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Effect of nanosilica/chitosan hybrid on leaf blast and blight diseases of rice in Vietnam

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Abstract

Nanosilica/chitosan (NSi/CTS) hybrid material was prepared using nanosilica (32.5 nm) from rice husk ash (RHA) and chitosan (CTS), and characterized by transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD). The obtained NSi/CTS was used for protection of rice leaf from blast disease (*Piriculariaoryzae*) and blight disease (*Xanthomonasoryzae*). Results indicated that foliar spraying of NSi/CTS with 100 ppm NSiand150 ppm CTS were effective against blast and blight diseases on rice (*Oryza* spp.). The leaf blast disease index (DI) (1.49 %) and the blight DI (1.45 %) were significantly decreased compared with control of 8.08 % and 9.29 %, respectively at 14th day after the first treatment. Thus, NSi/CTS hybrid material is promising to use for controlling plant diseases, particularly for rice.

Keywords. Nanosilica, chitosan, blast, blight disease, rice.

1. INTRODUCTION

Rice husk ash (RHA) with rich silica content (80-90%) is one of the agricultural by-products. Silica has been widely used in vegetable oil refining, medicine, detergents, adhesives, and ceramics [1]. Various methods have been reported for preparing silica nanoparticles materials in the literature, such as sol–gel [2-4], precipitation [5], hydrothermal and pyrolysis method [6, 7]. CTS attracted much attention from the research communities worldwide due to their unique properties such as biodegradability, bio-compatibility,… and was used in various practical application fields such as in medicine, in foodstorage [8-10],in agriculture [11]... CTS, a weak polybase, is an excellent candidate for the modification of acidic oxides to produce hybrid organic–inorganic composite materials [12]. The development of CTS and nanosilica (NSi) based novel hybrid materials opens the possibility to combine both the advantageous properties of the silica and the attractive features of CTS in one material [4, 8, 13, 14]. Prevention of disease infection for rice plant depends primarily on good agronomic practices. *Piriculariaoryzae* fungus and *Xanthomonasoryzae* bacteria caused severe blast disease and blight disease on rice, respectively.

The excessive use of agrochemicals in agriculture may lead to negative effects in food products and in environment that cause health hazard and eco-toxicity concerns. Chitosan and chitosan complexes have been extensively studied to replace the toxic agrochemicals.

The objective of this study is to explore effect of NSi/CTS hybrid materials in protecting rice plant against microbial pathogens, particularly *Piriculariaoryzae, Xanthomonasoryzae*.

2. EXPERIMENTAL

2.1. Materials

RHA was obtained by burning rice husk from Dong Nai province, Vietnam. Silica was extracted from RHA. CTS with molecular weight of ~100 kDa and deacetylation degree of about 85% was a product of Institute of Applied Material Science - VAST. NaOH, glycerin and HCl were purchased from Merck, Germany.

2.2. Preparation of NSi/CTS

NSi was prepared according to the procedure of Witoon et al. [14]. NaOH solution (3.5 M) was added to the RHA and boiled for 5 h in a Pyrex three-neck round-bottom flask equipped with a reflux condenser in a hemispherical heating mantle to produce a sodium silicate solution [3]. The solution was filtered and washed with boiling distilled water. The pH of sodium silicate was adjusted to about 6.5 by HCl 0.5 M.

CTS (2.0%, w/v) solution was prepared by dissolving chitosan in aqueous lactic acid solution (1.0%, w/v). Subsequently, RHA-derived sodium silicate was slowly added into the CTS solution, and the mixture was stirred at 60 °C. The resulting mixture was aged at 60 °C for 8 h. The aged silica/chitosan mixture was dried at 105 °C for 2 h. The dried silica/chitosan was calcined at 550 °C for 4 h in atmospheric condition [14]. The obtained product (NSi) was used to prepare NSi/CTS hybrid material.

Mixture of 300 mL 5 % CTS + 200 mL 5% NSi+ 500 mL water was stirred and then treated with ultrasonic cation at 60 ^{o}C for 15 min. Finally, the mixture of NSi/CTS hybrid material with concentration of 1 % NSi and 1.5 % CTS was obtained. The functional groups of the NSi/CTS hybrid were analyzed by FT-IR technique using KBr pellets on FTIR spectroscopy (FT-IR 8400S, Shimadzu) over the wave number range from 4000 to 400 cm^{-1}. The X-ray diffraction (XRD) of NSi/CTS was recorded on a X-ray diffractometer, D8 Avance A25, Bruker, Germany. The particle size of NSi/CTS was performed using transmission electron microscopy (TEM), model JEM1010, JEOL, Japan.

2.3. Effect of NSi/CTS against blast disease and blight disease on rice leaf

The experiment arranged in greenhouse consists of 5 treatments for each disease separately as follows: six treatments using NSi/CTS, treatment 7: Trizole 75WP (0.12% Tricyclazole) for leaf blast disease, treatment 8: Visen 20SC (0.002 % Saisentong) for blight disease, and control 1 (water) for leaf blast disease and control 2 for blight disease. The area of each treatment was of 30 m^2. The first foliar spraying was carried out 30 days after sawing. The second foliar spraying was after seven days for the first spraying. The DI was surveyed after seven days for the second spraying. The DI was calculated using the following formula [15, 16]:

$$\text{DI}\ (\%) = \frac{N_1 + 3N_3 + 5N_5 + 7N_7 + 9N_9}{9N} \times 100$$

Where: N is total number of survey leaves; N$_1$, N$_2$, N$_3$, N$_4$, N$_5$ are the infected leaves at each level 1, 2, 3, 4, 5, respectively.

Pathogen levels were divided:
Level 1: 0 - < 5 % of infected area of rice leaves
Level 3: 5 - < 10 % infected area of rice leaves
Level 5: 10 - < 25 % infected area of rice leaves
Level 7: 25 - < 50 % infected area of rice leaves
Level 9: $\geq$ 50 % infected area of rice leaves.

2.4. Statistical Analysis

The data was subjected to Analysis of Variance (ANOVA) using IRRISTAT software (IRRI, 2003). Treatment means were compared using Least Significance Difference (LSD) values at $P \leq 0.05$. Differences among treatments were tested by Ducan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

3.1. NSi/CTS hybrid material

NSi/CTS hybrid material was formed by the reaction of partially co-condensed NSi with CTS. The formation of the Si–O–C bonds and hydrogen (N:H) bonds and uniform dispersion of silica in CTS matrix were presented in Fig. 1 [10].

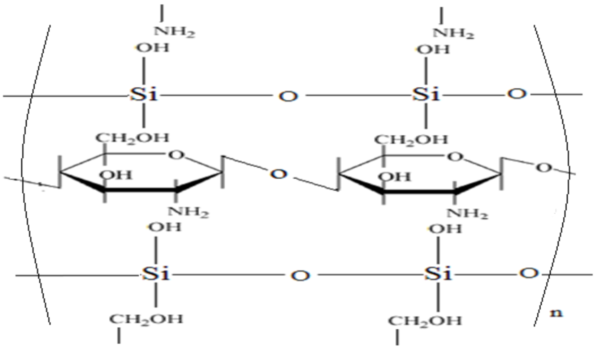

Figure 1: Schematic representation of interaction between nanosilica and CTS

In the FTIR spectrum of chitosan (Fig. 2a), the band at 3,461 cm^{-1} was assigned to the stretching vibrations hydroxyl groups (O-H) bound with carbon atoms. Intensive bands at 2,800 to 3,000 cm^{-1} were assigned to the C-H stretching vibrations. The band at 1,598 cm^{-1} corresponds to the deformation vibrations of -NH$_2$; 1,419 and 1,384 cm^{-1} for C-H bending vibrations, 1,321 cm^{-1} for asymmetric C-O-C stretching vibrations, and 1,080 cm^{-1} for C-O stretching vibration of CH-OH were detected. The FTIR spectrum of the synthesized composite (Fig. 2b) had a shift of the band 1,591 cm^{-1} of -NH$_2$ deformation vibrations in comparison with the spectrum of the initial chitosan. An intensive band at 1,087 cm^{-1} represents the Si-O stretching vibrations.

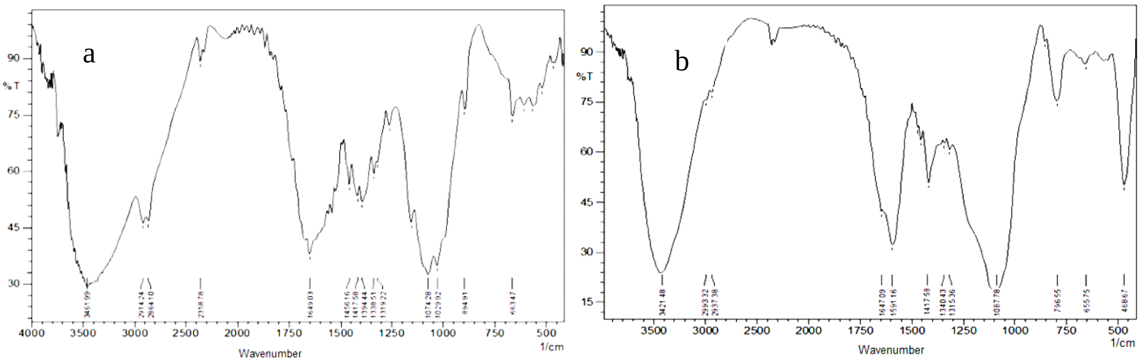

Figure 2: FTIR spectra of chitosan (a) and NSi/CTS (b)

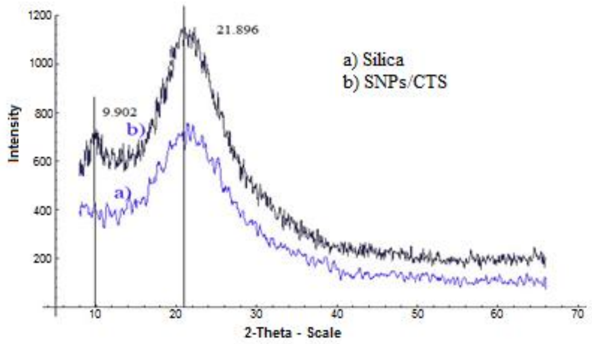

Figure 3: XRD patterns of nanosilica (a) and NSi/CTS (b)

XRD patterns of NSi and NSi/CTS were shown in Fig. 3. For NSi, there was only one peak at 2θ = 21.9° (Fig. 3a). It indicated an essentially amorphous form of silica [3, 5-7]. The XRD pattern of NSi/CTS in Fig. 3b showed one peak at 2θ = 9.9° (characteristic peak for CTS) and one peak at 2θ = 21.9° (characteristic peak for both CTS [17] and NSi). The average particle size of the NSi in NSi/CTS sample was of 32.5±13.8 nm measured by TEM in Fig. 4.

3.2. Effect of NSi/CTS against rice leaf blast disease

The results in table 1 showed that all treatments were significantly effective against leaf blast disease compared to control. DI of the treatments with 100 ppm NSi + 150 ppm CTS (1.58 %), 125 ppm NSi + 175 ppm CTS (1.49 %) and 0.12 % Tricyclazole (1.45 %) was not significant difference for 14 days after first spraying. Result indicated that NSi/CTS had equivalent effect to commercial pesticide (Trizole 75WP) against leaf blast disease. It was clear that foliar spraying of NSi/CTS suppressed remarkably blast disease for rice leaf in comparison to control (water) with DI of 8.08 %.

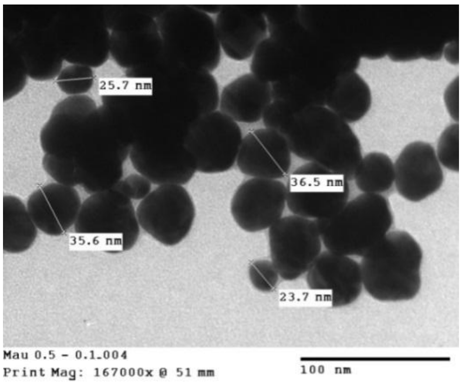

Figure 4: TEM images of NSi/CTS

Table 1: Effect of NSi/CTS on DI of leaf blast disease of rice

Treatment	Content of active ingredient	DI %	
		Pre-treatment	14 days after first treatment
NSi/ CTS	75 ppm NSi + 125 ppm CTS	0.68±0.02a	3.48±0.25b
	100 ppm NSi + 150 ppm CTS	0.59±0.01a	1.58±0.11c
	125 ppm NSi + 175 ppm CTS	0.58±0.01a	1.49±0.13c
Trizole 75WP	0.12% Tricyclazole	0.55±0.01b	1.45±0.13c
Control	Water	0.60±0.02a	8.08±0.59a
Least significant difference, 5%		0.11	1.54
Coefficient of variation, %		0.07	0.92

Different letters in the same column indicate significant differences at $P < 0.05$.

3.3. Effect of NSi/CTS against bacterial blight disease

The results in table 2 also showed that all treatments were significantly effective against leaf blight disease compared to control. DI of the treatments with 100 ppm NSi + 150 ppm CTS (1.50 %), 125 ppm NSi + 175 ppm CTS (1.66 %) and 0.002 % Saisentong (1.45 %) was not significant difference. The effect of NSi/CTS was as same as commercial pesticide (Visen 20SC) against blight disease. Thus, foliar spraying of NSi/CTS also suppressed remarkably blight disease for rice leaf in comparison to control (water) with DI of 9.29 %.

Table 2: Effect of NSi/CTS on DI of blight disease of rice

Treatment	Content of active ingredient	DI %	
		Pre-treatment	14 days after first treatment
NSi/CTS	75 ppm NSi + 125 ppm CTS	0.76±0.02a	3.33±0.74b
	100 ppm NSi + 150 ppm CTS	0.68±0.02a	1.50±0.12c
	125 ppm NSi + 175 ppm CTS	0.94±0.02a	1.66±0.13c
Visen 20SC	0.002% Saisentong	0.80±0.01b	1.45±0.11c
Control	Water	0.70±0.01a	9.29±1.29a
Least significant difference, 5%		0.17	1.54
Coefficient of variation, %		0.09	0.92

Different letters in the same column indicate significant differences at $P < 0.05$.

4. CONCLUSION

NSi/CTS hybrid material was prepared using nanosilica (32.5 nm) from RHA and chitosan from shrimp shells. NSi/CTS exhibited strong effect against leaf blast and blight diseases of rice. The prepared NSi/CTS hybrid material can be considered as an effectively new product for controlling plant diseases, particularly for rice plant.

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