	10.33 c	-
T_3=Seed Treated With 0.5% Chitosan	83.33 a	65.34	23.00 a	122.65
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	78.17 ab	55.10	22.00 a	112.97
T_5=Seed Treated With 0.1% Bavistin	78.50 ab	55.75	17.00 b	64.57
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	76.83 b	52.44	17.33 b	67.76

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

e) Effect of Chitosan and Bavistin on Enzyme Activity

Chitosan is known to have eliciting activities leading to a variety of defense responses in plants, in response to microbial infections to reduce the negative impact of diseases, on yield and quality of crops. Seed treatment with chitosan as an elicitor increased the CAT and POD activities (Table 6). The higher activities of CAT (256.88 μmol g^{-1} min^{-1}) and POD (0.9267 UA g^{-1} min^{-1}) were found in T4 followed by T3, CAT (235.28 μmol g^{-1} min^{-1}) and POD (0.7267 UA g^{-1} min^{-1}). Chitosan increased the CAT (113.48%) and POD (56.18%) activities in T4 over T2. The findings are in agreement with other investigators (Ortega-Ortiz *et al.,* 2007; Mandal, 2010; Mishra *et al.,* 2014) that chitosan treatment can cause induced resistance and increase enzyme activities in many plants. Chitosan also activates host defense genes leading to physical and biochemical changes in plant cells involved directly or indirectly in disease suppression.

Table 6: Effect of Chitosan on Enzyme Activity of Eggplant Leaves

Treatments	CAT (μmol g^{-1} min^{-1})	POD (UA g^{-1} min^{-1})
T_1=Untreated Seed (Control-1)	216.37 c	0.3833 d
T_2=Seed Treated With *P. vexans* (Control-2)	120.33 e	0.5933 c
T_3=Seed Treated With 0.5% Chitosan	235.28 b	0.7267 b
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	256.88 a	0.9267 a
T_5=Seed Treated With 0.1% Bavistin	175.56 d	0.4400 d
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	188.14 d	0.4067 d

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

f) Effect of Chitosan and Bavistin in Controlling Phomopsis Blight and Fruit rot of Eggplant in the Field

An attempt has been made for the management of Phomopsis leaf blight and fruit rot diseases of eggplant developed under inoculated condition through the application of chitosan. Phomopsis leaf blight and fruit rot disease incidence (DI) and percent disease index (PDI) were recorded from infected leaves and fruits at different growth stages from open field condition. Chitosan and fungicide treatments reduced leaf blight and fruit rot incidence and PDI in the field over T_2 where eggplant seeds were treated with *P. vexans* without using any biocontrol agent such as chitosan or any fungicide such as Bavistin (Table 7 and 8 and Fig. 4). The highest leaf blight (45.83%) and fruit rot (66.67%) incidence were found in treatment T_2 followed by leaf blight (37.5%) and fruit rot (45.83%) incidence in T_1. In contrast, treatment T_3 where seeds were treated with chitosan showed the lowest leaf blight (4.17%) and fruit rot (8.33%) incidence followed by T_4 and T_5 and no statistical difference was found among them. Similarly, significantly the highest leaf blight (46.67%) and fruit rot (61.67%) PDI were found in the treatment T_2 and the lowest leaf blight (10.00%) and fruit rot (8.33%) PDI were recorded in chitosan treated seed plot T_3. In the field, chitosan reduced disease incidence (leaf blight 81.82% and fruit rot 81.25%) and PDI (leaf blight 67.86% and fruit rot 78.38%) in T_4 over treatment T_2. The present experiment revealed that chitosan could be used as an alternative of fungicide to control Phomopsis blight and fruit rot of eggplant. This findings of the study justify with the statements of Benhamou *et al*., (1994) and O'Herlihy *et al*., (2003) that chitosan has been considered as an alternative to chemical fungicides.

Table 7: Effect of Chitosan and Bavistin on Leaf Blight and Fruit Rot Disease Incidence (DI) of Eggplant in the Field Condition

Treatments	Leaf Blight		Fruit Rot	
	% DI	% Reduction Over T_2	% DI	% Reduction Over T_2
T_1=Untreated Seed (Control-1)	37.5 a	18.18	45.83 b	31.26
T_2=Seed Treated With *P. vexans* (Control-2)	45.83 a	-	66.67 a	-
T_3=Seed Treated With 0.5% Chitosan	4.17 b	90.90	8.33 c	87.51
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	8.33 b	81.82	12.5 c	81.25
T_5=Seed Treated With 0.1% Bavistin	8.33 b	81.82	12.5 c	81.25
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	12.5 b	72.73	16.67 c	74.00

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

Table 8: Effect of Chitosan and Bavistin on Leaf Blight and Fruit Rot Disease Severity (PDI) of Eggplant in the Field Condition

Treatments	Leaf Blight		Fruit Rot	
	PDI	% Reduction Over T_2	PDI	% Reduction Over T_2
T_1=Untreated Seed (Control-1)	35.83 b	23.23	50.00 b	18.92
T_2=Seed Treated With *P. vexans* (Control-2)	46.67 a	-	61.67 a	-
T_3=Seed Treated With 0.5% Chitosan	10.00 d	78.57	8.33 d	86.49
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	15.00 cd	67.86	13.33 cd	78.38
T_5=Seed Treated With 0.1% Bavistin	11.67 cd	74.99	9.17 d	85.13
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	15.83 c	66.08	15.00 c	75.68

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

Fig. 4: Leaf Blight and Fruit Rot of Eggplant Caused by *P. vexans*.

g) Effect of Chitosan and Bavistin on the Yield of Eggplant

Results of the present study indicated that seeds treatment with chitosan and Bavistin 50 WP significantly increased fruit yield over the pathogen treatment (Table 9). The highest (16.51 t/ha) yield was found in the treatment T_3 followed by the treatment T_4 (15.77 t/ha) which were superior to all other treatments but these were statistically identical. The lowest yield (9.03 t/ha) was recorded in the treatment T_2 where eggplant seeds were treated with *P. vexans* spore suspension. Seed treatment with chitosan also increased the size of eggplant fruits. The bigger fruit size (60.27 g) was observed in T_3 which was statistically identical with treatment T_4 (59.83 g). In contrast, smaller fruit size (44.37 g) was found in T_2 followed by untreated control T_1 (50.23 g) and no statistical difference were found among them. Moreover, the maximum number of fruits were recorded in T_3 (164.73) followed by T_4 (158.15) and T_5 (151.33) and no statistical difference were found among them. Chitosan was found to increase 74.64% yield in T_4 over T_2 treatment. These results are approved by Mishra *et al*., 2014 who reported that seed treatment with chitosan increased number of fruits plant^{-1} and yield of tomato

Table 9: Effect of Chitosan and Bavistin on Yield of Eggplant

Treatment	Total Fruits/Plot	Weight (g)/Fruit	Yield (g/m^2)	Yield (t/ha)	% Increase Over T_2
T_1=Untreated Seed (Control-1)	133.17 d	50.23 bc	1116.58 c	11.17	23.69
T_2=Seed Treated With *P. vexans* (Control-2)	122.17 e	44.37 c	902.97 d	9.03	-
T_3=Seed Treated With 0.5% Chitosan	164.73 a	60.27 a	1651.09 a	16.51	82.83
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	158.15 ab	59.83 a	1576.61 a	15.77	74.64
T_5=Seed Treated With 0.1% Bavistin	151.33 bc	52.23 b	1318.65 b	13.19	46.06
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	145.94 c	51.53 b	1251.91 bc	12.52	38.65

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

IV. Conclusion

From this study it can be concluded that chitosan is a natural substance which biodegradable and non-toxic directly inhibited the growth and reproduction of fungus *P. vexans*. Seed treatment with chitosan induced the defense enzymes, reduced the disease incidence and PDI and increased growth and yield of eggplant. However, supplementary studies are required to confirm the optimal concentration of chitosan to control Phomopsis blight and fruit rot of eggplant caused by *P. vexans*.

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