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Antimicrobial property of chitosan and induction of systemic acquired resistance for the control of rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Swings *et al.*)

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ABSTRACT

Chitosan has been reported to have antimicrobial property to some pathogen species as well as an elicitor of resistance in plants , particularly Systemic Acquired Resistance (SAR). A bioassay of chitosan against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was conducted to determine its antimicrobial property against bacterial blight pathogen and the optimum concentration that is most inhibitory to the pathogen. Chitosan was also tested as foliar spray to rice plants to evaluate its potential to induce SAR against bacterial blight disease.

Chitosan treatments (300 ppm, 400 ppm, and 500 ppm) possessed antimicrobial property against *Xoo in vitro*, producing zones of inhibition which were generally significantly bigger than those of the control (streptomycin, acetic acid, and sterile distilled water) at 2, 4, and 6 days after inoculation. Chitosan-sprayed plants showed significantly shorter bacterial blight lesions which were comparable to the plants sprayed with streptomycin, and Boost, a commercial plant defense activator and a known inducer of SAR. Chitosan is found effective in reducing bacterial blight lesions in rice plants due to its antimicrobial property and also most likely due to the induction of SAR.

Keywords: *Rice, bacterial blight, resistance, induction, chitosan, antimicrobial, systemic acquired resistance (SAR)*

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INTRODUCTION

Rice is the major staple crop that is primarily consumed by humans. It has been estimated that half of the world's population subsist wholly or partially on rice. Ninety percent of the world's rice is grown and consumed in Asia (Columbia Electronic Encyclopedia, 2006).

Rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most economically serious diseases of lowland irrigated rice and can cause losses up to 50% (Gnanamanickam *et al*., 1999). Bacterial blight has high epidemic and destructive potential to high-yielding cultivars in both temperate and tropical regions especially in Asia. Problems were compounded by the fact that the disease becomes more virulent in the presence of susceptible hosts, especially under monoculture conditions (NIAS, 2004).

Chemical control of bacterial blight is impractical. Additionally, no effective bactericide is commercially available for disease control. The preferred strategy for disease control is through host plant resistance (Lee *et al*., 2002).

Plants possess a range of defenses that can be actively expressed in response to pathogen attack. This is termed as induced resistance. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are both induced resistance (Vallad and Goodman, 2004). The hypersensitive response (HR) precedes the secondary resistance response, the SAR and ISR. HR is characterized by immediate death of host tissues around the infection site that wall-off the pathogen and cause a localized lesion. This localized form of resistance can lead to resistance against subsequent infection with widely different pathogens which is known as SAR (Sticher *et al.*, 1997).

SAR, besides the constitutive defense, contributes to the overall resistance displayed by plants and may provide a selective advantage for survival. This resistance is expressed locally at the site of primary inoculation and also systemically in tissues remotely located from the initial point of attack. SAR is a long lasting and often confers a broad spectrum of resistance to different pathogens (Matthews, 2003).

Improved understanding of the underlying processes leading to the pre-conditioning of plants against pathogens has been largely driven by

the discovery of biological and chemical agents that are able to elicit the innate defenses of plants. Several biological and chemical elicitors are now commercially available for use in conventional agriculture (Vallad and Goodman, 2004). The possibility of stimulating internal plant defenses has become an interesting option for enhancing natural disease resistance (Barka *et al.*, 2004).

Among the elicitors of resistance known to date, chitosan, a polycationic-1, 4-linked-D-glucosamine polymer, has the best prospects as biocontrol agent (El Ghaouth *et al.*, 1994). Chitosan is produced commercially by deacytelation of chitin, which is the structural element in the exoskeleton of crustaceans (US Environmental Protection Agency, 2006).

Chitosan and its derivatives, such as glycol-chitosan and carboxymethyl chitosan, are known to form a semi-permeable film around plant tissues, are inhibitory to a number of pathogenic fungi, and also induce host-defense response (El Ghaouth *et al*., 1994). The application of chitosan solution may sensitize plants to respond more rapidly to pathogen attack by stimulating chitinase and glucanase production. Oligomers of chitosan (poly-N-glucosamine), which are likely to be released by the action of plant encoded-chitinase from walls of invading fungi can protect tomato roots against *Fusarium oxysporum* f.sp. *radicis-lycopresici* when applied to the seeds, roots or leaves (Lafontaine and Benhamou, 1996).

Barka *et al.* (2004) reported that chitogel a formulated chitosan solution either in the form of a chitogel supplemented medium or as a foliar spray in combination of chitogel-free medium can both successfully improve the growth of grapevine and protect it against gray mould disease.

In commercial agriculture, chitosan remains relatively unexploited, even though its specific properties, such as biodegradability, antimicrobial potential, and elicitor activity meet the criteria of a promising biocontrol agent (El Ghaouth *et al.*, 1992).

The potential use of chitosan as a plant activator that can induce SAR in rice plants may provide effective and economical alternative in controlling bacterial blight disease. However, studies on chitosan as an antimicrobial agent and as a plant activator against *Xoo* had not been

documented, hence, this study was conducted with the following objectives to: evaluate antimicrobial activity of chitosan for the control of *Xoo*; determine the optimum concentration of chitosan that is most inhibitory to *Xoo;* and evaluate the efficacy of chitosan for the induction of systemic acquired resistance (SAR) in rice plants for the control of bacterial blight disease.

MATERIALS AND METHODS

Preparation of chitosan solution

Low molecular weight chitosan was obtained from Saga University, Japan through the Dept. of Pure and Applied Chemistry, VSU, Visca, Baybay, Leyte.

One gram of Japanese chitosan was dissolved in 1 L of 1% acetic acid to prepare 1000 ppm stock solution. Three, four and five-hundred ppm chitosan concentrations were prepared from the stock solution with water as diluent.

In-vitro evaluation of antimicrobial property of chitosan against Xoo

Procurement and Preparation of Xoo culture

Bacterial blight pathogen (race 6-PXO99) was procured from the International Rice Research Institute (IRRI) through the Plant Disease Diagnostic Laboratory (PDDL), VSU, Visca, Baybay, Leyte. Wakimoto medium (Sucrose 20 g, Bactopeptone 5 g, Calcium Nitrate 0.5 g, Sodium Phosphate 0.82 g, Ferrous Sulfate 0.05 g, Agar (Difco) 19 g, distilled water 1000 mL) was used to culture medium for *Xoo* . The medium was dispensed in test tubes and flasks and were autoclaved at 15 psi for 15 min. Bacterial suspension was streaked onto the prepared Wakimoto medium and incubated upside down at room temperature for 24-48 hr. Colonies on the isolation plates were aseptically transferred to Wakimoto slants, which were maintained as pure culture.

Bioassay of chitosan against Xoo

Ten mL of sterile water was poured on slant culture of 48-hr old *Xoo* isolate and was aseptically scraped gently with wire loop. The concentration of the prepared suspension was standardized to 10^6 cfu/mL.

Approximately, 15 mL of previously sterilized and melted Wakimoto medium was poured into sterile plates and allowed to solidify. Bacterial suspension (0.1 mL) was pipetted and spread on the surface of agar plate using a sterilized L-shaped glass rod. A 5-mm diameter sterile Whatman filter paper disc was immersed in different concentrations of chitosan (300, 400, 500 ppm), respectively and was placed at the center of the medium. The plates were incubated at room temperature. The inhibition zone (in cm from the disc) was measured at 2, 4, and 6 days after treatment. The treatments were arranged in Completely Randomized Design (CRD) with 3 replications per treatment. The data were subjected to Analysis of Variance (ANOVA) and treatment means were computed using the Least Significance Difference (LSD).

The following were the treatments:

T_1 = sterile water (control)
T_2 = 300 ppm chitosan
T_3 = 400 ppm chitosan
T_4 = 500 ppm chitosan
T_5 = acetic acid (5000 ppm) (control)
T_6 = streptomycin (2000 ppm) (control)

Chitosan as SAR Inducer in rice plants

Effect of spray interval application on effectiveness of chitosan for SAR induction

The ability of chitosan to induce SAR to rice plants to protect it against bacterial blight was evaluated in the screenhouse using the most promising concentration (300 ppm) that showed antimicrobial property

in the laboratory experiment. Two intervals of application were compared in this experiment.

Preparation and maintenance of test plants

Seeds of IR24, known to be susceptible to the bacterial blight pathogen were disinfected with 1% NaOCl for 1 min. and rinsed in 4 changes of sterile distilled water. The seeds were pre-germinated in a Petri plate lined with moist tissue paper. After 10 days pre-germination, the seedlings were transferred individually to polyethylene pots and were maintained in the screenhouse (Fig. 1). The plants were provided with necessary care and maintenance until ready for chemical treatment and inoculation of the pathogen.

Application of chemical treatment

Thirty mL of each chemical treatment was sprayed per plant (Fig. 2). The treatments were sprayed at 5 and 10 days interval, starting 15 days after transplanting. The treatments were arranged in a Split Plot Design in RCBD with interval of application (5 and 10 days) as main plot and chemical treatment (300 ppm chitosan, with controls: 5000 ppm acetic acid, 2000 ppm streptomycin and sterile distilled water) as subplot. Two trials were conducted with 3 replications with 5 plants per replicate. Data were subjected to analysis of variance and treatment means were computed using LSD.

Preparation of inoculum and inoculation of the pathogen

A 48-hr old isolate of *Xoo* was added with 10 mL sterile water and the bacterial suspension was standardized to 10^8 cfu/mL. Inoculation of bacterial blight pathogen was done 40 days after transplanting following the leaf clipping method of Kauffman *et al.* (1973). Five fully expanded leaves were inoculated per plant.

Disease scoring

Disease scoring was done by measuring the length (cm) of blight lesion at 1, 2, 3, and 4 weeks after inoculation of the pathogen.

Figure 1. Experimental set-up in the screenhouse

Figure 2. Spray application of chemicals on rice plants.

Effect of different chitosan concentrations on SAR induction in rice

The original three concentrations of chitosan (300 ppm, 400 ppm and 500ppm) that were tested in the laboratory for antimicrobial property were further evaluated in the screenhouse for SAR induction to rice plants. This was because the concentration of chitosan that is inhibitory to *Xoo* may differ from the concentration of chitosan that may induce SAR.

Thirty milliliters of each chemical solution was sprayed per plant. The treatments were applied continuously at 10 days interval starting 15 days after transplanting up to 25 days after inoculation. The treatments were arranged in a Randomized Complete Block Design (RCBD) with 3 replications and 5 plants per replicate. Sterile water, acetic acid (5000 ppm), the solvent used in preparing the chitosan solution and Boost (100 ppm) from Syngenta were included as controls.

Treatments:

T_1 - sterile water
T_2 - 5000 ppm acetic acid
T_3 - 300 ppm chitosan
T_4 - 400 ppm chitosan
T_5 - 500 ppm chitosan
T_6 - 100 ppm Boost

Data were subjected to Analysis of Variance and treatment means were computed using LSD. The preparation of inoculum, inoculation of the pathogen and disease scoring were done as described in the previous experiment.

Effect of chemical treatments on plant height

As the experiment was on going, it was observed that the sprayed plants portrayed a difference in their growth so it was decided to measure the plant height (m) when the plants were 65, 85 and 105 days old. Data were subjected to Analysis of Variance (ANOVA). Means were compared

using Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Antimicrobial property of chitosan against Xoo

Results of the bioassay of low molecular weight (LMW) chitosan against *X. oryzae* pv. *oryzae* (*Xoo*) in vitro are summarized in Table 1. Data show the mean zones of inhibition of *Xoo* as affected by varying concentrations of chitosan at 2, 4 and 6 days after treatment. Chitosan at 300 ppm, produced the largest zone of inhibition (1.28 cm from the edge of the disc), followed by 400 ppm (1.14cm), streptomycin (0.85 cm) and 500 ppm chitosan (0.83 cm) (Fig. 3).

The three concentrations of chitosan used clearly showed zones of inhibition which were generally greater than the standard check (streptomycin). This indicates that chitosan controlled *Xoo* more effectively as compared to streptomycin. The zone of inhibition of chitosan at 300 ppm was the highest among all treatments.

The effect of acetic acid which was used as solvent of chitosan was not significantly different from that of sterile distilled water (control). This implies that acetic acid alone did not exhibit antimicrobial property and was not responsible for the antimicrobial property displayed by chitosan but only acted as a solvent for chitosan. Sterile water initially showed a little clear zone at 2 days after treatment (DAI) (with mean 0.03 cm) which could be just the wet area caused by the filter paper disc when it was immersed with sterile distilled water.

The zone of inhibition generally decreased from 2-6 days after treatment. This suggests a decreasing effectiveness of the chemical treatments with time. The chitosan treatments, however, were still more effective in controlling the growth of *Xoo* compared to the control checks with significantly wider zones of inhibition, even at 6 days after treatment.

It was also observed that as the concentration of chitosan increased, there was a corresponding reduction in the size of zone of inhibition of *Xoo*. Results of other investigators indicated that chitosan at lower concentration inhibited mycotoxin production and also created changes

Table 1. Zone of inhibition of *X. oryzae* pv. *oryzae* at 2, 4 and 6 days after treatment with varying concentrations of chitosan [1/]

TREATMENTS	ZONE OF INHIBITION (cm) Days after Treatment		
	2	4	6
300 ppm chitosan	1.28	1.05	0.93
400 ppm chitosan	1.14	0.99	0.65
500 ppm chitosan	0.83	0.72	0.60
2000 ppm streptomycin	0.85	0.60	0.43
5000 ppm acetic acid	0.17	0.07	0.00
Sterile water	0.03	0.00	0.00
LSD	0.08	0.09	0.62
CV (%)	6.34	8.7	8.03

[1/] Means in a column followed by common letter/s are not significantly different at 5% LSD.

in structure of mycelium hyphae (Redy *et al*., 1998). Similarly, the inhibitory influence of chitosan was observed in *Aspergillus niger* and *A. parasiticus* (Fang *et al.*, 1994).

These results were the reverse of the earlier investigation of El Ghaouth *et al*., (1992), that increasing concentration of chitosan causes greater inhibition of growth and formation of spores of *Rhizopus stolonifer* and *Botrytis cinerea*. Similarly, Benhamou *et al*. (1998) stated that at higher concentration of chitosan, the growth and development of *Alternaria alternata* f. sp. *lycopersici* were stimulated, but on the other hand, decreased the vitality of conidial spores.

Furthermore, Basurto *et al*. (2005) reported that higher concentration of chitosan resulted in stronger antimicrobial effects, although the lowest concentration (0.1%) of LMW chitosan appeared to be sufficient to kill all *Listeria* cells within 24 hr at room temperature. In this particular experiment, the reverse is true. Lower concentration of chitosan was more inhibitory probably because clumping of chitosan may had occurred at higher concentration making it less effective leading to decreased inhibition zone.

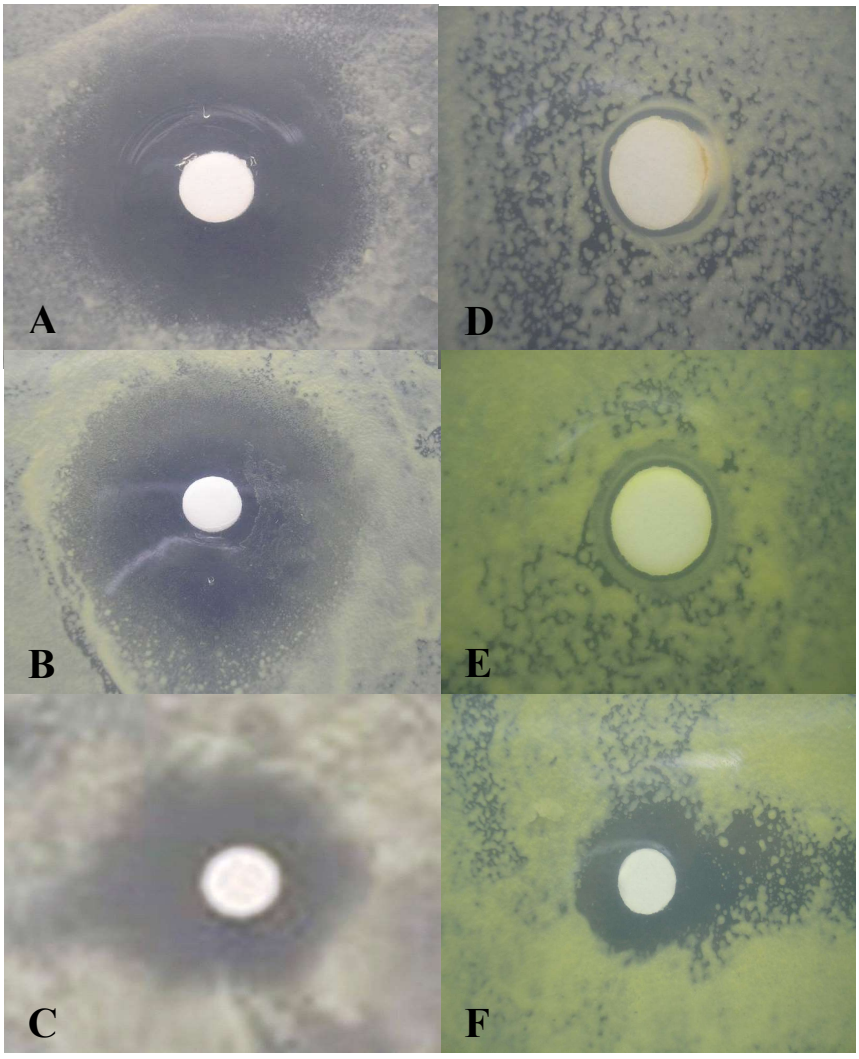

Figure 3. Close up of the zones of inhibition produced by chitosan at varying concentrations on *X. oryzae* pv. *oryzae* culture with sterile water, 5000 ppm acetic acid and 2000 ppm streptomycin as control checks

Legend:

A - 300 ppm chitosan
B - 400 ppm chitosan
C - 500 ppm chitosan
D - sterile water
E - 5000 ppm acetic acid
F - 2000 ppm streptomycin

Chitosan is soluble in acidic medium (pH=5.5) due to the presence of amino groups along the polymer chain. Uchida (1999) stated that chitosan oligomer I possessed weak antimicrobial activity at levels as high as 5000-6000 ppm, while oligomer II showed no activity. Furthermore, oligomer I inhibited the growth of *E. coli* at concentration of 3000 ppm and 5000 ppm, while oligomer II did not retard the growth of *E. coli* at equivalent concentration.

Chitosan has been shown to have some direct anti-fungal and antibacterial activities (Roller, 1999) and has even been proposed for use as a food preservative. Antimicrobial property of chitosan varies with the characteristics of the polymer, pH and the prevailing temperature (Basurto *et al.*, 2004). Chitosan has been shown to inhibit the growth of several food borne bacteria including *Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia. coli* and *Lactobacillus fructivorans*. However, reported minimum inhibitory effect varies widely from 0.01% to 5.0 % depending on the factors mentioned above.

Sudarshan *et al*. (1992) reported that low molecular weight chitosan like what was also employed in this experiment had stronger antimicrobial property regardless of bacterial species. Pieta *et al.* (2003) reported that dressing bean seeds with 0.1% chitosan resulted to the highest number of healthy seedlings. Chitosan induced the formation of phenolic compounds, which limited the growth and development of *Aspergillus flavus* and production of aflatoxin B1 (Fajardo *et al*., 1995). Its effect is on the limitation of ability to colonization of *Pythium aphanidermatum*. Moreover, it creates morphological changes in mycelium hyphae, which do not cause phytotoxic changes of cucumber (El Ghaouth *et a*l., 1994).

Chitosan as SAR inducer in rice plants

Effect of spray interval application on the effectiveness of chemical treatment for SAR induction

Table 2 shows the effect of different chemical treatments and

application interval on the length of bacterial blight lesions at 7, 14, 21, and 28 days after inoculation. At 5 days application interval, chitosan 300 ppm produced significantly shorter bacterial blight lesions (2.96 cm, 3.51 cm, 6.05 cm, and 8.26 cm at 7, 14, 21 and 28 DAI, respectively) compared to the untreated check (14.75 cm, 19.29 cm, 23.36 and 28.64 cm at 7, 14, 21 and 28 DAI, respectively). This result suggests that the chitosan treatment had either induced resistance in the treated plants as shown by the shorter blight lesions or had expressed its antimicrobial property so as to inhibit the pathogen resulting to reduced amount of disease.

The chitosan treatment was a little inferior to that of streptomycin (with lesion length of 2.15 cm, 3.38 cm, 5.49 cm and 6.72 cm at 7, 14, 21 and 28 DAI, respectively). The same trend of effect of the chemical treatments was observed in both 5 days and 10 days interval time of application (Fig. 4). It was generally observed however, that at 5 spray application interval, chitosan and streptomycin resulted to shorter lesions compared to 10 days application interval. This suggests that resistance induction as well as its antimicrobial action were greater at a more frequent application of the chemical.

Effect of different chitosan concentrations on SAR induction

Table 3 shows the length of bacterial blight lesions on rice as affected by varying concentrations of chitosan at 7, 14, 21 and 28 DAI. Analysis of variance revealed highly significant differences in mean lesion length among treated plants. At 7 DAI, plants sprayed with chitosan and Boost, produced shorter bacterial blight lesions compared to the control (Fig. 5). Plants sprayed with chitosan (300 ppm, 400 ppm and 500 ppm) produced shorter lesions (2.14 cm, 3.64 cm and 4.24 cm at 7 DAI, respectively) which was comparable to that sprayed with Boost (with mean lesion length of 2.63 cm). Plants sprayed with sterile water and acetic acid resulted to longer lesions (14.75 cm and 13.37 cm) at 7 DAI, respectively. The same trend was observed at 14, 21 and 28 DAI.

It was observed, however that the lesion length continuously increased from 14, 21 and 28 DAI in all the treatments. The plants sprayed with the three concentrations of chitosan and Boost still exhibited shorter

Table 2. Effect of different chemical treatments and varying intervals of application on bacterial blight lesion length of rice plants at 7, 14, 21and 28 days after inoculation

Spray Interval	Treatments	Lesion length (cm) Days after inoculation			
		7	14	21	28
5 days	sterile water	14.75	19.29	23.36	28.64
	5000 ppm acetic acid	13.37	18.24	23.31	27.43
	300 ppm chitosan	2.96	3.51	6.05	8.26
	2000 ppm streptomycin	2.15	3.58	5.49	6.72
10 days	sterile water	15.56	18.82	23.49	27.14
	5000 ppm acetic acid	14.18	18.82	22.90	26.65
	300 ppm chitosan	3.32	4.05	7.71	9.22
	2000 ppm streptomycin	2.65	4.65	7.83	9.12
LSD		0.31	0.25	0.2	0.25
CV	(%)	2.0	1.30	0.80	0.80

Means in a column followed by common letter/s are not significantly different at 5% LSD.

blight lesions (14.34 cm, 14.62 cm, 13.62 cm and 13.32 cm for chitosan 300 ppm, 400 ppm, Chitosan 500 ppm and Boost, respectively) compared to acetic acid (28.31 cm) and sterile water (27.99 cm) during the last day of data gathering which is 28 DAI.

The shorter lesions produced by Boost Bion treated plants were due to SAR induction since Benzothiadiazole (BTH), the active ingredient of Boost is a commercial plant defense activator and is known to induce SAR. Chitosan, on the other hand, had been reported by some authors to induce SAR. However under conditions of this experiment, measurable parameters to detect induction of SAR aside from resulting disease symptoms were not undertaken since facilities do not warrant. Thus, it cannot be ruled out that chitosan with its antimicrobial property could have also induced SAR in rice against bacterial blight disease.

Schneider *et al*. (1996) reported that various natural or synthetic substances are inducers of SAR and one of these is chitosan. Chitosan and its derivatives are known to form a semi-permeable film around plant

Figure 4. Bacterial blight lesions on IR24 as affected by different chemical treatments and varying intervals of application at 7 days after inoculation.

Legend:
A1 - 5 days interval
A2 - 10 days interval

T_1 - sterile water
T_2 - 300 ppm chitosan
T_3 - 5000 ppm acetic acid
T_4 - 2000 ppm streptomycin

Figure 5. Bacterial blight lesions on IR24 as affected by varying concentrations of chitosan at 7 days after inoculation.

Legend:
T_1 - sterile water
T_2 - 300 ppm chitosan
T_3 - 400 ppm chitosan
T_4 - 500 ppm chitosan
T_5 - 5000 ppm acetic acid
T_6 - 100 ppm Boost Bion

Table 3. Lesion length of rice as affected by varying concentrations of chitosan at 7, 14, 21, and 28 days after inoculation.

Treatments	Lesion length (cm) Days after inoculation			
	7	14	21	28
sterile water	7.42	19.62	24.49	27.99
5000 ppm acetic acid	9.66	18.65	24.41	28.31
300 ppm chitosan	4.24	9.11	13.68	14.34
400 ppm chitosan	3.64	8.84	13.72	14.62
500 ppm chitosan	2.14	8.52	12.12	13.62
100 ppm Boost Bion	2.63	7.66	9.49	13.32
LSD	0.25	0.28	0.35	0.77
CV (%)	2.08	1.3	1.2	2.3

Means in a column followed by common letter/s are not significantly different at 5% LSD.

tissues, which was inhibitory to a number of pathogenic fungi which also induced host-defense response according to El Ghaouth *et al*. (1994).

Application of chitosan to plants causes a number of different physiological responses. It reduces the stomatal apertures of the leaf, thus reducing the ability of pathogens to gain access into the plant (Lee, 1999), causes the production of phenolic compounds (Bhaskara, 1999), and increases crop yield (Dunand, 1995). The application of chitosan solution may sensitize the plants to respond rapidly to the attack of bacterial blight pathogen by stimulating chitinase and glucanase production (Lafontaine and Benhamou, 1996) which probably induced SAR. Chitosan induced plant resistance and protects against viral, bacterial and fungal infections. Several lines of evidence have shown that activation of natural plant defense systems could occur upon exogenous applications of chitin and chitosan oligosaccharides (Lafontaine and Benhamou, 1996). Szczeponek *et al.* (2006) said that the level of SAR induced by chitosan depends on plant species and kind of chitosan. Efficiency of chitosan can depend also on the manner of application.

Recent reports by Benhamou *et al*. (1998) have shown that chitosan

has the capacity to induce resistance to *Fusarium oxysporum* in susceptible tomato plants when applied as a root dressing, foliar spray and seed dressing by restricting the pathogen growth to the outer root tissues and eliciting a number of defense reactions including structural barriers. Chitosan can be active in soil as elicitor of resistance or can be absorbed by plant roots and utilized. In experiments conducted by Chang *et al*. (1998), chitosan was found to be a good elicitor of resistance in *Mentha piperita*, which protected plant against infections through increased production of menthol.

Effect of chemical treatments on plant growth

Table 4 shows the plant height of rice as affected by varying concentrations of chitosan at 65, 85 and 105 days after transplanting. Analysis of variance revealed that there was no significant difference of mean plant height of all treatments at 65 days after transplanting, however significant difference was observed at 85 and 105 days after transplanting. It was observed that plants treated with acetic acid were the tallest among other treatments with mean plant height of 0.9 m and 1.2 m at 85 and 105 days, respectively after transplanting. Acetic acid probably enhanced faster growth of the plants by stimulating the growth hormones present in the plants. It may have altered some physiological processes of the plant affecting its growth. Although it enhances faster growth in plants, it does not have the ability to control bacterial blight disease. On the other hand, chitosan and Boost induced resistance in rice plants and had no effect on plant height whose values did not differ significantly from the water-treated plants.

Acetic acid (CH_3COOH) is an essential component of plant growth substances like "auxin", which plays an essential role in coordination of many growth and behavioral processes in the plant life cycle. These include indole-3-acetic acid, 4-chloro-indoleacetic acid, phenylacetic acid (PAA) and indole-3-butyric acid (IBA). Synthetic auxin analogs include 1-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and others. Auxin is essential for cell growth, affecting both cell division and cellular expansion. Depending on the specific tissue, auxin may promote axial elongation (as in shoots), lateral expansion (as in root

Table 4. Plant height of rice as affected by varying concentrations of chitosan at 65, 85 and 105 days after transplanting

Treatments	Plant height (m) Days after transplanting		
	65	85	105
sterile water	0.72	0.88	1.04
5000 ppm acetic acid	0.78	0.90	1.20
300 ppm chitosan	0.69	0.86	1.03
400 ppm chitosan	0.69	0.86	1.02
500 ppm chitosan	0.70	0.86	1.02
100 ppm Boost	0.74	0.87	1.05
LSD	0.11	0.001	0.08
CV (%)	8.2	0.6	4.0

Means in a column followed by common letter/s are not significantly different at 5% LSD.

swelling), or isodiametric expansion (as in fruit growth) (http://en.wikipedia.org/wiki/Auxin, 2007).

CONCLUSION AND IMPLICATION

The study confirms the antimicrobial property of chitosan against *Xanthomonas oryzae* pv. *oryzae*, as well as its ability to induce Systemic Acquired Resistance (SAR) in rice plants to protect it against bacterial blight disease. The compound therefore is a very good alternative in the management of a very important disease of rice, i.e., bacterial blight. The findings of this research also have an environmental implication particularly concerning waste management since chitosan is derived and can be produced locally from crustacean wastes of discarded shrimp's heads and tails and crab's shells.

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