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*Short Communication*

# Application of chitosan solutions for rice production in Vietnam

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**Preparing chitosan solutions from shrimp shells for rice production was investigated. The chitosan produced from shrimp shells using dilute acetic acid proved effective in controlling plants infection by microbial agents leading to higher yields. The field data of our studies showed that the yields of rice significantly increased (~31%) after applying chitosan solution. In general, applying chitosan increased rice production and reduced cost of production significantly.**

**Key words:** Chitosan solution, rice production, common brown backed rice plant hoppers.

## INTRODUCTION

Chitin and chitosan are the structural components in the cuticles of crustacean, insects, mollusk and in the cell wall of fungi and plant pathogens (Boonlertnirun and Boonraung, 2008). Hirano (1996) reported that chitin and chitosan have various biological functions, for instance, antimicrobial activity, growth inhibitor of some pathogens, elicitor of phytoalexins, inducer of chitinase including accelerator of lignification in plants. Compared with chitin, chitosan has high degrees of deacetylation. Chitosan is highly soluble in dilute organic acid such as acetic acid, lactic acid, etc, making it easy and useful in applications in agriculture and in aqueous solutions at various concentrations. Data on applications of shrimp chitosan solutions in agriculture in Thailand were obtained by our colleagues in Bioprocess Technology Program, AIT-Bangkok, Thailand (Chandrkrachang, 2002). It has been applied successfully by Thai farmers with respect to its activity in self-defense system of plants and growth promoter to increase agricultural production.

While chitin is insoluble in most organic solvents, chitosan is readily soluble in dilute acidic solutions at pH below 6.0. Organic acids such as acetic, formic, and lactic acids are used for dissolving chitosan. The most commonly used is 1% acetic acid solution at about pH 4.0 as a reference. Chitosan is also soluble in 1% hydrochloric acid but insoluble in sulfuric and phosphoric acids. Solubility of chitosan in inorganic acids is quite limited. Concentrated acetic acid solutions at high temperature can cause depolymerization of chitosan (Stevens, 1996). Above pH 7.0, chitosan solubility’s stability is poor. At higher pH, precipitation or gelation tends to occur and the chitosan solution forms poly-ion complex with anionic hydrocolloid resulting in the gel formation. The solubility, however, is controlled by the degree of deacetylation and it is estimated that deacetylation must be at least 85% complete in order to achieve the desired solubility. Though several standard chemical procedures have been widely used to produce chitin and chitosan, and chitosan solutions utilizing shrimp waste as raw material, there is not much information about how to improve the production process so as to produce chitosan with consistent quality, especially high solubility. The dissolution of chitosan in dilute organic acid at low concentration has not been scientifically documented. This work determines the suitable process for preparing chitosan solution, and focuses on its applications in rice production, in particular with respect to its activity in self-defense system of plants and growth promoter to increase rice production.

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**Table 1.** Characteristics of chitosan powder

| Characteristics of chitosan sample | Content |
|---|---|
| Ash | 0.52 ± 0.15 (%) |
| Protein | 0.56 ± 0.22 (%) |
| Solubility | 99 ± 0.1 (%) |
| Degree of deacetylation | 88 ± 0.3 (%) |
| Turbidity | 20 ± 5.0 (NTU) |
| Viscosity | 4500 ± 300 (cps) |
| Molecular weight | 1.74 ± 0.01(Da x$10^{-6}$) |

## MATERIALS AND METHODS

High quality shrimp chitosan powder was prepared using Toan et al.'s method (2006), of which, the shrimp shells were preconditioned using dilute acetic acid. The preconditioned shrimp shells were transparent, had a clean surface and were susceptible to demineralization and deproteination using 0.68 mol$L^{-1}$ HCl and 0.62 mol$L^{-1}$ NaOH, respectively. The ash and protein residues in the final chitosan were about 0.5 and 0.55%, respectively; the viscosity was up to 4500 cps, and the solubility and transparency were ~100%. Characteristics of the chitosan powder and its powder sample are shown in the Table 1.

The prepared products of chitosan solutions with 1 to 5% concentration of chitosan in 1% acetic acid were used as stock solution for making dilution into different doses for various experiments. The molecular weight of the stock solutions was in the range 1.71 to 1.74 ± Da x $10^{-6}$. The degree of deacetylation was ~90% DD. The effect of chitosan treatment on the rice fields in different areas in Vietnam by spraying three times during rice growing period of 120 days in summer season was done with randomized complete block with three replications according to Boonlertnirun and Boonraung (2008). The applied doses were in the range of 10 to 15 ppm. Note that only 50% dose of organic fertilizers was used to prepare the cultivated land for rice plantation compared to the one without applying chitosan solutions.

The effect of chitosan treatment on common brown backed rice plant hoppers was tested by conducting six treatments (Tr1 to Tr6) with each block size area of 100 $m^2$. It is worth noting that the experimental design was set in the same area in order to make comparison between control and the applied samples easier. Treatments 1, 2 and 3 were set as control without chitosan application. Treatments 4, 5 and 6 were applied the prepared chitosan solution in doses of 50, 70 and 100 ppm, respectively every 10 days during the summer season.

### Statistical analysis

All data were subjected to analysis of variance according to the experimental design used in this study to compare the different means of treatment. All data were expressed as means ± standard deviation of representative of similar test carried out in triplicate.

## RESULTS AND DISCUSSION

### Effect of chitosan solutions on brown backed rice plant hopper attack

Reportedly, rice is attacked by a large number of insects. Among these insect pests, plant hoppers, stem borers, and gall midges are the most serious pests of rice (Brar et al., 2009). Among brown plant hoppers, a brown plant hopper is the most serious of the rice pests. It causes considerable damage by direct feeding. It also transmits grassy stunt and ragged stunt virus diseases. Comprehensive information is available on the taxonomy of brown plant hopper outbreaks, migration, and varietal resistance, including chemical, biological, and cultural control (IRRI, 1979). Brown plant hopper has been reported recently as the main cause of significant yield losses in rice. Brown plant hopper causes direct damage by sucking plant sap, causes hopperburn, and transmits viral diseases such as grassy stunt and ragged stunt. SBPH transmits rice stripe virus and black streak dwarf virus diseases, GRH transmits rice dwarf virus, and GLH transmits the virus that causes tungro disease.

In this study, percentage of common brown backed rice plant hopper attack in rice was found to be reduced from the control of 63.25% (without using chitosan solution) down to 35.15, 24.7 and 30.55%, corresponding to the use of chitosan solution with concentrations of 50, 70 and 100 ppm, respectively (Figure 1).

### Effect of chitosan solutions on rice yields

Data of rice yields using chitosan spray were collected from the local farmers in different areas in Vietnam and are shown in Table 2. Prepared chitosan products were applied by spraying three times during rice growing period of 120 days. The applied doses were in the range of 10 to 15 ppm. As demonstrated by results, it was found that the prepared chitosan solutions had strong effects in the biological activity of living tissues, antifungal activity and common brown backed rice hoppers. In addition, it also played a role of an elicitor to enhance the growth and self-defense system of rice plant. Moreover, chitosan was shown to be able to activate plant defensive genes through the octadecanoid pathway (Doares et al., 1995). These arguments are supported by Hui et al. (2004).

### Conclusions

The applications of chitosan solutions prepared in a

**Table 2.** The rice yields from using the prepared chitosan solutions in some areas in Vietnam.

| Place area | Number of spray | Yield (kg/ ha) | | Fertilizer (kg/ha) | | Percentage of rice increased in yield* (%) |
|---|---|---|---|---|---|---|
| | | Controlled sample | Applied sample | Controlled sample | Applied sample | |
| Van Binh | 3 | 450 | 606 | 244 | 125 | 34.70 ± 4.72 |
| Hanh Nhan | 3 | 480 | 627 | 242 | 120 | 30.60 ± 3.21 |
| Tuy An | 3 | 550 | 726 | 250 | 125 | 32.00 ± 3.40 |
| Cu Chi | 3 | 460 | 580 | 258 | 125 | 26.00 ± 4. 70 |

*The pesticides were applied only twice through the rice crop compared to five times one.

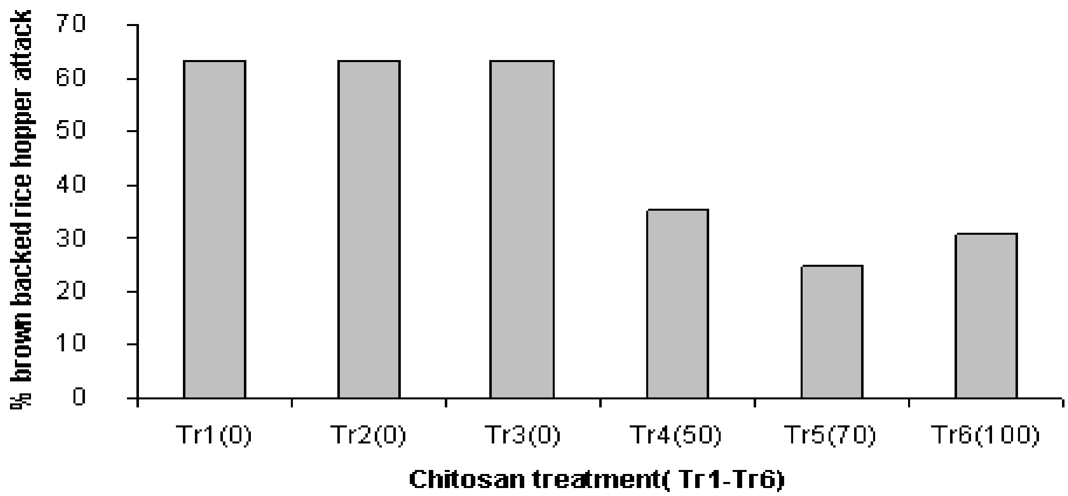


**Figure 1.** The effect of different chitosan doses on brown backed rice plant hoppers.

dedicated way under this study showed positive and promising effects in increasing rice yield in general and inhibiting commonly seen brown backed rice plant hoppers, in particular. Chitosan and chitosan solutions prepared following the proposed methods are more environmentally friendly, at low cost and can be applied at a large scale for mass production of rice and possibly for other agricultural plants.

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